Polyelectrolyte model of DNA

M. D. Frank-Kamenetskiĭ, V. V. Anshelevich, and A. V. Lukashin

Institute of Molecular Genetics, Academy of Sciences of the USSR

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The present state of the theory of strongly charged polyelectrolytes of the DNA type is reviewed. An infinitely long, uniformly charged cylinder immersed in a dielectric continuum is adopted as a model of DNA. Small mobile ions are treated as impermeable spheres. A comparison of the results of rigorous and approximate theoretical approaches to the description of this model shows that the self-consistent-field method, i.e., the Poisson-Boltzmann equation, is a reliable basis for deriving quantitative results. The theory of polyelectrolytes based on a solution of the nonlinear Poisson-Boltzmann equation is used to analyze the role played by electrostatic interactions in conformational changes in DNA. Transitions of two types are considered: a helix-coil transition and a transition between the ordinary right-hand-helix DNA (the B form) and the recently discovered left-hand-helix (the Z form). In the latter case the theory predicts a nonmonotonic behavior of the difference between the free energies of these conformations as a function of the salt concentration. It also predicts the existence of a critical point of a B-Z equilibrium for ionic strengths in the physiological region.

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1. INTRODUCTION; BASIC MODEL OF THE THEORY OF POLYELECTROLYTES OF THE DNA TYPE

Many polymers dissociate in solution, forming a charged polyion and small mobile ions (protons, hydroxyl ions, metal ions, etc.). In general, a solution may also contain other small ions of both signs. A system of this type, containing polyions and small ions, is called a "polyelectrolyte solution." The long-range electrostatic potential makes both the experimental behavior and the theoretical description of polyelectrolytes substantially different from those of ordinary uncharged polymers. The recent development of a theory of polyelectrolytes has been motivated by the circumstance that DNA is an example of a highly charged polyion in which there are two electron charges for each monomer unit (a pair of nucleotides).

The discovery of a completely new structure of the double helix, the so-called Z form, has caused a surge of interest in electrostatic effects in DNA. In contrast with the standard right-hand-helix of DNA of the B form, the Z form is a left-hand helix (Fig. 1). There are several other fundamental differences between the structures of the B and Z forms.[1] Sequences with a regular alternation of guanine and cytosine, poly(dGC)·poly(dGC),

. . . . GCGCGCGCGC . . . .
. . . . CGCGCGCGCG . . . . ,

convert most easily into the Z form. The unusual structural transition which occurs in this polymer as the ion strength is raised was discovered by Pohl and Jovin[2] by a circular-dichroism method. After Rich *et al.*[3] determined the structure of the Z form by x-ray structural analysis, studies by numerous methods (NMR, Raman scattering, etc.) showed that the transition observed in Ref. 2 did indeed correspond to the B-Z transition. The B-Z transition in DNA has now been studied in detail by a variety of experimental approaches (this work is reviewed in Refs. 1 and 4). A very interesting fact has emerged: A chemical modification of the alternating GC polymer consisting of a replacement of cytosine by methylcytosine [this compound is called poly(dGm^{5}C)·poly(dGm^{5}C)] sharply reduces the ionic strength at which the B-Z transition occurs.[5] Furthermore, it has recently been found that a polymer of this sort converts into the Z form not only as the ion strength is increased but also as it is lowered[6–8] (Fig. 2). These results make it a very urgent matter to develop a polyelectrolyte theory for the conformational transitions in DNA.

Electrostatic effects also play an important role in other structural transitions which are observed in DNA, the best-known and best-studied of which is the helix-coil transition. As is described in detail in the reviews in Refs. 9–11, the melting point of DNA depends linearly on the logarithm of the salt concentration in the solution; this simple relation has been a challenge to theoreticians for a long time now. Electrostatic interactions also play a very important role in

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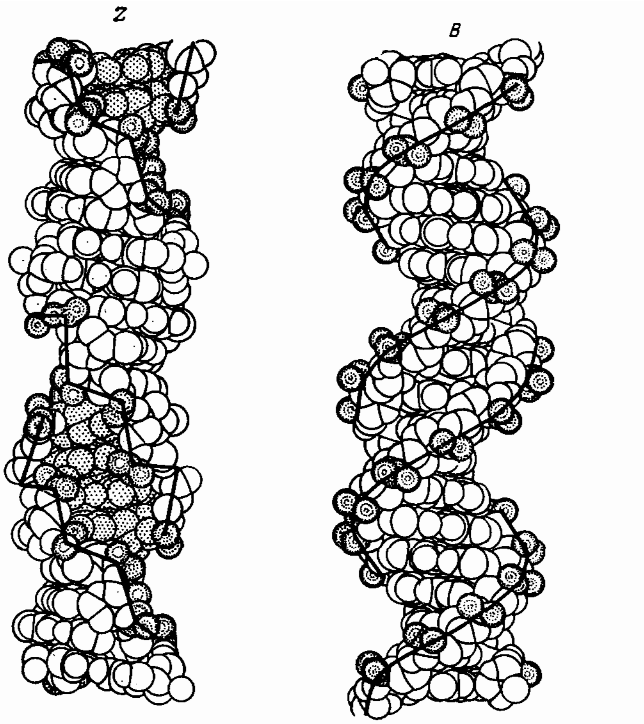

FIG. 1. Left-hand helix of the Z form (at the left) and right-hand helix of the B form (at the right), according to data from x-ray structural analysis.[1] The solid lines show the course of the sugar-phosphate chain. There is one electron charge on each phosphate group. The pitch of the helix of the Z form is 4.46 nm, and the number of pairs of nitrogenous bases per pitch of the helix is 12.0. For the B form, these values are 3.40 nm and 10.0.

the relaxation processes which are observed in the course of the helix-coil transition, which have recently attracted particular interest.[10-12]

In the theoretical work on electrostatic effects in DNA, the most popular model is the simple one (Fig. 3) in which DNA is an infinitely long, uniformly negatively charged cylinder of radius a with a surface charge density $-es$, where e is the charge of the proton. This cylinder is assumed to be immersed in an infinite medium with a constant dielectric constant $D \approx 80$ (an aqueous medium). Also in the volume are mobile ions, which are modeled by impermeable spherical particles of nonzero diameter d with a charge $ne (n = \pm 1)$. The concentrations by volume of these mobile ions at infinity are identical, equal to c_0 (a solution of a monovalent salt of the NaCl type).

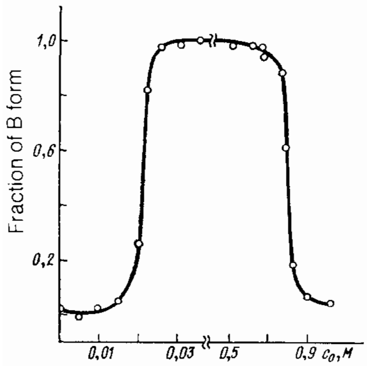

FIG. 2. Fraction of the DNA in the B form for poly(dGm^{5}C) as a function of the salt concentration c_0 according to measurements based on the circular-dichroism spectra.[6] At a low ionic strength ($c_0 < 0.01$M) the DNA is in the Z form. As c_0 increases, there is a transition to the B form (Z-B) and later, at $c_0 \approx 0.8$M, back to the Z form (B-Z). In Refs. 7 and 8, which were published later, the Z-B transition was observed at lower ionic strengths (~ 0.002M).

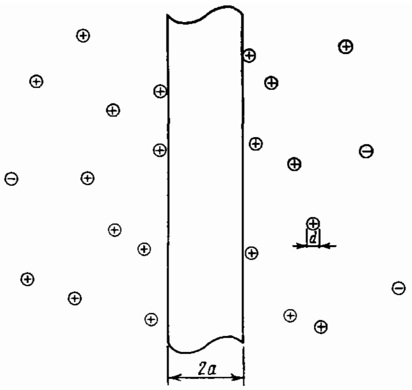

FIG. 3. Basic model of polyelectrolyte theory.

We are interested in determining certain characteristics of this electrostatic system: the equilibrium electrostatic potential $u(r)$ and the equilibrium concentrations of positively and negatively charged particles, $c^+(r)$ and $c^-(r)$, which depend (by virtue of the cylindrical symmetry only on the distance from the axis of the cylinder, r. These characteristics are related to each other by the Poisson equation

$$u''(r) + r^{-1}u'(r) = -4\pi l_B (c^+(r) - c^-(r)), \tag{1}$$

where the prime means differentiation with respect to the radial coordinate r, and $l_B = e^2/DkT$ is the Bjerrum length ($l_B = 0.7$ nm for an aqueous medium at room temperature). The dimensionless potential $u(r)$ in (1) is found in terms of the ordinary electrostatic potential $U(r)$ by means of the relation $u(r) = eU(r)/kT$. The boundary conditions on Eq. (1) are

$$u'(a) = \frac{2q}{a}, \quad u(R) = 0; \tag{2}$$

here R is a point which is infinitely remote from the axis of the cylinder. The boundary condition at this point formally makes the system electrically neutral at the macroscopic level. The most important parameter of polyelectrolyte theory is the quantity q, the dimensionless negative charge per unit length on the cylinder:

$$q = 2\pi a l_B s = \frac{l_B}{b}, \tag{3}$$

where b is the length of that section of the cylinder on whose surface the charge $-e$ is concentrated. In calculating q for a specific DNA structure (Fig. 1), we will take b to mean the average distance along the DNA axis between elementary charges. For the B form, for example, the distance along the axis of the helix between pairs of nucleotides is $2b = 0.34$ nm, and we have $q = 4.2$. For the Z form we have $2b = 0.37$ nm and $q = 3.9$.

This is the very simplest basic model of polyelectrolyte theory. Knowing the functions $u(r)$ and $c^n(r)$, we can calculate the electrostatic contributions to various thermodynamic characteristics of the DNA.

Other models constitute complications of one sort or another of this basic model. For example, it is natural to consider the bends of the cylinder; in this case it becomes possible to calculate the electrostatic contribution to the persistence length. Incorporating the interaction between different cylinders is necessary for analyzing three-dimensional effects and for dealing with concentrated polyelectrolyte solutions. One could take the path of adding details in the sense of incorporating the discrete nature of the charge distribution at a polyion, incorporating the molecular structure of the solvent, etc. However, there is no point in considering any of these complicating factors until we have found an appropriate method for solving the basic problem.

In this review we will focus on the problem of correctly analyzing the basic model of polyelectrolyte theory and the results of application of the theory to describe two types of conformational transitions in DNA: the helix-coil transition and the B-Z transition. We will also discuss the applicability of the basic model for analyzing specific situations.

