

Zero-phonon lines in the spectra of chlorophyll-type molecules embedded in low-temperature solid-state matrices

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A report is given of the results of investigations of electronic-vibrational spectra of chlorophyll molecules and their analogs, embedded in solid-state matrices, which were carried out using modern selective spectroscopic methods such as laser monochromatic excitation of the fluorescence and spectral hole burning. The fundamentals of the application of these methods to complex impurity molecules are considered together with the problems encountered in the analysis of the structure of the selective spectra, mechanisms of the appearance of stable and dynamic spectral holes, and determination of homogeneous spectral profiles and relaxation times. Special attention is given to a theory of the appearance of zero-phonon lines under energy transfer conditions and experimental detection of these lines in model and native systems.

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I. INTRODUCTION

Complex organic molecules play the key role in a very wide range of biophysical and biochemical processes. In the case of such molecules as chlorophyll, rhodopsin, phytochrome, etc., their functioning depends directly on the interaction of the electron system with light. The absorption and fluorescence spectra of chlorophyll-like molecules in solutions consist of wide structure-free bands formed as a result of transitions between electronic-vibrational (vibronic) levels. Since large molecules have a very large number of the vibrational degrees of freedom, the corresponding energy levels are densely and partly quasicontinuously spaced. Therefore, there has been even a view that, in principle, such molecules cannot have narrow spectral lines.

Observation of quasiline electronic-vibrational spectra of some aromatic molecules in frozen *n*-alkane matrices (representing discovery¹ of the Shpol'skiĭ effect) has been followed by a report of fine-structure spectra¹⁾ and also of some porphyrins in Shpol'skiĭ matrices.² This has been followed by a cycle of investigations of fine-structure spectra of the parent in this class of compounds, i.e., porphin and some of its derivatives (see Ch. 4 in Ref. 3). In the case of chlorophyll and its analogs the initial hopes of obtaining quasiline electronic spectra have also been based on the Shpol'skiĭ effect, but the usefulness of this method in the case of such large molecules has been found to be very limited.^{4,5}

It should be stressed that the appearance of fine-structure spectra of impurity molecules is due to the presence of zero-phonon lines in the spectra of solids and interpretation of the Shpol'skiĭ spectra has been the touchstone for the theory of zero-phonon lines.⁶ Only a few years after the development of the theory⁶⁻¹⁰ new important evidence has been obtained showing that it applies to such systems: selective excitation of the components of a multiplet has been achieved and the theoretically predicted phonon wings have been found in the excitation¹¹ and fluorescence²¹ spectra. Investigations have also been made of the thermal broadening and shift of zero-phonon lines in the Shpol'skiĭ spectra (see, for example, Refs. 13 and 14) which confirm all the fundamental conclusions of the theory.¹⁰

Investigations of zero-phonon lines of impurity centers in inorganic crystals have been proceeding in parallel (see, for example, Refs. 15–17). An important contribution to the understanding of the electronic-vibrational interaction processes has been made by studies of small molecular ions present as impurities in alkali halide crystals.¹⁸

In the late sixties the accepted view has been that the main factor preventing observation and investigation of narrower purely electronic zero-phonon lines in crystalline matrices is inhomogeneous broadening (see §30 in Ref. 10). The width of a purely electronic line in the spectrum of one center or a set of perfectly identical impurity centers (i.e., in a homogeneous spectrum) approaches the radiative width

($\Gamma_0 \leq 10^{-3} \text{ cm}^{-1}$) in the limit $T \rightarrow 0$. In real crystals, and even more in glasses, each impurity center is under the influence of various local fields because of the presence of defects, dislocations, other impurities, or simply a disordered structure of the matrix. These local differences result in stochastic shifts of the transition energies, which are manifested as inhomogeneous broadening of the spectra amounting to less than 1 cm^{-1} in the case of good single crystals and hundreds of reciprocal centimeters in the case of glasses.

The feasibility of partial suppression of inhomogeneous broadening of the spectrum by narrow-band excitation of the fluorescence was demonstrated in an earlier investigation¹⁹ using the example of solutions of coronene molecules at 77 K. Some narrowing of the lines of rare-earth impurities has been achieved by luminescence excitation using a monochromatized lamp²⁰ or nonresonance laser²¹ radiation. The almost complete suppression of inhomogeneous broadening has been achieved by resonance laser excitation of ruby crystals for which the electronic R_1 line has been narrowed down to 0.007 cm^{-1} (Ref. 22).

In the case of organic molecules in matrices which are not specially selected the situation is more complex because the spectra of the majority of frozen solutions are practically structure-free even at helium temperatures and the reason for the broadening is not clear. Selective laser excitation of such systems has revealed a striking effect of transformation of continuous into quasiline spectra, thus proving finally the decisive role of the inhomogeneous broadening.²³

Selective spectroscopic investigations of chlorophyll and its derivatives began with the work reported in Refs. 5 and 24. Soon after a theoretical analysis of the line widths and intensities in the case of selectively excited spectra, essential for interpretation of the observed complex structure, was provided.^{25,26} In particular, it was shown there that the purely electronic lines in the fluorescence spectra observed as a result of nonresonance monochromatic excitation assume the width of the corresponding vibronic absorption lines, i.e., they are still strongly broadened compared with the natural width of purely electronic lines predicted by the theory. The homogeneous profiles of the narrower purely electronic lines can be revealed by the method of optical burning of spectral holes or dips, which was proposed in Refs. 27 and 28 and has subsequently become highly popular.²⁹ The hole burning method has also been used to improve considerably the resolution of the spectroscopic data on chlorophyll-like molecules.

2. THEORY

2.1. Zero-phonon lines in the spectrum of a selectively excited inhomogeneous system

In the simplest model of an impurity center in a crystal, when only the shift of the adiabatic potentials as a result of an electron transition is taken into account (this corresponds to the linear electronic-vibrational interaction), purely electronic lines have the radiative width and they represent the optical analog of the Mössbauer lines.^{8,17} When an allowance is made for a change in the system of normal coordinates (corresponding to the quadratic electron-vibrational interaction) and anharmonicity of the vibrations, it is found that purely electronic lines becomes broader on increase in temperature.^{9,10,15} Vibronic lines, representing replicas of purely electronic lines associated with local vibra-

tions (which in the case of molecular impurities are of the internal molecular type), are broadened even at absolute zero because of the anharmonic decay of the relevant levels. All the zero-phonon lines are accompanied by phonon wings, which are manifested because of the difference between the adiabatic potentials of crystal vibrations. The line intensity, relative to the integral intensity of the wing plus the line itself, is known as the Debye-Waller factor. The manifestation of the electronic-vibrational interactions in fine-structure spectra have been considered, for example, in the review reported in Ref. 30.

Such "ideal" spectra of real systems are averaged over an inhomogeneous ensemble of impurity centers, which is manifested as inhomogeneous broadening. A thorough investigation³¹ has shown that inhomogeneous broadening may be characterized by different distributions in crystals, giving rise to Gaussian or Lorentz line profiles. The validity of the theory of Ref. 31 was demonstrated successfully in the case of the O_2^- centers.³²

Selective excitation of zero-phonon lines in inhomogeneous systems is based on the fact that these lines are extremely narrow so that they have an enormous peak intensity.³³ If, by way of example, we consider purely electronic lines in the limit $T \rightarrow 0$ with a radiative width of 10^{-4} cm^{-1} ($\tau_1 \approx 50 \text{ ns}$) and a wing of width 10^2 cm^{-1} , we find that when the Debye-Waller factor is $\beta = 0.1$, the ratio of the peak intensities of the line and wing should be 10^5 . Then, also in a monochromatically excited fluorescence spectrum the peak line intensity should be several orders of magnitude higher than the wing intensity. However, in the real experiments the temperature of a sample is not zero and the phonon wings as well as vibronic zero-phonon lines participate in the absorption, so that it is necessary to allow consistently for all the components of the spectra. A useful concept in the analysis of selectively excited spectra is an inhomogeneous broadening function representing the distribution of the number of impurity centers (molecules) arranged in accordance with the shifts $\Delta\nu$ of the frequency of a purely electronic transition.^{25,34} A monochromatically excited fluorescence spectrum can then be described by a convolution

$$F(\nu_e, \nu_f) = \eta_0 \int \rho(\Delta\nu) \varphi(\nu_f - \Delta\nu) \kappa(\nu_e - \Delta\nu) d\Delta\nu, \quad (1)$$

where $\rho(\Delta\nu)$ is the inhomogeneous distribution function; $\varphi(\nu)$ and $\kappa(\nu)$ are the homogeneous fluorescence and absorption spectra; ν_f and ν_e are the spectra at which the fluorescence is recorded and excited, respectively (Fig. 1). The coefficient η_0 represents a frequency-independent fluorescence efficiency. If $\nu_f = \text{const}$, Eq. (1) describes the excitation spectrum (also called the "selective absorption spectrum").

Separation of homogeneous spectra into zero-phonon lines and their phonon wings by means of Eq. (1) leads to the following conclusions.^{25,26} Firstly, the observed line width Γ_{se} consists of homogeneous fluorescence and absorption line widths:

$$\Gamma_{se} \approx \Gamma_e + \Gamma_0. \quad (2)$$

Secondly, excitation in the range of vibronic transitions gives rise to a multiline structure in a purely electronic band.

