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Proteins as specific polar media for charge transfer processes

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Contents

Abstract. A great many of biological processes involve the transfer of charged particles: electrons and protons. The electrostatic interaction of a moving charge with its surroundings, primarily with protein, is the major factor determining the thermodynamics and kinetics involved. This paper treats protein as a pre-organized highly-polar low-dielectric medium that possesses a pre-existent intraprotein electric field, a low reorganization energy, and a wide range of dielectric relaxation times. These specific protein features are the factors controlling the catalytic acceleration of biochemical reactions. Methods for quantitatively calculating the energetics of a number of typical processes are considered, and examples of their application are given.

1. Introduction

Protein functions in living nature are numerous and diverse. One of the main properties of protein is its high enzymatic activity, i.e., the ability to catalyze a variety of biochemical processes, with reactions being markedly accelerated under rather mild external conditions (temperature, pressure, pH values, etc.) characteristic of living organisms. Various physicochemical aspects of enzymatic processes are considered in many reviews, including some recent ones [1–10].

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A characteristic property of enzymes is specificity, i.e., the ability to accelerate reactions of a given (usually very narrow) class involving a limited number of substrates. Evidently, such behavior of enzymes is related to the specific structure of concrete classes of proteins. At the same time, enzymes having different chemically active sites and spatial structure share the property of high catalytic activity, which suggests that some essential features of enzymes are due not only to their specific structural features but also to certain general physical principles underlying their action and stemming from peculiarities of their architectonics.

The overwhelming majority of enzymatic reactions include the stage of charge transfer, e.g., such formally electroneutral processes as hydrolysis of amide bonds or cis-trans isomerization of unsaturated compounds. These reactions proceed through consecutive attachment and detachment of protons. Clearly, the physical principles of enzyme functioning can be explained in terms of the modern theory of charge transfer in condensed media [11–24]. Two types of processes are considered in the framework of this theory: the motion (transfer) of so-called quantum (fast) particles (electrons and sometimes protons) and the motion of slow classical particles (transfer of heavy ions, re-orientation of solvent dipoles, etc.). In the bound state, the former and the latter are characterized by high and low eigenfrequencies, $\hbar\omega \gg k_B T$ and $\hbar\omega \ll k_B T$, respectively. The present review is focused on widespread cases of electron and proton transfer. It should be borne in mind that reactions accompanied by the transfer of classical particles are not infrequently coupled to the transfer of quantum particles.

A theoretical analysis of the reaction rate implies calculation of two quantities: free activation energy and the pre-exponential factor. The free activation energy determines the probability of achieving a classical subsystem configuration corresponding to the top of the energy barrier while the pre-exponential factor is determined by the probability of transfer

of the quantum particle at a given transition configuration. One of the main matter-of-course classical generalized coordinates of the system in charge transfer reactions is electrical polarization of the medium; this polarization interacts with the charge being transferred.

According to the charge transfer theory, the activation energy of the elementary electron or proton transfer act proper, $\Delta G^{\neq}$, is determined by two parameters, viz. the reorganization energy of the classical subsystem λ and the free energy of the elementary act ΔG:

$$\Delta G^{\neq} = \frac{(\lambda + \Delta G)^2}{4\lambda} . \quad (1)$$

The relationships described by Eqn (1), or the Marcus equation, are illustrated by Fig. 1. The reorganization energy is the energy necessary to pass from the equilibrium coordinates of the initial state to those of the final one, with the charge distribution corresponding to the initial state. For example, the reorganization energy for reaction $A^+ + B = A + B^+$ is the difference between the non-equilibrium energy of state $A^+ + B$ at coordinates matching the equilibrium state $A + B^+$ and the equilibrium energy of state $A^+ + B$ at the respective equilibrium coordinates.

The free energy of an elementary act ΔG is the difference between equilibrium energies of the states immediately after charge transfer and just before it. For the simplest case of a reaction like $A^+ + B = A + B^+$, the energy ΔG coincides with the standard free energy ΔG_0 (abstracting for a while from reactant approaching energy). Evidently, in the general case, ΔG must be independent of reactant concentration. This property is intrinsic in the so-called configurational free energy ΔG_c, i.e., the free energy lacking in the component associated with permutation entropy. The configurational free energy differs from the standard energy ΔG_0 when the number of molecules changes during the reaction or when different standard states are chosen for the reactants and products [25]. Equation (1) holds for non-adiabatic charge transfer processes (prevailing in biological systems). In adiabatic processes characterized by strong donor-acceptor interactions, the considerable splitting of terms takes place in the area where the curves of initial and final states intersect; it results in a substantial reduction of the activation energy.

Equation (1) implies the parabolic dependence of the energy on all classical coordinates. Under this condition, both λ and ΔG_0 can be represented as the sums of contributions attributable to the inherent properties of reactants (λ_i and ΔG_i) and contributions from the interaction of the charge and its environment (λ_s, ΔG_s). The first constituent is the same for reactions proceeding in an aqueous medium and enzymes (reactions of identical active groups being compared), whereas the second constituent (both λ_s and ΔG_s) is essentially dependent on the ambient environment. Because enzymatic reactions, as a rule, occur within a protein macromolecule, proteins function not only as reactants but also as reaction media [26]; hence, the necessity to consider the properties of proteins as a specific dielectric medium.

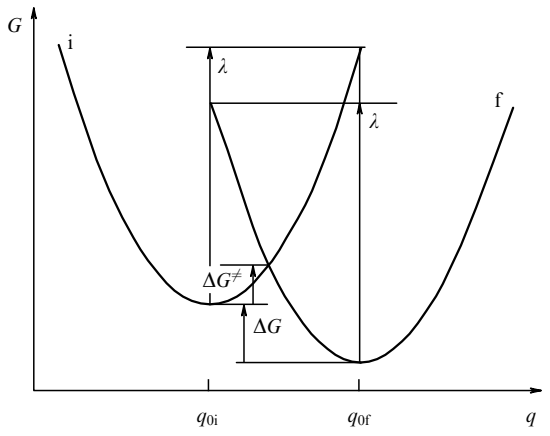

Figure 1. Energetic profile of a charge transfer reaction. The curves of initial (i) and final (f) states, q_{0i} and q_{0f}, are equilibrium coordinates of the classical subsystem (medium polarization) in the initial and final states.

Section 2 of this review provides a general description of the properties of proteins as specific condensed polar media. Sections 3 and 4 deal with the influence of these properties on the equilibrium energies of charging processes, as well as the energies of reorganization and activation of charge transfer processes. Section 3.3 overviews methods for the calculation of equilibrium energies, Section 3.3.1 describes the general approach to such calculation, and Section 3.3.2 focuses on the estimation of parameters of electrostatic computation (it is designed for readers interested in the technical aspects of such calculations). Theoretical and experimental findings are compared in Sections 4.4 and 4.4.3, both starting from a discussion of main results and conclusions followed by examples of the application of general principles to concrete biological systems. Section 5 summarizes the results of the discussion.

2. Proteins as polar media for charge transfer reactions

The main principle underlying protein architectonics is the formation of a linear sequence of amino acid residues linked together by strong peptide bonds and rather tightly packed into secondary and tertiary structures. The main elements of this structure, peptide groups, are characterized by a large dipole moment. For example, dipole moments of many free amides are close to 4 D [27]; there is evidence that the formation of certain secondary structures, such as α-helices, leads to mutual polarization of peptide bonds and enhancement of their dipole moment up to $\sim$ 6 D [28–30].

Tight packing of peptide sequences and polar side chains inside certain structures substantially restricts the ability of protein dipoles to change orientation (to reorient) under the action of an external electric field. Of course, the dipoles retain some mobility, as shown both in experiment and by molecular dynamic simulations, but their orientation varies within fairly narrow limits. This has two important consequences [31].

First, the dielectric response of a protein to the external electric field proves very weak, and its static dielectric permittivity is low (for certain dry proteins, dielectric permittivity was found to be $\sim 3.5-4.0$ [32–36]). Similar values were obtained with different variants of molecular dynamic modeling for selected regions inside protein molecules [37–43]. At the same time, liquid low-molecular weight amides with practically the same or a lower concentration of polar amide groups are characterized by high dielectric permittivity, amounting in N-methylformamide to 180 [44]. As shown in Section 4, the low static dielectric permittivity of proteins accounts for a sharp decrease in the reorganization energy of charge transfer reactions in these media.

Second, an important difference between proteins and low-molecular weight polar solvents lies in the existence of a

permanent intraprotein electric field [31, 45–47]. The electric potential created by dipoles at any point of a low-molecular weight liquid at an arbitrary moment of time generally differs from zero. However, random fluctuations of dipole orientation make the time averaged potential equal to zero. The averaged dipole orientation ceases to be arbitrary only after an ion is introduced into a given point; then, the potential created by the dipoles takes a definite value. Unlike dipole orientation in liquids, that in proteins is determined by their structure. Certainly dipole orientation in proteins undergoes fluctuations (otherwise, their static dielectric response would be absent), but the permissible variations are very limited, which accounts for the existence of a permanent time-averaged electric field at any point in the protein molecule. The field is present prior to the introduction of a new free charge; therefore, it may be called a pre-existent field. Dipoles in proteins are intrinsically ordered, whereas in liquids their ordering develops only after the introduction of a charge. For this reason, proteins can be described as 'pre-organized polar media' [31].

The energy of interaction between charge q and point dipole μ is expressed as $q\mu\cos\theta/R^2$, where R is the distance between the charge and the dipole, and θ is the angle between the dipole moment and the radius-vector $\mathbf{R}$. $n\pi R^2$ dipoles are localized R apart from the charge (n is the mean concentration of dipoles per cross section area). Thus, the total contribution of a certain dipole layer to the interaction energy is $q\mu n\pi\cos\theta(R)$, where $\cos\theta(R)$ describes the mean dipole orientation at the distance R. Dipole orientation in a liquid dielectric is determined by competition between the effects of the external field and the thermal motion. Because the field decreases in inverse proportion to the squared distance, $\cos\theta(R)$ rapidly decreases with distance from the free charge. As a result, the greater the distance between the dipoles and the charge the smaller the total dipole contribution to the dipole-charge interaction energy.

The case of a pre-organized dielectric is altogether different. The pre-organized dipole orientation in such a medium stipulated by the structure is charge field-independent. Therefore, dipoles localized far from the charge are likely to have a more advantageous orientation than those close to the charge. Because the number of dipoles R apart from the charge increases with R, their contribution to the energy of interaction between the charge and the pre-existent field may be greater than that of the nearby dipoles and even have a different sign. This simple point dipole model qualitatively adequately reflects the main difference between pre-organized and structureless media. As regards quantitative analysis, the calculation of potentials created by partial charges of protein atoms at different points of the active site of α-chymotrypsin revealed both the prevalence of the contribution from distant amino acid residues in certain cases and the change of the potential sign [47–49].

It follows from the foregoing that proteins are highly-polar low-dielectric media, i.e., they exhibit a combination of properties that can never be found in low-molecular weight liquids showing practically unlimited dipole mobility. Therefore, there is no sense, in the case of proteins, in setting high polarity against low dielectric permittivity.

In real biological systems, proteins practically never function as the sole dielectric phase; they are usually in contact with other dielectrics, e.g., water or lipids. The long-range action of Coulomb forces accounts for the meaningful interaction of charges with a non-protein phase that must be necessarily taken into consideration in the quantitative description of charge transfer processes. The introduction of certain effective dielectric permittivity sometimes practiced to provide an averaged description of the response of the two phases is a rather rough approximation having no clear physical sense (see, for instance [50]).

The aqueous environment influences both the energy of interaction between the charge and dipoles created by polarization (the presence of water is known to always enhance such interaction by improving ion solvation) and the strength of the pre-existent field. The latter is due to the shielding of the charge field in a protein by polarization of its aqueous environment, characterized by high dielectric permittivity. This effect was first reported in quantitative terms in the classic work of Tanford and Kirkwood [51] for the field of charged ionogenic groups in proteins and taken into account for dipole fields in the microscopic model of Warshel and Levitt [46]; it was described in the framework of a semi-continuum model in Refs [49, 52]. At present, a few programs, e.g., DelPhi [53] and MEAD [54], are available for the electrostatic calculation of arbitrarily shaped macromolecules dipped into an electrolyte solution. The point in question is the numerical solution of the Poisson–Boltzmann equation (solution of the Poisson equation taking account of the Boltzmann distribution of electrolyte ions in the Debye–Huckel approximation).

An interesting feature worthy of note is that shielding always weakens the field of a unit charge, whereas the dipole field in a certain space region may be strengthened. It is easy to see if charge shielding by polarization of the external aqueous phase is described as the superposition of the charge field and the field opposite to the sign of the image in the aqueous phase. If one of the dipole poles, e.g., the positive one, is closer to the interface than the other, the negative charge of its image is also closer to the interface than the positive charge of the other image. Then, the potential created by the image dipole at any point in the protein molecule is negative. In the region of the protein phase where the primary dipole potential is positive, the superposition of the image field decreases the potential; in the region with the negative primary potential, it assumes an even higher negative value under the effect of the image field, i.e., its absolute value increases. A few cases of such behavior were revealed by calculation of the partial charge field in α-chymotrypsin, in the presence of the aqueous environment and without it [49].

Structural constraints on the mobility of dipoles affect not only the permissible degree of their orientation but also their rotation rate. A wide variety of different-scale movements are known to occur in proteins, with different activation barriers and, therefore, with different characteristic times [55–57]. Dipole rotation associated with different types of protein movements correspond to different dielectric response rates reflected in the broad spectrum of dielectric relaxation times (from hundreds of femtoseconds to a few seconds). This situation can be phenomenologically described as a set of effective dielectric permittivities, each corresponding to a certain time interval [58, 59].

To sum up, the consequences of common principles of protein architectonics are low dielectric permittivity, the presence of a constant intraprotein electric field, and a wide variety of dielectric relaxation times. How these specific features of proteins as dielectric media manifest themselves in the catalytic activity of enzymes will be shown in Sections 3 and 4.