2. POISSON-BOLTZMANN EQUATION

Equation (1) relates three functions which are to be determined: $u(r)$, $c^{+}(r)$, and $c^{-}(r)$. The particle concentration functions $c^{n}(r)$ can be written as

$$c^{n}(r) = c_0 \exp\left[-\left(f_r^{n} - f_R^{n}\right)\right], \tag{4}$$

where the difference $f_r^{n} - f_R^{n}$ is the free energy (in units of kT) which is required to move a particle of diameter d and charge ne from an infinitely remote point R to the point r. The basic problem in calculating the functions $u(r)$ and $c^{n}(r)$ is that in the basic model the free energy f_r^{n} is not expressed exclusively in terms of $u(r)$ and the first correlation functions of $c^{n}(r)$. A systematic derivation of the unknown characteristics $u(r)$ and $c^{n}(r)$ reduces to solving a chain of coupled equations which are equivalent to a hierarchy of Bogolyubov-Born-Green-Kirkwood-Yvonne (BBGKY) equations (Refs. 13 and 14, for example). A correct truncation of this system of equations unavoidably requires the introduction into the basic model of some additional small parameters.

It becomes significantly simpler to solve the original problem if we switch from a discrete description of the mobile ions to a model of a charged continuous medium. Specifically, we let the diameters of the mobile ions and their charges approach zero, and we let the number of ions of each species approach infinity, in such a way that the charge density at each point remains fixed. When we follow this procedure we find in particular that the free energy f_r^{n} can be replaced by the energy of a particle of species n in an average electrostatic potential $u(r)$. We can then write $c^{n}(r) = c_0 \exp[-nu(r)]$, and from Eqs. (1)-(4) we find

$$u''(r) + r^{-1}u'(r) = \varkappa^2 \operatorname{sh} u(r), \tag{5a}$$

$$u'(a) = 2qa^{-1}, \quad u(R) = 0, \tag{5b}$$

where

$$\varkappa^{-1} = r_D = (8\pi l_B c_0)^{-1/2} \tag{6}$$

is the Debye length.

Expression (5) is the well-known Poisson-Boltzmann equation for the case of cylindrical symmetry (Ref. 15, for example). For the basic model, the Poisson-Boltzmann equation corresponds to the self-consistent field approximation, while for the model of a charged continuous medium this equation is rigorous. An explicit analytic solution of the nonlinear equation (5) for arbitrary values of the parameters q, a, and c_0 is not known. Ramanathan[16] recently derived the potential at the surface of the cylinder $u(a)$, making use of the asymptotic behavior in the case of the small parameter $\varkappa a$, i.e., for extremely low ionic strengths:

$$u(a) = 2q \ln(\varkappa a), \qquad q < 1, \tag{7a}$$

$$u(a) = 2\ln(\varkappa a) - \ln\left[4(q-1)^2\right], \quad q > 1. \tag{7b}$$

Comparison of expressions (7) with the exact values found for $u(a)$ through a numerical solution of Eq. (5) shows that at $q < 1$ and $q > 2$ expressions (7a) and (7b) are essentially rigorous even at $c_0 < 0.01$M ($\varkappa a < 0.3$), while at $c_0 \sim 0.1$M the difference is no more than 5%.

Equation (5) describes the case of a so-called salt excess. Another case which might be considered is that in which there is no supporting electrolyte in the solution (an infinite dilution in terms of the salt). Equation (5) does not give a correct description of this limit, since the excess-salt condition played a fundamental role in its derivation. The concentration of mobile ions (the ionic strength) can be arbitrarily low, but the total number of these ions in the volume must be much greater than the number of charges on a polyion. Making the same assumptions as were used in the derivation of Eq. (5), Katchalsky *et al.* proposed a correct method for taking the limit of extreme dilution (see Ref. 17 and the papers cited there). They considered a model of cells in which ions of only one sign (counter-ions) are in a cylindrical ring of finite radius R_0 around a polyion; these ions neutralize the charge on the polyion. In this case the Poisson-Boltzmann equation becomes

$$u''(r) + r^{-1}u'(r) = -\frac{p^2}{2} e^{-u(r)}. \tag{8}$$

Equations of the same type describe diffusion and heat transfer in cylindrically symmetric cases.[18]

In the polyelectrolyte problem, the parameter p in Eq. (8) is unambiguously related to the outer radius R_0: $p^2 = 8\pi l_B c^{+}(R_0)$. The boundary conditions for the cell model are

$$u'(a) = \frac{2q}{a}, \quad u'(R_0) = 0, \quad u(R_0) = 0. \tag{9}$$

This model is attractive in that, in contrast with (5), an explicit solution of nonlinear equation (8) can be found (Refs. 19 and 20; see also the recent paper by LeBret and Zimm[21]) for arbitrary values of the parameters of the problem:

$$u(r) = \ln\left[\left(\frac{p}{2|z|}\right)^2 r^2 \cos^2\left(z \ln\frac{r}{R_M}\right)\right]. \tag{10}$$

Here z and R_M are integration constants; z^2 is a real quantity; and we have $R_M > 0$. Boundary conditions (9) give us a system of equations for finding z and R_M in terms of the parameters p and q:

$$1 - z \operatorname{tg}\left(z \ln\frac{a}{R_M}\right) = q, \tag{11a}$$

$$1 - z \operatorname{tg}\left(z \ln\frac{R_0}{R_M}\right) = 0, \tag{11b}$$

$$p^2 R_0^2 = 4(1 + z^2). \tag{11c}$$

Since the 1950s, the Poisson-Boltzmann equation has been used widely to analyze the polyelectrolyte properties of polymer chains.[15-17,19-49] Until very recently, the results derived with the help of this equation were not completely trusted since this equation was derived in the self-consistent-field approximation. There are many examples in which the use of this method leads to incorrect results. As for the polyelectrolyte theory, we note that again in this case the suitability of the self-consistent-field method was by no means obvious.

3. DEBYE-HÜCKEL APPROXIMATION; THEORY OF THE CONDENSATION OF COUNTER-IONS

The situation simplifies significantly if the condition

$$|u(r)| \ll 1 \tag{12}$$

holds at any point r. Under this condition, Eq. (5) becomes linear and converts into the well-known Debye-Hückel equation:

$$u''(r) + r^{-1}u'(r) = \varkappa^2 u(r), \tag{13a}$$

$$u'(a) = \frac{2q}{a}, \quad u(R) = 0. \tag{13b}$$

Linearized equation (13) is rigorous not only for the model of a charged continuous medium but also for the basic model in general, since under condition (12) the self-consistent-field method becomes rigorous. A solution of Eq. (13) is (Ref. 50, for example)

$$u(r) = -2q\,(\varkappa a K_1(\varkappa a))^{-1}\,K_0(\varkappa r). \tag{14}$$

Here $K_m(x)$ is a modified Bessel function.

The Debye-Hückel equation is used very widely and successfully in the theory of simple—nonpolymer—electrolytes. It leads to the well-known Debye screening of a Coulomb potential: an effect whose importance extends far beyond the theory of electrolytes. In polyelectrolyte theory the Debye-Hückel equation is also used widely. For example, in cases in which the charges are distributed sparsely on the polyion the Debye-Hückel equation can be applied directly. This topic is the subject of a fairly extensive literature (among the recent papers, see, e.g., Refs. 51 and 52).

In the present review we are interested in the case of highly charged polyelectrolytes, for which the Debye-Hückel equation is definitely not valid near a polyion. At a sufficiently large distance from a polyion, on the other hand, condition (12) becomes valid, and we are tempted to use the Debye-Hückel equation in this region. However, in order to find the potential far from a highly charged polyion on the basis of the Debye-Hückel equation we need to know boundary conditions at the boundary of the region in which the Debye-Hückel equation is applicable. Consequently, until the actual form of these boundary conditions has been found, the approach based on the use of the Debye-Hückel equation will not be productive for finding the potential $u(r)$ even far from a polyion, since this approach gives the value of the potential only within an unknown constant factor.

An elegant way to avoid this difficulty was proposed by Manning.[53] Manning's idea was to use the internally consistent Debye-Hückel equation in the theory and to find boundary conditions in terms of a condensation of counter-ions on a polyion. The condensation idea is simply the most brilliant physical idea in all of polyelectrolyte theory. This idea can be credited to Onsager,[53] who discussed it as follows.

We consider a negatively charged polyion consisting of an infinitely thin rectilinear filament with a charge per unit length $-e/b$. We assume that the mobile ions are point charges. Here r is the distance from the filament to the nearest counter-ion. At sufficiently small values of r, the electrostatic energy of this counter-ion is determined by the unscreened Coulomb potential of the filament:

$$u(r) = 2q \ln r. \tag{15}$$

That component of the complete partition function which comes from configurations in which the nearest counter-ion lies at a distance $r < r_0$ from the filament, while all the other mobile ions are at distances greater than r_0, can be written within a finite factor as

$$Z = \int_0^{r_0} e^{-u(r)} r\,\mathrm{d}r = \int_0^{r_0} r^{1-2q}\,\mathrm{d}r. \tag{16}$$

We see that for $q > 1$ the partition function diverges at the lower limit. Onsager and later Manning concluded from this fact that if the charge per unit length on a polyion is such that the relation $q > 1$ holds then the counter-ions should "condense" on the polyion, thereby reducing its charge density to the critical value corresponding to $q = 1$.

On the basis of these considerations Manning proposed a procedure for calculating the potential around a polyion. Specifically, the potential is to be calculated from the Debye-Hückel equation with the following boundary condition at the surface of the polyion:

$$u'(a) = \frac{2q^*}{a}, \tag{17a}$$

$$q^* = q, \quad q < 1; \qquad q^* = 1, \quad q \geqslant 1. \tag{17b}$$

In Manning's theory the boundary-condition problem is thus resolved without reference to the magnitude of the initial charge density on the polyion. The case $q < 1$, in which Manning's theory reduces to the conventional Debye-Hückel theory, corresponds to the case of a weakly charged polyion, while the case $q > 1$ corresponds to that of a highly charged polyion. In Manning's theory no formal distinction is made between these two cases.

Manning's theory is attractive because of its physical transparency and because it allows the derivation of some simple explicit expressions, since a linear equation is used for the potential. At one time, these factors won the theory widespread popularity in research on various physicochemical properties of polyelectrolyte solutions.[53–70] Many experimental confirmations of the theory have been found (see the reviews in Refs. 63 and 64, for example). The success of the theory has been attributed to the concept of a condensation of counter-ions, which is part of the basis of this theory. It gradually came to be believed that all the conclusions of Manning's theory are consequences of the condensation idea. Furthermore, the theory itself came to be called "condensation theory." It also gradually came to be believed that the picture of condensation which was introduced in the theory on the basis of the formal mathematical arguments presented above corresponds to physical reality. In other words, the counter-ions surrounding a polyion decompose into two phases: Some of them "settle" on the surface of the polyion, while others form a diffuse cloud around it.

