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To the bright memory of our teacher
Academician A. A. Krasnovskii

ELECTRON TRANSPORT IN PHOTOSYNTHESIS†

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A review is presented of the data obtained on the mechanisms and kinetics of electron transport in the primary processes of photosynthesis. It is shown that tunnel electron transfer in the chain of photosynthesis is clearly linked with passage between the conformational states in the protein parts of the carriers. The harmonized intramolecular dynamics of the protein complexes of the reaction centres, including movements with a set of different characteristic times, is the necessary condition for the manifestation of functional activity.

Photosynthesis is based on a complex chain of reactions of electron transport between the macromolecular complexes of carriers localized in the photosynthetic membranes. Determinant in the primary stages of photosynthesis is the role of chlorophyll whose energy of the electron excitation initiates the functioning of the electron transport chain. The pioneering investigations of Krasnovskii [1] were the first to demonstrate the capacity of photo-excited chlorophyll for reversible photoreduction (the Krasnovskii reaction) which underlies ideas on photosynthesis as a system of redox reactions. In solution, this reaction passes through the triplet state of the pigment which maintains the energy of electron excitation for a sufficiently long time, and makes possible the reaction in this time of the colliding molecules in solution. However, in the solid photosynthetic membrane the situation is much more complex. In this case it is important to understand the influence exerted by the protein–lipid matrix on the transformation of light energy in the pigment apparatus of photosynthesis. Already by the end of the 1940s and at the beginning of the 1950s, Krasnovskii [1] was the first to draw attention to this side of the problem. He took up investigation of the native state of the pigments showing the leading role of pigment–pigment and pigment–protein interactions. The problem of electron transport in photosynthetic membranes has been the subject of research undertaken in the Department of Biophysics of the Biology Faculty of Moscow State University into the

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kinetics and mechanisms of electron transfer over a wide time range from a few pico- and nanoseconds to micro- and millisecond time intervals. Parallel study of the physicochemical mechanisms of these processes has concentrated on elucidation of the role of protein in ensuring the efficacy of directed electron transport in photosynthetic membranes. This approach is coupled with development of theoretical models for determining from the experimental data the parameters of the intramolecular mobility of protein. The present paper is devoted to a brief exposition of the results of these investigations.

GENERAL CHARACTERIZATION OF ELECTRON TRANSPORT

The main feature of the initial stages of photosynthesis and accordingly the difficulty in solving these problems consists in the high speed of electron transfer which is necessary to ensure effective primary charge separation. In the lifetime of the singlet excited state of chlorophyll (bacteriochlorophyll) which in solution is $\tau \sim 5$ ns, the energy of electron excitation must be utilized in the photosynthetic membrane with a quantum yield close to 100%. From this it follows that the primary charge separation constant K_{ph} must be $\sim 10^{11}$ s^{-1} and, consequently, the initial act of photosynthesis in the reaction centres must occur in several picoseconds (ps). However, on coupling of the reaction centres with mobile carriers at the ends of the electron transport chain, the transfer times amount to 10^{-4}–10^{-3} s, which already corresponds to the usual enzymatic processes, thereby ensuring the normal interaction of the fast initial stages of photosynthesis with its dark processes. Thus, in the photosynthetic reaction centres processes of electron transfer differing in their rates by many orders are brought together within a single structuro-functional organization. The question arises of the mechanisms ensuring coupling, the high efficacy and directivity of the reactions of electron transfer and their connection with other processes of energy transformation in photosynthesis. In solving this problem, we started from the premise that the protein components of the macromolecular pigment–protein complexes may play an active role in electron transport. This means that protein not only ensures a definite spatial localization of the carriers in the photosynthetic membrane but itself takes a direct part in the reactions of electron transfer. As we shall show, electron transfer is determined by the character of the conformational changes of the protein of the electron transport chain carriers over a wide time range. These electron-conformational interactions underlie not only the elementary acts of electron transfer but also influence its directivity and regulation in the photosynthetic membrane. We would note that the electron-conformational interactions in a broad sense form a common mechanism of functioning of the macromolecules also in other biological processes [2–4]. Thus, in the act of enzymatic catalysis, initial changes occur in the stereoelectron interactions in the active centre of the enzyme–substrate complex and, as a consequence, the cascade of conformational conversions leading to the formation and release of the free product and regeneration of the enzyme. However, it is understandable that in the usual "dark" enzymatic processes, it is difficult to isolate experimentally and really study the mechanisms and kinetics of these electron-conformational interactions. The rates of delivery of the substrate and the formation of its complex with the enzyme maximally attainable in experiments are far slower ($\sim 10^{-6}$–10^{-5} s) than the rates of the subsequent electron interactions in the substrate–enzyme configuration in the active centre ($\sim 10^{-5}$–10^{-10} s). Because of this, the kinetics and details of the mechanisms of electron interactions in the active centre cannot be resolved in

time in a direct experimental fashion and their study calls for special procedures. In photosynthesis the light quanta serve as "substrate" triggering the chain of reactions in the photo-active enzyme whose role is played by the macromolecular pigment–protein complex of the reaction centre. The possibility of extremely rapid excitation of the active pigment or the primary electron donor (P) in the reaction centres using laser flashes (10^{-9}–10^{-11} s and up to 10^{-15} s) is now provided for researchers by a new approach to a direct study of the kinetics and mechanisms of energy transformation and electron transport in macromolecular complexes. The reaction centres isolated from the chromatophores of purple photosynthesizing bacteria provide a convenient object for solving these problems.