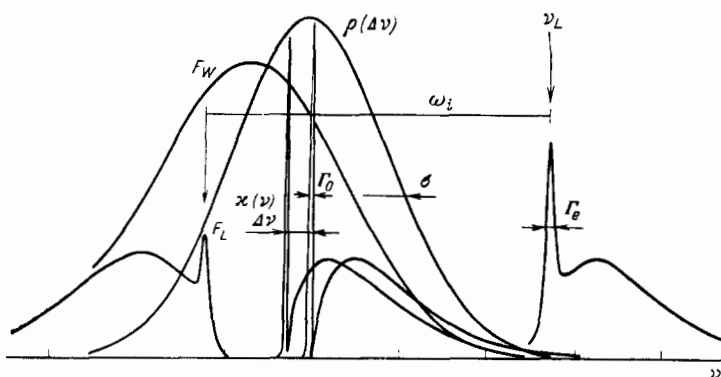


FIG. 1. Inhomogeneous distribution function $\rho(\Delta\nu)$ of a Gaussian profile with a half-width σ and two homogeneous absorption spectra $\kappa(\nu)$ with a purely electronic line width Γ_0 shifted by the frequency $\Delta\nu$. Here, F_W is an inhomogeneous fluorescence spectrum obtained using white excitation and F_L is the corresponding spectrum obtained using monochromatic excitation in resonance with a vibronic zero-phonon line of width Γ_e corresponding to a local mode ω_i .

The separations of these "pseudolines" from the excitation line, $\nu_e - \nu_f$, are equal to the frequencies of local vibrations ω_i in an excited electronic state. Therefore, these frequencies can be found directly from the fluorescence spectra. Such a multilined structure has been reported for Shpol'skii systems²⁶ and for molecules in glassy matrices.³⁷ Its manifestation is typical of systems exhibiting a strong inhomogeneous broadening (see Sec. 3.2).

Thirdly, the relative intensities of the line and wing depend strongly on the shift $\Delta\nu$ of the excitation frequency within the limits of the inhomogeneous distribution function: zero-phonon lines are more likely to participate in the absorption at the low-frequency edge than at the high-frequency end where summation of the phonon wings is the dominant feature (so that a pseudowing is observed). This conclusion has been confirmed in greater detail by model calculations.³⁶ Recently such numerical calculations were made for various spectral profiles and ratios of the parameters of the model.³⁷ The inhomogeneous distribution functions of excited molecules and the selectivity function were considered and the effective Debye-Waller factor was calculated.

In this connection we should point out that the mirror symmetry between the fluorescence and absorption spectra is lost because of inhomogeneous broadening in the selective spectra. As shown in Ref. 38, if $\rho(\Delta\nu)$ is a symmetric curve and the homogeneous spectra $\kappa(\nu)$ and $\varphi(\nu)$ have a mutual mirror symmetry, Eq. (1) yields a two-dimensional symmetry between the fluorescence and excitation spectra:

$$\mathcal{E}(\nu, \nu^*) = F(\nu_e = -\nu^*, \nu_f = -\nu), \quad (3)$$

where $\mathcal{E}(\nu, \nu^*)$ is the excitation spectrum recorded at a frequency $\nu_f = \nu^*$ and the frequencies are measured from the unshifted ($\Delta\nu = 0$) purely electronic line. Hence, we obtain a simple relationship between the values of the Debye-Waller factor for these two spectra:

$$\beta_e(\nu_f = \nu^*) = \beta_f(\nu_e = -\nu^*), \quad (4)$$

which reflects the circumstance that the intensity of a line in the fluorescence spectrum increases for excitation at the "red" edge of the inhomogeneous distribution function ($\nu^* < 0$), whereas in the excitation spectrum this happens at the "blue" edge ($\nu^* > 0$).

In this simple model the inhomogeneous distribution function is defined for a single specific electronic transition. It is shown in Ref. 39 that inhomogeneous distributions of

various electronic transitions may in practice be uncorrelated and, therefore, for example the narrowing of the fluorescence spectra has become possible only as a result of the direct $T_1 \leftarrow S_0$ excitation.⁴⁰ An additional small inhomogeneous broadening is experienced also by vibrational sublevels of the state with a fixed frequency of the purely electronic transition.⁴¹

The solution of the direct problem of calculation of the observed spectral curves and of the parameters on the basis of model concepts is tackled in a number of papers in which methods are developed for the determination of the inhomogeneous distribution function itself and of homogeneous spectra from the experimental results.⁴²⁻⁴⁴ The problems in a unified theory of resonance secondary radiation under conditions of inhomogeneous broadening have also been considered.⁴⁵

2.2. Influence of energy transfer on the excitation selectivity

The published calculations have been made for the case of a low concentration when the transfer of the excitation energy between impurity molecules can be ignored. Investigations of the spectra of chlorophyll in model and native systems have made it necessary to consider also selective spectra in the case of fast energy transfer. This situation was considered in Ref. 38.

We shall begin with the model of Sec. 2.1 and calculate the probability of energy transfer between impurities in an inhomogeneous ensemble using the dipole-dipole approximation in accordance with the treatment of Förster and Galanin⁴⁶:

$$W(\nu_d, \nu_a, R) \propto R_0^6(\nu_d, \nu_a) R^{-6}, \quad (5)$$

where ν_d and ν_a are the shifts of the frequencies of the 0-0 transition in a donor and an acceptor, respectively; R is the distance between them. The transfer radius is governed by the overlap integral

$$R_0^6 \propto S(\nu_d - \nu_a) = \int \varphi(\nu_t - \nu_d) \kappa(\nu_t - \nu_a) d\nu_t. \quad (6)$$

Nonresonance transfer due to the overlap of phonon wings in the fluorescence spectrum of a donor and the absorption spectrum of an acceptor causes the zero-phonon line to disappear in the case of a wide inhomogeneous distribution function (Fig. 2).

It is useful to consider the energy transfer function $p(\nu_d)$ representing the average probability of transfer from a

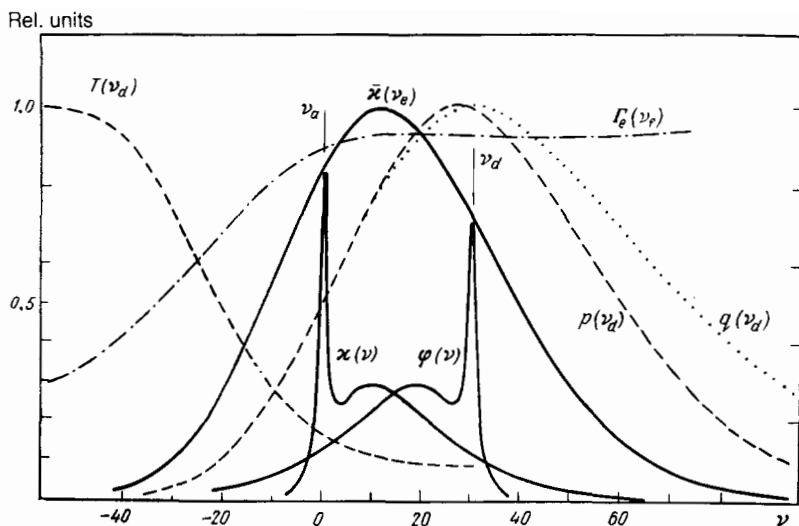


FIG. 2. Schematic representation of energy transfer in an inhomogeneous band ($\sigma = 42$) from centers excited resonantly at a frequency ν_d to acceptors with a zero-phonon line frequency ν_a because of overlap of the corresponding fluorescence $\varphi(\nu)$ and absorption $x(\nu)$ spectra. The phonon wing in $x(\nu)$ is modeled by a profile $Q(\alpha, \nu) = \nu\alpha^{-2}\exp(-\nu/\alpha), \nu > 0; Q = 0$ for $\nu < 0$. The zero-phonon line is modeled by a Lorentzian ($\alpha = 10, \beta = 0.2$). Calculated results: inhomogeneous absorption spectrum $\bar{x}(\nu_e)$, transfer functions $p(\nu_d)$ and $q(\nu_d)$, factors representing attenuation of the zero-phonon line in the fluorescence $T(\nu_d)$ and excitation $\Gamma_e(\nu_f)$ spectra [denoted by $\Gamma_e(\nu_f)$.]

given donor to all possible acceptors inside an inhomogeneous system. Assuming that the averaging over the distances is independent of the frequency distribution, we obtain

$$p(\nu_d) = p_0 \int \Gamma(\nu_a) S(\nu_d - \nu_a) d\nu_a. \quad (7)$$

If rigorous averaging is carried out over the distances and frequencies, the transfer function becomes modified somewhat to

$$q(\nu_d) = q_0 \int \rho(\nu_a) [S(\nu_d - \nu_a)]^{1/2} d\nu_a. \quad (8)$$

Figure 2 shows the results of a model calculation of the functions $p(\nu_d)$ and $q(\nu_d)$ which reflect the general tendency for the rate of transfer to decrease in the region of the low-frequency edge of an inhomogeneous spectral band. This is due to a reduction in the number of suitable [in the sense of the magnitude of the overlap integral of Eq. (6)] acceptors on reduction in ν_d . The functions p and q differ little, so that in practice we can use the simpler expression given by Eq. (7).

The fall of the relative intensity of the zero-phonon line is then manifested by a frequency dependence of the donor fluorescence efficiency

$$T(\nu_d) = [1 + p_1 \tau_0 p(\nu_d)]^{-1}; \quad (9)$$

here, the parameter p_1 represents the maximum transfer rate (at a given concentration) expressed in terms of the reciprocal of the decay time τ_0^{-1} of the excited state.