By the beginning of the 1980s, the following situation had thus developed in the theory of highly charged polyelectrolytes (see the review in Ref. 36). There were two independent approaches: one which used the Poisson-Boltzmann equation,[15-17,19-49] and one which reduced the problem to a Debye-Hückel equation through the use of the concept of condensation.[53-70] Since each of these approaches starts with an *ad hoc* postulate, the only way out of the situation was to derive a more rigorous theory.

4. RIGOROUS METHODS FOR ANALYZING THE BASIC MODEL

The most direct approach which might be taken in an attempt to find accurate values for the potential and density of the mobile ions for the basic model is the Monte Carlo method or, more precisely, that version of the Monte Carlo method which is known as the Metropolis method.[71,72] This method has been used in several studies,[73-76] and the results of direct simulations have been compared with the solution of the Poisson-Boltzmann equation both for the cell model[73,74] and for the case of a salt excess.[75,76] A general conclusion which follows from these studies is quite encouraging: The differences between the results found by the Monte Carlo method and those found from the Poisson-Boltzmann equation are slight. The discrepancy in even such a sensitive characteristic as the density of counter-ions near the surface of the polyion does not exceed 10% in the worst cases.

On the other hand, for the time being the results found for a given problem by the Monte Carlo method should be interpreted somewhat cautiously, especially in the case of a salt excess.[75,76] In practice, the convergence rate of the Metropolis method is such that the actual number of ions involved in the process cannot exceed a few hundred. On the other hand, the long-range nature of the electrostatic potential, which is used without screening in this approach, has the consequence that the "cell" volume in the Monte Carlo method must be extremely large. This comment applies in particular to the size of a cylindrical "cell" along the axial direction.[75] Accordingly, the number of particles which should be taken into account for a correct solution of the problem is considerably higher than can be handled by existing computers, so it becomes necessary to appeal to approximate methods, which lack a solid basis for solving this problem.[75,76]

Some theoretical papers of a general nature appeared in parallel with the Monte Carlo calculations and furnished results which, in our opinion, overlap the results of the Monte Carlo work for the basic model of polyelectrolyte theory.

The first study which took up the question of a statistical-mechanics foundation for the Poisson-Boltzmann equation was a paper by Fixman.[77] Fixman developed a method for making correlation corrections to the Poisson-Boltzmann equation, and for a certain particular model he numerically calculated the expected deviation from the results given by the Poisson-Boltzmann equation. Specifically, the error in the value of the electrostatic potential found from the Poisson-Boltzmann equation can be of the order of 20% at ionic strengths $c_0 < 0.1$M, according to Fixman.[77] A point which should be emphasized here is that although just how general the calculations carried out by Fixman were, remained unclear, this study changed the course of the debate over the Poisson-Boltzmann equation and Manning's approach (see the review in Ref. 36) and spurred the subsequent widespread use of the Poisson-Boltzmann equation.

A rigorous analysis of this problem in the case of extremely low ionic strengths was carried out by Ramanathan and Woodbury.[78] They took an approach based on an analysis of a chain of coupled BBGKY equations. For the basic model of polyelectrolyte theory, a correct method for truncating this chain can be implemented by retaining the leading terms in the expansion in terms of additional small parameters which arise because of the small values of the ionic strengths: $\varkappa a \ll 1$, $\varkappa d \ll 1$, $\varkappa l_B \ll 1$. The basic result of Ref. 78 is the demonstration that the Poisson-Boltzmann equation is rigorous for this particular limiting case. The question of the suitability of the Poisson-Boltzmann equation at nonvanishing values of the ionic strengths, on the other hand, remains open. Bacquet and Rossky[79] examined the problem of finite ionic strengths, also working from an analysis of a chain of coupled equations for correlation functions. Their analysis, however, cannot be accepted as convincing since it was based on a technique for splitting the equations which, although it is a standard technique (the hyperchain approximation), has absolutely no justification for this particular problem.

In Ref. 80 we carried out a statistical-mechanics analysis of the basic model of polyelectrolyte theory for finite ionic strengths. That approach made it possible to avoid the problem of finding an "exact" solution of a chain of coupled equations. Specifically, closed equations, amenable to effective solution, were found for functions $u_{\max}(r)$ and $u_{\min}(r)$ such that they bracket the unknown equilibrium electrostatic potential $u(r)$ which satisfies relations (1)–(4):

$$u_{\min}(r) \leqslant u(r) \leqslant u_{\max}(r). \tag{18}$$

Analysis based on a numerical solution of these equations showed that the "gap" $u_{\max}(r) - u_{\min}(r)$ is fairly small over essentially the entire region of values of the parameters a, q, d, and c_0 which is of interest. Here we will simply outline the basic idea of the method and then look at the final results.

Returning to the initial relations, (1)–(4), we write the free energy required for the transfer of a particle of diameter d with charge ne from an infinitely remote point R to the point r as

$$f_r^n - f_R^n = (f_r^0 - f_R^0) + (f_r^n - f_r^0) - (f_R^n - f_R^0); \tag{19}$$

here $f_r^0 - f_R^0$ is the free energy required for the formation of a spherical cavity of diameter d which is free of ions and which is centered at a point r. This free energy is reckoned from the free energy of the formation of an equivalent cavity at an infinitely remote point R. We denote by P_r the probability that the center of no particle lies within a spherical cavity of radius d which is centered at the point r. We then have

$$f_r^0 - f_R^0 = -(\ln P_r - \ln P_R). \tag{20}$$

The difference $f_r^n - f_r^0$ is then the free energy required for a change in the charge in a spherical cavity from zero to ne. To calculate it, it is convenient to use the standard charge-variation technique. We fix the center of a test particle of diameter d and charge nte $(0 < t < 1)$ at point r, and we denote by $u(r|r,nt)$ the equilibrium potential which is set up at point r by all the mobile particles and the polyion. We then write

$$f_r^n - f_r^0 = n \int_0^1 u(r|r,\ nt)\, dt. \tag{21}$$

For the function $c^n(r)$ we thus have

$$c^n(r) = c_0 \frac{P_r}{P_R} \exp\left\{ - n \int_0^1 [u(r|r,\ nt) - u(R|R,\ nt)]\, \mathrm{d}t \right\}. \tag{22}$$

We wish to call attention to the fact that the equilibrium potential $u(r|r,nt)$ is calculated under the condition that the test particle is fixed at the point r. The presence of this particle changes the statistical weights of the configurations of the mobile ions; in general the potential $u(r|r,nt)$ will be different from $u(r)$. These quantities are equal to each other only in the approximation of a charged continuous medium. Pursuing the discussions in this direction, we find that in order to obtain the potentials $u(r|r,nt)$ we need to carry out a variation of the charges of *two* test particles; etc. Accordingly, and as we have already mentioned, the systematic derivation of the unknown characteristics $u(r)$ and $c^n(r)$ reduces to the problem of solving an infinite chain of coupled equations which is equivalent to the BBGKY equations. However, a different approach can be taken here.

Let us take a more detailed look at what the difference between the potentials $u(r|r,nt)$ and $u(r)$ actually is. We first note that the placing of a test particle of diameter d and charge nte at point r is accompanied by a displacement of a charge $E(r,c)$ from the corresponding spherical region. The magnitude of this displaced charge can be easily calculated from the given equilibrium charge density $ec(r)$, where $c(r) = c^+(r) - c^-(r)$ in the neighborhood of the point r. After we have placed the test particle at point r, the equilibrium charge density must change in such a way that the charge $nte - E(r,c)$ which is placed at point r is screened. We denote by $I(r,nt)$ the component of the electrostatic potential $u(r|r,nt)$ at point r which stems from this additional screening. The potential $u(r|r,nt)$ can then be written in the form

$$u(r \mid r,\ nt) = u(r) - v(r,\ c) + I(r,\ nt), \tag{23}$$

where $v(r,c)$ is the component of the electrostatic potential $u(r)$ which comes from particles in the spherical region around point r before the test particle is placed there. This quantity, like the charge $E(r,c)$, is completely determined by the equilibrium charge density $ec(r)$ near point r.

The basic problem is to find the additional screening function $I(r,nt)$. A rigorous calculation of this characteristic of course runs into the same difficulties as the solution of the original problem, but for it we can find upper and lower estimates. Specifically, we know the net charge which sets up the additional screening: It is equal to the negative of the screened charge. The maximum possible screening is reached when we place all of the screening charge at the smallest possible distance (d) from the screened charge. The minimum possible screening is the removal of the screening charge to an infinite distance from the screened charge. This type of analysis of the screening function yields rigorous upper and lower estimates of the integrals of the potential difference $u(r|r,nt) - u(R|R,nt)$ which appear in the expression for the particle concentration functions $c^n(r)$. There is no difficulty in finding upper and lower estimates of the ratio P_r/P_R.

These estimates lead to inequalities involving the concentration functions $c^n(r)$, which serve in turn as the basis for a derivation of closed equations for the potentials $u_{\min}(r)$ and $u_{\max}(r)$ satisfying inequalities (18). These equations are

$$u''_{\mathrm{m}}(r) + r^{-1}u'_{\mathrm{m}}(r) = -4\pi l_{\mathrm{B}}(c^+_{\mathrm{m}}(r) - c^-_{\mathrm{m}}(r)), \tag{24a}$$

$$u'_{\mathrm{m}}(a) = \frac{2q}{a}, \tag{24b}$$

$$u_{\mathrm{m}}(R) = (-1)^m \left(\frac{l_{\mathrm{B}}}{4d} - \ln\left(1 - \frac{8\pi d^3 c_0}{3}\right)^{1/2}\right), \tag{24c}$$

$$c^+_{\mathrm{m}}(r) = c_0 G^+_{\mathrm{m}}(r,\ c^+_{\mathrm{m}},\ c^-_{\mathrm{m}}) \exp(-u_{\mathrm{m}}(r) - L^+_{\mathrm{m}}(r,\ c^+_{\mathrm{m}},\ c^-_{\mathrm{m}})), \tag{24d}$$

$$c^-_{\mathrm{m}}(r) = c_0 G^-_{\mathrm{m}}(r,\ c^+_{\mathrm{m}},\ c^-_{\mathrm{m}}) \exp(+u_{\mathrm{m}}(r) + L^-_{\mathrm{m}}(r,\ c^+_{\mathrm{m}},\ c^-_{\mathrm{m}})). \tag{24e}$$

The subscript m in these expressions has the meaning m = min if we are calculating $u_{\min}(r)$ or m = max in the opposite case. In boundary condition (24c) we have $m = 1$ for $u_{\min}(R)$ and $m = 2$ for $u_{\max}(R)$. The functions $G_{\mathrm{m}}(r)$ and $L_{\mathrm{m}}(r)$ are simultaneously functionals of the particle concentrations $c^n_{\mathrm{m}}(r)$ and can be expressed in terms of them explicitly.[80] In addition to the difference in boundary conditions (24c), the systems of equations with "min" and "max" differ in the specific form of these functionals. On the whole, relations (24) constitute a closed system of nonlinear integrodifferential equations for the three unknown functions $u_{\mathrm{m}}(r)$, $c^+_m(r)$ and $c^-_m(r)$. The specific features of these equations suggest use of the effective procedure of Ref. 80 for a numerical solution. That procedure can be implemented on a minicomputer at roughly the same speed at which Poisson-Boltzmann equation (5) can be solved.