3. Equilibrium free energy of charge transfer in proteins

3.1 Electrostatic features of proteins as pre-organized media

As shown in Section 2, the free energy of a charge transfer elementary act is the sum of the component depending on the inherent properties of reactants (ionization, the breakdown or change in the length of bonds, etc.) and the energy of interaction between the charge and the environment. The latter energy, in turn, comprises two components. One is the energy of polarization by the charge of the dielectric medium, ΔG_{pol}. The polarization energy generated by the dielectric response of the medium is manifested in both low-molecular weight liquids and proteins, where it differs only quantitatively in agreement with the difference between static dielectric permittivities.

Usual polarization of the medium by the charge is supplemented in protein media by a qualitatively new effect absent in liquid dielectrics; namely, the pre-existing field effect mentioned in Section 2. A charge introduced into a protein (or transferred from one of its points to another) interacts not only with the polarization it induces but also with the pre-existing field:

$$\Delta G_{\text{s}} = \Delta G_{\text{pol}} + \Delta G_{\text{p.f.}} . \qquad (2)$$

The effect of the pre-existing field of dipoles (and not only of superficial charged groups) described in the early 1970s [31, 45–47] was later investigated by many authors. However, the analysis of electrostatic effects in proteins has until recently been inconsistent due to the ambiguity of the notion of dielectric permittivity used in these calculations. Specifically, it was shown that the effective dielectric permittivity of proteins differs depending on the objectives of research, which renders this parameter physically senseless (see, for instance [37, 50, 60, 61] and the comprehensive Ref. [62]).

Ideally, a fully microscopic description of a protein should yield electron density (hence, electric field) distribution over the entire protein molecule, avoiding the use of the notion of dielectric permittivity (actually, at a vacuum value of $\varepsilon = 1$). However, the baffling complexity of the system dictates the necessity of simplifications. We substitute the true electron density distribution over all atoms of the protein by certain effective partial atomic charges obtained in quantum chemical calculations of small molecules or their fragments and roughly describing the field outside these molecules (see Section 3.3.2 for parametrization of these charges). Further on, we have to take into account that the field of each fragment distorts the electron density distribution of all other fragments by inducing respective dipole moments. Sequential microscopic calculation requires the self-consistent finding of these moments by means of multiple iterations [46, 63]. This approach allows the intraprotein field at fixed atomic coordinates to be found. Thereafter, the introduction of a new free charge will require not only recalculation of the field of induced electron dipoles but also consideration of a shift of heavy atoms toward new equilibrium coordinates. Such a procedure of purely microscopic calculation is highly laborious and requires a number of simplifications and approximations, even if powerful computers are used.

Another, semi-microscopic, method implies a much smaller amount of computation and has (in our opinion) a clearer physical sense. Also, it more naturally agrees with the calculation of reorganization energy (see Section 4.4.1).

In this review, we mainly rely on the results of the semi-microscopic (also termed semi-continuum) method. Certainly, the two methods (fully microscopic and semi-microscopic) yield, in principle, correct quantitative results, provided all attendant factors are taken into account. Their practical application inevitably involves a number of approximations, which are different for different approaches. It is therefore essential to have the possibility of comparing the data obtained by both methods (such a comparison is reported in Section 4.4.3).

In the framework of the semi-continuum approximation, mutual electronic polarization of protein molecule fragments is averaged and described by a certain mean electronic (optical) dielectric permittivity ε_{o}; in other words, protein is described as a system of partial charges embedded in a continuous medium with permittivity ε_{o}.

Characteristically, it is exactly optical permittivity ε_{o} that is used to calculate the pre-existent field, because the coordinates of all the atoms are specified and their shift under the action of the pre-existing field should not be taken into consideration (the atoms came to equilibrium positions determined by their interaction during formation of the protein structure).

This approach is semi-continuum, because the medium is described continually taking account of definite discrete localization of point-like partial charges. The appearance of a new free charge in such a medium (attachment to a group of electrons or protons) causes both displacement of heavy atoms (and/or dipole rotation, an analog of atomic and orientational polarization of ordinary dielectrics) and polarization of all electron shells. In this case, responsiveness being inherent in all types of polarizability, the effect of the interaction of the new charge with the polarization it induces is determined by total, i.e., static, dielectric permittivity ε_{s}. This brings us to an algorithm of ΔG_{s} calculation with which $\Delta G_{\text{p.f}}$ and ΔG_{pol} are computed using protein ε_{o} and ε_{s}, respectively.

Let us consider a case when a certain group, e.g., one of the redox centers, remains uncharged under conditions in which the protein structure is determined. Charging this group would cause a change in protein dipole orientation. Therefore, the potential created by its charge at other points must be calculated with the use of static permittivity ε_{s}. This potential is added to the one created by all the remaining charges (total and partial) of the pre-existent field and computed based on optical permittivity ε_{o}.

The approach proposed in our papers [64, 65] resolves the above uncertainty arising from the attempt to describe proteins using effective dielectric permittivity alone. The computational method proposed in Refs [64, 65] permits avoiding the contradiction (noticed earlier by Warshel et al. [41]) inherent in the previously applied algorithm in which ε_{s} was used to calculate the two components of the energy. Indeed, ε_{s} is used on the assumption that permanent dipoles in both cases not only create an intraprotein field but also screen it. The fact of the dipole field being screened by electronic polarization alone described by ε_{o} eliminates the physically unsound proposition that the permanent dipole field is shielded by the dipoles themselves.

A similar idea to use static dielectric permittivity to calculate only polarization energy was suggested by Simonson et al. [66]. However, these authors disregarded the role of

optical permittivity and calculated the pre-existent field effect at $\varepsilon \approx 1$; in other words, they practically neglected the influence of electron polarizability.

One more circumstance is worthy of note. In real biological systems, a protein is surrounded by a different medium, in the first place water; therefore, calculations must be performed for a heterogeneous system. Unlike atomic coordinates in proteins, the coordinates of the surrounding water molecules are not fixed, and its dielectric response includes all types of polarizability, meaning that the pre-existing field must be calculated based on ε_o for the protein but on static permittivity ε_{sw} for water.

As shown above, ΔG_{pol} is computed using the static permittivity of both the protein and the surrounding water. Since the ε_s of protein is much smaller than that of water, the absolute value of energy ΔG_{pol} in the protein-water system is significantly lower than in the purely aqueous environment. This makes charge transfer from water into a low-dielectric medium energetically disadvantageous. An ion can exist in the protein environment only when the loss of the solvation energy is compensated for by the action of the intraprotein pre-existing field. Such more or less full compensation was first reported in Refs [67, 68] and thereafter has been observed many times by different authors [69–73]. A drawback of these studies is that the pre-existing field was calculated with the use of protein static permittivity; the use of optical permittivity enhances the field and makes compensation for the low solvation energy a more reliably established fact. As shown in Section 3.4, it is the use of optical permittivity alone that leads to the agreement between theory and experiment.

3.2 Continuum calculation of ion solvation energy in model solvents

The title of this section reflects a very extensive issue. This review is focused on two aspects. First, certain solvents can be used as models to estimate validity limits of continuum electrostatics for some ions. Second, a usual application of electrostatic calculations to proteins is the computation of the energy of ion transfer into proteins from a solution for which the thermodynamic properties of a given ion are known from experiment. This approach needs to be verified.

The dielectric response of proteins, generally speaking, may be different from the strictly continuum one due to the effect of medium discreteness [74]. The validity limits of macroscopic determination of protein dielectric permittivity remain to be estimated theoretically, even if the use of the notion of dielectric permittivity sometimes allows adequately describing the situation of interest [4]. Anyway, there is little doubt that a necessary condition for the application of continuum description to a selected ionogenic group of proteins is that they must hold for the same group in a simpler, but also discrete, medium, e.g., liquid solvents.

Electrostatic computations make it possible to find the so-called chemical energy of ion solvation, i.e., the difference between the ion chemical potential in the solvent and the gaseous phase. However, direct experimental measurement of this characteristic is impossible. Any thermodynamically substantiated measurement of the work of a process with ion participation reduces to the measurement of the electrochemical potential difference:

$$\tilde{\mu} = \mu \pm e\varphi\,. \tag{3}$$

Here, the plus and minus signs refer to the cation and the anion, respectively. The electrochemical potential has the chemical constituent μ, totally determined by the properties of the ion, including its interaction with the medium, and the energy in the electric field $\pm e\varphi$, where φ is the potential of a given phase with respect to a certain reference value, and e is the electron charge (for simplicity, we shall consider singly charged ions). In this case, φ is determined by the properties of the phase as a whole, rather than by the properties of an individual ion. μ and φ can not be measured separately; to this end, one has to resort to extrathermodynamic assumptions.

As is known, the Born equation most widely used in continuum electrostatics is inapplicable to the quantitative description of small ions, because the electric field near an ion is very high. On the one hand, it causes dielectric saturation of the medium. On the other hand, a field rapidly changing with distance significantly enhances the effect of medium discreteness. In an ideal continuum, polarization at each point is totally determined by the field at a given point; i.e., it is local. In a discrete medium represented by a real solvent, polarization at a given point depends also on the fields at the neighboring points, because orientation of a single dipole (polar bond) of the molecule is correlated with orientation of another dipole of the same molecule (non-local dielectric response) [75–77]. The characteristic correlation length is on the order of the radius of a solvent molecule (or molecular cluster), giving reason to expect that the system's behavior becomes closer to the continuum one when the ion size is much greater than the size of solvent molecules (small field variations over the correlation length). A large low-charge ion does not cause an appreciable dielectric saturation, either.

It proved possible to verify the applicability of continuum electrostatics by experimental measurement of the sum of chemical energies of cation and anion transfer. The main result of these studies [78–85] is that the Born equation quantitatively describes the solvation energy of large compact ions, e.g., metallocenes, in aprotic solvents. The validity of this inference is confirmed at different values of dielectric permittivity down to the low ones (7.2), similar to those characteristic of protein active sites. This provides a basis for the application of continuum electrostatic techniques to large prosthetic groups in proteins. The redox potential of metallocenes (ferrocene, cobaltocene) can be used as an internal standard for the comparison of potentials of different redox-pairs in aprotic media.

Marked deviations of energy values for anions calculated by the Born equation from the respective experimental data occur in the presence of proton donors, especially water [82, 85–87]. These deviations are due to the formation of a hydrogen bond between the donor molecule and anion.

Unlike most solvents, water has a well-developed three-dimensional network of hydrogen bonds accounting for the strong correlation between orientations of neighboring dipoles at which the characteristic correlation length is significantly greater than the molecule size. As a result, the simple continuum description may prove inadequate, even for rather large particles. In particular, we demonstrated the inapplicability of the continuum description of water for large molecules of proflavin dye [88].

Experimental assessment of the influence of water structure on the redox-potential of cobalticinium was undertaken in a study on the effect of water supplementation with 30 volume percent of aprotic solvents destroying the hydrogen bond network [89]. This addition causes a considerable positive shift of the potential that is an order of magnitude or more greater than the one expected in accordance with the

Born equation due to the reduced dielectric permittivity of the mixed solvent. Detailed analysis of the data obtained allows the effect of water structure to be estimated at roughly 0.07 V.

Calculations based on the cobalticinium standard (including correction for the water structure) disregard one more factor, i.e., the pre-existing electric field. Numerous studies modeling aqueous solutions by molecular dynamics methods have revealed an asymmetric orientation of water molecules around a neutral molecule of dissolved matter creating a positive potential [90–96]. This potential, unlike the pre-existing field of proteins determined by their own structure, is caused by the asymmetry of short-range forces rather than by the solvent structure; hydrogen atoms have a spatially less extended electron shell and are, therefore, subject to weaker repulsion from a molecule of dissolved matter approaching it more closely than an oxygen atom [94]. Analysis of experimental findings in Ref. [97] made it possible to estimate this intraphase potential of water for cobalticinium ions at 0.1 V and to show that such a potential is negligibly small for certain aprotic solvents, including dimethylformamide, chemically close to proteins.

The continuum electrostatics approximation is extensively used in protein biophysics, e.g., for the calculation of redox-potential of the corresponding sites and pK of ionogenic groups (actually, variations of these quantities during the transfer of model compounds from water to protein are calculated). The above analysis shows that such an approximation is by no means satisfactory. Electrostatic calculations can be recommended for the comparison of the energies of sufficiently large ions and molecules in proteins and aprotic media based on experimental data obtained with appropriate solvents. Examples of such calculations are presented in Section 3.4.

3.3 Methods for the calculation of equilibrium charge transfer energies in proteins

3.3.1 General approach to the calculation of equilibrium energies. Calculated equilibrium energies in proteins are the sums of two components: the known energy of the process in a given medium and the energy of reactant transfer into the protein. The medium is either a vacuum or a solvent.

The energy of a vacuum process is either measured in experiment (e.g., ionization potential) or calculated by quantum chemical methods. Because the ionization potential relates to a process giving rise to a free electron, and because we are interested in the redox-potential with respect to the selected aqueous reference electrode (usually a normal hydrogen electrode), the vacuum energy should be compared with the respective energy for this reference electrode (the so-called absolute electrode potential of the reference electrode). For reasons analogous to those expounded in Section 3.2, the absolute electrode potential can not be measured directly; one has to resort to extrathermodynamic assumptions. The use of the so-called Trasatti potential, equal to 4.30 V, is especially well substantiated (see Section 3.4.2 for details).

As shown in Section 3.2, water is unsuitable as a model solvent due to its structuredness, marked intraphase potential, and capability of forming (in certain cases) hydrogen bonds with reactants. All these effects are beyond a simple continuum electrostatic description of the medium. Therefore, the solvents of choice are aprotic solvents, like dimethylforamide, chemically similar to the peptide bond. However, the use of non-aqueous solvents poses the problem of experimentally unmeasurable interphase potential at the solvent-water interface. This problem is also resolved with the use of extrathermodynamic assumptions. This goal is reached with minimum errors by using metallocene/metallocinium electrodes as the internal standard. Examples of such calculations are given in Section 3.4.2.

Calculations of pK encounter similar problems. Here, the absolute proton hydration energy and the energy of proton transfer from water into an aprotic solvent must be known too. These quantities are found based on the same extrathermodynamic assumptions (see Section 3.4.2).