The actual form of the fluorescence spectrum is then calculated by solving the integral equation for $f(\nu_e, \Delta\nu)$, which represents an inhomogeneous spectral distribution of the population of the excited state on the assumption that the excitation is monochromatic. The fluorescence spectrum can now be described by

$$F_f(\nu_e, \nu_f) = \int (\nu_e, \Delta\nu) \varphi(\nu_f - \Delta\nu) d\Delta\nu. \quad (10)$$

The results of model calculations (Fig. 3) show that al-

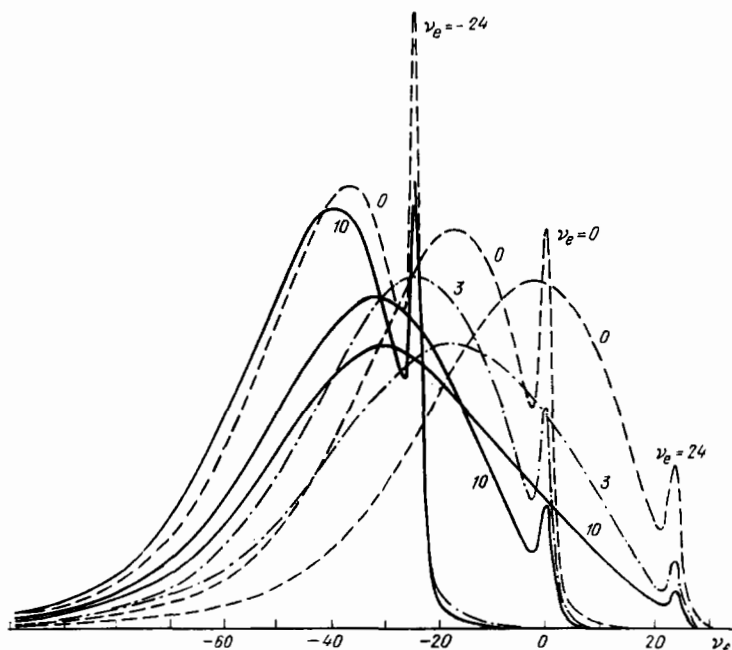


FIG. 3. Selectively excited fluorescence spectra calculated³⁸ allowing for energy transfer in the model of Fig. 2 for $\nu_e = 24, 0,$ and -24 assuming that the transfer parameter has the values $p_1 \tau_0 = 0, 3,$ and 10 , which are given alongside the curves. The spectra are reduced to the same area.

though the reduction in the zero-phonon line is considerable near the maximum of the inhomogeneous band, at the low-frequency edge we can still obtain clear lines even when the concentration reaches the critical value $C \approx \langle R_0 \rangle^{-3}$. An analysis of the associated excitation spectra shows that Eqs. (3) and (4) are violated by the transfer process. In the case of the fluorescence spectra the frequency dependence of the Debye-Waller factor becomes stronger, whereas for the excitation spectra the frequency dependence becomes flatter. In this sense the zero-phonon lines in the excitation spectra are less sensitive to energy transfer than those in the fluorescence spectra, where the lines in the high-frequency part of the inhomogeneous distribution function disappear rapidly. These trends are manifested by a frequency dependence of the relative reduction in the effective Debye-Waller factor as a result of energy transfer. In the case of the fluorescence spectra the ratio in question is simply equal to the fluorescence efficiency of the initially excited donors $T(\nu_e)$, whereas in the case of the excitation spectra, we have

$$r_e(\nu_f) = \frac{T(\nu_f)}{F_t^{(w)}(\nu_f)}, \quad (11)$$

where $F_t^{(w)}(\nu_f)$ represents the fluorescence spectrum obtained as a result of excitation with white light. It is clear from Fig. 2 that the frequency dependences of the curves $T(\nu_e)$ and $r_e(\nu_f)$ are indeed very different.

Since these calculations were carried out for a resonance 0-0 group, in real experiments when the vibronic absorption region is also excited, we have to allow for the superposition of several (pseudo)lines and their wings also in the purely electronic region. Hence, we can draw the general conclusion that the most suitable procedure for observing the zero-phonon lines is determination of the excitation spectrum of the fluorescence corresponding to the short-wavelength edge of the 0-0 band.

3. FINE-STRUCTURE SPECTRA OF CHLOROPHYLL-LIKE MOLECULES

3.1. Experimental techniques

Spectroscopic measurements are now usually made employing laser radiation sources. The first experiments on suppression of inhomogeneous broadening were carried out by exciting the fluorescence with individual lines emitted by cw gas lasers.^{23,24,26} Tunable dye lasers⁴⁷⁻⁴⁹ make it possible to avoid limitation to several fixed excitation frequencies. The signal/noise ratio is better in the case of cw lasers, but tuning over a wide spectral interval is easier in the case of pulsed lasers. The requirements are even more stringent in the case of determination of the excitation spectra.

In experiments of this type a suitable light source is a dye laser pumped by an argon or a krypton laser. In the case of excitation in the region of vibronic transitions it is sufficient to use powers of 1-10 mW when the line width is $\sim 1 \text{ cm}^{-1}$. The output power from a laser should be kept constant over the whole spectrum and should be stabilized in time, which can be achieved by (for example) a feedback loop controlling the current in the pump laser. Stabilization of the output power by an electro-optic noise suppression system is also possible.⁵⁰ Accurate calibration of the wavelength scale is ensured by recording reference lines of neon in addition to the excitation spectrum with the aid of the optogalvanic effect.⁵¹

When the fluorescence spectra are determined as a result of excitation inside the 0-0 band, bearing in mind the intensity and small width of two electronic lines, it is frequently necessary to deal with rapid deexcitation of resonance centers and a corresponding reduction in the intensities of the lines in the course of the measurements. These intensities can be restored by periodic wide-band illumination of a sample.⁴⁷ A reduction in the excitation power density to $100 \mu\text{W}/\text{cm}^2$ is also sufficient in such measurements.⁴⁹ In the case of irreversible de-excitation it is possible to scan the excitation frequency in a narrow part of an inhomogeneous band. For example, if the line width is 0.5 cm^{-1} , scanning of ν_e within 10 cm^{-1} reduces the degree of deexcitation by a factor up to 20. If we assume that the inhomogeneous distribution function is represented by a smooth curve, such changes have little influence on the nature of the spectrum, but the necessary corrections should be made to the frequency scale.

In the experiments involving spectral hole burning the power required depends largely on the burning mechanism, the system in question, line width, etc. It is important to limit then the intensity "dose" of the radiation so as to avoid saturation effects. All these topics are discussed in detail in Ref. 29.

Selectively excited spectra are usually determined at liquid helium temperature (4.2 K) or in helium vapor ($\approx 5 \text{ K}$). For the majority of organic systems, including chlorophyll-like molecules, the zero-phonon lines in such spectra disappear at temperatures above 40 K (Refs. 23 and 49). Even lower temperatures are needed when use is made of the hole burning method, which makes it possible to study extremely narrow purely electronic lines.

Typically the spectra of chlorophyll-like molecules are recorded when the concentration of these molecules in a solution is $\sim 10^{-5} \text{ M}$. In the case of chlorophylls, which have a magnesium atom at the center of the molecule, magnesium monosolvates can be obtained in solvents such as diethyl ether, triethylamine, or dimethylformamide; disolvates are obtained using solvents such as pyridine, tetrahydrofuran, and other strong electron donors. Hydrogen bonds with the C=O groups of pigment molecules may form in alcohol solutions.

3.2. Selective fluorescence and excitation spectra

The main fluorescence band of the principal photosynthetic pigment chlorophyll-*a* (CL-*a*) in a frozen solution kept at liquid helium temperature transforms into a quasi-line spectrum under monochromatic excitation conditions^{5,24} (Fig. 4). According to Sec. 2.1, all the observed zero-phonon lines are the 0-0 pseudolines and the frequency interval up to the exciting line can be used to determine the frequencies of internal molecular (intramolecular) vibrations ω_1 in an excited state S_1 . The background under the lines is the result of summation of the phonon wings of all the excited centers (excitation occurs at the zero-phonon lines and in the wings). Nevertheless, some of the background may be due to "bad" centers in which the electron-phonon interaction is for some reason (association or complexation) enhanced. Moreover, line broadening and a reduction in their intensity with temperature until the structure disappears at $T \gtrsim 40 \text{ K}$ has been observed.⁴⁹

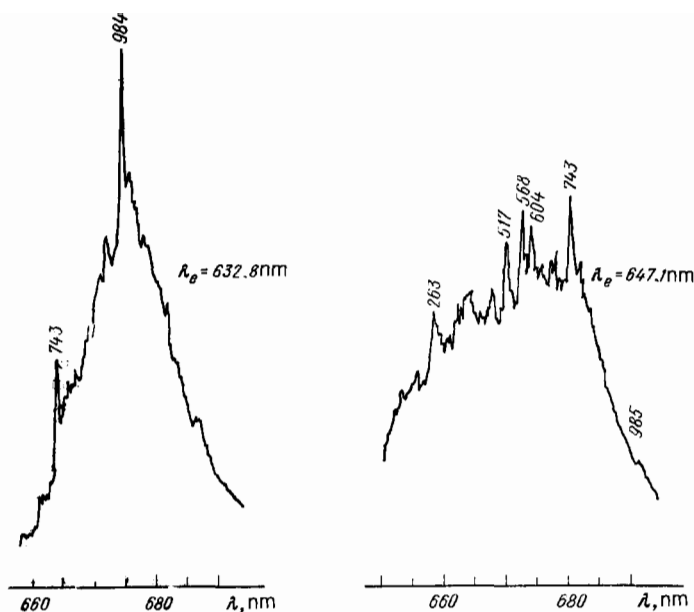


FIG. 4. Fluorescence spectra of chlorophyll-*a* in toluene ($C = 10^{-5}$ M) excited with laser lines 632.8 and 647.1 nm; $T = 4.2$ K (Ref. 53). The frequencies of vibrations of the S_1 state $\omega_i = \nu_e - \nu_r$ are given alongside the lines.