The protein part of these reaction centres (*Rhodobacter sphaeroides*, R-26) is represented by three polypeptides with molecular weights 19,000, 22,000 and 28,000 Daltons respectively, on which are located four molecules of bacteriochlorophyll (BChl), two bacteriopheophytin molecules — BPheo (H), two molecules of a quinone nature (ubiquinones) Q and also an iron atom. In different laboratories, measurement of the photo-induced differential absorption spectra (light minus dark) has established that two BChl molecules serve as primary electron donor (P), one of the two BPheo molecules in 200 ps transfers an electron from this special pair P to the primary quinone (Q_a), which then donates an electron in 10^{-4} s to a secondary quinone (Q_b). The positive "hole" formed on the primary donor as a result of charge separation and electron transfer in 4 ps to the primary acceptor H is filled in $\sim 10^{-6}$ s by an electron from the secondary donor — cytochrome (C). Between the dimer P and BPheo (H) is also situated one molecule of bacteriochlorophyll (B) which serves as an intermediate carrier where the electron is held for a time shorter than 4 ps. The general scheme of the primary processes of electron transport in bacterial photosynthesis has the form:

$$\begin{aligned} &CPBHQ_aQ_b \xrightarrow{h\nu} CP^*BHQ_aQ_b \xrightarrow{4\,ps} CP^+BH^-Q_aQ_b \xrightarrow{200\,ps} \\ &\xrightarrow{200\,ps} CP^+BHQ_a^-Q_b \xrightarrow{100\,\mu s} CPBHQ_aQ_b^- \end{aligned} \qquad (I)$$

$$CP^+BHQ_a^-Q_b \xrightarrow{1-2\,\mu s} C^+HBHQ_a^-Q_b$$

$$CP^+BHQ_a^-Q_b \xrightarrow{100\,ms} CPBHQ_aQ_b$$

After acceptance of the first electron and proton the secondary quinone in semiquinone form accepts a second electron and attaches a proton passing to the form Q_8H_2 and then diffuses in the membrane for further participation in the redox processes. Scheme I shows that with movement along the electron transport chain the reactions of electron transfer slow, and it is stabilized with corresponding loss of part of the energy on the intermediate acceptors. This principle of coupling the initial fast stages of charge separation followed by slowing of the electron flux is also realized in the reaction centres of higher plants in photosystems I and II (PS I and PS II). A simplified general sequence of the processes in the electron transport chain of photosynthesis may be represented as:

$$PIQ \xrightarrow{h\nu} P^*IQ \underset{1\,ns}{\overset{3-8\,ps}{\rightleftharpoons}} PI^-Q \xrightarrow{100-200\,ps} P^+IQ^- \qquad (II)$$

$$\uparrow \quad 10 - 20\ ns \quad \downarrow$$

where P is the primary donor (P_{870} in purple bacteria, P_{700} in PS I and P_{690} in PS II), I is the primary acceptor (H is bacteriopheophytin, Chl_{696} in PS I and pheophytin in PS II), Q is the secondary acceptor (Q_a — ubiquinone, iron sulphur centres in PS I, Q_a is plastoquinone in PS II).

Current data on X-ray structural analysis of crystalline protein samples of the reaction centres [5] and also data obtained on different mutants by gene engineering give quite a full idea of the character of the immediate protein environs of the prosthetic groups of the electron transport chain carriers. Without going into details of the structural organization of the reaction centres, we would note that the carriers are tightly bound to the amino acid residues of protein through the hydrogen bonds, hydrophobic and van der Waals interactions. For example, in the reaction centres of bacterial photosynthesis (*Rhodospeudomonas viridis*) the primary donor P is bound to two parallel sequences of carriers (B and H) arranged symmetrically on two subunits (L and M). However, only the sequence of carriers on the L-subunit is functionally active and leads to the reduction of Q_a and then Q_b. The site of binding of Q_a consists of nine amino acid residues. Hydrogen bonds form between the oxygens of the carbonyl groups of the quinone head of Q_a and the heteroatoms of some of them (imidazole ring of histidine 217 and the NH-group of the peptide bond of alanine M_{258} in *Rhodospeudomonas viridis*). It is interesting to note that at first sight the insignificant difference between the inactive M- and active L-chains of the carriers consists in the presence in the structure of the latter of a hydrogen bond between BPheo and the protein environs (with glutamine L104). We shall return later to the discussion of the role of the hydrogen bonds in the stabilization of the electron on the molecule of the carrier on its reduction. Other structural features of the protein environs in the reaction centre also exist but their role in the act of electron transfer is still not clear. The current experimental method of solving the problem of the link between the structure and function of protein in biological membranes is to modify its amino acid composition by gene engineering. In photosynthesis this approach has proved highly fruitful, since it allowed the presence of a particular amino acid residue to be linked with the possibility of electron transfer in the reaction centre. However, this link may also have a mediated character in view of change in the general structural features of the reaction centres on their modification and does not necessarily demonstrate the direct participation of a given amino acid residue in the act of electron transfer. Another powerful tool of modern biophysics exists — numerical modelling of the molecular dynamics of protein [6] using the computer technique for solving a large number of equations of motion of the system of particles (atomic groups of the protein molecule). However, at present, it allows one to decipher the dynamics of intramolecular movements only in a time of ~100–200 ps on introducing significant simplifying assumptions on the structure and properties of the system. The use of this method for modelling the functional activity of protein is only in the initial stage in biophysics. Together with these approaches, a fruitful line is parallel study of the physico-chemical mechanisms and kinetic features of electron transfer and the mobility of protein by radiospectroscopy, and also the construction on the basis of data obtained of a general semiquantitative theory of protein dynamics. In experimental investigations for these purposes we modified the structuro-functional state of active photosynthetic membranes and their fragments (reaction centres) of the cells of different organisms chiefly by lowering the temperature, and changing the state of water (systems of hydrogen bonds) in the preparations studied. Comparison in these conditions of the parameters of the movement of protein and the functional activity of the samples in electron transfer provided the basic material for solving the problem posed.