The energy of transfer from a model medium into protein is computed electrostatically. The electrostatic energy in the model medium consists only of the energy of dielectric response (the model media are chosen such that the continuum calculation yields correct values). The protein electrostatic energy includes the dielectric response energy and the charge energy in the pre-existing field. As shown in Section 3.1, each of these constituents in the protein as a pre-organized medium must be calculated with the use of the respective dielectric permittivity, either static or optical.

3.3.2 Parameters of calculation of protein electrostatics. The optical dielectric permittivity of certain liquid amides is close to 2 [44]. However, at the practically identical polarizability of each link of the polypeptide chain, protein density is approximately 30% higher. According to the Clausius–Mosotti equation, that elevates ε_o to ~ 2.5 K. Extrapolation of refraction indices of α-chymotrypsin aqueous solutions yields similar values [88].

As mentioned in Section 2, experimental ε_s values for dry proteins are close to 3.5-4.0. Calculations give similar values for the core of the globule but much higher ones (~ 10) for the globule periphery of certain proteins [37–43]. Although these calculations are largely based on molecular dynamics methods and rather inconsistently take into account the electronic polarizability of proteins, they provide a general view of the magnitude of the static dielectric response. Some calculations have yielded substantially higher constants of up to ~ 30 [42]. However, being due to superficial high-mobility ionized groups, they do not actually characterize the dielectric medium inside proteins; in real systems, these groups are essentially shielded by the aqueous environment [43].

Active sites are frequently localized not in the center of the globe but closer to its surface, i.e., in those regions with somewhat enhanced dielectric permittivity. In the absence of exact data on the spatial distribution of dielectric permittivity, its averaged value may be taken as 5. Examples of the use of this approximation are presented in Section 3.4.2; also shown is the possibility of estimating (in certain cases) local dielectric permittivities based on independent experimental data.

If static protein dielectric permittivity ε_s is close to 4, then ε_i of the 'body' of this group for which pK or redox-potential is calculated must be similar to the respective optical value, because it is difficult to imagine an appreciable orientational polarization inside this group. Thus, we have a system of at least three dielectrics, viz., the group, the surrounding protein, and external water.

One more important problem is the choice of partial charges that are unmeasurable in experiment but determined as certain effective values describing the extramolecular field corresponding to the results of quantum chemical calculations. A consistent microscopic description of multiatomic (and multimolecular) systems encounters serious difficulties.

Therefore, various systems of parametrized partial charges have been proposed so as to describe in the best possible way experimental data by some simplified methods.

We deem it necessary to choose, in the first place, such values of partial charges that correspond to the experimentally measurable characteristic of a given molecule directly related to its charge density distribution, i.e., dipole moment. We note that the ambiguity of extraction of partial charge values from quantum chemical data accounts for the fact that different charge systems described in the literature are characterized by somewhat different partial charge distributions, even after reduction to similar dipole moments. Second, quantum chemical calculations give the molecule field in a vacuum with ε inside the molecule as unity, too. Because we describe proteins as continuous media with ε_o when calculating the pre-existent field, the same optical permittivity is automatically attributed to the inner region of each molecular fragment. Such 'filling' of a fragment with a medium having $\varepsilon_o > 1$ weakens its field. In order to retain the right field value outside the fragment, 'vacuum' partial charges should be multiplied by a coefficient close to $(\varepsilon_o + 2)/\varepsilon_o$ (the field of a dipole placed in a sphere with ε_o is $\varepsilon_o/(\varepsilon_o + 2)$ times weaker than the field of the same dipole in a vacuum). This coefficient should be derived only for the partial charges of dipoles or other electroneutral groups. Free charges must not be corrected (the field outside the charged sphere being independent of dielectric permittivity inside the sphere).

3.4 Comparison of calculated protein equilibrium properties with experimental findings

3.4.1 Overview of results. Section 3.4.2 contains examples of calculations for certain real proteins. We deemed it necessary to summarize the results of these studies for readers not interested in a detailed consideration of concrete systems.

We calculated redox-potentials of all eight co-factors of Photosystem I (PSI), ferredoxins, originating from two different organisms and the Rieske iron-sulfur protein. Moreover, we calculated pK of histidines in α-chymotrypsin. The calculations were made based on the experimental data obtained for solutions in dimethylformamide and ionization potentials in a vacuum computed by quantum chemical methods. In all cases, a reasonable agreement between theory and experiment was reached. Note that when experimental data is lacking or is of poor accuracy, the calculation permits drawing more definitive conclusions about reaction mechanisms.

It should be emphasized that the agreement with experimental findings was reached due to the consistent application of our new approach, i.e., the use of static dielectric permittivity in the computation of dielectric response energy and protein optical permittivity for finding the pre-existent field. The traditional approach taking into account only one (static) permittivity is fraught with serious errors amounting to 1 V. The accuracy of calculations is substantially improved by the correct use of extrathermodynamic assumptions described in this review and allowing us to move from the data obtained in a vacuum or a non-aqueous solvent to those in proteins. For the computations described in the literature, the errors inherent in this problem amount to ~ 0.3 V.

3.4.2 Examples of calculation of concrete systems. The examples below contain neither details of calculations nor a detailed discussion of experimental data; we confine ourselves to a description of the systems of interest and the main results.

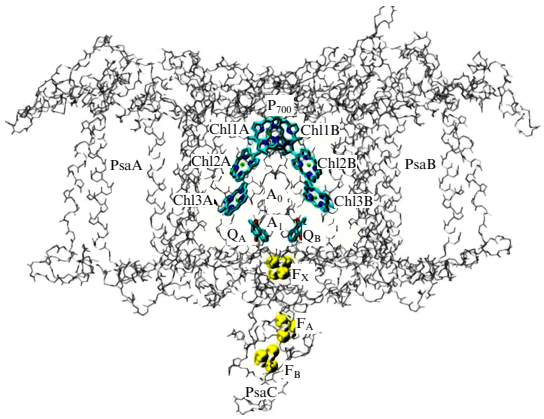

Figure 2. PFI structure (see in color online). The thin lines show the polypeptide backbone of three proteins, the thick color lines and symbols denote co-factors (indicated nearby). Proteins PsaA and PsaB are embedded into the lipid membrane, protein PsaC is outside it.

I. *Redox-potential of PSI co-factors. Calculations based on a comparison with experimental redox-potentials* [98].

PSI is one of the two electron transport systems intrinsic in photosynthesizing organisms that produce oxygen. PSI contains several redox-centers (co-factors) fixed within the protein matrix (Fig. 2). The co-factors are arranged into a chain extending from one side of the chloroplast membrane to the opposite one. The primary electron donor is the so-called P700 special pair, a dimer of two chlorophyll molecules, ChIIA and ChIIB (the letters A and B indicate that part of the co-factors are aligned in the form of parallel and largely symmetric chains A and B). As the pair absorbs light (absorption maximum at 700 nm), it becomes excited P^*_{700}. The high electron energy in P^*_{700} (large negative redox-potential) makes possible electron transport to the primary acceptor A_0 made up of two chlorophyll molecules, Chl2A and Chl3A (or Chl2B and Chl3B). Then, the electron is transferred to acceptor A_1 (one of the two plastoquinone molecules, Q_A or Q_B), and further on successively to three acceptors, F_X, F_A, and F_B, that are tetranuclear iron-sulfur clusters bound to the protein matrix through four amino acid (cysteine) residues $(RS)_4Fe_4S_4$ (here, RS briefly denotes a cysteine residue RSH in which hydrogen is substituted by iron). Thereafter, the electron is transferred from F_B to the soluble protein ferredoxin containing two analogous iron-sulfur clusters. Ferredoxin is involved in the subsequent series of synthetic reactions. On the other (donor) side of the membrane, the cation (P^+_{700} radical) formed after the special pair loses an electron is reduced by another soluble protein plastocyanin. The cycle being over, PSI reverts to the original state.

There are publications reporting unsuccessful calculations of redox-potentials for certain co-factors. Our calculations considering all cofactors in the framework of a consistent approach differ from those described in the literature in three aspects. First, the electrostatic energy of transfer was computed as substantiated above based on two dielectric permittivities, static and optical. Second, we took into account the heterogeneity of static dielectric permittivity of the protein complex. Third, we practiced successive transition from redox-potentials measured in a non-aqueous solvent, dimethylformamide (DMF), to the potentials with respect to an aqueous reference electrode, i.e., the standard hydrogen electrode (SHE) relative to which protein redox-center potentials are measured.

Measurements of electrogenesis, i.e., the potential difference arising from charge transfer between two co-factors, make it possible to estimate relative dielectric permittivity [99]. Indeed, the said potential difference is proportional to the charge transfer distance (normal to the membrane), which is known from X-ray structural analysis, and is inversely proportional to the dielectric permittivity of the respective region. Similar data have recently been reported for FSI [100, 101]. With the method described, dielectric permittivity is assessed in the framework of the model of several parallel layers with different ε_s. It can be expected that the relatively low-mobility polypeptide backbone makes a roughly identical contribution to the static polarizability of different protein regions. Essential differences are introduced by more mobile polar amino acid side chains. There is a significant correlation between the mean concentration of polar groups and ε_s values obtained by measurement of electrogenesis. In the middle part of a protein complex with a low concentration of polar groups, $\varepsilon_s = 3$; ε_s increases to 6–10 at the periphery of the complex. Co-factors P_{700}, A_0, and A_1 are localized in the region with $\varepsilon_s = 3$ and iron-sulfur clusters in the region with higher ε_s values.

Redox-potentials in non-aqueous solvents are usually measured relative to the aqueous reference electrode. However, such experimental values can not be directly used as reference points for the calculation of protein redox-potentials. As shown in Section 3.2, the quantity being sought contains an unmeasurable component, the interphase potential of liquid–liquid junction φ. Neglect of this constituent or incorrect attempts to obviate this problem are fraught with serious errors. Suffice it to say that the error in the estimated value of redox-potential Q/Q^{*-} in Refs [102, 103] amounts to 0.34 V.

The following approach was used in our calculations. We determined the potential of the electrode of interest, $X/X^{\pm}$, with respect to the ferrocene/ferricenium (Fc/Fc^+) electrode in the same solvent, DMF. Then, we moved to the potential with respect to the aqueous Fc/Fc^+ electrode, whose potential relative to the SHE was known (0.4 V). The extrathermodynamic estimates described in Section 3.2 were used for such a transition. As a result, the Fc/Fc^+ potential in DMF with respect to aqueous SHE was estimated at 0.43 V. This allowed the potential of the $X/X^{\pm}$ pair in DMF to be reduced to potentials with respect to the SHE.

The main results of calculations for PSI and soluble ferredoxins for two different organisms are presented in the table.

There is one more problem that was not discussed in the foregoing. Chlorophyll molecules are arranged close to one another in parallel planes, which creates conditions for conjugation of their π-orbitals and the related reduction in energy. The effect of conjugation for a special pair is the subject of many discussions, but the problem for A_0 remains unresolved. We undertook quantum chemical calculations for the mutual arrangement of Chl molecules known from X-ray structural analysis. The respective corrections are introduced into averaged potentials of each pair of Chl molecules; the result is presented in the table as the dimer potential.

Experimental values of the redox-potentials were obtained by direct redox-titration only for P_{700} and soluble ferredoxins; other potentials were found by indirect methods, which accounts for the wide scatter of the values. Making allowance for this fact and possible calculation errors, the agreement with experiment is quite satisfactory.

The Table shows contributions of various effects to the total potential. The contribution from the intraprotein field

Table. Redox-potentials of FSI co-factors and soluble ferredoxins. Major contributions to estimated values.

Reaction	Co-factor	*E* in DMF with respect to SHE, V	Effect of variations of dielectric response, eV	Itraprotein field effect, eVB	*E* (theory), V	*E* (experiment),
$P_{700} \rightarrow P_{700}^+$	Chl1A	0.54	0.30	−0.29	0.55	
	Chl1B	0.54	0.27	−0.23	0.58	
	Dimer				0.45	0.45
$A_0(A) \rightarrow A_0(A)^-$	Chl2A	−1.16	−0.35	0.09	−1.42	
	Chl3A	−1.16	−0.37	0.26	−1.27	
	Dimer				−1.23	−1.07÷−1.29
$A_0(B) \rightarrow A_0(B)^-$	Chl2B	−1.16	−0.36	0.08	−1.44	
	Chl3B	−1.16	−0.38	0.22	−1.32	
	Dimer				−1.27	−1.07÷−1.29
$A_1(A, B) \rightarrow$ $\rightarrow A_1(A, B)^-$	Q_A	−0.80	−0.41	0.54	−0.67	−0.75÷−0.81
	Q_B	−0.80	−0.41	0.37	−0.84	< -0.7
$Fe_4S_4^{2-} \rightarrow Fe_4S_4^{3-}$	F_X	−1.35	−0.94	1.70	−0.59	−0.65÷−0.71
	F_A	−1.35	−0.70	1.57	−0.48	−0.44÷−0.54
	F_B	−1.35	−0.73	1.53	−0.55	−0.47÷−0.59
Ferredoxin, Fe_4S_4-clusters						
A. vinelandii		−1.35	−0.44	1.14	−0.65	−0.64
C. acidiurici		−1.35	−0.38	1.27	−0.46	−0.43

depends on those from the fields of permanent partial atomic charges, charged amino acid side chains, and charged co-factors (P_{700}^{+}, $Fe_4S_4^{2-}$). The first contribution predominates, even though other contributions (for A_0 and A_1) may be comparable in terms of absolute value. It is noteworthy that field effects and changes in dielectric response (Born solvation energy) have opposite signs. These effects are on the whole mutually compensated for chlorophylls and quinones, but the field effect for iron-sulfur clusters remains uncompensated.

Let us consider conclusions about the mechanism of FSI action based on these calculations. The electron energy in the excited state of P_{700}^{*} is 1.77 eV higher than that in the ground state. Accordingly, the redox-potential shifts to the region of negative values: $0.45 - 1.77 = -1.32$ V, and its absolute value surpasses the estimated potential of the A_0 dimer (-1.23 V or -1.27 V). This means that the electron transfer from P_{700}^{*} to A_0 ('downhill') is energetically advantageous. However, this process would be disadvantageous if the primary acceptor were one of the monomers, either Chl2A (-1.42 V) or Chl2B (-1.44 V), rather than the dimer. This testifies to the necessity of conjugation between two chlorophylls.