In Ref. 80 we gave a rigorous proof that the functions $u_{\min}(r)$ and $u_{\max}(r)$, which are solutions of systems of equations (24), satisfy inequalities (18). The only fundamental restriction on the approach taken in Ref. 80 is the requirement that there be no short-range order (or, even more so, no long-range order) in the arrangement of the mobile ions in the system (this is the "gas" approximation). The formal expression of this fact is that absence of short-range order is necessary if we wish to find unambiguous estimates of the screening function $I(r,nt)$ and to prove inequalities (18). The gas approximation is valid for mobile-particle concentrations below a certain critical c_{cr} which depends on the diameter of the mobile ions, the temperature, and other parameters of the system. The following crude estimate, clearly on the low side, was found in Ref. 80:

$$c_{\mathrm{cr}} = \left(\frac{4\pi d^3}{3} + 2\pi d^2 l_{\mathrm{B}}\right)^{-1} \tag{25}$$

Figures 4 and 5 illustrate the situation with values found for the potentials $u_{\min}(r)$ and $u_{\max}(r)$ at the surface of a polyion ($r = a$) through a numerical solution of systems (24) for two values of the diameter of the mobile parts: $d = 0.8$ nm and $d = 0.2$ nm. These values are typical of the cases of hydrated and nonhydrated ions. The radius of the polyion is $a = 1$ nm; this figure corresponds to the case of DNA in the B form. The results are compared with the solution given by the Poisson-Boltzmann equation over broad ranges of the parameters c_0 and q. These results show that the "gap" between the limiting values of $u_{\max}(r)$ and $u_{\min}(r)$ is small enough to permit a reliable estimate of the actual value of the potential $u(r)$. Completely similar results are found for the potentials at any distance from the polyion.

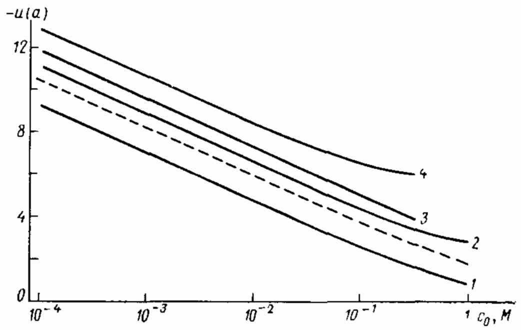

FIG. 4. Maximum and minimum values of the electrostatic potential at the surface of a polyion as functions of the ionic strength of the solution for two diameters of the mobile ions. Solid lines: 1—$u_{\max}$ ($d = 0.2$ nm); 2—$u_{\min}$ ($d = 0.2$ nm); 3—$u_{\max}$ ($d = 0.8$ nm); 4—$u_{\min}$ ($d = 0.8$ nm). Dashed line: The result of a solution of the Poisson-Boltzmann equation. The other parameter values are $l_B = 0.7$ nm, $q = 4.2$, and $a = 1.0$ nm.

Our data agree completely with the estimates of Fixman,[77] the results of the study by Ramanathan and Woodbury,[78] and the conclusions which follow from Monte Carlo studies.[73–76] Our data established the range of applicability of the Poisson-Boltzmann equation for finite values of the ionic strengths. Within the context of this review we can conclude that the approach based on the Poisson-Boltzmann equation is completely adequate for our problem, especially for small diameters of the mobile ions.

The primary result of the recent theoretical work of a general nature has thus been to explain the fact that, in contrast with the situation in many other cases of the use of the self-consistent-field method, the Poisson-Boltzmann equation is an extremely good approximation for the basic model of polyelectrolyte theory. The Poisson-Boltzmann equation is thus regaining its status as the basis for theoretical research on polyelectrolytes, now with a complete foundation and a clearly defined range of applicability.

5. PRESENT STATUS OF CONDENSATION THEORY

In the course of the "rehabilitation" of the Poisson-Boltzmann equation, old results derived back in the 1950s by Katchalsky *et al.*[17] were reinterpreted, and also new results were obtained, which revealed in particular the reasons for the success of Manning's condensation theory.[16,21,77,78,81,82] In light of the Poisson-Boltzmann equation, these successes are at first glance surprising, since the Poisson-Boltzmann equation does not predict the existence of a phase separation of counter-ions for a finite polyion radius. Indeed, the very form of the Poisson-Boltzmann equation rules out the possible existence in this case of solutions with singularities (such a solution arises only in the limit of an infinitely thin filament[81]). In this connection we should recall that the condensation concept arose specifically in the study of an infinitely thin charged filament. One might suspect that this phenomenon is in general an artifact of this particular model and disappears for any finite radius of the polyion. Let us examine this fundamental question in more detail.

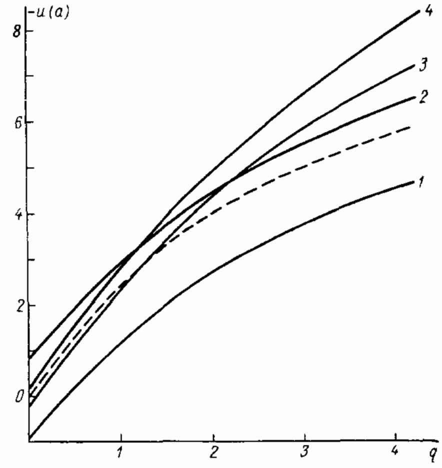

FIG. 5. Maximum and minimum values of the electrostatic potential at the surface of a polyion as functions of the charge per unit length on the polyion q. Solid lines 1–4 have the same meaning as in Fig. 4; the dashed line is a solution of the Poisson-Boltzmann equation. All the curves were obtained for $c_0 = 0.01$M.

We first consider the case of a limiting dilution in terms of the salt, in which case the solution essentially contains only counter-ions. This case is important from the conceptual standpoint since it allows us to see the real meaning of "condensation" for a polyion of finite radius, as was originally pointed out by Zimm and LeBret.[82]

Following Ref. 82, we take up the solution of the Poisson-Boltzmann equation for cell model (10), (11). We note that for the case of interest to us, $q > 1$, the integration constant R_M has a clear physical meaning. We denote by $W(r)$ the number of counter-ions which lie in a cylindrical shell of height l_B with outer radius $r < R_0$. For $W(r)$ we find from (10) and (11)

$$W(r) = \frac{p^2}{4} \int_a^r e^{-u(r)}\, r\, dr = z \operatorname{tg}\left(z \ln \frac{r}{R_M}\right) + q - 1. \tag{26}$$

It follows that the number of counter-ions which lie within a cylindrical shell with an outer radius R_M is

$$W_M = q - 1. \tag{27}$$

This is precisely the number of counter-ions which according to Manning's theory should "settle" on the surface of the polyion (in other words, which should be in a cylindrical shell which has a thickness of the order of the radius of the polyion and which is adjacent to the polyion).

For any W in the interval $0 < W < q$ the outer radius of a cylindrical shell enclosing a given number of counter-ions W is given by

$$r(W) = R_M \exp\left(z^{-1} \operatorname{arctg} \frac{W - W_M}{z}\right). \tag{28}$$

We now take the limit $p \to 0$ ($R_0 \to \infty$). In this limit, the asymptotic form of function (28) is different in the cases $W < W_M$, $W = W_M$ and $W > W_M$:

$$\lim_{p \to 0} r(W) = a \exp\left[\frac{W}{W_M (W_M - W)}\right], \quad W < W_M, \tag{29}$$

$$\lim_{p \to 0} r(W_M) = \lim_{p \to 0} R_M = \left(\frac{2a}{p}\right)^{1/2} \exp \frac{W_M - 1}{2W_M}, \tag{30}$$

$$\lim_{p \to 0} r(W) = \frac{2}{p} \exp\left(1 - \frac{1}{W - W_M}\right), \quad W > W_M. \tag{31}$$

According to Manning's theory, the radius R_M which bounds the cylindrical shell enclosing the counter-ions which neutralize the charge on the polyion to $q = 1$ does not depend on p; it has a value of the order of the polyion radius a. Actually, however, as can be seen from (30), the value of R_M in the case of infinite dilution tends toward infinity as $(a/p)^{1/2} = (aR_0)^{1/2}$. We see that the two-phase model does not hold up, and the concept of a condensation of counter-ions in the sense treated by Manning is incorrect.

Zimm and LeBret showed,[82] however, that in a certain sense one can speak in terms of if not a condensation, then a "confinement" of counter-ions by a polyion. This confinement effect becomes particularly transparent when we compare the behavior of counter-ions around charged objects of three types—a plane, a cylinder, and a sphere—in the limit of infinite dilution. In the first of these cases, we know quite well that all the counter-ions will condense on the plane, and an electrical double layer will form. In the case of the sphere, in contrast, all the counter-ions will go off to infinity; i.e., a sphere is incapable of confining a cloud of counter-ions around itself. The same comments apply to the case of a weakly charged cylinder ($q<1$). In the case of a highly charged cylinder ($q>1$), however, some of the ions go off to infinity, while the others form a diffuse cloud around the cylinder, so that the concentration of counter-ions falls off smoothly with distance from the cylinder. Expression (29) gives the limiting expression for the distribution function of counter-ions in the case $q>1$. Interestingly, however, the total number of confined ions is precisely equal to the number of "condensed" ions in Manning's theory [see (26), (27)].

We wish to stress again that this confinement of counter-ions is completely different in nature from Manning's condensation, since there is no finite radius within which these confined ions would be enclosed.