LOW TEMPERATURE REACTIONS OF ELECTRON TRANSFER

Study of the spectral properties of the pigments in the membranes of photosynthesizing organisms by the low temperature fixation method has for long been used in biophysics. In the work of Krasnovskii and his followers this approach played an important role in study of the native forms of chlorophyll and the identification of the intermediate forms of the pigments in the photochemical conversions of protochlorophyll and later the photocycle of bacteriorhodopsin (Litvin). In the 1960s a new trend took shape in the biophysics of photosynthesis — study of electron transport in low temperature conditions excluding the usual diffusion of the carrier molecules in the photosynthetic membranes.

In the work of De Vault and Chance [7], lowering the temperature of the purple bacteria (*Chromatium*) made it possible for the first time to identify and measure the temperature dependence of the rate of such a reaction of oxidation of the cytochromes by the photooxidized dimer of bacteriochlorophyll $C \rightarrow P^+$. It turned out that with fall in temperature from 300 to 120 K, the characteristic time of the reaction increases from 1 μs to 2.5 μs, remaining practically unchanged with further fall in temperature. The fundamental conclusion stemming from these experiments is that oxidation of the cytochromes in photosynthetic membranes does not require the usual activational mechanisms similar to redox reactions in solutions, but proceeds by other laws. The fundamental concept advanced as a result of analysis of the effect discovered was based on the physical phenomenon of tunnel electron transfer. Electron tunnelling may occur between the molecules of the donor and acceptor in conditions when the energy of the electron is less than the height of the activational barrier separating them. According to quantomechanical notions a definite probability of overlap of electron wave functions of the initial and final states exists depending on the height and width of the barrier. The electron, as it were, "escapes" or tunnels under the barrier by virtue of the wave nature. According to the usual variant of the tunnel transfer theory on resonance concordance of the electron levels in the donor (D) and acceptor (A) together with forward $D^{\cdot} \rightarrow A$ back tunnel transfer ($D \leftarrow A^{\cdot}$) is also possible. The electron will perform quantomechanical oscillations between D and A and true irreversible passage $D^{\cdot} \rightarrow A$ does not occur which plainly contradicts the experimental data on the work of the electron transport chains where the quantum efficiency of electron transfer in the primary stages approaches 100%. The main idea allowing this paradox to be solved is that in the time of stay of the electron on the donor molecule there is loss of part of the electron energy and as a consequence disturbance of the resonance concordance of the position of the A and D levels. As a result, back tunnelling is practically impossible and electron transfer becomes irreversible. Dissipation of the electron energy through the electron oscillatory interactions occurs in 10^{-12}–10^{-13} s, with excitation of the oscillatory accepting modes in the donor–acceptor complex in the $DA^{\cdot}$ state.

The role of the accepting modes on to which is run off the excess of electron energy may be played by the vibrations of the hydrogen atoms in the chemical groups C–H, O–H and N–H. Thus, the condition of irreversibility in the final state of the tunnel transfer is the ratio between the time of electron transition (τ_{el}) depending on the overlap of the wave functions of the initial and final states and the time of oscillatory relaxation τ_{rel} in the final state: $\tau_{el} >> \tau_{rel}$ (Fermi's golden rule).

The literature contains extensive material on the low temperature oxidation of low potential (C_l) and high potential (C_h) cytochromes by photoactive bacteriochlorophyll (P).

Figure 1*a* gives a typical biphasic curve of the oxidation of low temperature (C_1) cytochrome and the diagrams of the potential surfaces of electron energy U_i and U_f of the initial (i) and final (f) states of the donor–acceptor pair (DA).