It was hypothesized that the primary donor is Chl2 rather than P_{700}^{*} and the primary acceptor is Chl3 [104]. However, our calculations showed that such a process would be thermodynamically disadvantageous and, therefore, hardly probable (the respective values are not included in the Table). The primary donor for the photosystem II (FSII) with a similar structure is the special pair P_{680}. In the presence of a charge on Q_A, P_{680} remains the primary donor at $T = 278$ K, but this role is played by the chlorophyll monomer at $T = 77$ K, because the appearance of a new charge on Q_A creates an additional field determined by the static dielectric permittivity of protein as described in Section 3.1. The effective ε_s value at $T = 278$ K is roughly 4 but approaches 2.5 at $T = 77$ K when all atomic movements are practically frozen. Chl is closer to Q_A than P_{680}; therefore, the Chl field is stronger. At $T = 278$ K, the field is low and has practically no effect on the potential difference between P_{680} and Chl; however, this correction for the potential becomes essential at $T = 77$ K, and the absolute value of the Chl negative potential is higher than that of the potential of P_{680}. It is due to this that Chl becomes the primary donor [105].

Further electron transfer to quinones and F clusters proceeds 'downhill'. The transfer from Q_B to F_X is more advantageous than from Q_A. This inference agreeing with kinetic data would be impracticable based on experimental redox-potentials because of their ambiguity. However, the results of calculations, even if they do not pretend to accurately reflect absolute values, reliably demonstrate the relative values of the potentials of interest that are obtained in the framework of the same approximations and with the same parameters.

In order to assess the importance of new approaches to the computation of electrostatic energies proposed in our work, we made calculations by a traditional method, i.e., assuming common dielectric permittivity $\varepsilon = 4$ for all components. Such a comparison is especially demonstrative for co-factors with the largest electrostatic constituents, i.e., for ion-sulfur clusters. The following results were obtained: -1.46 V for F_X, -1.55 V for F_A, -1.44 V for F_B, -1.42 V and -1.20 V for *A. vinelandi* and *C. acidiurici*, respectively. Thus, the traditional approach resulted in errors on the order of 0.8–1.0 V, i.e., in utterly unacceptable results.

Preliminary results of our calculations for the structurally similar bacterial photosynthetic reaction center are also in reasonable agreement with experiment. The use of two dielectric permittivities proved necessary in this system.

II. *Redox-potential of the Rieske iron-sulfur protein. Absolute calculation of the potential* [106].

The Rieske iron-sulfur protein is a component of electron transport chains in practically all living organisms. Its physiological role consists of participation in the first stage of hydroquinone (QH_2) oxidation (different plant and animal hydroquinones) with subsequent electron transfer to cytochrome c_1 or *f*. The iron-sulfur cluster Fe_2S_2 is liganded through two cysteines and two histidines $(RS)_2FeS_2Fe(RIm)_2$ (here, Im is imidazole). In addition, one sulfur atom forms a hydrogen bond with serine. Unlike the tetranuclear clusters described above, there are no experimental data for the potential of an isolated Rieske cluster in any solvent. Therefore, an absolute calculation of the electrode potential is needed.

The overall electrode process

$$\mathrm{Red_{prot}} = \mathrm{Ox_{prot}} + \mathrm{e_{vac}}$$

can be represented as a sequence of three stages:

$$\mathrm{Red_{prot}} = \mathrm{Red_{vac}}\,, \quad \mathrm{Red_{vac}} = \mathrm{Ox_{vac}} + \mathrm{e_{vac}},$$

$$\mathrm{Ox_{vac}} = \mathrm{Ox_{prot}}\,.$$

Here, Red and Ox stand for the reduced and oxidized forms of the reactant, respectively, e is the electron, and the subscripts prot and vac point to particle localization in the protein and vacuum.

The standard electrode potential E^0 with respect to SHE can be calculated by the formula

$$FE^0 = \Delta G^0_{\mathrm{ion}}(\mathrm{Red}) - \left[\Delta G^0_{\mathrm{sol}}(\mathrm{Red}) - \Delta G^0_{\mathrm{sol}}(\mathrm{Ox})\right] - FE(\mathrm{H})\,, \tag{4}$$

where F is the Faraday constant, $\Delta G^0_{\mathrm{ion}}$ and $\Delta G^0_{\mathrm{sol}}$ are the standard Gibbs free ionization energies in a vacuum and solvation energies in the protein, respectively, and the constant $E(\mathrm{H})$ is given by the following relation:

$$-FE(\mathrm{H}) = \frac{1}{2}\,\Delta G^0_{\mathrm{dis}}(\mathrm{H_2}) + \Delta G^0_{\mathrm{ion}}(\mathrm{H}) + \Delta G^{\mathrm{chem}}_{\mathrm{hyd}}(\mathrm{H^+})\,, \tag{5}$$

where $\Delta G^0_{\mathrm{dis}}(\mathrm{H_2})$ is the Gibbs free dissociation energy of hydrogen atoms. The 'chemical' hydration energy is the free proton hydration energy $\Delta G^{\mathrm{chem}}_{\mathrm{hyd}}(\mathrm{H^+})$.

The so-called absolute hydrogen electrode potential defined by Reiss and Heller [107] is very frequently (practically always in the biophysical literature) used as $E(\mathrm{H})$. As shown in Ref. [108], this potential is actually indistinguishable from potential E_K suggested for the first time by Kanevskii [109]. The 'absolute' potential can be strictly computed thermodynamically from experimental data as the sum of the metal work function and the potential difference at the metal-solution interface at a given electrode potential, specifically the SHE potential. As shown in Ref. [110], calculations based on equations (4), (5) use the electrode potential depending on the 'chemical' hydration energy. Unlike this potential, Kanevskii's potential is determined by the 'real' hydration energy, including the water surface potential $\chi(\mathrm{H_2O})$:

$$\Delta G^{\mathrm{real}}_{\mathrm{hyd}}(\mathrm{H^+}) = \Delta G^{\mathrm{chem}}_{\mathrm{hyd}}(\mathrm{H^+}) + F\chi(\mathrm{H_2O})\,. \tag{6}$$

The 'chemical' hydration energy enters the so-called Trasatti potential E_T [111] differing from the Kanevskii potential by an addition equaling the water surface potential: $E_K = E_T + \chi(H_2O)$ (see the monograph by A N Frumkin [112]). This means that the Trasatti potential should be employed as $E(H)$ in calculations with the use of formula (4).

Determination of the water-gas potential jump $\chi(H_2O)$ implies the use of extrathermodynamic assumptions. One of them was applied in Ref. [97] to find the value of $\chi(H_2O) = 0.14$ V. In conjunction with the known experimental value of $E_K = 4.44$ V, it yields $E_T = 4.30$ V for the Trasatti potential.

The solvation energy in a protein has two components: dielectric response energy and the energy of charge introduction into the pre-existing field. The former was calculated in two variants, at $\varepsilon_{sp} = 4$ and $\varepsilon_{sp} = 5$, while the latter was calculated with the use of optical permittivity $\varepsilon_{op} = 2.5$. Permittivity inside a reactant $\varepsilon_i = 2.5$.

The ionization energy of the Rieske complex (as well as partial atomic charges) was calculated by the quantum chemical method of the density functional theory (DFT) and coupling between iron atomic spins by the broken symmetry (BS) method. Calculations were made for different atomic basic sets, such as Triple Zeta Valence plus Polarization (TZVP) and 6-31 + + G(d,p), which proved helpful in the analysis of anions and systems with low ionization energy.

The ligands of iron atoms being amino acid residues linked by covalent bonds to the rest of the protein molecule, interactions inside the active site are not the sole factor determining their coordinates. Accordingly, optimization of Fe and S coordinates was performed at the fixed coordinates of external ligands. This variant of calculation was regarded as the principal one. At the same time, the quantum chemical calculation of the structure of $Im_2Fe_2S_2(SCH_3)_2$ molecules with full optimization of the coordinates of all atoms yielded a structure substantially different from the experimental structure of this complex in protein.

The Rieske protein redox-potentials were calculated to be 0.19 and 0.26 V at $\varepsilon_{sp} = 4$ and 0.26 and 0.34 V at $\varepsilon_{sp} = 5$ (the former values were obtained using TZVP and the latter with 6-31 + + G(d,p)). The experimental value of the Rieske protein redox-potential is 0.308 V, in good agreement with the theoretical values. The sole publication reporting results of quantum chemical/electrostatic calculation of the Rieske protein redox-potential gives the negative value −0.01 V, which is much lower than the experimental one. The main cause of this discrepancy is the use of the Kanevskii potential in [113] and static dielectric permittivity in the calculation of the intraprotein field.

As mentioned in a preceding paragraph, full optimization of the coordinates of all active site atoms leads to a structure significantly different from its structure in which ligand coordinates are fixed by the protein matrix. Calculations for a fully optimized complex differing from the one discussed above both in geometry and partial charge distribution give potentials shifted 0.27–0.32 V toward negative values. This suggests an important role of intracomplex stresses created by the protein in the adjustment of the necessary redox-potential values.

Besides the above characteristics of the wild-type protein, we calculated its redox potential in an alkaline solution where imidazole ligands lose two protons each and thereby shift the potential to −0.13 V; similar calculations were made for three mutants. All these data are in reasonable agreement with experiment.

III. *pK of ionogenic protein groups, an example of calculation for α-chymotrypsin* [114].

The calculation of pK is made according to the same principles as calculation of redox-potentials, i.e., on the combination of the experimentally found pK value in a model solvent, e.g., DMF, and the energies of transfer of the acid (XH^n) and basic (X^{n-1}) forms from DMF into the protein calculated based on continuum electrostatics. A specific feature of the acid dissociation of protein groups is that both forms, the acid and the base, are inside the protein, whereas the hydrogen ion enters water (subscripts prot and aq, respectively):

$$XH^n_{prot} = X^{n-1}_{prot} + H^+_{aq}\,.$$

This implies consideration of the energy of H^+ transfer from DMF into water, besides the energies of transfer into protein:

$$2.3RT\,pK = 2.3RT\,pK_0 - \Delta G^{tr}_{XH^n} + \Delta G^{tr}_{X^{n-1}} + \Delta G^{tr}_{H^+}\,. \quad (7)$$

Here, pK_0 is the pK value in DMF, and ΔG^{tr} with different subscripts are the respective transfer energies.

Hydrogen bonds created in the protein by the acid (and the conjugate base) are taken into account explicitly in electrostatic calculations (calculations of the pre-existent field), in contrast to the continuum calculation of the charging energy in DMF. For this reason, the energy of the hydrogen bond between the acid and DMF must be subtracted from the energy of its transport from DMF into protein.

The continuum calculation of the energies of acid and base transfer from DMF into the protein is made in the same manner as described above for the redox-potential. The hydrogen bond energy is assumed to be 20 kJ mol^{-1} and the energy of H^+ transfer from DMF into water is 28 kJ mol^{-1} [97], with the experimental value of $pK_0 = 6.4$.

The calculation of pK for one of the histidine residues (His-57) determining the pK of the active site of the enzyme yielded [1] 6.2 at $\varepsilon_{sp} = 4$ and 7.9 at $\varepsilon_{sp} = 5$. Experimental pK = 7. Thus, it should be assumed that $\varepsilon_{sp} \approx 4.5$ if quantitative agreement with experiment is to be reached, i.e., a somewhat greater value than for dry proteins (3.5–4.0) should be used. A higher ε_{sp} value was found for other ionogenic groups localized closer to the globule surface, in excellent agreement with the results of molecular-dynamic modeling of protein electric properties discussed in Section 3.3 [37–43].

It should be noted that the accuracy of the above absolute pK values is hardly higher than 1.0, first and foremost due to the inaccuracy of energy values for H^+ transfer. However, it does not refer to the comparison of relative pK values of different groups and, therefore, to the aforementioned enhancement of effective dielectric permittivity closer to the globule surface. The above calculations were made with respect to the Michaelis complex of α-chymotrypsin with a small substrate (dipeptide). In a complex with a large protein, the shielding of the active site (hence, the dielectric response energy) is altered. Moreover, the dipole field of this protein is added. Our calculation for the complex with a protein inhibitor gave a 1.4 lower pK value, in agreement with the experimental data ($\Delta pK = 1.5$).

[1] After the adjustment of the energy of H^+ transfer, these values proved to be 0.7 higher than reported in Ref. [113].

The traditional semi-continuum calculation method for pK of protein groups disregards the effects of water structure and hydrogen bonds between water and dissolved substances; moreover, it employs one and the same (static) dielectric permittivity for the computation of the pre-existing field and dielectric response. The use of static dielectric permittivity results in substantial underestimation of the former component. It requires assuming an unreasonably high value of protein dielectric permittivity to ensure agreement between theoretical and experimental results. The calculation of His-57 pK in the framework of the traditional approach gave values in agreement with experimental findings only at $\varepsilon_{sp} \geqslant 20$. Practically the same result was obtained in Ref. [115]; it is hardly possible to provide a physical substantiation for such high ε_s value.

4. Reorganization energy and activation energy

4.1 Protein as a medium with low reorganization energy

Let us start by considering the simple case of charge e transfer in a homogeneous medium with optical and static dielectric permittivities ε_o and ε_s. According to the Marcus equation, the medium reorganization energy

$$\lambda_s = e^2\left(\frac{1}{\varepsilon_o}-\frac{1}{\varepsilon_s}\right)\left(\frac{1}{2a_1}+\frac{1}{2a_2}-\frac{1}{R_{1,2}}\right), \qquad (8)$$

where a_1, α_2 are the radii of reactants, and $R_{1,2}$ is the distance between their centers.