The analysis offered in Ref. 82 refers to the case of an extreme dilution. To study the distribution of counter-ions near a polyion in the case of a salt excess, described by Eq. (5), it is necessary to resort to numerical calculations. Such calculations were carried out by Gueron and Weisbuch,[32] who subjected the question of the existence of a condensation of counter-ions to a careful analysis. In general, the transition to finite concentrations does not change the results. In particular, for small ionic strengths ($\varkappa\to 0$) the numerical calculations yielded for R_M the asymptotic behavior $R_M\sim(a/\varkappa)^{1/2}$. In the context of the condensation problem there is accordingly no fundamental distinction between the case of a limiting dilution in terms of the salt and the case of finite ionic strengths in terms of the nature of the distribution of counter-ions around the polyion.

The recent work has thus placed polyelectrolyte theory on a reliable theoretical foundation. It has been found that of the two theoretical approaches which have been available (the Poisson-Boltzmann equation and Manning's theory) preference should be given to the Poisson-Boltzmann equation, which has a fairly broad range of applicability. Manning's theory, on the other hand, now appears not as a rival of the Poisson-Boltzmann equation but as a rather crude approximation of the picture drawn by the Poisson-Boltzmann equation. Using this approximation may result in some particularly large errors in calculations of characteristics in which the distribution of ions near the polyion plays an important role.

6. APPLICATION OF THE THEORY TO THE ANALYSIS OF CONFORMATIONAL TRANSITIONS IN DNA

6.1. Electrostatic free energy

During the 1970s, Manning's condensation theory dominated polyelectrolyte theory almost completely. Many theoretical and experimental studies permeated by the ideas of this theory were carried out during this period; many have now become classics and have even found their way into monographs, textbooks, and lecture courses. This is true in particular of the work on DNA. Now that it has been learned that Manning's theory is only a crude approximation of more-rigorous approaches based on the Poisson-Boltzmann equation and on the even more general theories discussed above, it is clear that we need to reexamine all the theoretical results derived from Manning's theory for the case of highly charged polyelectrolytes.

According to the above analysis of the status of Manning's theory in the light of the present understanding, those conclusions of the theory which deal with the behavior of the free ions at a significant distance (essentially infinite) from the polyion apparently remain valid, although this question has not been studied exhaustively, to the best of our knowledge. On the other hand, all the conclusions without exception of Manning's theory for which the behavior of the potential and/or the density of the ions near the polyion is important come under doubt and must be reexamined. An example is the question of the electrostatic contribution to the rigidity of a polymer chain. This problem has been analyzed in detail[38,39] on the basis of the Poisson-Boltzmann equation (see Ref. 83 for an analysis of the experimental situation). An analysis of this question, however, goes beyond the scope of the present review, since it involves leaving the basic cylinder model and incorporating bends or breaks.

Here we wish to analyze in detail another very important aspect of polyelectrolyte theory which concerns the role played by electrostatic interactions in conformational changes of the DNA molecule.

In the basic model of polyelectrolyte theory, a conformation of DNA is characterized by two parameters: the radius of the cylinder which serves as a model of the polyion, a, and the charge per unit length of this cylinder, q. To find the relative stability of two conformations it is necessary to calculate the difference between their free energies. The total free energy of the system consisting of a polyion with given parameters a and q and mobile ions, for which the concentration by volume of the particles of each species at infinity (the ionic strength), c_0, can be written as

$$F=F^0+F^{\mathrm{el}},\qquad(32)$$

where F^0 is the free energy of this system at $q=0$, and F^{el} is the electrostatic part of the free energy. Since the free energy increases linearly with increasing length of the cylinder, it is natural to look at the specific free energy. In the case of DNA, it is convenient to consider the free energy per pair of nucleotides, i.e., the free energy corresponding to that length of the cylinder on which a charge of $-2e$ is concentrated.

The term F^{el} is equal to the work required to change the charge on the cylinder from zero to the value corresponding to a given charge per unit length q. Using the standard charge-variation technique we have

$$F^{\mathrm{el}}(a,\ q,\ c_0)=-2kT\int\limits_0^1 u(a,\ tq,\ c_0)\,\mathrm{d}t;\qquad(33)$$

here k is the Boltzmann constant and $u(a,tq,c_0)$ is the dimensionless electrostatic potential at the surface of a polyion which has a charge per unit length tq, for a given ionic strength c_0. This quantity cannot be calculated correctly in the condensation theory. As is shown by the result presented in Sec. 4 (Refs. 73–80), Poisson-Boltzmann equation (5) can serve as a reliable basis for a quantitative analysis of this problem. This assertion is true in particular if we are dealing not with the absolute values of the potentials at the surface of the polyion but with their dependence on the ionic strength and on the charge on the polyion (Figs. 4 and 5). This is the approach which we will take below toward the analysis of the dependence of conformational changes in DNA on the ionic strength.

6.2. Helix-coil transition in DNA

The role played by electrostatic interactions in the melting of the DNA double helix has been the subject of very many studies (see, e.g., Refs. 23–25 and 84), but Manning's approach has enjoyed the greatest popularity here, as elsewhere. That approach is presented, in particular, in the fundamental textbook by Cantor and Schimmel.[85] Here we will analyze this question on the basis of the Poisson-Boltzmann equation. In the theoretical work on the ionic-strength dependence of the thermodynamic characteristics of the helix-coil transition, the conventional approach, dating as far back as the work by Kotin,[23] has been to model both conformations of DNA—helical and molten—as infinitely long, uniformly charged cylinders with parameter values which remain constant as the ionic strength of the solution changes. We will also use this model here, although its validity for describing single-strand DNA is not completely obvious (more on this below).

To calculate the melting point T_m of DNA as a function of the ionic strength c_0 we begin with the equation expressing the equality of the free energies of the molten (C) and helical (H) states per pair of nucleotides:

$$F_{HC} = F^0_{HC} + F^{el}_{HC} = 0, \tag{34}$$

where $F^0_{HC} = F^0_C - F^0_H$ is the difference between the nonelectrostatic parts of the free energies,

$$F^{el}_{HC} = F^{el}(a_C, q_C, c_0) - F^{el}(a_H, q_H, c_0), \tag{35}$$

and F^{el} is given by integral (33). The quantities a_C, q_C and a_H, q_H are the parameters of the cylinders which model the DNA in the single- and double-strand conformations. Equation (34) is conveniently rewritten as

$$F_{HC} = U^*_{HC} - TS^*_{HC} + \Delta F^{el}_{HC} = 0, \tag{36}$$

where U^*_{HC} and S^*_{HC} are respectively the transition enthalpy and transition entropy at a certain standard value of the ionic strength, $c_0 = c_0^*$, and F^{el}_{HC} is the change in the transition free energy due to the difference between c_0 and c_0^*. We take the standard value to be $c_0^* = 0.1\text{M}$, for which we have $U^*_{HC} = 8500$ cal/mole and $S^*_{HC} = 23.7$ cal/(mole·deg) (Refs. 86–88; for this particular problem, the heterogeneity in the stability of pairs of basis can be ignored). Our calculations show that the quantity ΔF^{el}_{HC} can in practice be approximated very accurately by a linear function of the temperature for all possible values of the structural parameters over the temperature interval from 290 to 370 K. Writing this functional dependence as

$$\Delta F^{el}_{HC} = F^{el}_{HC}(c_0) - F^{el}_{HC}(c_0^*) = \Delta U^{el}_{HC} - T\,\Delta S^{el}_{HC}, \tag{37}$$

where ΔU^{el}_{HC} and ΔS^{el}_{HC} are the changes in the electrostatic parts of the corresponding quantities upon a change in the ionic strength, we find the following expression for the melting point from (36):

$$T_m = \frac{U^*_{HC} + \Delta U^{el}_{HC}}{S^*_{HC} + \Delta S^{el}_{HC}}. \tag{38}$$

The calculation of the melting point can thus be outlined as follows: The parameters a_H and q_H are fixed at values corresponding to the data from x-ray structural analysis for the B form of DNA: $a_H = 1.0$ nm and $q_H = 4.2$. For single-strand DNA the radius of the cylinder modeling the DNA, a_C, is an adjustable parameter. Although we do not know q_C precisely, it lies between 1.05 (a chain which has been stretched out completely) and 2.1 (single-strand DNA has the same length as double-strand DNA). By specifying certain values of these two parameters for several values of the ionic strength over the interval $-3.0 \leqslant \lg c_0 \leqslant -0.5$ we can work from the solution of Poisson-Boltzmann equation (5) through the use of (33) and (35) to calculate the temperature dependence of the change in the electrostatic component of the free energy, ΔF^{el}_{HC} (the temperature factor enters the problem through the Bjerrum length $l_B = e^2/DkT$). Over the interval of interest here, the temperature dependence of the dielectric permittivity of water can be approximated by the power law[69,89,90] $D(T) = D(T_0)(T_0/T)^{1.4}$, where $T_0 = 293$ K. We then determine ΔU^{el}_{HC} and ΔS^{el}_{HC} in (37) for the given ionic strength and use (38) to find the melting point.

Figure 6 shows curves of the melting point of DNA as functions of the ionic strength of the solution calculated by this procedure for four sets of parameters chosen for single-strand DNA. The results are compared with experimental data on the melting point of DNA having identical concentrations of AT and GC pairs. The experimental data for this case can be approximated very accurately by[91]

$$T_m = 100.3 + 14.8 \lg c_0, \tag{39}$$

where the temperature is expressed in degrees Celsius, while the ionic strength is expressed in moles per liter.

We see that the theoretical dependence of T_m on the logarithm of the ionic strength by no means necessarily re-

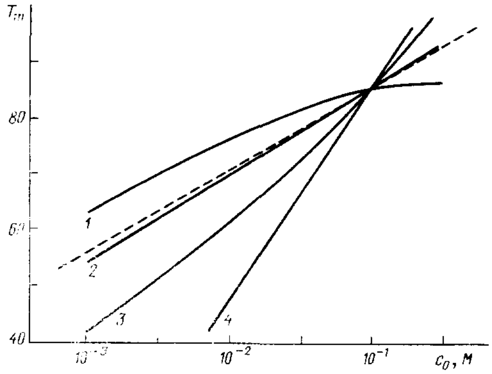

FIG. 6. The melting point of DNA, T_m, as a function of the ionic strength of the solution, c_0, for four selected sets of parameters of single-strand DNA. *The parameter values used in finding the solid lines are as follows:* 1—$q_C = 2.1$, $a_C = 0.25$ nm; 2—$q_C = 2.1$, $a_C = 0.50$ nm; 3—$q_C = 2.1$, $a_C = 1.00$ nm; 4—$q_C = 1.1$, $a_C = 0.50$ nm. The dashed line is a plot of expression (39), which is an approximation of the experimental data.

mains linear over the entire range of ionic strengths. In addition, there is a completely definite pair of plausible values of the adjustable parameters a_C and q_C for which this dependence is essentially linear and has a slope very close to the experimental value.