In the case of some related compounds and derivatives of chlorophyll (Fig. 5) the results are similar and sets of vibrational frequencies have been determined.^{48,49,52,53} Measurements of this kind have also been made at other laboratories using both amorphous and polycrystalline matrices.⁵⁴⁻⁵⁶

Since for the majority of porphyrins one can find a suitable Shpol'skiĭ matrix, selective spectroscopy has been applied to these molecules somewhat later. It is reported in

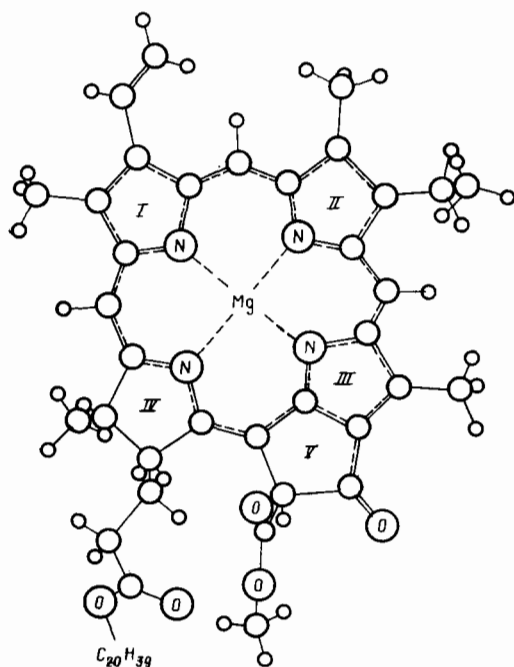


FIG. 5. Molecular structure of chlorophyll-*a*. The medium sized circles denote carbon atoms and the smallest ones are hydrogens. In the case of protochlorophyll (PCL) the π -electron system (dashed lines) includes also the ring IV because there are no protons in this ring. In pheophytins (PP) an atom of Mg is replaced with two protons coordinated with N_I and N_{II} . In bacteriochlorophyll the ring II is reproduced by protons. Free porphyrin is a tetrapyrrole skeleton without side groups.

Ref. 57 that the method of selective laser excitation of porphyrin and its derivatives in polymer films was used to determine the polarization characteristics of the $S_1 \leftarrow S_0$ vibronic transitions and to determine the types of their symmetry. Selective excitation has also made it possible to obtain quasi-line fluorescent spectra of the ionic forms of some biogenic porphyrins.^{58,59} Studies have been made of the characteristics of the spectra of metal porphyrins excited with monochromatic light in the region of overlap of the $S_1 \leftarrow S_0$ and $S_2 \leftarrow S_0$ vibronic transitions.⁶⁰ In the case of protoporphyrin a detailed study has been made of the dependence of the structure of the spectrum on the excitation frequency within the limits of the inhomogeneous distribution function.⁶¹

Relatively simple porphyrins can be studied also by the method of recording fine-structure spectra when molecules are cooled in supersonic jets.⁶²

The vibrational structure of quasiline spectra of porphyrins is discussed in detail in a monograph of Solov'ev *et al.*⁶³

Information on vibrational sublevels of the ground electronic state of molecules is frequently obtained by the method of Raman spectroscopy. In particular, this method has been applied to porphyrins⁶³ and chlorophylls.⁶⁴ It should be pointed out that there is a close relationship between the resonance Raman scattering and the vibronic absorption and fluorescence spectra; in particular, the excitation profiles of the resonance scattering are obtained from the absorption spectra by the transformation analysis method.^{65,66}

The vibrational frequencies of the ground electronic state are manifested also in the fluorescence spectra as a result of selective excitation in the 0-0 band. However, in this case serious difficulties occur because of burning a spectral hole at the excitation frequency. Various methods for reconstructing the initial inhomogeneous profile have been used to obtain such spectra for CL-*a*, some of its derivatives, and also porphyrins.^{47,49,57,58}

In the case of the frequencies of the excited electronic state S_1 , their complete set can be obtained using a series of fixed excitation frequencies throughout the vibronic $S_1 \leftarrow S_0$ absorption region. This method has been used to obtain ex-

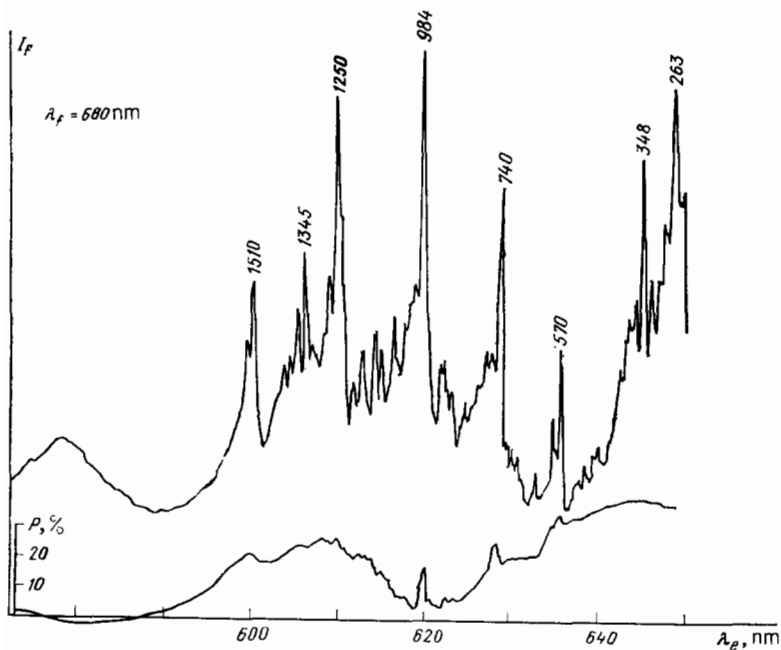


FIG. 6. Excitation spectrum of the fluorescence of chlorophyll-*a* in ether recorded selectively at 660 nm indicating the vibrational frequencies.⁴⁹ The lower part of the figure shows the polarization spectrum in a methanol-ethanol solvent; $T = 5$ K.

act values of ω_1 for CL-*a* and six of its closest analogs.⁴⁹ The advantages of these spectra are the relative simplicity of the method used to determine them, the high sensitivity (one can use a solute in amounts of $\sim 10^{-12}$ M and an average laser power 10 mW), and the feasibility of identification of molecules on the basis of the characteristic multiplet of pseudolines. On the other hand, the relative intensities of the vibronic transitions, as well as their frequencies and polarizations for centers with a given frequency of a purely electronic transition, can be determined most directly from the excitation spectra (Fig. 6). Narrow-line selective excitation spectra have also been determined for a number of chlorophyll-like molecules.^{48,49}

These investigations have been carried out on chlorophyll in higher plants and its derivatives. On the other hand, the primary processes of bacterial photosynthesis have been investigated and understood much better (see Ref. 67 and the literature cited there), compared with the two-system

photosynthesis in plants. The interest has therefore increased in spectroscopic studies of bacteriochlorophyll (BCL). The first high-resolution spectra of BCL were reported recently.⁵⁰

Figure 7 shows selective fluorescence and excitation spectra of the monosolvate form of BCL. We can see that the spectra are characterized by a high contrast between the lines and the background, which leads to the first conclusion that protonation of the second pyrrole ring does not increase the electron-phonon interaction compared with that in CL-*a*.

3.3. Analysis of the structure of the spectra

Since the first satisfactory theoretical interpretation of the vibrational spectra of metal complexes of porphin,⁶⁸ considerable progress has been made in semiempirical calculations of vibrations of porphyrins on the basis of the experi-

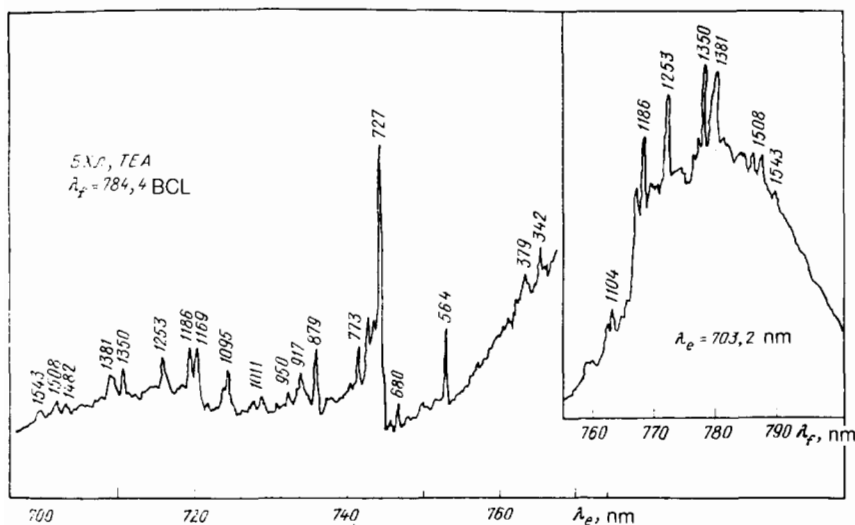


FIG. 7. Selective excitation spectrum (on the left) and fluorescence spectrum (on the right in the inset) of bacteriochlorophyll in triethylamine; $T = 5$ K.

mental spectra.⁶³ These results demonstrate that the vibrations in such a large π -electron system are usually weakly coupled to the individual structure elements of the molecule. On the other hand, although in the case of some simple porphyrins the form of the normal vibrations has been determined, this has not yet been done quantitatively for chlorophyll and its analogs. A vibrational analysis has been based mainly on a comparison of similar derivatives with one another and with appropriate porphyrins.

The total number of normal vibrations of the chlorophyll molecule exceeds 400, but the majority of the peripheral groups does not interact with optical π -electron transitions, which are localized in the tetrapyrrole ring. Therefore, the qualitative similarity with the vibronic structure of the spectra of porphyrins is fully expected. In view of the low symmetry, there are no rigorous selection rules governing the appearance of vibrations in the spectra: all the vibrations are totally symmetric (no motion of atoms can disturb the symmetry, because there is no symmetry). However, it is reasonable to begin with the symmetry approximations, in particular the polarization of vibronic transitions may differ from that for purely electronic lines, if in accordance with the approximate symmetry the corresponding vibrations are not totally symmetric. More rigorously, the difference between the polarizations of the individual lines is evidence that the angle between the moments of the observed transitions differs from zero.