The low static dielectric permittivity of proteins has a dramatic effect on their reorganization energy. Indeed, $\varepsilon_s \gg \varepsilon_o$ for usual polar solvents; therefore, the so-called polaron coupling constant (Pekar constant) $C = 1/\varepsilon_o - 1/\varepsilon_s$ weakly depends on static permittivity. For example, for water, $\varepsilon_o = 1.8$, $\varepsilon_s = 78$, and $C = 0.54$ (sometimes, the so-called quantum boundary value should be used instead of optical dielectric permittivity, equaling roughly 2.1 for water [116], i.e., $C = 0.46$); for DMF, $\varepsilon_o = 2.04$, $\varepsilon_s = 36.7$, $C = 0.46$. Similar values of optical and static permittivity (roughly 2.5 and 4) characteristic of proteins are responsible for low $C \approx 0.15$. Accordingly, the protein reorganization energy can be expected to be thrice as low as in water and other polar solvents. The low reorganization energy of charge transfer in proteins is one of the main physical causes of low activation energy and, therefore, of high catalytic activity of enzymes [117, 118].

At the outset, we came to the conclusion of low protein reorganization energy from qualitative considerations: low mobility of protein dipoles accounts for a negligible alteration of their orientation during charge transfer, i.e., weak reorganization [31]. The limited dipole mobility is actually responsible for both weak dielectric response and low reorganization energy. Their quantitative relationship is expressed through the coupling constant.

The lower reorganization energy of proteins themselves does not comprehensively characterize the process of charge transfer, because they are, as a rule, surrounded by a medium (usually water) with different dielectric properties (the aqueous environment was taken into consideration in the calculation of equilibrium energies in Section 3). For this reason, the quantitative analysis of the problem requires that a more general expression taking account of the reorganization of the aqueous environment be used instead of the simplified formula (8). In Sections 4.4.1 and 4.4.2, we shall consider general derivation of the equation describing the reorganization energy for a real protein system of arbitrary geometry, along with the methods for its numerical computation. But we shall start by discussing a simplified model to elucidate some general trends.

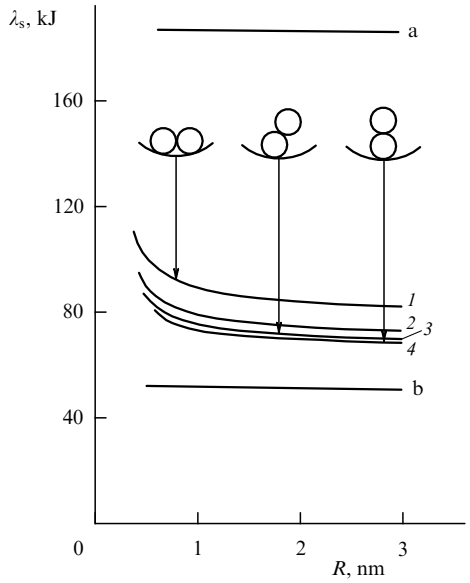

Figure 3. Dependence of the reorganization energy on the globule radius for the following model. Two spherical reactants of radii 0.2 nm in contact with each other are inside a spherical globe, so that reagent 1 touches its surface and the center of reagent 2 is 0.2 nm (in touch with the surface, curve *1*), 0.3, 0.4, and 0.6 nm apart from the surface (curves *2–4*, respectively). Positions of reactants depicted on schemes correspond to curves *1*, *3*, and *4*. Lines a and b correspond to reactants in water and an infinite protein, respectively (data borrowed from [122]).

Let us consider two spherical reactants of radius α_1 and α_2, respectively, localized at points r_1 and r_2 inside a spherical globule with radius R [117, 118] (see also [119–122]). The results of the calculations for a set of parameters reasonable in terms of the order of magnitude are presented in Fig. 3. The upper and lower horizontal straight lines represent reorganization energies of reactions in the aqueous environment and the infinite homogeneous protein, respectively. The curves correspond to a protein globule at different values of its geometric parameters. It can be seen that the reorganization energy in the protein globule is significantly lower (by 95–120 kJ mol^{-1}) than in water. The physical cause of this is obvious: part of the solvent near the reactants is replaced by the poorly reorganizing medium (protein), thus decreasing the reorganization energy. On the other hand, the reorganization energy in the globule is much higher than in the infinite protein; this suggests a certain contribution from the globule's aqueous environment.

Naturally, the interaction of the charge being transferred with the protein and the aqueous environment depends on the system's geometry, as is well illustrated by the curves in Fig. 3. The closer the two reagents to the protein-water interface, the higher the reorganization energy. An increase in the globule radius at a given distance of reactants from its surface increases charge screening by the protein and thereby decreases the reorganization energy. This dependence is more pronounced at small radii; as the radius of the globule grows, the water layers increasingly farther apart from it are

excluded from interaction with the charge, and the effect of its substitution by the protein becomes less apparent.

Low values of the protein reorganization energy were also obtained for a few concrete reactions by means of microscopic modeling and in later studies by the molecular dynamics method (see, for instance, [123–135]). Importantly, some of them revealed the quadratic dependence of the energy on the respective microscopic parameter, i.e., the dependence underlying the Marcus equation (see [129, 133]). Thus, conclusions drawn from the semi-continuum theory agree with the results of microscopic modeling.[2]

It follows from the foregoing, as well as from theoretical and experimental data for a number of concrete systems (see Section 4.4.3), that the reorganization energy essentially depends on the system's geometry (reactant size and position relative to each other and the protein-water interface), which means that the use of the notion of 'protein reorganization energy' as a certain constant parameter frequently encountered in the literature makes no physical sense.

4.2 Charge transfer activation energy and protein globule geometry

The objective of this section is to elucidate the dependence of the activation energy on the geometric parameters of our heterogeneous dielectric (protein + water). Let us assume λ_i to be constant (such an assumption does not practically affect the form of the dependence). The quantity ΔG includes the essential intramolecular contribution ΔG_i that is constant for a given reaction and the contribution due to the interaction between the charge and its environment, i.e., ΔG_{pol} and $\Delta G_{p.f}$. Because polarization components λ_s and ΔG_{pol} are independent of the pre-existent field (see [64] and Section 4.4.1), ΔG_i and $\Delta G_{p.f}$ may be combined into a quantity ΔG_{str} determined by the structure of reactants and protein; this quantity can be regarded as a parameter. Certainly, the strength of the pre-existing field differs for real globules of different size being determined, not only by the dipoles closest to the reaction center but also by more distant protein layers. However, the contribution from other regions markedly decreases starting from a certain distance from the center. Therefore, the pre-existing field can be regarded as constant in the first approximation. This permits qualitatively assessing the tendency for variation of the activation energy with alteration of the globule size. In the examples below, we varied ΔG_{str} in an interval of ± 10 kcal mol^{-1} encompassing the usual scatter range of activation energy values. Results of some model calculations are presented in Figs 4, 5.

These data show that the activation energy decreases when charge transfer in the reaction is directed from the

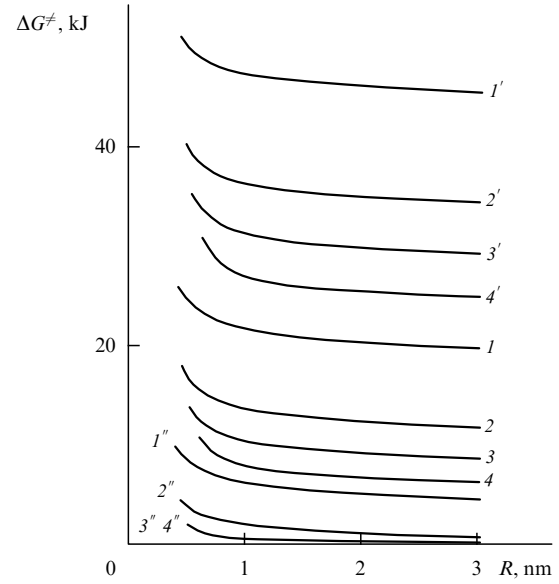

Figure 4. Dependence of the activation energy on the globule radius for the model of Fig. 3. Charge transfer from reagent 2 to reagent 1, i.e., from the inside of the globule to its surface. For curves *1–4*, $\Delta G_{str} = 0$, for curves *1′–4′*, $\Delta G_{str} = 41.8$ kJ mole^{-1}, for curves *1″–4″*, $\Delta G_{str} = -41.8$ kJ mole^{-1} (curves *3″* and *4″* practically merge). (Data borrowed from [112].)

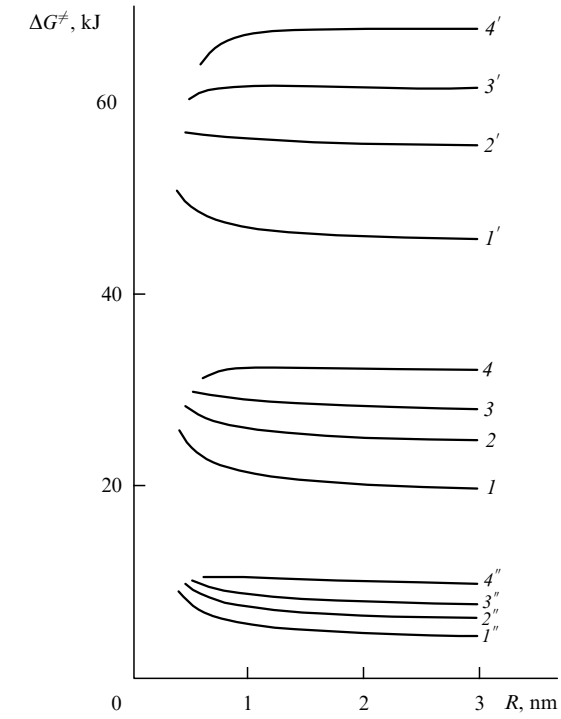

Figure 5. Dependence of the activation energy on the globule radius for the model of Fig. 3. Charge transfer from reagent 1 to reagent 2, i.e., from the surface inside the globule. For curves *1–4*, $\Delta G_{str} = 0$, for curves *1′–4′*, $\Delta G_{str} = 41.8$ kJ mole^{-1}, for curves *1″–4″*, $\Delta G_{str} = -41.8$ kJ mole^{-1}. (Data borrowed from [112].)

[2] The role of low reorganization energy was stressed by Warshel et al. in 1989 [129]. Unfortunately, these authors describe their conclusion, which is actually in full agreement with our earlier observations, as altogether different. It is stated in Ref. [129, p. 4805]: "This conclusion might not surprise those who are familiar with earlier macroscopic arguments of Krishtalik and co-workers who tried to estimate the reorganization energies for various enzymatic reactions on the basis of a macroscopic model that considered the protein active site as a structureless low dielectric environment... . Unfortunately, nonpolar protein sites would slow rather than accelerate charge-transfer reactions due to the loss in self energy (solvation energy)... . This point was in fact recognized by Krishtalik in subsequent studies (see Ref. 12 [in this paper, Ref. 137 — L.I.K.])". This argument, repeated by Warshel in many papers (see, for instance, [3]) stems from the confusion of the notions of 'non-polar' and 'low-dielectric' (see Section 2). In our opinion stated many times long before Ref. [129] was published, proteins have a pre-organized structure, and only the joint action of low dielectric permittivity (low reorganization energy) and the intraprotein electric field accounts for the main energy characteristics of enzymatic catalysis (see, for instance, [137] and Section 4.3). This line of reasoning eliminates the contradiction mentioned by Warshel and co-workers.

inside to the outer boundary of the globule, all other factors being equal. This is understandable, because such a transfer direction makes interaction between the charge and the aqueous environment energetically advantageous [136]. The charge transfer inevitably occurs in two directions, because in the complete catalytic cycle the charge eventually returns to the starting position, meaning that at the energetically least advantageous stage of the process (maximum positive ΔG_{str}) the charge should be transferred from the inside to the outside of the globule if catalysis is to be optimized. We analyzed the structure of certain globular enzymes with the known mechanism of reaction and found that the slow phase of the reaction was in all cases associated with a shift of the charge from inside to outside. This inference holds for serine and cysteine proteinases, ribonuclease, lysozyme, and NAD-dependent [3] dehydrogenases [120, 122].

Charge transfer at an acute angle to the globule surface is energetically advantageous; in this case, the activation energy is almost minimal (see Fig. 4), but the initial charge is not deeply (energetically disadvantageously) submerged in the protein. This observation is confirmed by the structure of the active center of the aforementioned enzymes.

The results of our calculations indicate that both reorganization energy and activation energy of charge transfer decrease with increasing globule radius, first rapidly then slowly (see Figs 3, 4), which suggests the existence of a range of optimal radii within which the activation energy is almost minimal, as is the consumption of 'building material', i.e., protein. The initially fast decrease in the activation energy with increasing radius accounts for the experimentally observed fact that the size of enzyme globules is, as a rule, greater than the size of the active site proper.

We estimated the optimal radius (at which the dynamics of R-dependence of $\Delta G^{\neq}$ slows down) in the framework of an approximate model (a spherical globule and reactants) in which the size of the reactants, the distance between them, and their position relative the globule surface were specified. It yielded the right order of magnitude and relative sizes of enzymes (the calculated radii proved somewhat smaller compared with their real length, which is natural due to the approximateness of the model).

It is interesting to compare three serine proteases, viz., chymotrypsin, subtilisine, and carboxypeptidase having a similar structure and size of the active site. The globule radii of the two former enzymes are virtually identical, whereas carboxypeptidase, differing from them only in active group orientation with respect to the globule surface, has a much longer radius due to the altered geometric conditions for interaction with the aqueous environment [120, 122].