Electron tunnelling occurs when the energies of the states i and f become equal which occurs at the point R^* of the nuclear coordinate where the curves $U_i(R)$ and $U_f(R)$ intersect:

$$(D^{\cdot}A)_i \rightarrow (DA^{\cdot})_f$$

In the state $(DA^{\cdot})_f$ the system is at one of the higher vibratory sublevels from which runs off the excess of energy with passage to lower vibratory sublevels and excitation of the accepting modes. The approach along the nuclear coordinate to the point R^* occurs with rise in the temperature and population of the high vibratory sublevels of the initial state. The width of the activational barrier of the transition, as Fig. 1*b* shows, diminishes and, consequently, the rate of tunnelling grows with rise in temperature. At low temperatures in the state i only the lower vibratory sublevels are populated and the point R^* can be reached only through tunnelling of the nuclei themselves (the nuclei are quite light particles). Here, however, the width and height of the barrier are greatest and, therefore, the rate of tunnelling is minimal but no longer depends on temperature. Thus, the biphasic character of the temperature dependence of oxidation of cytochrome is explained by the influence of temperature on the process of rearrangement of the nuclear configuration which is characterized by the size of the shift $\Delta R = R_{of} - R_{oi}$ of the initial equilibrium nuclear configuration R_{oi} to the new equilibrium value R_{of} on electron transfer. The quantitative theory developed of tunnel transfer in a protein medium gives expressions for the rate constant of tunnelling in the case of a strong and weak electron-vibratory link, allowing one to find the parameters of the process of electron transfer and, in particular, the frequencies of the vibrations of the accepting mode which also explains a number of experimental findings [7–16]. These frequencies are characterized by the values ~300–400 cm^{-1} and the vibrations of the water molecules in the hydrate sheaths of the porphyrin molecules may act as accepting mode [15, 16]. The low temperature limit of the reaction rate is determined by tunnelling of the H_2O molecules as a whole over the distances $\Delta R \sim 0.15$–0.2 Å.

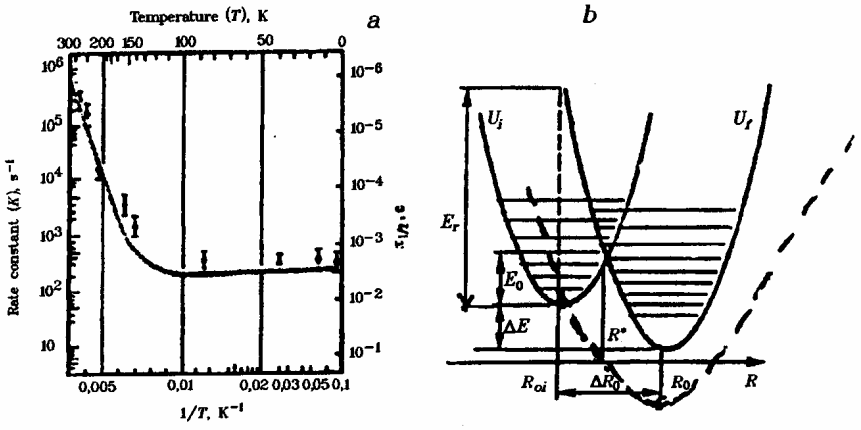

Fig. 1. Temperature dependence of the rate of oxidation of cytochrome in photosynthesizing bacteria (*a*); electron-vibratory interactions on electron tunnelling (*b*); E_0 is the activational energy, E_r is the energy of reorganization of the medium, ΔE is the thermal effect of the electron transfer reaction, ΔR_0 is shift in the position of equilibrium of the nuclei on passing from the initial (i) to the final (f) state. Broken curve corresponds to non-activational electron transfer (E_a).