A theory based on the Poisson-Boltzmann equation thus gives a satisfactory description of the experimental dependence of the stability of the DNA double helix on the ionic strength.

How does the analysis above compare with the analysis of the same question on the basis of Manning's theory? We first note that there is no hope that Manning's theory would be successful in finding this functional dependence except in the case of extremely low ionic strengths. Even for that limiting case, however, an analysis based on condensation theory involves several assumptions. Manning actually breaks up the free-energy difference $F^{\mathrm{el}}_{\mathrm{HC}}$ into two components: one from the entropy of mixing of the counter-ions, which are liberated as a result of the decrease in the charge per unit length during denaturation, with ions in a free phase, and one from the change in the electrostatic free energy calculated from the Debye-Hückel equation (the procedure for calculating $F^{\mathrm{el}}_{\mathrm{HC}}$ on the basis of Manning's condensation theory is given in detail in Ref. 85). The resulting expression is

$$\Delta F^{\mathrm{el}}_{\mathrm{HC}} = kT\,(q_{\mathrm{C}}^{-1} - q_{\mathrm{H}}^{-1})\,(\ln c_0 - \ln c_0^{*}). \tag{40}$$

This expression leads to a linear dependence of T_{m} on the logarithm of the ionic strength. The slope of this dependence is essentially the same as the asymptotic value of the slope at $c_0 < 0.01\mathrm{M}$ which we found above. The terms considered by Manning, however, are only part of the overall change in the free energy. Within the framework of condensation theory there is also a term corresponding to the electrostatic free energy of the binding of condensed ions with the polyion. It follows from the considerations which led to the concept of condensation that this free energy is infinitely large. A difference between infinitely large quantities of this type should appear in $F^{\mathrm{el}}_{\mathrm{HC}}$. If this difference is zero or if it is independent of the ionic strength, then it will not contribute to expression (40). However, Manning proved neither of these conditions.

This example clearly demonstrates the advantages of the approach based on the Poisson-Boltzmann equation. It becomes possible to incorporate correctly all contributions to the electrostatic free energy, and we run into none of the internal contradictions which arise in condensation theory whenever we take up the problem of calculating the free energy of a polyion. At the same time, we must not forget that the approach taken in this problem is based on some nontrivial assumptions. First, there is the replacement of the actual structure of the polyion by a continuously charged cylinder. Furthermore, there is the assumption that the geometric parameters of the macromolecule are independent of the ionic strength. For double-strand DNA this model seems completely acceptable. To demonstrate this point, we note that the persistence length of DNA, i.e., the length scale over which the macromolecule may be regarded as rectilinear—is of the order of 50 nm even at high ionic strengths.[92] This length is considerably greater than the other length scales of the system (the radius of the polyion, the Debye length, the distance between charges on the DNA, etc.). At the same time, the theoretical analysis undertaken in Refs. 43 and 74 shows that replacing the discrete configuration of charges on the polyion by a continuously charged cylinder does not significantly change the mean value of the potential or of the distribution of counter-ions near the polyion. Furthermore, there are no direct indications of a significant change in the distance between the charged groups in DNA upon a change in ionic strength.

Single-strand DNA presents a completely different situation. This conformation obviously has a far greater structural lability, and there is essentially no justification for the model of a cylinder in this case.

6.3. Polyelectrolyte theory of the B-Z transition[93]

A transition induced between the ordinary right-hand-helix B form and the left-hand-helix Z conformation by a change in ionic strength (Fig. 1) is one of the most vivid effects associated with electrostatic interactions in DNA. A description of this phenomenon on the basis of the basic model of polyelectrolyte theory is completely justified, since both forms of DNA have a fairly long persistence length[1] and can be approximated by infinitely long, continuously charged cylinders.[43,74]

As in the case of the helix-coil transition, to analyze the behavior of the B-Z equilibrium we need to calculate the difference between electrostatic free energies:

$$F^{\mathrm{el}}_{\mathrm{BZ}} = F^{\mathrm{el}}(a_{\mathrm{Z}},\ q_{\mathrm{Z}},\ c_0) - F^{\mathrm{el}}(a_{\mathrm{B}},\ q_{\mathrm{B}},\ c_0), \tag{41}$$

where the free energies F^{el} are given by (5) and (33). We wish to emphasize that in this problem, in contrast with the helix-coil transition, all the parameters of the theory are known from x-ray structural analysis[1]: $q_{\mathrm{B}} = 4.2$, $a_{\mathrm{B}} = 1.0$ nm, $q_{\mathrm{Z}} = 3.9$, and $a_{\mathrm{Z}} = 0.9$ nm.

Before we look at the results of rigorous calculations of $F^{\mathrm{el}}_{\mathrm{BZ}}$ we wish to present a simple qualitative theory of the B-Z transition based on the use of the Debye-Hückel equation. Although the Debye-Hückel theory does not give a correct quantitative description of electrostatic interactions in DNA, as has been stated repeatedly, that theory is still useful since the simple explicit expressions which it yields help us understand the results of rigorous numerical calculations. We will thus work from Debye formulas (13) and (14), considering separately the two limiting cases $r_{\mathrm{D}} \gg a$ and $r_{\mathrm{D}} \ll a$.

In the first of these cases (low ionic strengths) the asymptotic expression of the Bessel functions in (14) in the parameter $\varkappa a < 1$ gives us

$$u(a) = 2q \ln \frac{a}{r_{\mathrm{D}}}. \tag{42}$$

Substituting (42) into (33), we find

$$F^{\mathrm{el}} = -2kTq \ln \frac{a}{r_{\mathrm{D}}}. \tag{43}$$

Retaining in this expression only the term which depends on the ionic strength, we find

$$F^{\mathrm{el}}_{\mathrm{BZ}} = kT\,(q_{\mathrm{B}} - q_{\mathrm{Z}}) \ln c_0. \tag{44}$$

This simple theory thus predicts that the quantity $F^{\mathrm{el}}_{\mathrm{BZ}}$, and with it the relative stability of the B form, should increase with increasing ionic strength at low values of the ionic strength, by virtue of the relation $q_{\mathrm{B}} > q_{\mathrm{Z}}$. The same conclusion is reached in Manning's condensation theory. Here the resulting expression is [cf. (40)].

$$F^{\mathrm{el}}_{\mathrm{BZ}} = kT\,(q_{\mathrm{Z}}^{-1} - q_{\mathrm{B}}^{-1})\ln c_0. \tag{45}$$

All the experimental data on the B-Z transition in regularly alternating GC sequences of DNA (see the review by Rich *et al.*[1]) provide evidence that an increase in the ionic strength stabilizes the Z form. On this basis, Behe and Felsenfeld[5] expressed doubt regarding the applicability of the basic model of polyelectrolyte theory for describing the B-Z transition.

We turn to another limiting case. At high ionic strengths, ($r_{\mathrm{D}} < a$) the asymptotic expression for the Bessel functions in (14) in the parameter $\varkappa a > 1$ gives us

$$u(a) = -\frac{2r_{\mathrm{D}}q}{a}. \tag{46}$$

The same result can be derived in a more graphic way. Since the characteristic radius of the electrostatic interactions in this limit is much shorter than the radius of curvature of the charged surface, the problem becomes equivalent to the problem of a charged plane, for which the Debye-Hückel equation is

$$u''(x) = r_{\mathrm{D}}^{-2}u(x), \tag{47a}$$

$$u'(0) = 4\pi l_{\mathrm{B}}s. \tag{47b}$$

Here x is the distance from the plane, and $-es$ is the surface charge density. From (47) we find

$$u(0) = -4\pi r_{\mathrm{D}} l_{\mathrm{B}} s, \tag{48}$$

which corresponds precisely to (46) since the ratio q/a is proportional to the surface density s [see (3)]. From (46) and (33) we find

$$F^{\mathrm{el}}_{\mathrm{BZ}} = 0{,}6kT\left(\frac{q_{\mathrm{Z}}}{a_{\mathrm{Z}}} - \frac{q_{\mathrm{B}}}{a_{\mathrm{B}}}\right)c_0^{-1/2}. \tag{49}$$

It follows from (49) that at high ionic strengths the free-energy difference $F^{\mathrm{el}}_{\mathrm{BZ}}$ continues to increase with increasing salt concentration, as at low ionic strengths, if not only the charge per unit length but also the surface charge density is higher in the B form. In the opposite case, however, i.e., under the condition

$$\frac{q_{\mathrm{Z}}}{q_{\mathrm{B}}} > \frac{a_{\mathrm{Z}}}{a_{\mathrm{B}}}, \tag{50}$$

the quantity $F^{\mathrm{el}}_{\mathrm{BZ}}$ decreases with increasing salt concentration at high ionic strengths. The geometric parameters of the B and Z forms satisfy the latter inequality (the surface charge density is higher in the Z form). This simple theory thus predicts that the quantity $F^{\mathrm{el}}_{\mathrm{BZ}}$ and thus the stability of the B form with respect to the Z form should reach a maximum at ionic strengths for which the relation $r_{\mathrm{D}} \sim a$ holds, i.e., at $c_0 \sim 0.1$M.

Figure 7 shows the results of rigorous calculations of $F^{\mathrm{el}}_{\mathrm{BZ}}$ as a function of the ionic strength. In complete accordance with the simple theory, $F^{\mathrm{el}}_{\mathrm{BZ}}$ increases monotonically over the entire range of ionic strengths under the inequality $q_{\mathrm{Z}}/q_{\mathrm{B}} < a_{\mathrm{Z}}/a_{\mathrm{B}}$ (curves 1 and 2) and goes through a maximum as soon as condition (50) becomes satisfied (curves 3 and 4). The position of the maximum also agrees with the rough estimate found above.