In fact, in the case of the $S_1 \leftarrow S_0$ excitation spectrum of CL-*a* all the lines and the background are polarized parallel to a purely electronic transition (see Fig. 6). The spectra of protochlorophyll (PCL) and pheophytin-*a* (PP-*a*) include some negatively polarized lines,⁶⁹ in agreement with the existence of more definite symmetry elements in the case of these molecules. Wide polarization minima are clearly visible in the excitation spectra of CL-*a* and PCL. The contrast with the superimposed positively polarized lines makes it possible to attribute them to the second electronic transition $S_2 \leftarrow S_0$. This confirms the smallness of the S_2 - S_1 splitting, which amounts to $\Delta E_{21} \approx 800 \text{ cm}^{-1}$ for CL-*a* and $\approx 400 \text{ cm}^{-1}$ for PCL, discussed on many previous occasions.^{70,71} The degree of polarization at the minima shows that the angle between the moments of the electronic transitions to the states S_1 and S_2 amounts to about 60° for CL-*a* and is somewhat larger for PCL, in agreement with the higher approximate symmetry of the latter.

The spectra of $S_1 \leftarrow S_0$ vibronic transitions consist of a series of strong lines typical of specific molecules. For example, in the case of all the chlorophylls and pheophytins there is a strong line near $560\text{--}570 \text{ cm}^{-1}$ and it can be attributed to the presence of a cyclopentane ring, because it is absent in the case of porphyrins. Very characteristic vibronic lines near 750 cm^{-1} are exhibited by all tetrapyrrole compounds and these are associated with vibrations of the C-C-N and C-N-C types.⁶³ A line at 980 cm^{-1} is sensitive to small changes of the molecular structure: it is very strong in the spectrum of CL-*a*, much weaker in the case of PP-*a*, and frequency-shifted in the case of other compounds. Clearly, this frequency corresponds to the pyrrole C-C vibrations the activity of which changes because of a redistribution of the π -electron density in the course of complexation with a metal.

Near 1250 cm^{-1} there are several vibrations with dif-

ferent approximate symmetries and these can be attributed to C-N-H vibrations in porphyrins. At higher frequencies ($1320\text{--}1360$ and $1510\text{--}1550 \text{ cm}^{-1}$) the vibrations are mainly of the skeletal type, which are not very sensitive to small changes in the molecular structure.

Vibrations sensitive to solvation are of special interest because chlorophyll in photosynthetic units exhibits specific interactions with the lipoprotein environment via coordination and hydrogen bonds. Solvates with one or two extra ligands of the central magnesium atoms exhibit the following characteristic features: in the case of PCL there is a $\omega_1 = 1208 \text{ cm}^{-1}$ vibration in disolvates and the frequency shift is 330 cm^{-1} , whereas in the case of CL-*a* there are frequencies $570, 600, 880, 910,$ and 1005 cm^{-1} in the spectrum of monosolvates, 590 and 615 cm^{-1} in the case of disolvates, and a peak at 984 cm^{-1} becomes weaker on transition to disolvates. All these vibrations should involve the central part of the molecule and provide information on its interaction with the surrounding matrix.

4. OPTICAL BURNING OF SPECTRAL HOLES

4.1. Photochemical processes

In selective fluorescence spectroscopy the excited molecules either emit light or return nonradiatively to the initial state, so that there is no change in the steady-state inhomogeneous distribution function. However, some fraction of the excited centers may exhibit changes in the molecular structure or its environment, and may have a slightly different unexcited state. At the end of the excitation process this fraction of molecules absorbs at the shifted frequency of the electronic transition, whereas at the frequency of the exciting laser line a hole or a dip appears in the inhomogeneous distribution function and this hole is usually a long-lived residue of selective excitation. In the case of monochromatic illumination of an inhomogeneous system the absorption via a purely electronic resonance line creates a very narrow hole (with the minimum width $2\Gamma_0$) at the excitation frequency.

One of the principal classes of the processes responsible for hole burning is represented by low-temperature photochemical transformations.^{27,29} In the case of porphyrins it has been found that the components of the Shpol'skiĭ spectrum exhibit mutual transformation and this is attributed to tautomeric photochemistry.⁷² The same mechanism was invoked in the first study of photochemical hole burning in the case of phthalocyanine molecules²⁷ and also in the case of porphyrin in *n*-alkane matrices.⁷³ The tautomers of these molecules are almost equivalent and the shifts of the frequencies of purely electronic lines are small ($\sim 100 \text{ cm}^{-1}$). In the case of H_2 -chlorin (dihydroporphyrin), in which one pyrrole ring is protonated, the frequency of the $S_1 \leftarrow S_0$ transition due to rotation of the central NH bonds is shifted by 1500 cm^{-1} (Ref. 74). Manifestations of inequivalent NH isomers can be expected also in the case of pheophytins. In fact, hole burning in the main band of PP-*a* near 670 nm gives rise to a new fluorescence band at 650 nm (Ref. 75). A moderate shift by $\Delta\nu_{00} \approx 500 \text{ cm}^{-1}$ is explained by the partly compensated influence of the rings IV and V (Fig. 8). The isomeric form of PP-*a* is stable in darkness at 4.2 K , but very photolabile: the efficiency of its photodissociation is two orders of magnitude higher than the quantum yield of the process, which amounts to 10^{-3} . Figure 8 shows the potential curves

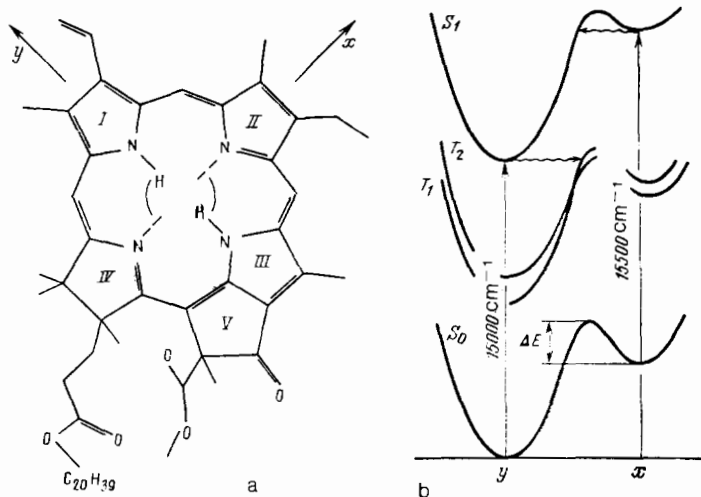


FIG. 8. Structural formula of pheophytin-*a* showing rotation of NH bonds (a) and schematic representation of corresponding potential curves (b).

of the configurational coordinate of the tautomeric NH vibration. The height of the potential barrier ΔE in the case of the ground electronic state can be estimated from the temperature dependence of the thermal decomposition of the photoproduct. The initial parts of the dissociation curves give $\Delta E = 50 \text{ cm}^{-1}$. The nonexponential nature of the dissociation indicates that the barrier heights ΔE are subject to a considerable scatter because of the inhomogeneous interaction with the solvent molecules. A similar phototautomerization has been observed also in the case of PP-*b*. Recently, NH tautomers have been identified also in the case of some porphyrins with an isocycle and their dimers.⁷⁶

Little is known so far about the details of the photo-transformation mechanism. The method of optical detection of magnetic resonances has been used to establish that there are no proton jumps, at least from the zeroth vibrational sublevel of the triplet state T_1 , both in the case of porphin⁷⁷ and in some bioporphyrins.⁷⁸ It is most natural to assume that the process is completed in the course of vibrational relaxation, i.e., relaxation from a "hot" state assisted by vibronic mixing of singlet and triplet states.

Photochemical spectral hole burning has been used to determine the rates of vibrational relaxation in an excited electronic state. Phase relaxation due to the electron-phonon coupling slows down as a result of cooling so that the corresponding modulation broadening does not exceed 1 cm^{-1} at

temperatures $T < 5 \text{ K}$. Therefore, the homogeneous width Γ_e of vibronic levels is governed primarily by the energy relaxation rate and these two quantities are related by the indeterminacy principle.

The values of Γ_e have been determined by the hole burning method in the case of phthalocyanine²⁹ and porphin⁷⁹ in Shpol'skiĭ matrices. Homogeneous widths of individual vibronic levels in systems with a strong inhomogeneous broadening can be found by recording first the selective excitation spectrum and then inducing hole burning at the position of a vibronic line.⁸⁰ The difference between these two spectra gives the required homogeneous profile (Fig. 9). This method has been used to find the values of Γ_e and the corresponding relaxation times for the majority of vibronic-active vibrations of the PP-*a* molecule in the S_1 state.⁸⁰ The vibrational relaxation times have been found to be within the range 1–7 ps, i.e., of the same order—on the average—as those reported for porphin.⁷⁹ There is a general trend for an increase in the rate of relaxation on increase in the vibration frequency, which is due to the appearance of additional channels for decay to other vibrational states.