The experimental checks made in many laboratories of the above outlined ideas confirmed the basic proposition of the theory that electron transitions in macromolecular complexes are inseparably connected with transformation of the nuclear subsystem. However, in the last few years, a whole number of new data has accumulated. They do not fit into the framework of merely electron-vibratory interactions according to which, as stated above, the observed effects are entirely determined by the dependence on temperature of the rate constant of the elementary act of electron transfer. For example, it is difficult to explain in this way the very large differences in the function $K(T)$ observed for preparations of different bacteria [11] and also the break in the curve of the function $K(T)$ far sharper than follows from the formulae of the theory of electron-vibratory transitions in the low temperature region (Fig. 1*a*). In our investigations [12] we found that the rate of oxidation of high potential cytochrome (C_h) in the reaction centre (*Ectothiorhodospira shaposhnikovii*) does not depend at all on temperature in the region 300–120 K ($\tau \sim 2.5$ μs). However, in the range 210–120 K, for an unchanged rate of oxidation, fall in the amplitude of the signal was observed, i.e. fall in the quantity of oxidizable cytochromes. The nature and mechanisms of this amplitude-kinetic effect were later investigated by us in more detail (see below). However, it is now important to emphasize that it cannot be understood within the framework of ideas on the temperature dependence only of the rate constant of electron tunnelling but demands that other processes in the protein matrix be taken into consideration. We would note that, from comparison of the times of the forward and back reactions in scheme II, it will be seen that stabilization of the electron in the electron transport chain reaches several orders although this transfer is characterized by relatively minor thermal effects. The dielectric polarization processes developing as a consequence of change in the charge state of the carriers in the protein matrix may prevent recombination of the separated charges in the reaction centre, raising the efficiency of electron transfer [13, 14]. Evidently, in the electron transport chain the role of the relaxation processes in protein does not merely add up to acceptance of the excess electron energy but may be connected with the more profound reorganization of the donor–acceptor complex over isolated degrees of freedom. We were also brought to this conclusion by the results of the influence of dehydration on the photo-induced oxidation of high potential cytochromes [17]. It has been shown that for dehydration of film samples of the reaction centre from the level of normal relative humidity to 0.1–0.3 P/P_s (0.15–0.25 g water per g of the dry weight of the preparation, g/g) in H_2O, there is a reduction in the number of oxidizable molecules of high potential cytochrome, in the same way as when the temperature is reduced for a relatively unchanged rate of oxidation of the C_h molecules remaining in the active state. On hydration of dehydrated preparations sharp increase in the mobility of the water protons and non-water molecules was observed recorded from the appearance of the slow components of the drop in the spin echo $T_2 \simeq 1$ ms [18]. This increase in mobility in the membrane correlates with rise in the functional activity of photo-oxidation of C_h for humidities from 0.5 to 0.8 P/P_s (0.25–0.47 g H_2O/g) where the mobile molecules of weakly bound water appear. Remaining in the sphere of the above-outlined theory of tunnel transfer, according to calculations, in the conditions of low humidity the distance between C_h and P ought to rise to 20 Å which is clearly unrealistic. Evidently, the influence of hydration is connected not with changes in the parameters of the barrier of any one elementary act of tunnelling ($C_h \rightarrow P$). With change in the state of water the structure of the whole complex changes as a result of modification of the relative position of the donor–acceptor pairs in the protein matrix. This influences the formation of the contact state between them within which interprotein electron transfer from cytochrome to bacterio-

chlorophyll is accordingly possible. The role of water in this process of rearrangement of the membranes is considerable since the native conformation of the complex of the cytochromes with the reaction centres depends on the hydrophobic interactions. The character and the direction of the structural rearrangements in the membranes are determined by the relationship of the factors of orderliness of the aqueous surroundings (entropic factor) and by the formation of hydrogen bonds between the water molecules and the hydrophobic bonds between the molecular groups (enthalpic factor). The rearrangement of the membranes accompanying these transitions is complex in character and occurs differently in the lipid and protein phases in different organisms [19, 20]. Thus, in *Rhodospirillum rubrum* the appearance of mobility in the non-polar region on wetting the samples is accompanied by formation of water bridges between the polar heads of the lipids and rise in the orderliness of their arrangement and the rigidity of the bonds between them. However, in these conditions movement of the non-polar tails of the molecules is facilitated. In membrane proteins an appreciable increase in the internal dynamics is already recorded at the initial stages of wetting ($P/P_s \geqslant 0.15$). In *Rhodobacter sphaeroides* anomalous increase in the rigidity of the bonds is not observed in the polar region at the initial stages of wetting. In both cases for $P/P_s = 0.80$ the mobility of all the membrane components sharply increases with rise in the number of sorbed water molecules and their lateral mobility becomes possible. Functional activity in all parts of the electron transport chain here reaches normal values. Below we go into similar relations in the donor–acceptor part of the reaction centre in the region of reduction of the quinone acceptors

$$(\mathrm{PBH}) \rightarrow \mathrm{Q_a} \rightarrow \mathrm{Q_b}.$$

It may be concluded that electron transfer in the reaction centre and its immediate environs is accompanied by complex structural rearrangements into which are drawn not only the high-frequency vibrations of the molecular groups of the accepting mode but other types of low-frequency movements of protein associated with the conformational degrees of freedom. Understanding the mechanisms of interaction of the electron transitions and different types of intramolecular movements firstly calls for determination in direct experiments of their parameters — characteristic times and amplitudes of movements at different temperatures and humidities in comparison with the functional activity of the preparations in the same conditions.

ASPECTS OF THE RELAXATIONAL PROCESSES IN THE REACTION CENTRES

Transfer of an electron from the bacteriochlorophyll dimer to bacteriopheophytin and then to the primary quinone $\mathrm{Q_a}$ comes about at high speed and practically does not depend on temperature [21–25]. Evidently, in this system the mutual orientation of the donor–acceptor groups of the carriers is optimal ensuring effective electron transfer. Within the theory of electron-vibratory interactions, the independence of the rate constant from temperature means that the point R^* of the intersection of the potential curves $U_i(R)$ and $U_f(R)$ (Fig. 1*b*) is located in the vicinity of the apex of the parabola $U_i(R)$ in the region of lower vibratory sublevels of the initial state, i.e. it is in a region accessible for the nuclear subsystem at the usual temperatures. In this case the process of electron transfer will also be non-activational. Moreover, lowering of temperature may cause a shift of the point R^* of the intersection of the

curves directly to the apex of the parabola $U_i(R)$. This must lead to "anomalous" acceleration of electron transfer with fall in temperature as a result of contraction of the barrier size. Such an acceleration of 1.5–2 times is actually observed in experiments [20, 21].