4.3 High catalytic activity of enzymes as a result of a combination of low protein polarizability and a pre-existent intraprotein electric field

As shown in the foregoing (Sections 4.1, 4.2), low static dielectric permittivity of proteins results in a low reorganization energy. On the other hand, low dielectric permittivity accounts for the disadvantage of the presence of charged groups in a protein that may, in principle, cause a rise in the reaction activation energy compared with its value in an aqueous medium, especially when the process is associated with the increase in the total charge of reactants. Moreover, consistent comparison of two media implies taking account of the energy of charge transfer ΔG_{tr} from one medium to the other (or the difference between charge formation energies in them). The effective activation energy is expressed as

$$\Delta G_{eff}^{\neq} = \Delta G_{tr} + \Delta G^{\neq} . \quad (9)$$

We calculated activation energy for certain typical reactions (separation, neutralization, transport of charges from an initially charged reactant to an uncharged one or from two donors to one acceptor, etc.). Various models were employed for the quantitative assessment of the activation energy (see Section 4.2 and [122, 137, 138]), but the general conclusions do not depend on the concrete model used for the purpose [122]. In accordance with these conclusions, the activation energy of some elementary acts in a medium with low dielectric permittivity is lower than in water in some cases, and greater in others. However, the effective activation energy in the low-dielectric medium is invariably higher than in the high-dielectric one. In other words, an additional energy expenditure necessary to transfer a charge into a low-dielectric permittivity medium (or generate a charge in such a medium) always outweighs the respective decreases in the activation energy of the elementary charge transfer act $\Delta G^{\neq}$.

This inference holds for a structureless dielectric for which polarization interaction between the charge and its environment (Born solvation energy) is the sole contribution to the electrostatic energy of the system. However, proteins are highly structured systems having a permanent electric field at each point that pre-existed before a free reactant charge was introduced. The energy of this charge in a given field is an essential constituent of the system's electrostatic energy; it may compensate for the loss of the Born solvation energy given a proper potential in the region of reactant localization. Examples of such compensation are presented in Sections 3.1 and 3.4.

The compensation effect of the intraprotein field may be either slightly smaller or greater than the loss of the Born solvation energy; it is important that the total ΔG value must be reduced to the acceptable level. Excessive stabilization of the charge is sometimes unfavorable; it may decrease ΔG at a given stage and hamper realization of the next stage related to neutralization of an intermediate product (or release of a free ion).

Many authors argue that the intraprotein field stabilizes the transient state. They have good reason to hold such a view, e.g., so far as heavy particle transfer reactions are concerned, but it does not encompass all aspects of this problem [138]. The notion of transient state charge makes no sense for non-adiabatic electron (and proton [24]) transfer, widespread in biochemical processes, because the charge is wholly concentrated in one of the reactants either in the initial or final states of the reaction. In such a situation, one may speak of electrostatic stabilization of the reaction product, which lowers the activation barrier. In other words, a decrease in the transient state energy is an indirect result of reduced ΔG of the reaction rather than the consequence of the direct field action on the transient state charge that is not a definite quantity (see above). To recall, the transient state includes not only the reactive groups proper but also the surrounding medium in a non-equilibrium configuration. The behavior of this medium is governed by its reorganization energy, which does not depend on the intraprotein electric field; its low value is due to the character of the dielectric response.

[3] NAD — nicotinamide adenine dinucleotide.

To summarize, low dielectric permittivity of proteins is responsible for their low reorganization energy and thereby promotes the enhanced catalytic activity of enzymes. At the same time, this factor exerts an unfavorable action on the equilibrium energy of the process by lowering the solvation energy of charged reactants. However, this action can be compensated by the intraprotein electric field. Both effects, low dielectric permittivity and intraprotein field, stem from the common principle of protein architectonics, i.e., fixation of highly-polar groups within a definite structure, which creates a specific highly-polar medium with low dielectric permittivity. Only the joint action of these factors can maintain a high catalytic effectiveness of enzymes. We believe that it is these structural features of proteins that ensured evolutionary advantages of enzymes as biological catalysts.

A new class of biocatalysts, ribozymes, whose chemical nature is altogether different from that of proteins, have recently been discovered (see a review, e.g., in [139]). At the same time, ribonucleic acid (RNA) and proteins exhibit an essential structural analogy: the RNA backbone contains a regular sequence of highly-polar phosphodiester groups, while side chains are also polar purine and pyrimidine bases. X-ray structural analysis has shown that these elements make up a rather dense structure [140]. Therefore, it can be expected that ribozymes are highly-polar low-dielectric media, too.

4.4 Calculation of the charge transfer reorganization energy in proteins

Described in this section are the methods for the calculation of the charge transfer reorganization energy and the results of their application to concrete systems in comparison with experimental data.

4.4.1. Derivation of the general equation for reorganization energy. Let us start with a brief description of the method designed to derive the general equation for the reorganization energy of a structureless dielectric. According to Marcus [142, 143], this energy can be found using a two-step charging process:

(1) equilibrium charging in a medium with static dielectric permittivity ε_s at which all types of polarization, viz., slow (inertial) atomic and fast (inertialess) polarizations, manifest themselves in full measure. The energy of this process is W_I;

(2) rapid discharging during which the coordinates of all heavy particles (nuclei) remain unaltered and only the electron density distribution follows the variation of reactant charges. In other words, only electronic polarization of the medium, i.e., its optical permittivity ε_o, responds to the reactant charge redistribution. The corresponding energy is denoted by W_{II}.

This two-step process completed, the reactant charge distribution corresponds to the initial state, and the inertial polarization of the medium corresponds to the charges in the final state. In other words, the total energy of the process is nothing but the medium reorganization energy:

$$\lambda_s = W_I + W_{II}\,. \tag{10}$$

Let us consider a dielectric with the arbitrary distribution of dielectric permittivities $\varepsilon_s(\mathbf{r})$ and $\varepsilon_o(\mathbf{r})$. The initial reactant charge distribution is $\rho_i(\mathbf{r})$, and the potential created by these charges in a medium with static permittivity is $\varphi_{si}(\rho_i, \mathbf{r})$. Now, let us charge the reactants; their final charge density has the form $\rho_f(\mathbf{r}) = \rho_i(\mathbf{r}) + \Delta\rho(\mathbf{r})$. Omitting the details of the calculation, here is the final expression for the charging energy:

$$W_I = \int_V \varphi_{si}(\rho_i, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r} + \frac{1}{2}\int_V \varphi_s(\Delta\rho, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r}\,. \tag{11}$$

Let us introduce special notations for each term on the right-hand side of (11):

$$W_I = W_c + W_s\,. \tag{11a}$$

Here, W_c (first integral) the energy of the charge $\Delta\rho(\mathbf{r})$ introduced into field $\varphi_{si}(\rho_i, \mathbf{r})$, and W_s is the charging energy proper determined by the introduced charge $\Delta\rho(\mathbf{r})$.

Let us assume for calculating W_{II} that the reactants have identical charges of an opposite sign, $\rho_i - \rho_f = -\Delta\rho$, in a medium with ε_o (such a change in the reactant charges leads to the initial charge distribution in the system). The corresponding charging energy consists of two components: the energy of charge $\rho_i - \rho_f$ introduced into the field $\varphi_s(\rho_f, \mathbf{r})$ created after charging at stage 1, and the energy of electronic polarization response to the introduction of charge $\rho_i - \rho_f$,

$$W_{II} = -\int_V \varphi_s(\rho_f, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r} + \frac{1}{2}\int_V \varphi_o(\Delta\rho, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r}\,, \tag{12}$$

where $\varphi_o(\Delta\rho, \mathbf{r})$ is the field of optical response to the charge density re-distribution $\Delta\rho(\mathbf{r})$ in a medium with ε_o.

After charging stage 1, the field $\varphi_s(\rho_f, \mathbf{r}) = \varphi_{si}(\rho_i, \mathbf{r}) + \varphi_s(\Delta\rho, \mathbf{r})$. Then,

$$W_{II} = -W_c - 2W_s + W_o\,. \tag{12a}$$

The sum of (11) and (12) gives

$$\lambda_s = \frac{1}{2}\int_V \varphi_o(\Delta\rho, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r} - \frac{1}{2}\int_V \varphi_s(\Delta\rho, \mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r} = W_o - W_s\,. \tag{13}$$

Equation (13) looks like the difference between charging energies (dielectric response) in media with ε_o and ε_s (W_o and W_s) when the reactants in both cases are originally uncharged. However, the negative sign of the second term results simply from summation of W_s from Eqn (11) and $-2W_s$ from Eqn (12) (W_c and $-W_c$ from these equation cancel each other); therefore, the total energy λ_s is actually the sum of W_I and W_{II}.

It follows from (13) that the reorganization energy, unlike charging energies W_I and W_{II}, depends only on the variation of reactant charges, not on their absolute values.

As mentioned above many times, proteins are pre-organized polar media in the sense that their dipoles are arranged as a certain definite structure that existed prior to charging the reactants [31]. Such pre-organization results in the formation of pre-existent field $\varphi_{p.f}$. Taken it into account in calculation of W_I we should add potential $\varphi_{p.f}$ that is independent of $\rho_i(\mathbf{r})$ and $\Delta\rho(\mathbf{r})$ to the initial potential $\varphi_{si}(\rho_i, \mathbf{r})$. Accordingly, an additional integral appears in the expression for W_I:

$$W_{p.f} = \int_V \varphi_{p.f}(\mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r}\,,$$

reflecting the dependence of the reaction equilibrium energy, including W_I, on the pre-existent field.

The same integral, but with a minus sign, appears in the calculation of W_{II}, because $\Delta\rho(\mathbf{r})$ has the opposite sign:

$$-W_{p.f} = -\int_V \varphi_{p.f}(\mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r}\,.$$

These integrals cancel each other when the expressions for W_I and W_{II} are summed up and the same equation (13) is obtained for λ_s. Thus, the reorganization energy does not depend on the pre-existing field [64].

In the framework of the semi-continuum formalism, the field of pre-oriented dipoles affects ΔG^0. However, the absolute value of this field is by no means as important for the reorganization energy as its variation in the charging process. The variation is calculated in the framework of the dielectric formalism. As shown above, the charge transfer reorganization energy in proteins is independent of the pre-existing field. Therefore, the computation methods are the same for pre-organized and usual media. The independence of the intraprotein field makes the continuum calculation of the reorganization energy more accurate than the semi-continuum calculation of the reaction energy; in the latter case, both the coordinates and partial charges of all protein atoms must be known.

In microscopic modeling, the charge-dipole interaction energy is calculated in the explicit form. The difference between the energies of interaction of dipoles with initial charge distribution in the starting and final coordinates (or vice versa) gives the reorganization energy (see [129–135]). Thus, in the framework of this approach, the reorganization energy depends only on the difference between charge-dipole interaction. Not surprisingly, both approaches yield similar values (see Section 4.4.3).

One important feature of molecular dynamic calculations is worthy of note. In most of them, parametrization disregarding electronic polarization of atoms in the explicit form has been used to date. Today, this factor is beginning to be considered (the so-called polarizable force field approximation) (see, for instance, [134]). It should be noted that quantitative results of microscopic calculations are susceptible to the parametrization used.

4.4.2. Methods for the calculation of the reorganization energy. We represented Eqn (13) in a form convenient for the calculation of the reorganization energy using programs for numerical solution of the Poisson–Boltzmann equation (preferably programs allowing describing heterogeneous systems with more than two regions differing in dielectric permittivity). It is desirable to choose the approximation in which the continuous charge density distribution is replaced by a set of partial point charges of each atom. In other words, integrals over volume $\int_V \varphi(\Delta\rho,\mathbf{r})\,\Delta\rho(\mathbf{r})\,d\mathbf{r}$ are substituted by the sums over all reactant atoms $\sum_i \varphi_i\,\Delta q_i$, where Δq_i is the change in the charge of the i-th atom during the reaction and φ_i is the potential at the i-th atom created by the changes in the charges of all other atoms.

Partial atomic charges are calculated by the quantum chemical method. The most suitable of the available variants are the so-called electrostatic potentials (ESPs), selected so as to optimally approximate the extra-atomic electrostatic field created by the electron density distribution. Various ESP approximations are feasible; it is desirable to compare different variants. The results of concrete calculations of the reorganization energy suggest its low sensitivity to the choice of partial charge system.

Reactants are described as bodies possessed of optical dielectric permittivity; that takes into account modulation of their internal orbitals under the effect of non-equilibrium polarization of the medium (electronic polarization of the dielectric cavity (Mertz et al. [144])). The dielectric permittivity of a reactant ε_{or} is usually estimated at 2.0 to 2.5.

Programs for the numerical solution of the Poisson-Boltzmann equation (e.g. DelPhi [53]) permit us to calculate the so-called reaction field (dielectric response energy, Born solvation energy). The charging energy in a medium is the algebraic sum of the charging energy in a vacuum and the reaction field energy, meaning that the reorganization energy, i.e., the difference between the charging energy in optical and static media, equals the difference between reaction field energies in these media.

Dielectric response energies in a static medium are calculated with static permittivities $\varepsilon_{sp} = 4-5$ for protein and $\varepsilon_{sw} = 78$ for water with regard for the Boltzmann distribution of electrolyte ions in the framework of the Debye-Huckel approximation. In an optical medium, $\varepsilon_{op} = 2.5$, $\varepsilon_{ow} = 1.8$; charging in this medium, i.e., a rapid process, does not involve slow ion redistribution. Formally, it can be described by the introduction of a very low concentration of ions (e.g., 0.00001 mol) at which the Debye length proves to be a few orders of magnitude greater than the size of the box containing the protein and its aqueous environment.

4.4.3 Comparison of calculated and experimental reorganization energies.

I. *An overview of results.*

Reorganization energy was calculated for systems of different types, viz., globular proteins (native and modified cytochrome *c*, azurin, ferredoxin), intramembrane proteins (bacterial photosynthetic reaction center, cytochrome *c*-oxidase), and interprotein complex. In all cases except where otherwise specified below, a reasonable agreement with experiment was achieved. It should be noted that the experimental assessment of reorganization energies frequently very roughly yields only intervals of probable values. The theoretical values fall within these ranges.

An important result of calculations agreeing with experimental data is the strong dependence of the reorganization energy on the degree of proximity of the reaction centers to the aqueous phase. For example, the reorganization energy for reactions involving ruthenium-amine complexes covalently bound to cytochrome *c* and surrounded on all sides by water is almost six times that of electron transfer between two hemes embedded deep in the intramembrane cytochrome *c*-oxidase complex. This result confirms that the use of 'protein reorganization energy' as a constant parameter frequently reported in the literature is invalid.