4.2. Burning by saturation of transitions

Formation of saturation dips or holes is well known from the spectroscopy of free atoms and molecules.⁸¹ In the

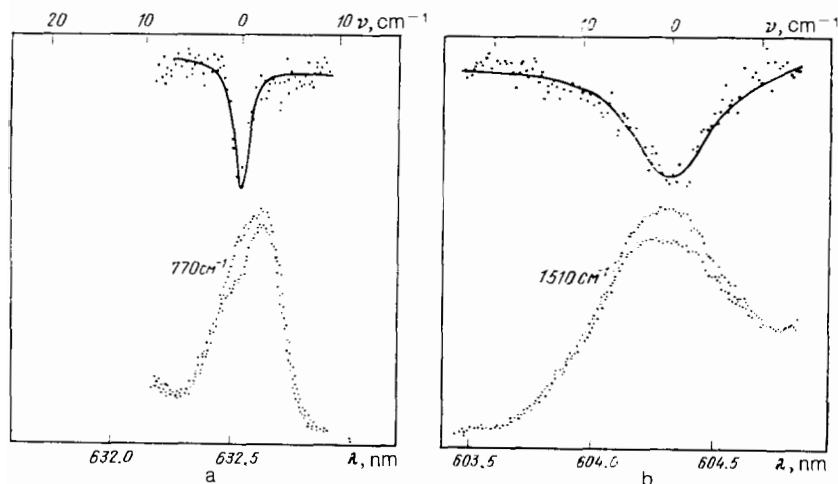


FIG. 9. Photochemical holes formed at vibronic lines in the excitation spectrum of pheophytin-*a* in ether at 5 K, $\lambda_r = 665 \text{ nm}$ (Ref. 80). The difference profiles are approximated by Lorentzians (continuous curve) of width 1.6 cm^{-1} (a) and 9.8 cm^{-1} (b).

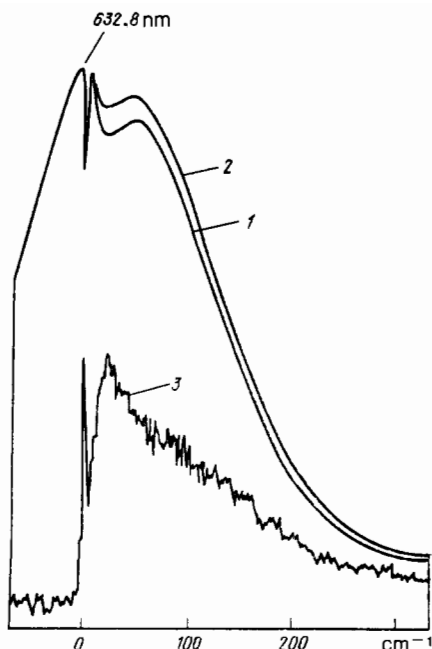


FIG. 10. Resonance metastable hole together with the phonon wing in the fluorescence spectrum of protochlorophyll in an ether-butanol matrix at $T = 5$ K (Ref. 90). Curves 1 and 2 were obtained using a delay of 15 ms between the moments of recording; 3) difference between curves 1 and 2 but shown on a larger scale.

case of solids the direct saturation of optical transitions by various competing relaxation processes is difficult. However, saturation is achieved much more readily in a three-level system. A simple estimate shows that even low-power (~ 10 mW) laser excitation via a strong singlet-singlet transition can create a perceptible population of the triplet state ($\tau_T \sim 10^{-3}$ – 10^{-2} s) of complex molecules. The absorption via a narrow zero-phonon line results in the formation of a metastable hole in an inhomogeneous band because of filling of such a triplet reservoir.

The first attempt to observe a hole characterized by saturation of the triplet level has confirmed the effect, but the holes have remained wide because the CL-*a* molecules have been excited via vibronic levels of the S_1 – S_0 transition.⁸² Further improvements in the method made it possible to observe resonance dips in the case of purely electronic transitions in PCL, CL-*a*, etc.⁸³ The difference spectrum reveals clearly a purely electronic line with an instrumental width of 0.3 cm^{-1} and its pseudowing (Fig. 10). The kinetics of burning and filling of a hole is in good agreement with the decay parameters of the T_1 state ($\tau_T \approx 5$ ms). Metastable holes in the spectra of Zn porphyrin dissolved in *n*-octane⁸⁴ and of Zn tetrabenzoporphyrin in a polymer matrix⁸⁵ have been observed in a similar manner.

Saturation of the triplet reservoir must definitely be allowed for in photochemical hole burning as a possible cause of the broadening limiting the excitation density used in the hole burning procedure.^{80,85,86}

In spite of these difficulties, the use of a high-power pulsed dye laser has made it possible to observe holes also for the direct S_1 – S_0 saturation of perylene impurity molecules in benzophenone and in *n*-hexane.⁸⁷

4.3. Nonphotochemical transformation of centers

Burning of holes in the spectra of molecules regarded as photochemically stable has been observed in glassy matrices.^{28,88} Therefore, a mechanism has been suggested for the so-called nonphotochemical spectral-hole burning⁸⁹ representing photostimulated transformation of centers in a metastable system of two-well potentials. The photoreaction clearly alters the local forces of the interaction between the impurity and the matrix, but detailed data on its mechanism at microscopic level are still lacking. A sign of nonphotochemical hole burning is that the shift of the frequency of the 0–0 transition $\Delta\nu_{00}$ of the photoproduct does not go beyond the limits of the initial inhomogeneous distribution function.

In the case of chlorophylls (which do not have central protons responsible for the photochemistry in the case of pheophytins), hole burning because of the presence of a triplet reservoir as well as slower nonphotochemical burning processes have been observed.⁸³ The stability of the holes in darkness has made it possible to determine the homogeneous width of a purely electronic line of CL-*a* at $T = 1.8$ K: the value obtained in Ref. 90 is $\Gamma_0 \leq 0.005$ cm^{-1} . This is just one order of magnitude greater than the width governed by radiative decay and it includes a contribution of the electron-phonon broadening, which could be frozen out only at much lower temperatures.

It has been possible also to obtain information on the essence of the burning mechanism, which is the recovery.⁹¹ Firstly, it has been found that the frequencies of the burnt-out centers are distributed uniformly over the whole inhomogeneous profile proportionally to the initial inhomogeneous distribution function (Fig. 11). Secondly, irreversibility of the thermal filling of a hole as a result of cyclic variation of temperature has shown that for each center there are many matrix-molecule configurations covering on the energy scale an interval in excess of 100 cm^{-1} . Clearly, the model of two-well potentials is inapplicable to systems of this kind: each molecule has access to a large number of potential wells. Recent nonphotochemical spectral hole burning in the case of CL-*a* in polystyrene⁹² has revealed that the holes are clearly broadened because of saturation or insufficient resolution and the temperature dependences obtained are not fully reliable.

The photoburning of spectral holes is being used also to determine homogeneous spectral profiles, including the phonon wing. In the study reported in Ref. 87 use has been made of the fact that saturation of the S_1 – S_0 transition for a purely electronic line occurs (on the intensity scale) several orders of magnitude earlier than in the case of the phonon wing. Homogeneous profiles and inhomogeneous distribution functions of perylene have been obtained for two matrices.

Combination of the methods of selective excitation and hole burning has made it possible to develop a new fine and experimentally simple method for the determination of homogeneous spectra and inhomogeneous distribution functions.⁴⁴ Once again use is made of the enormous difference between the peak intensities of purely electronic lines and of the phonon wing, which justifies the neglect of burning-out in the wings during the stage of saturation of a resonance hole. Strictly speaking, the pseudowing which then appears in the absorption spectrum is of intensity comparable with the real wing, but it can be eliminated by subsequent selec-

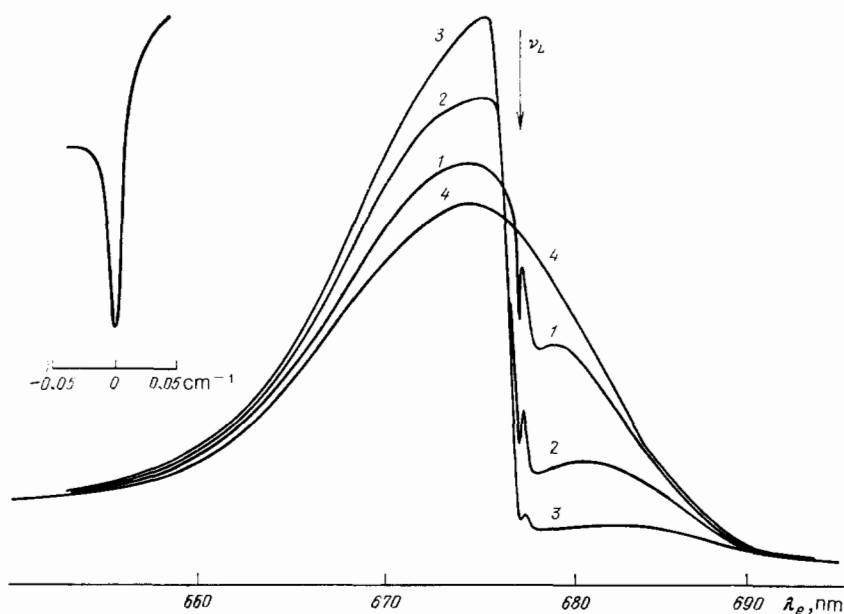


FIG. 11. Transformation of the excitation spectrum of the fluorescence of chlorophyll-*a* in an ether-butanol matrix as a result of irradiation with a 676.4 nm laser line (200 mW/cm^2)⁹¹ for periods of 30 s (1), 5 min (2), and 30 min (3). Curve 4 represents the initial spectrum. The profile of a hole is shown separately with a high resolution.

tive excitation. The whole procedure is as follows: fluorescence spectra recorded twice using monochromatic excitation so weak that hole burning during measurements can be ignored, whereas between these two measurements a hole is burned at the same frequency ν_e . The difference between the two spectra obtained in this way yields the homogeneous fluorescence spectrum for impurities with a purely electronic line at a frequency ν_e , with the exception of the profile of the resonance line itself. One can similarly derive a homogeneous absorption spectrum using the difference between selective excitation spectra recorded employing intermediate hole burning at the recording frequency. The inhomogeneous distribution function can be calculated if we determine additionally the inhomogeneous spectrum (of fluorescence or absorption), which mathematically speaking is a convolution of the corresponding homogeneous spectrum and the inhomogeneous distribution function. A convenient deconvolution algorithm given in Ref. 44 can be used to find the inhomogeneous distribution function from such a convolution.