Investigation of the nature of the relaxational processes in the highly ordered complex of carriers (PHQ_a) was undertaken by us using various agents modifying the system of hydrogen bonds of the reaction centre. In the experiments we used the methods of isotope replacement of H_2O by D_2O, modification of the hydrogen bonds in the samples by cryoprotectors, multiatomic alcohols (ethylene glycol, propylene glycol) and aprotonic solvents (dimethylsulphoxide). It was found that these agents sharply change the recorded absorption and fluorescent parameters characterizing the rate and direction of electron transfer in the reaction centre [25, 26].

Figure 2 presents the changes in absorption at 665 nm corresponding to the appearance and disappearance of reduced bacteriopheophytin (H) under the influence of isotope replacement of D_2O by H_2O and the action of dimethylsulphoxide. It will be seen that this modification of the system of hydrogen bonds slows electron transfer at the stages $P \rightarrow H$ and $H \rightarrow Q_2$. The degree of the effect caused by the action of the cryoprotector increases as a function of its hydrophobicity and correlates with the depth of its penetration into the internal regions of the photosynthetic membranes. Simultaneously with slowing of forward transfer $P \rightarrow H$, the component of reduction P^+ appears in the course of recombination $P^+ \leftarrow H^-$ with the time $\sim 0.7-1$ ns, which is accompanied by the appearance of delayed luminescence with the same characteristic time.

The observed kinetic effects indicate that tunnelling of an electron on bacteriopheophytin is accompanied by fall in its level in the processes of molecular relaxation with the participation of intraprotein hydrogen bonds. Electron transfer $P \rightarrow H$ changes the charge state in the donor–acceptor complex (P^+H^-) as compared with the initial (PH). The balance of forces determining the equilibrium conformation of the dark-adapted system changes. The system will now tend to a new equilibrium. In the course of subsequent relaxation with the participation of the system of hydrogen bonds the non-equilibrium stressed state $(P^+H^-)^v$ appears on illumination and a new equilibrium state of the ion-radical pair PH is reached

$$P^*H \rightarrow (P^+H^-)^v \rightarrow (P^+H^-)_{relax}.$$

Then there is further electron transfer to Q_a

$$(P^*H)Q_a \rightarrow (P^+H^-)^vQ_a \rightarrow (P^+H^-)_{relax.}\ Q_a \rightarrow (P^-H)Q_a.$$

The decisive factor is the relationship between the characteristic times of the electron transitions (τ_{el}) and the relaxational processes accompanying them (τ_{rel}). We would recall that the ratio $\tau_{rel} << \tau_{el}$ necessary for irreversible electron transfer is usually fulfilled in normal conditions since the processes of vibratory relaxation occur quite rapidly (1–2 ps) as compared with the electron transitions and do not influence their character. However, at the stages of initial electron transfer in the PBH complex the times of the electron transitions amount to several picoseconds and are comparable with the times of the vibratory relaxation $\tau_{rel} \simeq \tau_{el}$. Modification of the hydrogen bonds slows to an even greater degree the relaxation of the original stressed state of the complex $(P^+H^-)^v$ so that $\tau_{rel} \geqslant \tau_{el}$. As a result the electron does not have time to stabilize to a sufficient degree on bacteriopheophytin before its further transfer from the non-relaxed state $(P^+H^-)^v$ occurs. From this state $(P^+H^-)^v$ the electron now