Molecular-dynamic simulations of several systems studied in our work is described in the literature. The degree of agreement of these data with experiment is no better than that of the results of our semi-continuum calculations (except one type of amine-ruthenium cytochromes *c* for which the molecular dynamic estimates are essentially different from both experimental findings and our calculations). Molecular-dynamic modeling requires a few orders of magnitude more computer time than continuum electrostatic calculations.

Rather accurate experimental estimates were obtained for cytochrome *c* and its amine-ruthenium derivatives. The

calculated reorganization energy in such systems proved to be roughly 0.2 eV lower than the experimentally measured one. This discrepancy can be accounted for by the fact that the redox reaction of the active site (heme, i.e., iron-porphyrin) is accompanied by a marked shift of the adjacent amino acid side chains, which can not be depicted as a linear response in the framework of the dielectric description. Quantum chemical calculation of the energy consumed to modify the configuration of these amino acid residues yielded the value of 0.2 eV, practically coinciding with the difference between experiment and continuum calculations.

A special case is electron transfer in an interprotein complex of methylamine dehydrogenase and amicyanin. Its reorganization energy was experimentally measured to be 2.3 eV or more than twice the theoretical value. Such a discrepancy clearly surpasses the possible calculation error and needs explanation. The mutual orientation of two proteins within a stable interprotein complex does not ensure long-range electron transfer (see the Conclusion for a brief discussion of the long-range electron transfer problem). For this reason, the experimentally found activation energy of the process includes not only the activation energy of the electron transfer elementary act described by the Marcus formula (1), but also the energy spent to alter the mutual orientation of the two proteins (the 'surface diffusion' phenomenon). Thus, calculation of the reorganization energy revealed one more factor influencing the rate of this reaction.

II. *Calculation of reorganization energy of concrete systems with different classes of proteins.*

1. *Globular proteins. External charge transfer.*

Cytochrome c. A small water-soluble protein, cytochrome *c* transfers electrons between various electron-transporting chains. Its redox-center is a heme with axial ligands, histidine (simulated by imidazole), and methionine (simulated by dimethylsulfide).

The results of calculations proved a low sensitivity (a few millielectronvolts) to variations of the optical permittivity of the reactant and a partial charge distribution within a reasonable range. The same is true of the other systems described below.

The calculated reorganization energies of the medium turned out to be 0.45 eV at $\varepsilon_{sp} = 4.0$ and 0.48 eV at $\varepsilon_{sp} = 5.0$ (the latter value appears to be more realistic, bearing in mind the higher mobility of protein fragments near the globule surface (see Section 3.3)). Thus, variation of protein static permittivity within a reasonable range did not significantly affect the final result. Similar data were obtained for other proteins.

The reorganization energy for a liganded heme in an infinite protein is 0.19 eV (at $\varepsilon_{sp} = 4.0$) and 0.28 eV (at $\varepsilon_{sp} = 5.0$). The difference between this quantity and the reorganization energy for a heterogeneous protein-water system (0.26–0.22 eV) is a rough measure of the contribution from the aqueous environment. In other words, the contributions from protein and water are of the same order.

We calculated the reorganization energy for reaction

$$\mathrm{Cyt}\,c\,(\mathrm{Fe^{II}}) = \mathrm{Cyt}\,c\,(\mathrm{Fe^{III}}) + \mathrm{e}\,,$$

where electrons are assumed to move off to infinity. The experimental estimates [145, 146] were obtained in the study of the dependence of the electron exchange rate on the potential of the electrode modified by long-chain aliphatic alcohol. This dependence is nothing but the dependence of the activation energy on reaction ΔG_0 that gives [using Eqn (1)] the reorganization energy λ, equaling 0.58 eV. Under experimental conditions, electrons do not move off to infinity but enter the metal. Therefore, this value should be corrected for the energy of electron interaction with image forces. Such a correction calculated by the formula derived in Ref. [147] is 0.06 eV; thus, the reference value of $\lambda = 0.64$ eV.

It is natural to attribute the difference between the experimental total reorganization energy and the calculated reorganization energy of the medium (0.19–0.16 eV) to the contribution of other reorganization processes. The intrasphere reorganization energy of a liganded heme does not exceed 0.04 eV [148]. There is one more component of the medium reorganization energy associated with marked displacement of water molecule 156, as well as Asn-152, Tyr-57, and Thr-78 adjoining the heme [149–153]. This shift can not be described in the usual dielectric approximation, suggesting a linear response practically feasible only at small displacements. Our quantum chemical calculations yielded a value on the order of 0.28 eV for this 'non-linear dielectric reorganization' of the environment; it is close to the aforementioned difference.

Microscopic modeling of cytochrome *c* reorganization yielded $\lambda = 0.40-0.65$ eV [132] and 0.77 eV [133] (the latter study being more detailed), in reasonable agreement with the results of experimental measurements and semi-continuum calculations.

2. *Globular proteins. Intraglobular electron transfer.*

Modified cytochrome c. Gray and co-workers [154, 155] developed an original method for studying electron transfer in proteins. In this approach, a redox-active inorganic complex is covalently attached to one of the superficial amino acids (usually histidine). After reaction in the solution changes the valence state of the complex, the electron exchange rate between the complex and the redox-active site of the protein is measured. Using a series of such complexes with different redox-potentials, it is possible to find the dependence of the free activation energy on the standard free reaction energy, and thereafter determine the reorganization energy from the Marcus equation.

We calculated the reorganization energy for representatives of two series of cytochrome *c* molecules with histidine-32 residues modified by ruthenium-amines. One series was represented by $Ru(NH_3)_5$ and $Ru(NH_3)_4Py$, the other by $Ru(BPy)_2Im$. Here, Py is pyridine, BPy is bipirydine, Im is imidazole. For $Ru(NH_3)_5$, $\lambda = 1.12$ eV (at $\varepsilon_{sp} = 4$) and 1.18 ($\varepsilon_{sp} = 5$), and for $Ru(NH_3)_4Py$, 0.95 eV and 1.01 eV, respectively. Estimation of the water effect, as before, gives 0.57–0.67 eV for the first derivative and 0.47–0.55 eV for the second one. Such a behavior of λ could be expected since the charge on the Ru complex is much less protected from interaction with water than on the heme localized inside the protein. The experimental estimate of the reorganization energy 1.20 ± 0.05 eV is based largely on the data obtained using a more voluminous Py-type ligand; therefore, it should be compared with 0.95–1.01 eV. The difference, on the order of 0.2 eV, is almost the same as for unmodified cytochrome *c* and appears to be due to the same cause (non-linear reorganization of the immediate surroundings).

The calculated reorganization energy for $Ru(BPy)_2Im$ is 0.70–0.74 eV. It was found to be 0.75 eV in experiment [156] with BPy-substituted series (i.e., more voluminous ligands), which suggests some difference between dielectric and total reorganization energies.

The fully microscopic modeling of substituted cytochromes was undertaken in Ref. [134]. It yielded the total $\lambda = 1.34$ eV for $Ru(NH_3)_5$, which agrees with both experimental result and our continuum calculations. Importantly, the authors of [134] used the polarizable atom model because the model of unpolarizable atom overestimates the reorganization energy by approximately 40%. They found $\lambda = 1.26$ eV for $Ru(BPy)_2Im$. This value agrees with experimental data worse than the results of continuum calculations do, the cause of so great a discrepancy being unclear.

Azurin. Farver and Pecht [157] investigated the reaction between the disulfide anion-radical and the mononuclear copper reaction center. The anion-radical formed in the reaction with anion-radical CO_2^-, a product of pulsed radiolysis of an aqueous solution. Azurines isolated from various organisms and their mutants were used to study their kinetics in a range of redox-potentials. The reorganization energy was found to be 1.39 eV.

The calculated reorganization energy of the medium was 1.24–1.34 eV. The effect of the aqueous environment was estimated at 0.54–0.64 eV, i.e., higher than for cytochrome *c*, because reactants in azurine are closer to the globule surface.

The intrasphere reorganization energy for a $Cu(Im)_2(SCH_3)(S(CH_3)_2)$ complex (a reaction center analog) is close to 0.32 eV [158, 159]; therefore, the anticipated total reorganization energy is 1.5–1.6 eV. This value is in acceptable agreement with experimental data, bearing in mind that the results of [158] are obtained in a narrow range of energies and subject to wide scatter.

Ferredoxin. Ferredoxins contain two iron-sulfur clusters. Comparison of ferredoxin redox-centers from different mutants of *Chromatium vinosum* allowed the reorganization energy to be approximately evaluated as 0.2–0.5 eV [160]. Our calculations gave values of 0.50–0.58 eV, practically coinciding with the experimental upper limit. The aqueous environment proved to be less significant for ferredoxins than for all other globular proteins (0.16–0.19 eV).

3. *Electron transfer in intramembrane protein complexes*.

Bacterial photosynthetic reaction center (BPRC). In analogy with FSI described in Section 3.4.2, BPRC contains a few co-factors arranged in two parallel chains across the membrane. The transmembrane electron transfer is accomplished by translocation from one co-factor to the next. The so-called special pair P (bacteriochlorophyll dimer $BChl_2$) serves as the primary electron donor after its photoexcitation. The primary acceptor is a bacteriochlorophyll monomer (BChl). The electron is further transferred to bacteriopheophytin (BPhe), and thereafter to quinone. The reorganization energies of these transfer reactions were calculated in Ref. [59] in the framework of the three-layer membrane model; co-factors were simulated by ellipsoids of proper size [161].

A primary separation of charges ($P^+ - BChl^-$) and subsequent electron transfer to BPhe take 1–2 ps with possible oscillations in the subpicosecond range [162, 163]. This time is insufficient for the dielectric response to fully develop, as appears from the difference between the levels of electron energies in P^* and $BPhe^-$ determined by the delayed fluorescence technique after a lapse of time following formation of $BPhe^-$. Immediately after formation ($\sim$2–3 ps), the difference is $-.08 \div -0.18$ eV [164–167]; it is $-0.20 \div -0.23$ eV in 10 ps [165–167], -0.26 eV in 100 μs [168], and -0.30 eV after a few milliseconds [169]. Such variations of the reaction energy can be described in the framework of the continuum dielectric model as resulting from the change in static (quasistatic) dielectric permittivity ε_s [58, 59]. Changes in ε_s affect both reaction energy ΔG^0 and reorganization energy λ. In the charge separation reaction, these two effects fully compensate each other, while in the charge translocation reaction the mutual compensation is almost complete. Due to this, the conclusion about the activationless nature of this reaction based on the results of semi-continuum calculations is practically independent of the effective ε_s value [58, 59].

The equality of calculated and experimental (over a proper time interval) reorganization energies with the reaction energies was confirmed by the molecular dynamic method [127–127, 130, 170]. Thus, microscopic modeling yields results in agreement with those obtained by continuum electrostatic methods.

Because we are dealing with different ε_s, i.e., different λ over different time intervals, polyexponential kinetics appear possible [171]. Rapid activationless transition occurs at small times. But the small energy gap (ions are poorly solvated for the lack of time) gives rise to the reverse reaction, leading to equilibrium. Solvation increases with the strengthening of the dielectric response, and equilibrium shifts toward the increasingly complete separation of charges. At this stage, the process rate as a whole is governed by the speed of protein relaxation to a new equilibrium state but not the electron transfer mechanisms. An alternative explanation of polyexponential kinetics is based on such factors as the system's microheterogeneity and a set of conformational sub-states differing in kinetic parameters (although their reorganization energies can hardly be identical) [172, 173]. If the transition between these states occurs at a rate comparable to the reaction rate, this variant is actually similar to the one considered earlier. Such a situation is feasible at physiological temperatures, whereas at low temperatures transitions between the sub-states are frozen [174].

Electron transfer from $BPhe^-$ to quinone takes some 100 ps; it is associated with a marked energy drop by 0.52–0.54 eV [58, 59]. Such a difference is necessary to prevent the return of electrons to the initial state. The process (activationless) is maintained at a high speed if the reorganization energy virtually equals the absolute reaction energy. Such a value was obtained by semi-continuum calculations; a rise in the reorganization energy was achieved by using a quinone receptor, i.e., a co-factor of a smaller size than BPhe, and increasing the distance between reactants.

The above reaction energy corresponds to the situation in which the semiquinone anion-radical is stabilized only due to dielectric relaxation. Charging quinone at physiological pH results in protonation of the adjacent amino acid residues, and thereby promotes additional stabilization of the anion-radical (see [171] for a more detailed discussion of this 'proton relaxation' effect).

Cytochrome c oxidase (C*c*O). A variety of processes proceed in the C*c*O complex, but we confine ourselves to two electron transfer reactions, between *a* and a_3 hemes and between binuclear copper center Cu_A and the *a* heme.

The temperature dependence of electron transfer rate between *a* and a_3 hemes in the former reaction was studied in Ref. [175]. The data obtained were used to estimate the reorganization energy as equaling 0.05–0.20 eV (the upper limit appears more realistic). Since the two hemes are localized in the very midst of the membrane, static dielectric permittivity is most likely to be low. Calculations at $\varepsilon_{sp} = 3.5$ and 3.0 yielded λ_s values of 0.24 and 0.17 eV, respectively,

which are close to the most probable experimental ones (intrasphere heme reorganization may be neglected). The contribution of the aqueous environment to the energy balance is only 0.06–0.07 eV because the hemes are fairly well protected from interaction with water. Microscopic modeling by Wikström et al. [135] yielded $\lambda_s = 0.2$ eV. This value practically coincides with the results of semi-continuum calculations cited above.

Experimental estimates of reorganization energies for electron transfer between Cu_A and the *a* heme are especially variable (from 0.15 to 0.5 eV) [176, 177]. The calculated reorganization energy of the medium is 0.48 eV at $\varepsilon_{sp} = 4$ and 0.30 eV at $\varepsilon_{sp} = 3$; the effect of the aqueous environment is estimated at 0.11 eV and 0.15 eV, respectively. In this case, Cu_A is less shielded than the heme. The spatial distribution of dielectric permittivity in this complex remains unknown, but a higher ε_{sp} value appears to be more probable, because the copper center is localized farther apart from its middle part. The intrasphere component, estimated at 0.22 eV, should be added to the medium reorganization energy [159, 178]. The expected total reorganization energy of 0.5–0.7 eV is close to the experimental upper limit.