Polyelectrolyte theory thus predicts that the relative stability of the B and Z forms of DNA will be a nonmonotonic function of the salt concentration. This conclusion leans heavily on the specific numerical values of the geometric parameters of these structures, which we have taken from x-ray structural analysis. We are naturally led to ask how much these parameter values will change upon a change in conditions? In particular, does the structure of DNA in solution differ from that in a crystal or a fiber? The data available on these questions imply that the assumption that the parameters remain constant appears to be quite realistic. Indeed, exceedingly precise measurements of the number of pairs per turn of the helix of the B form of DNA in solution[94,95] yield the number 10.5, which is close to the value of 10.0 which follows from x-ray data. The situation with regard to the Z form is similar.[96–98]

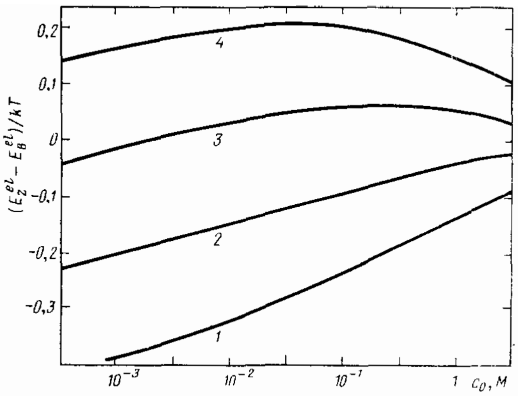

FIG. 7. Difference between the electrostatic free energies of the Z and B forms of DNA as a function of the ionic strength according to calculations from Eqs. (5), (33) and (41). The parameter values are $q_{\mathrm{B}} = 4.2$, $q_{\mathrm{Z}} = 3.9$ and $a_{\mathrm{B}} = 1.0$ nm, which are taken from x-ray structural analysis.[1] The curves correspond to various values of the parameter a_{Z} (nm): 1—1.00; 2—0.95; 3—0.90; 4—0.85. The data of the x-ray structural analysis[1] correspond to line 3. According to condition (50), the maximum should appear at $a_{\mathrm{Z}} < 0.93$ nm.

Let us look at the experimental data available on the B-Z transition in light of the discussion above.

The Z form of DNA was originally discovered for alternating GC polymers at anomalously high ionic strengths[2] (of the order of 2.5M). As soon as the three-dimensional structure of the Z form was deciphered[3] it was learned that charged phosphate groups of the different strands are closer to each other in this conformation than in the B form (Fig. 1). The distance between the nearest phosphates of the different strands in the B form is 0.8 nm, in contrast with 1.2 nm in the B form.[1,3] For a long time the decrease in the electrostatic repulsion between phosphates caused by screening by counter-ions upon an increase in the salt concentration was regarded as the main cause of the increase in the relative stability of the Z form as the ionic strength was increased (see the review by Rich *et al.*[1]). However, the long-range nature of the electrostatic interaction and the specific polyelectrolyte properties of this system (the concentration of counter-ions near a polyion depends weakly on the ionic strength of the solution[16,21,32]) cast doubt on the validity of that explanation. On the basis of the results presented above, the following mechanism might be suggested as the reason for the transition of DNA to the Z form upon an increase in the ionic strength: The B-Z transition is accompanied by an increase in the length of the polymer and by a decrease in its effective radius, but the relative decrease in the radius exceeds the relative increase in length, with the result that the surface charge density in the Z form becomes greater than that in the B form—although the opposite relation holds for the charge per unit length. It is the higher surface charge density of the Z form which determines the growth of its relative stability upon an increase in the ionic strength at high salt concentrations.

A fundamental conclusion which follows from the polyelectrolyte theory of the B-Z transition is the existence of a maximum in the relative stability of the B form at ionic strengths in the physiological region ($c_0 \sim 0.1$M). Experimentally it is customary to measure the point of the B-Z transition along the scale of the ionic strength; this point is determined by the equality of the total free energies of the conformations, $F^{\mathrm{el}}_{\mathrm{BZ}} + F^{0}_{\mathrm{BZ}} = 0$. If we vary the nonelectrostatic component of the free energy by varying some experimental parameter (e.g., the concentration of a nonelectrolyte), then our theory predicts the presence of a critical point for the B-Z equilibrium. At a favorable value of F^{0}_{BZ} one can observe two transitions as the salt concentration is raised: a Z-B transition at low ionic strengths and a B-Z transition at high ionic strengths (overall, we have a Z-B-Z transition).

The value of F^{0}_{BZ} may be influenced by the chemical modification of the nitrogenous bases, the binding of the polymer with ligands of various types, a change in the solvent composition, etc. Since the work by Behe and Felsenfeld,[5] there is no further doubt that the Z form can exist under low-salt conditions (see also Refs. 99–102). However, finding convincing evidence that this effect is of a purely electrostatic nature required detecting the Z-B-Z transition through a variation of the ionic strength alone. The most convenient system for such an experiment is poly(dGm^{5}C)$\cdot$poly(dGm^{5}C), for which F^{0}_{BZ} is far smaller than for the ordinary poly(dGC)$\cdot$poly(dGC) by virtue of a chemical modification of the cytosine, and both transitions occur at ionic strengths which are accessible to measurements. The Z-B-Z transition for poly(dGm^{5}C)$\cdot$poly(dGm^{5}C) was recently detected simultaneously in three studies[6–8] (Fig. 2). These studies were criticized in a very recent paper,[103] but we find the criticism unconvincing.

For a quantitative comparison of theory and experiment we need the entire experimental curve of the dependence of $F^{\mathrm{el}}_{\mathrm{BZ}}$ on the ionic strength. Obtaining this information requires varying the quantity F^{0}_{BZ} by changing, for example, the properties of the solvent and finding the points of the Z-B and B-Z transitions along the scale of the ionic strength. Measurements of this type were recently carried out by V. I. Ivanov and É.I. Minyat; the results agree well with the theory (Fig. 8).

In concluding this section of the paper we note that our examination of the problem of the B-Z equilibrium in DNA has made extensive use of the basic model of polyelectrolyte theory. As has been shown by the many studies discussed in the preceding sections of this paper, this basic model gives a reliable description of the electrostatic properties of double-helix DNA at low and intermediate values of the ionic strengths, up to a salt concentration of the order of 1M. As the salt concentration is raised, the deviations from this model become more and more obvious. The "gas" approximation (Sec. 4) fails, and the concentration of mobile ions around a polyion becomes so high that these mobile ions may form something of the nature of a "liquid" or even "crystalline" phase. Just how and under what conditions these events occur we can only guess at, since at this point we have no methods for constructing a reliable theoretical description of such a situation. What apparently happens is that such factors as the microstructure of the solid, the discrete nature of the charge distribution on the polyion, the details of the polyion surface, the nonelectrostatic interactions of mobile ions with each other and with the polyion, etc., come into play simultaneously. We are therefore unconvinced by attempts to analyze, say, the B-Z equilibrium by approaches in which some of these factors are taken into account as rigorously as possible, while others are arbitrarily ignored.[104,107]

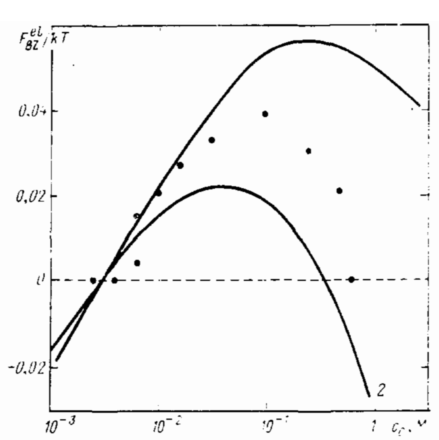

FIG. 8. Difference between the free energies of the Z and B forms, reckoned from the value of this difference at $c_0 = 0.0025$M, as a function of the ionic strength. The points are experimental data obtained by V. I. Ivanov and É. I. Minyat; the solid lines are results calculated from Eqs. (5), (33), and (41). The parameter values in the theory are $q_{\mathrm{B}} = 4.2$, $q_{\mathrm{Z}} = 3.9$, and $a_{\mathrm{B}} = 1.00$ nm. 1—$a_{\mathrm{Z}} = 0.90$ nm; 2—$a_{\mathrm{Z}} = 0.85$ nm.

7. CONCLUSION

We have examined the theoretical foundations and practical applications of the polyelectrolyte model of DNA. This model treats the DNA molecule as a cylinder which has a charge smeared uniformly over its surface and which is immersed in a continuous dielectric medium. An alternative approach to this model is to treat the system consisting of DNA + small ions + water in all its complexity by Monte Carlo or molecular dynamics methods.[108–112] That approach requires powerful computers (a recent study used a CRAY supercomputer[112]). That approach is of course extremely promising for studying the fine structural details of DNA and the solvent around it under fixed conditions. However, such an approach could hardly be realistic for calculating the free energy of this system, and it is specifically the free energy which we need in order to analyze conformational transitions. The circumstance is illustrated particularly clearly by the data on the B-Z transition which we have presented in this review. As can be seen from Figs. 7 and 8, the electrostatic effects which substantially influence the B-Z equilibrium have a magnitude of the order of a few hundredths of kT per pair of DNA bases. Clearly, no first-principles calculation for such a complex system is now or will ever be capable of guaranteeing such an accuracy. On the other hand, the model approach offers the indisputable advantages that its results can be interpreted clearly and that their qualitative reliability can be confirmed by simple physical considerations. This is precisely what was demonstrated above for the case of the B-Z transition.

[1] A. Rich, A. Nordheim, and A. H.-J. Wang, Ann. Rev. Biochem. **53**, 791 (1984).

[2] F. M. Pohl and T. M. Jovin, J. Mol. Biol. **67**, 375 (1972).

[3] A. H.-J.Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, and A. Rich, Nature **282**, 680 (1979).

[4] T. M. Jovin, L. MacIntosh, H. van de Sande, and D. M. Soumpasis, Ann. Rev. Phys. Chem. (in press), (1986).

[5] M. Behe and G. Felsenfeld, Proc. Natl. Acad. Sci. USA **78**, 1619 (1981).

[6] P. K. Latha and S. K. Brachmachari, FEBS Lett. **182**, 315 (1985).

[7] B. G. Feuerstein, L. J. Marton, M. A. Keniry, D. L. Wade, and B. H. Shafer, Nucl. Acids Res. **13**, 4133 (1985).

[8] W. C. Krueger and M. D. Prairie, Biopolymers **24**, 905 (1985).

[9] A. A. Vedenov, A. M. Dykhne, and M. D. Frank-Kamenetskiĭ, Usp. Fiz. Nauk **105**, 479 (1971) [Sov. Phys. Usp. **14**, 715 (1972)].

[10] A. Wada, S. Yabuki, and Y. Husimi, CRC Crit. Rev. Biochem. **9**, 87 (1980).

[11] R. M. Wartell and A. S. Benight, Phys. Rept. **126**, 67 (1985).

[12] V. V. Anshelevich, A. V. Vologodskii, A. V. Lukashin, and M. D. Frank-Kamenetskiĭ, Biopolymers **23**, 39 (1984).

[13] T. L. Hill, *Statistical Mechanics*, McGraw-Hill, New York, 1956 (Russ. transl., IL, M., 1960).

[14] G. V. Ramanathan and C. P. Woodbury, J. Chem. Phys. **77**, 4120 (1982).

[15] S. A. Rice and M. Nagasawa, *Polyelectrolyte Solutions*, Academic Press, Orlando, 1977.

[16] G. V. Ramanathan, J. Chem. Phys. **78**, 3223 (1983).