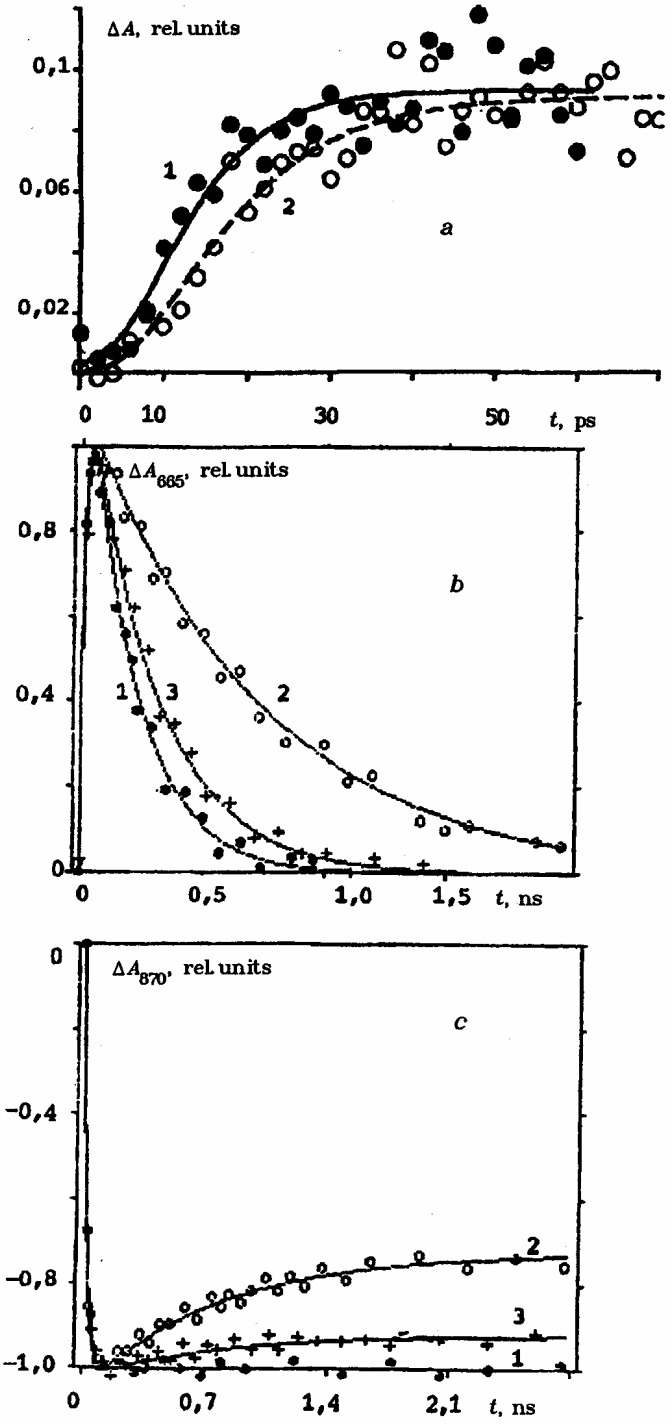

Fig. 2. Kinetics of change in absorption at 665 nm in the reaction centres of *Rh. sphaeroides* reflecting transfer $P^*H^* \xrightarrow{k_1} P^+H^-$ and excitation at $\lambda = 590$ nm (*a*): *1*, control; *2*, after addition of 40% DMSO. $\tau_1 = 1/k_1 = 5.7$ ps; $\tau_2 = 10.8$ ps. Kinetics of electron transfer in the reaction $H^-Q_a \rightarrow HQ_a^-$ (*b*). Kinetics of recombination $P^+H^- \rightarrow P^*H \rightarrow PH + h\nu$ (*c*). *1*, control; *2*, after isotope replacement of H_2O by D_2O; 3, after back replacement of heavy by ordinary water.

has time to return back recombining with P^+ in the time ~1 ns which is accompanied by delayed luminescence. There may also be further electron transfer from the state $(P^+H^-)^v$ to Q_a but at a lower speed (Fig. 3). Thus, in the relaxational processes with the condition $\tau_{rel} > \tau_{el}$ in the donor–acceptor complex conformational substates are reached differing in their functional activity (rates and direction of electron transfer). The state of the hydrogen bonds here influences the dynamics of the photo-induced relaxational processes in the course of which different conformational states appear in the system. However, in the dark, too, as a result of spontaneous low-frequency movements of protein (α-helices) conformational states may also form, characterized by a different rate of electron transfer on illumination. Since the energies proper of such states differ at different temperatures of the sample the population of the states and the transitions between them will change in the dark as will also the total rate of electron transfer on subsequent illumination [27, 28]. In both cases, nevertheless, the basic proposition of an inseparable link between the functional activity of protein of the reaction centre and its intramolecular mobility must hold.

Below we outline the results of investigations with a direct experimental check of this proposition.