4. *Interprotein electron transfer*.
Electron transfer was experimentally investigated in a number of complexes formed by two contacting proteins. The study of ΔG^0-dependence of $\Delta G^{\neq}$ showed that the reorganization energy of the reaction between cytochrome b_5 and cytochrome *c* equals 0.8 eV [179]. Practically identical λ values (about 0.38 eV) were found for reactions of cytochrome *c*-551 with plastocyanin and rusticyanin; they fall in a range characteristic of protein reactions [180].

Of special interest is the reaction among methylamine dehydrogenase and amicyanin, including its mutants, characterized by an unusually high reorganization energy, $\lambda = 2.3 \pm 0.1$ eV [181]. The semi-continuum calculation yielded 0.68–0.72 eV. The total value remains much lower than the experimental one, even after the addition of the intrasphere reorganization energy of the copper center (about 0.3 eV). Davidson [181] explains the large value of λ by the reorientation of two water molecules close to the active site of dehydrogenase. However, this explanation is hardly adequate. We calculated the reorganization energy for an imaginary system in which protein is completely replaced by water to obtain a greatly exaggerated effect. The value sought after proved equal to 1.72 eV, i.e., also much lower than the experimental one.

We explain this anomaly as follows. The value of $\lambda =$ 2.3 eV was obtained based on the activation energy determined in the study of the temperature dependence of the reaction rate. However, this 'apparent' activation energy comprises not only the activation energy of the elementary act proper, but also the energy consumed for the formation of the reaction complex itself, in which electron transfer takes place. In the original Marcus theory, it is the energy of convergence, by which mutual repulsion of reactants, e.g., like-charged ions, is overcome. Important in a reaction between two proteins is the achievement of such mutual orientation of the macromolecules, which maintains the optimal electron transfer pathway. Transition from one configuration to another (so-called 'surface diffusion' [179]) may require a high energy expenditure, associated with a marked increase in the activation energy. Most probably, this factor plays the key role in this case, because the dehydrogenase-amicyanin complex is very stable.

4.5 Low protein reorganization energy: investigation by fluorescence spectroscopy

The application of fluorescence spectroscopy for the study of Stokes shifts [88] makes it possible to characterize the protein reorganization energy without using kinetic data on enzyme-catalyzed charge transfer reactions. Photoexcitation of a chromophor group results in electron density redistribution and rearrangement of the surrounding medium to match the new charge distribution patterns. In a classical system, the Stokes shift is equivalent to the doubled reorganization energy [182, 186] (Fig. 6). Taking account of variations in the oscillation levels of the ground and excited states leads to an appropriate correction [184].

Proflavin dye (PF) was chosen as a fluorophor whose fluorescence spectrum is resistant to possible complicating effects [185]. PF is a competitive inhibitor of α-chymotrypsin (CT), filling the so-called binding pocket at the enzyme active site (conditions for its complete binding are described in [186]). Thus, the reorganization energy can be determined directly within the enzyme active site.

Figure 7 shows the final result of the experiment without details of set-up and data treatment. The circles denote Stokes shift of PF in certain aprotic solvents. These values significantly correlate with the medium reorganization energy calculated from the quantum chemical data on the charge density redistribution during photoexcitation of the molecule. They may serve as a reference for the comparison of protein-bound PF spectra. The upper horizontal arrow indicates the Stokes shift value for the PF-CT complex in an aqueous solution; this value is close to that for PF in a moderately polar solvent but much smaller than for PF in a purely aqueous environment (denoted by the rhombus in the figure). Clearly, the shift for PF in water is much greater than could be expected in a solvent with the macroscopic dielectric characteristics of water (water exhibits an abnormally high reorganization potential). The Stokes shift of the complex in the aqueous solution reflects the contribution of both the protein and its aqueous environment. In order to

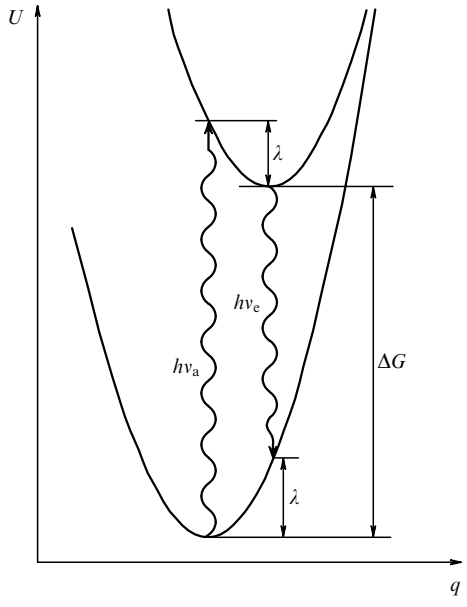

Figure 6. Potential curves for the ground (lower curve) and excited (upper curve) electron states. $h\nu_a$ — maximum absorption energy, $h\nu_e$ — maximum emission energy.

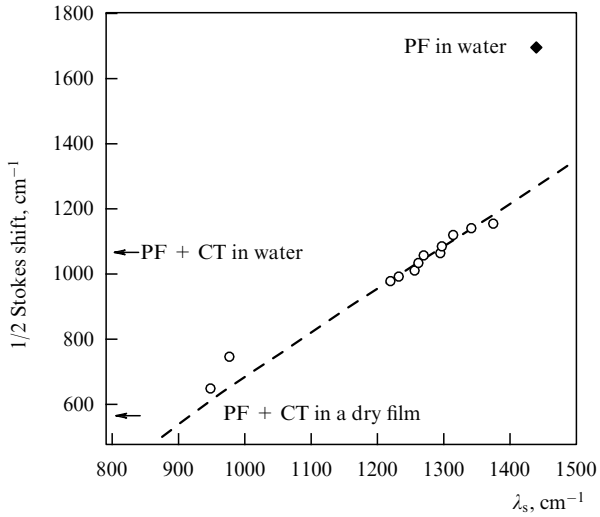

Figure 7. Dependence of 1/2 Stokes shift of the proflavin (PF) fluorescence spectrum on the outer-sphere reorganization energy calculated in the framework of continuum electrostatics (partial charge redistribution induced by optical excitation is calculated by the quantum chemical method, taking into account the dielectric permittivity of the environment). Circles are data for some aprotic solvents and their mixtures; the solid rhombus denotes the respective value for water. The upper horizontal arrow indicates the Stokes shift value for the α-chymotrypsin-PF complex in an aqueous solution; the lower arrow indicates this value for the same complex in the form of a dry film (data borrowed from [88]).

estimate the purely protein contribution, the fluorescence spectra of the complex were measured in a dry protein film (the Stokes shift is shown by the lower horizontal arrow). In this system, the reorganization energy is even smaller than in such low-polar solvent as dichloromethane ($\varepsilon_{sp} = 9$, lower point).

A study of the fluorescence of the protein-bound dye also revealed relaxation effects in globular proteins [88, 187]. Characteristic relaxation times varied from a few picoseconds to several nanoseconds [187]. Interestingly, relaxation times in excess of nanoseconds make a very small contribution (several percent) to the total protein reorganization energy [88]. In this respect, a globular protein (in the present case, α-chymotrypsin) is markedly different from the photosynthetic reaction center (see above) probably due to both a more compact structure of the globule and the absence of appreciable proton relaxation.

5. Conclusion

Specific features of proteins as dielectrics ensue from the general principles of their structural organization, such as fixation of a large number of highly-polar groups within a rigid structure. Fixation, i.e., a rather low mobility, of dipoles accounts for the very weak dielectric response of proteins. The set orientation of dipoles (within certain limits) is responsible for the presence of a strong intraprotein electric field. These two effects, reflecting the difference between proteins and usual polar solvents, characterize them as highly-polar, low-dielectric media.

The low static dielectric permittivity of proteins has two important consequences. On the one hand, it is the low reorganization energy of the medium, leading to reduced activation energies of most reactions. On the other hand, it is the poor solvation of charged particles, making charge transfer into a protein medium energetically disadvantageous and thereby slowing the process. The latter energetically disadvantageous process prevails in structureless dielectrics. However, it can be made up for by the intraprotein electric field. Only the joint action of two factors, the low reorganization energy and the intraprotein electric field, ensures a substantial reduction in the activation energy, i.e., the high catalytic activity of enzymes. Both effects directly follow from the general principle of protein structural organization.

The well-known structural specificity of enzymatic catalysis is due to the structure of active sites that ensures selective substrate binding, convergence, and mutual orientation of the substrate and the respective catalytic groups. In addition, such a structural factor as the spatial configuration of the intraprotein field is needed to ensure a favorable energy effect of the charge transfer process. This field depends not only on the amino acid composition of the active site but also on a large number of dipoles of peptide groups and side chains surrounding it.

For the purpose of calculation of the intraprotein field, the coordinates and partial charges of all atoms are assumed to be known. The pre-existing field of partial charges is shielded only by electronic polarization of the protein (its optical dielectric permittivity) due to the fixed position of all atoms in a given structure. In contrast, the introduction of a new charge absent in the initial structure causes the displacement of atoms, and thereby changes the structure. The necessity of using two different dielectric permittivities in the semi-continuum calculation of equilibrium energy effects is a specific property of proteins as structured polar media.

The complicated structure of proteins accounts for a variety of characteristic dielectric relaxation times for its different elements; this property affects, in the first place, the kinetics of fast electron transfer reactions, the velocity of which is comparable to the dipole relaxation rate.

Finally, the heterophasicity of biological objects (specifically, the aqueous environment of many proteins) plays an important role. This factor should be taken into account in any quantitative analysis of charge transfer processes in proteins.

We have considered the influence of protein dielectric properties on charge transfer reactions. However, it is only one aspect of the question of proteins as a specific reaction milieu. Proteins exhibit other peculiar features irreducible to dielectric properties, but their discussion is beyond the scope of this review, and we confine ourselves to their brief enumeration.

Some systems undergo large-scale conformational changes resulting from domain motion in definite directions, in addition to the dielectric response, i.e., small atomic displacements practically proportional to the electric field (in a sense, one can consider as a non-linear dielectric response the energy change due to marked changes in the orientation of individual amino acids residues). The possibility of such motion gave impetus to the formulation of the notion of proteins as molecular machines [188, 189].

Domain movements may be different. A Rieske protein bound to a bc_1-complex via the hinge region performs periodic movements from the ubiquinol-binding site (electron donor) to the cytochrome c_1 (acceptor) [190]. This device does not utilize energy from a definite chemical reaction and can hardly be called a machine in the strict sense of the word. Machines utilizing the energy from adenosine triphosphate

(ATP) are linear motors in which conformational changes cause the displacement of some proteins relative to others, e.g., myosin filaments relative to actin or kinesin molecules relative to tubuline microtubules [191]. Even more complicated relations are characteristic of rotating molecular motors, such as ATP synthase, the most important and efficacious of them [192]. In this device, the accumulated chemical energy equivalent to the proton electrochemical potential difference is converted into mechanical rotational energy of complex F_0, which is transferred to complex F_1; the resulting conformational changes induce ATP synthesis, i.e., transformation of mechanical to chemical energy. This system may function in the reverse direction as a proton pump. Electrostatic interactions play an important role also in these processes.

Some enzyme-catalyzed proton transfer processes are associated with effects usually rare in homogeneous reactions in liquids. Both donors (acids) and acceptors (bases) of protons in solutions normally have a hydrogen bond and readily come closer to one another than the equilibrium distance. This results in an appreciable reduction of the proton coordinate barrier, which enables the proton to pass to the final state in a classical (suprabarrier) manner. In many proteins, the possibility for proton donors and acceptors to draw together is restricted by their binding to the protein matrix. In these cases, the corresponding barrier is high and the sub-barrier (tunneling) transition prevails. The probability of tunneling depends on the particle mass, which accounts for the well apparent kinetic isotope effect in such reactions [24].

Long-range electron transfer, characteristic of a wide class of biological redox-processes, has an appreciable effect in the value of the pre-exponential factor (see reviews [193, 194]). Electron transfer is realized via the superexchange mechanism with an overlap between wave functions of the upper occupied and lower unoccupied orbitals of the molecules localized between the donor and the acceptor [194]. Distances between atoms having covalent bonds are much shorter; therefore, the overlap of the wave function is significantly greater than for atoms in the van der Waals contact, which explains why long-range electron transfer occurs much more frequently in proteins with an extended network of covalent bonds than in low-molecular weight solvents. This is an essential property of proteins as an electron transfer reaction medium.

The probability of electron transfer decreases exponentially with an increasing number of intermediate bonds. The distance traveled by electrons changes symbatically with a rise in the number of bonds, which accounts for the exponential decrease in probability with increasing distance [193]. Sometimes, this dependence is described as the dependence of the probability of tunneling on the barrier thickness. However, there is actually no unambiguous distance dependence of the pre-exponent, even if such empirical dependence is justified in many cases.

Proteins exhibit a variety of parallel transfer pathways. It was shown by Niki et al. [195] that electron transfer from a metal coated with a layer of long-chain carboxylic acid to cytochrone *c* involves only one of the many lysine residues (Lys-13) surrounding a heme. This residue is bound to the heme by the shortest chain of covalent bonds, which is, therefore, the shortest electron transfer pathway. Geometric distances between other lysines and the heme are practically the same, but their covalent bond chains are much longer.

Another example is electrochemical reduction of hemin absorbed on a carboxylic acid monolayer. An alteration in the orientation of hydrocarbon chains of carboxylic acid upon a change in pH of the solution results in a two-fold decrease in the layer thickness, but the reaction rate remains practically unaltered, since the number of covalent bonds between the electrode and hemin does not change, either [196].

The structure of proteins predetermines their ability to perform a large variety of functions. Much attention in this review was given to the structure-functional relationship between the general principles of protein architectonics and their properties as dielectric media for charge transfer. Certainly, the role of structural factor extends farther than that: it determines the specificity of interaction of proteins between themselves and low-molecular weight substances, mechanochemical transformations, long-range electron transfer pathways, etc. The issue of structure-functional relationships is central in molecular biophysics, and investigations into its various aspects are attracting great interest from researchers.

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