This method has been used to determine the required characteristics of PCL impurity molecules⁹³ for ten values of ν_e . Figure 12 shows an example of homogeneous spectra obtained at one of the selected frequencies. We can see that in the case of the coupled $S_1 \leftrightarrow S_0$ transitions the absorption and fluorescence spectra no longer exhibit the mirror symmetry²⁾ expected on the basis of the basic model of an impurity center.¹⁰ We can suggest several mechanisms capable of inducing such asymmetry: deviation from the Condon approximation, mixing of normal coordinates (equivalent to a change in the force constants in the local pattern) as a result of an electronic transition, and nonadiabatic interaction. In this case the important contribution is made by the nonadiabaticity due to a small S_2-S_1 splitting, which may be accompanied also by a deviation from the Condon approximation. The perturbing role of the S_2 state is supported also by the dependence⁹³ of the profile of the absorption wing on the frequency ν_e (this is true, for example, of the values labeled 1 and 2 in Fig. 12b), because the S_2-S_1 splitting also depends

on $\Delta\nu$ (Ref. 69). The inhomogeneous distribution functions obtained for this system represent smooth and slightly asymmetric curves with a half-width of 176 cm^{-1} .

It should also be mentioned that if the inhomogeneity includes not only the shift of the electronic transition fre-

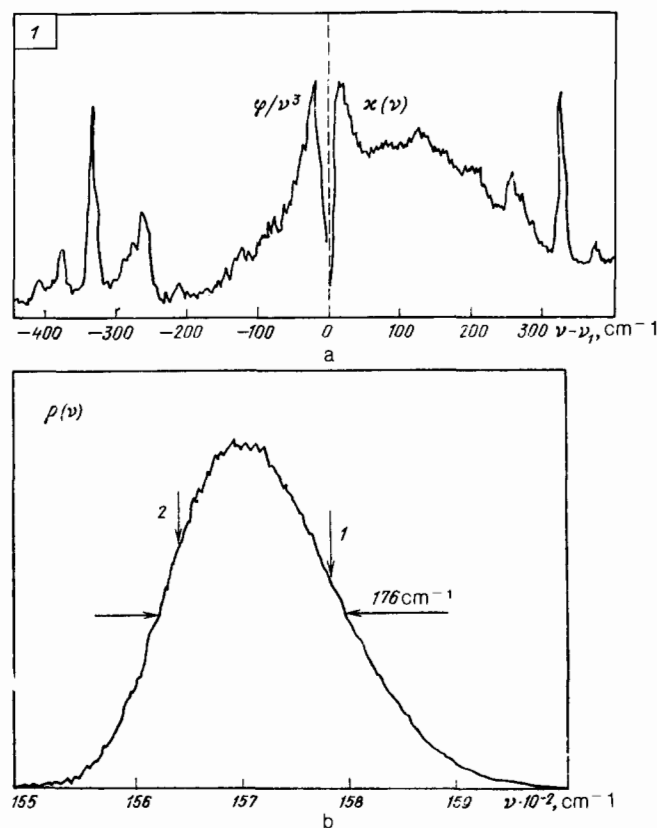


FIG. 12. Homogeneous phonon wings together with low-frequency vibrational replicas (a) for $\nu_{00} = 15782 \text{ cm}^{-1}$ in the fluorescence (φ/ν^3) and absorption (χ) spectra of PCL impurity molecules in ether-butanol glass ($T = 5 \text{ K}$) and calculated inhomogeneous distribution function (b).⁹³

quency, but also variations of the electronic-vibrational interaction, the resultant spectra are averaged over such characteristics as the Debye-Waller factor and the structure of the wing in the case of centers characterized by a given frequency of a purely electronic line. In fact, repeated hole burning at different frequencies has made it possible to reveal the latent structure of similar averaged phonon wings in the spectrum of Zn tetraphenylporphin.⁹⁴

5. HIGH-RESOLUTION SPECTRA OF REAL AND MODEL SYSTEMS

In view of the importance of studies of primary photosynthetic processes, the application of selective spectroscopy methods to ever more complex (including native) objects is attracting considerable interest. Much attention has recently been given to model systems, such as dimers, pigment-protein complexes, etc.

Selective spectra of synthetic isobacteriochlorin have been investigated⁹⁵ and used to determine ways of biosynthesizing vitamin B₁₂. The excitation and fluorescence spectra have been used to determine the frequencies of vibrations in the S₁ state in the interval 400–820 cm⁻¹.

Selective laser excitation has yielded fine-structure fluorescence spectra of metal-free cytochrome C representing a complex of porphyrin with a polypeptide chain.⁹⁶ Multiline spectra, similar to those observed in the case of chlorophyll (Fig. 4), were recorded for a glassy matrix at 4.2 K. A similar interpretation of the structure of the spectra was based on the model of inhomogeneous broadening ($\sigma \approx 200$ cm⁻¹). In the case of CL-*b*, introduced into membranes of lecithin vesicles, the structure of selectively excited spectra is less clear.⁹⁷

Investigations of selective spectra of model pigment-protein complexes of tetraphenylporphin with phenylalanine in ortho- and paraforms and of pheophorbide with a polytripeptide chain were begun recently.⁹⁸ In particular, it has been shown that the width of the inhomogeneous distribution function and the position of its maximum are very sensitive to the nature of the substituent: in the case of a polypeptide complex of pheophorbide the function in question is shifted by 150 cm⁻¹ toward longer wavelengths and it is broadened by 200 cm⁻¹ compared with a free pigment.

Promising results have been obtained by applying the method of optical burning of spectral holes to native systems. In the case of chlorella cells (at 4.2 K) the spectrum exhibits holes^{99,100} attributed to phototransformation of the reaction centers of the photosystem I (P700). The width of a hole (after allowing for the instrumental function) yields an estimate of the relaxation time of the excited state in a reaction center, amounting to $\tau \approx 50$ ps. The duration of the primary photoprocesses $\tau \sim 4$ ps in the photosystem II has been determined similarly.¹⁰¹

The hole burning method has also been employed in a study of native pigment-protein complexes of blue-green and red algae (phycobilins),¹⁰² the chromophores of which consist of noncyclic (linear) tetrapyrrole molecules. Hole formation is due to reversible proton transfer. The large width of the holes (> 1 cm⁻¹ at 1.8 K) has not yet been adequately explained.

In the case of green plants, where the bulk of chlorophyll is in the form of what is known as a light-gathering antenna, attempts to apply selective spectroscopy methods

have not been successful. Several possible explanations have been proposed to account for the absence of a line structure in the fluorescence spectra of green leaves subjected to monochromatic excitation, including the loss of selectivity because of fast energy migration.¹⁰³ On the basis of papers cited above⁹⁹⁻¹⁰² the pigment-protein and pigment-pigment interactions should not necessarily destroy zero-phonon lines in low-temperature spectra of chromophores, so that energy transfer is the most probable reason for the loss of selectivity by such systems. A theoretical analysis of selective spectroscopy under energy transfer conditions was given in Sec. 2.2. A fall of the relative intensity of zero-phonon lines in the spectra of inhomogeneous systems because of the transfer of electronic excitations can be illustrated by the excitation spectra of the fluorescence of CL-*a* present in two concentrations differing by more than two orders of magnitude (Fig. 13).³⁸ In the case of the concentration $C = 6 \times 10^{-5}$ M the value of the transfer parameter is $p_t = 23\tau_0^{-1}$, so that there is no line structure at all at the center of an inhomogeneous band, but at the short-wavelength edge of the band (this was why the wavelength was selected to be 659.9 nm) there is only a moderate reduction in the intensity of the vibronic lines relative to the background, in good agreement with the conclusions reached in Sec. 2.2. In real photosynthetic systems the concentration of chlorophyll should reach 10⁻¹ M, so that the absence of lines in their spectra is fully expected. Naturally, this does not exclude the possibility that in some parts (for example, reaction centers) of chloroplasts the conditions are more favorable for the observation of zero-phonon lines.

The hypothesis of the decisive role of the rate of energy transfer in the loss of excitation selectivity by photosynthetic

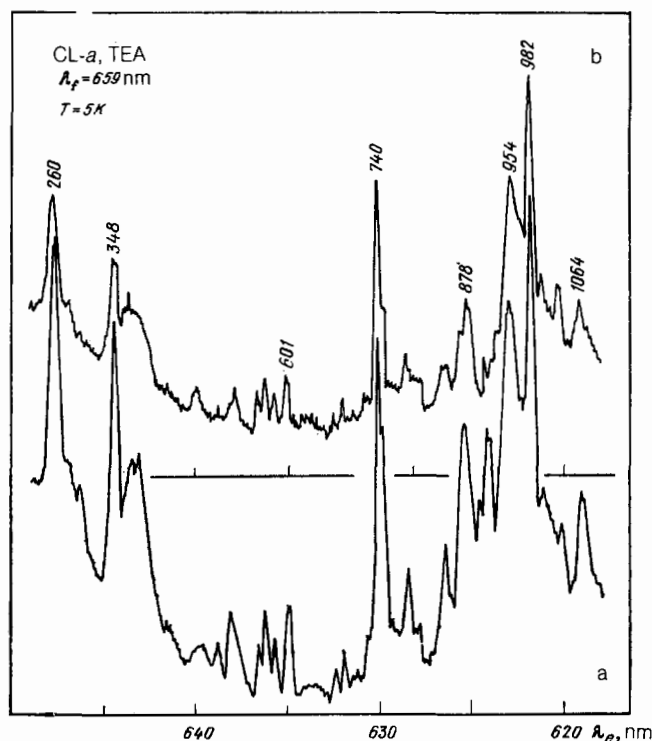


FIG. 13. Excitation spectra of the fluorescence of chlorophyll-*a* in triethylamine with concentrations $C = 2 \times 10^{-5}$ M (a) and 6×10^{-5} M (b) recorded at the short-wavelength edge of the 0-0 band.³⁸

systems is supported by experiments on etiolated (grown in darkness) leaves and turning leaves green.¹⁰⁴ The concentration of pigments in these leaves is so low that energy transfer can be, to some extent, neglected. Etiolated sheets initially have two forms of protochlorophyllide P630 (inactive) and P650 (photoactive). In the case of the fluorescence spectrum of the inactive form, representing an inhomogeneous assembly of monomer molecules of protochlorophyllide, selective laser excitation at $T = 5$ K produces a typical pseudo-line structure and the vibrational frequencies of the S_1 state are close to those observed for PCL in solutions. A similar structure is observed in the selective excitation spectrum. However, in the case of the band due to the photoactive form P650 there is no line structure as a result of excitation of its vibronic transitions, which may be attributed to a strong exchange interaction in this aggregated pigment-protein complex. However, in the case of excitation in the 0-0 band, when the influence of vibrations of the vibronic exciton type is suppressed, it has been possible to obtain the line structure in the fluorescence spectrum of P650 using the vibrational replicas of the S_1-S_0 transition.¹⁰⁵