[17] A. Katzir-Katchalsky, *Biophysics and Other Topics: Selected Papers*, Academic Press, Orlando, 1976.

[18] D. A. Frank-Kamenetskiĭ, Diffusion and Heat Transfer in Chemical Kinetics (in Russian), Nauka, M., 1967.

[19] R. M. Fuoss, A. Katchalsky, and S. Lifson, Proc. Natl. Acad. Sci. USA **37**, 579 (1951).

[20] T. Alfrey, P. W. Berg, and H. Morawetz, J. Polym. Sci. **7**, 543 (1951).

[21] M. LeBret and B. Zimm, Biopolymers **23**, 287 (1984).

[22] L. Kotin and M. Nagasawa, J. Chem. Phys. **36**, 873 (1962).

[23] L. Kotin, J. Mol. Biol. **7**, 309 (1963).

[24] A. D. MacGillivray and A. I. MacMullen, J. Theor. Biol. **12**, 75 (1966).

[25] A. D. MacGillivray and A. I. MacMullen, J. Theor. Biol. **12**, 260 (1966).

[26] A. D. MacGillivray, J. Chem. Phys. **56**, 80 (1972).

[27] A. D. MacGillivray, J. Chem. Phys. **57**, 4071 (1972).

[28] D. Stigter, J. Colloid and Interface Sci. **53**, 296 (1975).

[29] J. A. Shellman and S. Stigter, Biopolymers **16**, 1415 (1977).

[30] D. Stigter, Biopolymers **16**, 1435 (1977).

[31] M. Gueron and G. Weisbuch, J. Phys. Chem. **83**, 1991 (1979).

[32] M. Gueron and G. Weisbuch, Biopolymers **19**, 353 (1980).

[33] C. F. Anderson and M. T. Record, Biophys. Chem. **11**, 353 (1980).

[34] G. Weisbuch and M. Gueron, J. Phys. Chem. **85**, 517 (1981).

[35] B. K. Klein, C. F. Anderson, and M. T. Record, Biopolymers **20**, 2263 (1981).

[36] C. F. Anderson and M. T. Record, Ann. Rev. Phys. Chem. **33**, 191 (1982).

[37] J. Granot and D. R. Kearns, Biopolymers **21**, 219 (1982).

[38] M. LeBret, J. Chem. Phys. **76**, 6243 (1982).

[39] M. Fixman, J. Chem. Phys. **76**, 6346 (1982).

[40] W. B. Russel, J. Polym. Sci. **20**, 1233 (1982).

[41] J. Granot, Biopolymers **22**, 1831 (1983).

[42] A. Delville and P. Laszlo, Biophys. Chem. **17**, 119 (1983).

[43] B. J. Klein and G. R. Pack, Biopolymers **22**, 2331 (1983).

[44] G. R. Pack, Intern. J. Quant. Chem: Quant. Biol. Symp. **10**, 61 (1983).

[45] T. Odijk, Chem. Phys. Lett. **100**, 145 (1983).

[46] D. Bratko and D. Dolar, J. Chem. Phys. **80**, 5782 (1984).

[47] L. Belloni, M. Drifford, and P. Turq, Chem. Phys. **83**, 147 (1984).

[48] M. A. Lampert and R. Martinelli, Chem. Phys. **88**, 399 (1984).

[49] G. R. Pack and C. V. Prasad, Intern. J. Quant. Chem.: Quant. Biol. Symp. **11**, 287 (1984).

[50] C. Tanford, *The Physical Chemistry of Macromolecules*, Wiley Press, New York, 1961 (Russ. transl., Khimiya, M., 1965).

[51] A. R. Khokhlov and K. A. Khachaturian, Polymer **23**, 1742 (1982).

[52] A. R. Kholodenko and K. F. Freed, J. Chem. Phys. **78**, 7412 (1983).

[53] G. S. Manning, J. Chem. Phys. **51**, 924 (1969).

[54] G. S. Manning, J. Chem. Phys. **51**, 3249 (1969).

[55] G. S. Manning, Biopolymers **11**, 937 (1972).

[56] G. S. Manning, Biopolymers **11**, 951 (1972).

[57] G. S. Manning, Ann. Rev. Phys. Chem. **23**, 117 (1972).

[58] J. C. T. Kwak, M. C. O'Brien, and D. A. MacLean, J. Phys. Chem. **79**, 2381 (1975).

[59] G. S. Manning, Biophys. Chem. **7**, 95 (1977).

[60] N. Ise and T. Okubo, Macromolecules **11**, 439 (1978).

[61] M. Kowblansky, M. Tomasula, and P. Ander, J. Phys. Chem. **82**, 1491 (1978).

[62] Y. M. Joshi and J. C. T. Kwak, J. Phys. Chem. **83**, 1978 (1979).

[63] M. T. Record, C. F. Anderson, and T. M. Lohman, Quart. Rev. Biophys. **11**, 103 (1978).

[64] G. S. Manning, Quart. Rev. Biophys. **11**, 179 (1978).

[65] R. Trifiletti and P. Ander, Macromolecules **12**, 1197 (1979).

[66] G. S. Manning, Acc. Chem. Res. **12**, 443 (1979).

[67] R. W. Wilson and V. A. Bloomfield, Biochemistry **18**, 2192 (1979).

[68] G. S. Manning, J. Phys. Chem. **88**, 6654 (1984).

[69] S. Paoletti, O. Smidsrod, and H. Grasdalen, Biopolymers **23**, 1771 (1984).

[70] M. Satoh, J. Komiyama, and T. Iijima, Macromolecules **18**, 1195 (1985).

[71] N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller, and E. Teller, J. Chem. Phys. **21**, 1087 (1953).

[72] K. Binder (ed.), *Monte Carlo Methods in Statistical Physics*, Springer-Verlag, New York, 1979 (Russ. transl., Mir, Moscow, 1982).

[73] D. Bratko and V. Vlachy, Chem. Phys. Lett. **90**, 434 (1982).

[74] M. LeBret and B. Zimm, Biopolymers **23**, 271 (1984).

[75] C. S. Murthy, R. J. Bacquet, and P. J. Rossky, J. Phys. Chem. **89**, 701 (1985).

[76] P. Mills, C. F. Anderson, and M. T. Record, J. Phys. Chem. **89**, 3984 (1985).

[77] M. Fixman, J. Chem. Phys. **70**, 4995 (1979).

[78] G. V. Ramanathan and C. P. Woodbury, J. Chem. Phys. **77**, 4133 (1982).

[79] R. J. Bacquet and P. J. Rossky, J. Phys. Chem. **88**, 2660 (1984).

[80] V. V. Anshelevich, A. V. Lukashin, and M. D. Frank-Kamenetskiĭ, Chem. Phys. **91**, 225 (1984).

[81] M. A. Lampert, Biopolymers **21**, 159 (1982).

[82] B. Zimm and M. LeBret, J. Biomol. Struct. Dyn. **1**, 461 (1983).

[83] M. Tricot, Macromolecules **17**, 1698 (1984).

[84] C. DeLisi and D. M. Crothers, Biopolymers **10**, 2323 (1971).

[85] C. R. Cantor and P. R. Schimmel, Biophysical Chemistry, Freeman, San Francisco, 1980 (Russ. transl., Mir, M., 1984, Ch. 22).

[86] P. L. Privalov, O. B. Ptitsyn, and T. M. Birshtein, Biopolymers **8**, 559 (1969).

[87] D. W. Gruenwedel, Biochem. and Biophys. Acta **340**, 16 (1974).

[88] D. R. Monaselidze and G. N. Mgeladze, Biofizika **22**, 950 (1977).

[89] H. Fröhlich, *Theory of Dielectrics*, Clarendon Press, Oxford, 1958 (Russ. transl., IL, M., 1960).

[90] D. Eisenberg and W. Kazmann, *The Structure and Properties of Water*, Clarendon Press, Oxford, 1964.

[91] M. D. Frank-Kamenetskiĭ, Biopolymers **10**, 2623 (1971).

[92] P. J. Hagerman, Biopolymers **20**, 1503 (1981).

[93] M. D. Frank-Kamenetskiĭ, A. V. Lukashin, and V. V. Anshelevich, J. Biomol. Struct. Dyn. **3**, 35 (1985).

[94] L. J. Peck and J. C. Wang, Nature **292**, 375 (1981).

[95] D. S. Horowitz and J. C. Wang, J. Mol. Biol. **173**, 75 (1984).

[96] J. C. Wang, L. J. Peck, and K. Becherer, Cold Spring Harb. Symp. Quant. Biol. **47**, 85 (1983).

[97] L. J. Peck and J. C. Wang, Proc. Natl. Acad. Sci. USA **80**, 6206 (1983).

[98] I. Panyutin, V. Lyamichev, and S. Mirkin, J. Biomol. Struct. Dyn. **2**, 1221 (1985).

[99] B. Malfoy, B. Hartmann, and M. Leng, Nucl. Acids Res. **9**, 5659 (1981).

[100] H. H. Chen, M. J. Behe, and D. C. Rau, Nucl. Acids Res. **12**, 2381 (1984).

[101] T. J. Thomas and V. A. Bloomfield, Biochemistry **24**, 713 (1985).

[102] M. J. Behe, G. Felsenfeld, S. C. Szu, and E. Cnarney, Biopolymers **24**, 289 (1985).

[103] A. Woisard, W. Guschelbauer, and G. V. Fazakerley, Nucl. Acids Res. **14**, 3515 (1986).

[104] G. R. Pack, Intern. J. Quant. Chem.: Quant. Biol. Symp. **9**, 81 (1982).

[105] D. M. Soumpasis, Proc. Natl. Acad. Sci. USA **81**, 5116 (1984).

[106] J. B. Matthew and F. M. Richards, Biopolymers **23**, 2743 (1984).

[107] G. R. Pack and B. J. Klein, Biopolymers **23**, 2801 (1984).

[108] E. Clementi, in: *Structure and Dynamics: Nucleic Acids and Proteins* (ed. E. Clementi and R.H. Sarma), Adenine Press, New York, 1983, p. 321.

[109] O. L. Olatunji and S. Premilat, Biochem. and Biophys. Res. Commun. **126**, 247 (1985).

[110] J. L. Finney, J. M. Goodfellow, P. L. Howell, and F. Vovelle, J. Biomol. Struct. Dyn. **3**, 599 (1985).
[111] U. C. Singh, S. J. Weiner, and P. A. Kollman, Proc. Natl. Acad. Sci. USA **82**, 755 (1985).
[112] G. L. Seibel, U. C. Singh, and P. A. Kollman, Proc. Natl. Acad. Sci. USA **82**, 6537 (1985).

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