During the initial stage of illumination at physiological temperatures the photoactive form P650 is converted into chlorophyllide, which after passing through several intermediate forms, is converted into chlorophyll. The excitation spectrum of the band of the newly formed chlorophyllide also has a line vibronic structure¹⁰⁴ if the spectrum is recorded at the short-wavelength edge of the fluorescence band (Fig. 14). After a shift of the recording frequency ν_f of the fluorescence toward longer wavelengths or after prolonged illumination in the case of pigment accumulation the structure of the spectrum disappears, in good agreement with the conclusions reached on the influence of the energy transport process on the intensity of zero-phonon lines in inhomogeneous systems (Sec. 2.2).

In the case of the 0-0 band of the photoactive form of etiolated leaves it has been possible to burn narrow holes (of width $\delta\nu < 0.3$ cm⁻¹ at 5 K).¹⁰⁵ Second burning at similar frequencies (Fig. 15) shows that there is no significant filling of the "old" holes when new ones are formed. This is evidence in support of the photochemical mechanism of spectral hole formation, which is probably associated with the accumulation of a nonfluorescing intermediate product of chlorophyllide because further thermal reactions stop at such a low temperature, in agreement with nonselective measurements.¹⁰⁶ Fairly deep holes (Fig. 15) are formed indicating a weak electron-phonon coupling (comparable with that in solid solutions of chlorophyll molecules) in the case of this complex, which is a dimer or tetramer of protochlorophyllide attached to large protein particles. A high intensity of a purely electronic line (manifested in the form of a resonance hole) demonstrates the rigidity of the environment of the chromophore. A study of the temperature dependence of the width of a hole can provide valuable information on the nature of the polypeptide matrix: in glassy media this dependence is very strikingly different from that for crystals.²⁹

Very recently attempts have been made to burn a hole in the absorption band of the reaction centers of bacterial photosynthesis. In two investigations^{107,108} involving laser excitation in the band of the P870 reaction centers of purple bacteria *R. Sphaeroides* ($T = 1.5$ K) reversible attenuation of this band has been induced without formation of narrow holes. The authors of these papers considered possible reasons for such a large width of the "holes" (200-400 cm⁻¹), not excluding the possibility that this is the homogeneous width due to ultrafast processes ($\tau < 10^{-13}$ s) of charge separation in the reaction centers. However, it has been reported recently¹⁰⁹ that a narrow hole ($\delta\nu \approx 5$ cm⁻¹) can be formed in the same system at $T = 4.2$ K. Its width can be

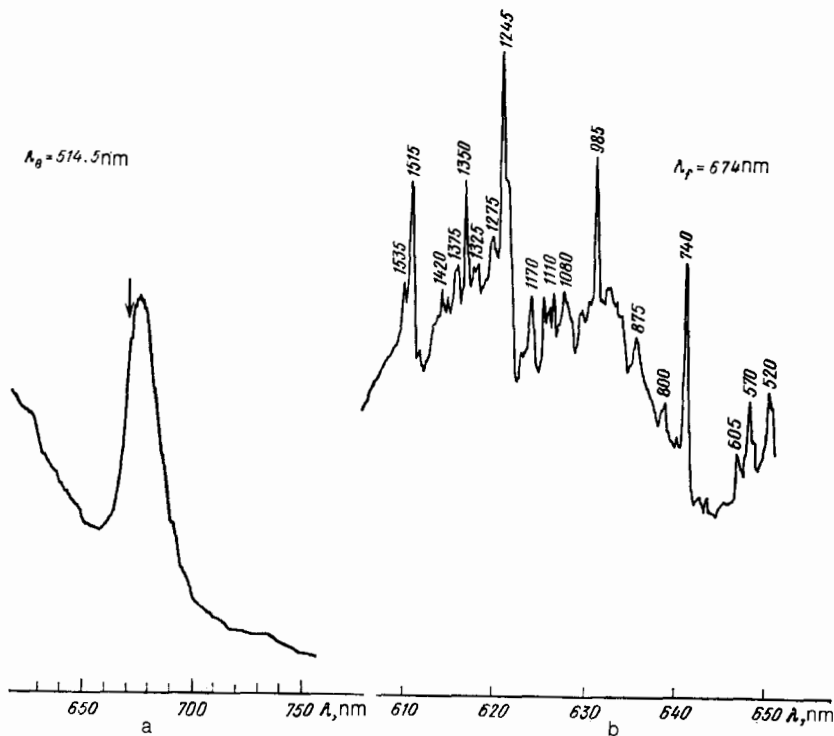


FIG. 14. Spectra of a leaf turning green of barley at $T = 5$ K (Ref. 104): a) fluorescence excited at 514.5 nm + b) excitation spectrum of this fluorescence for $\lambda_f = 674$ nm.

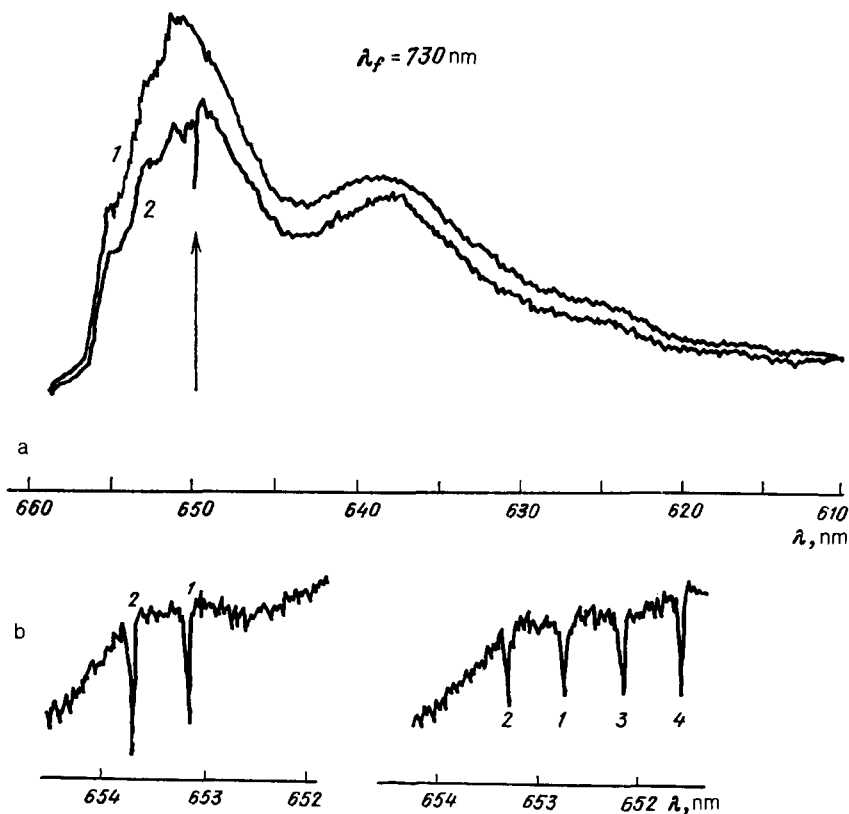


FIG. 15. Excitation spectra of an etiolated leaf before (1) and after (2) burning of a spectral hole by a laser at a wavelength 649.05 nm (a) and second burning of holes in the sequence from 1 to 4 (b).¹⁰⁵

used to obtain a more reasonable estimate of the electron transfer time $\tau = 1.4 \pm 0.7$ ps (see Ref. 67). Clear contradictions between the results of earlier investigations demonstrate the need to continue these experiments using selective spectroscopy methods and also careful analysis of the results.

6. CONCLUSIONS

The methods of selective laser spectroscopy have been used for a decade to study electronic-vibrational spectra of photosynthetic pigments. This has made it possible to solve the problem of interpretation of the structure and to accumulate large amounts of spectroscopic data. It is now reasonable to expect further applications of these methods in biophysics, photochemistry, etc. The first examples of selective spectra and hole burning in the case of pigment-protein complexes and chlorophyll *in situ* have already been discussed above.

We shall conclude by mentioning some reviews on related subjects of selective spectroscopy,¹¹⁰ hole burning,^{29,111-113} spectroscopy of chlorophyll,¹¹⁴ and zero-phonon lines.^{33,115}

¹¹The width of the lines in such spectra is usually 1–10 cm⁻¹ at temperatures from 4.2 to 77 K.

²²The apparent symmetry of the selective spectra (see Sec. 2.1) is already eliminated.

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