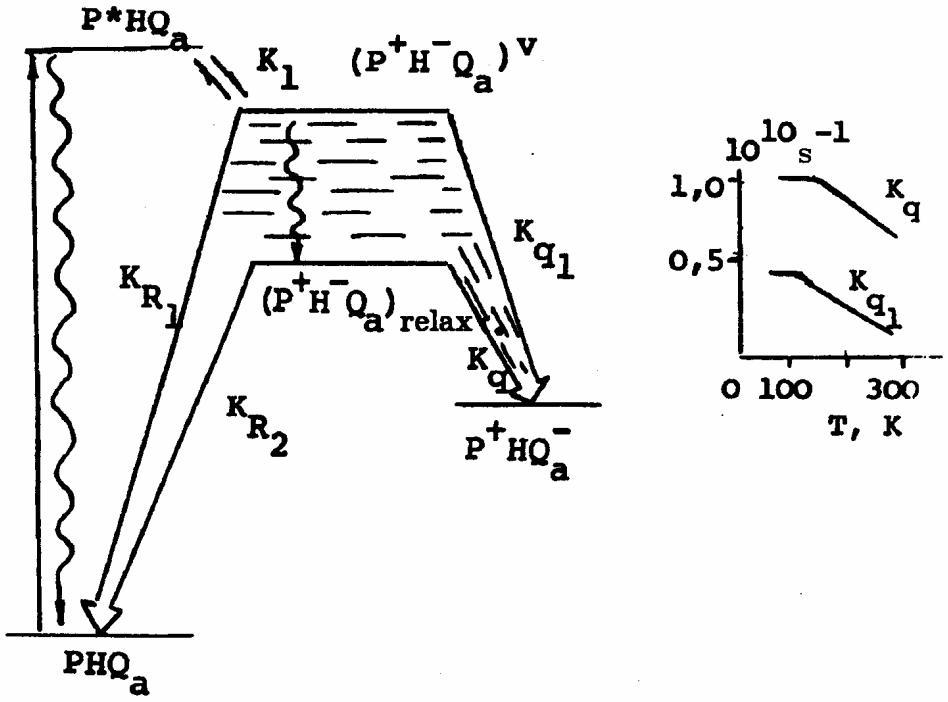

Fig. 3. Scheme of electron transfer with reference to conformational relaxation of the complex (PHQ_a). K_1 is the transfer constant of $PH \rightarrow P^+H^-$; $(P^+H^-Q_a)^v$ and $(P^+H^-Q_a)_{equib}$ are conformationally non-equilibrium and relaxed ion-radical pairs; K_q and K_{q_1} are constants of transfer of $P^+H^-Q_a \rightarrow P^+HQ_a^-$ from these states; K_{R_1} and K_{R_2} are constants of passage to the ground state of (PHQ_a). Inset: temperature dependences of the transfer constants K_q and K_{q_1} of $^+H^-Q_a \rightarrow P^+HQ_a^-$.

ELECTRON TRANSFER AND CONFORMATIONAL MOBILITY OF THE REACTION CENTRES

In the reaction centre preparations their modification may result in conditions when in the system all the pathways of electron transfer are blocked apart from the processes of forward transfer from P to Q_a and Q_b and recombination of the ion-radical products formed:

$$P \rightarrow Q_a \rightarrow Q_b$$
$$\uparrow \_\_\_\_\_ \downarrow$$

In this case, in the experiment two components of reduction of P^+ after switching off the light are observed: fast, corresponding to recombination $P^+ \rightarrow Q_a^-$, and slow, through recombination $P^+ \rightarrow Q_b^-$. The ratio of the amplitudes of these components depends on the steady distribution of the electrons on illumination between the quinone acceptors and reflects the efficiency of electron transfer from Q_a to Q_b^* [29]. In the system of quinone acceptors, electron transfer between the primary Q_a and secondary Q_b quinones depends on temperature.

The temperature of the process of $Q_a^- Q_b \rightarrow Q_a Q_b^-$ transfer presented in Fig. 4 were compared with the dependences of the correlation time of rotatory diffusion (τ) of the spin probe (on the basis of lysolecithin or stearic acid with nitroxyl fragments) introduced into the lipid phase and the spin label covalently attached to the sulphydryl groups of the protein-chromatophores of *R. rubrum* [30, 31]. As may be seen, with rise in temperature the efficiency of electron transfer increases with simultaneous intensification of intramolecular mobility in the surface layers of the protein macromolecules, as may be seen from shortening of the correlation time of the spin label in the time region $\tau_s \sim 10^{-7}$–10^{-8} s. The profile of the temperature mobility of the lipid probe correlates to a lesser degree with that for electron transfer. In this temperature region ($T > 200$ K), symbatically with increase in the efficiency

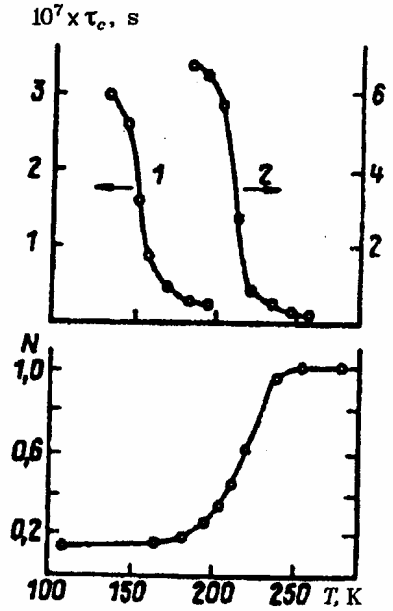

Fig. 4. Correlation time τ_s of rotatory diffusion of spin probes (*1*) — analogues of typical membrane lipids and the protein iodacetamide label (*2*) and also the efficiency (N) of electron phototransfer $Q_a^- Q_b \rightarrow Q_a Q_b^-$ in the chromatophores of *R. rubrum* as a function of temperature.

of electron transfer in the system Q_aQ_b there is also on unfreezing of the reorientational movements of the protein–lipid matrix with times shorter than 10^{-8} s. This effect was recorded from the shift in the position of the maximum of the fluorescence spectrum ($\Delta\lambda \sim 20$ nm) of the chromophore label (eosin thiocyanate) covalently attached to the surface amino groups of the membrane proteins. A similar correlation was established between electron transfer $Q_a \rightarrow Q_b$ and the mobility of the protein of the reaction centres for different humidity of the preparations. The mobility of protein was recorded from shift in the fluorescence maximum of the tryptophan residues forming part of the subunits (L, M) in the reaction centres of *Rhodospirillum rubrum*. It was established that at $P/P_s > 0.5$ the efficiency of transfer $Q_a \rightarrow Q_b$ and the intramolecular mobility of protein simultaneously increase with rise in the mobility of the water molecules, as may be seen from increase in the time T_2 of spin-spin relaxation of the water protons. The correlation found between electron transfer and the intramolecular movements does not signify direct coupling of electron transfer with those conformational degrees of freedom, which determine the mobility of the labels in a given time range, depending on the resolving capacity of the method used. We would recall that, in protein, intramolecular movements simultaneously occur, differing in their times by several orders. The dependences obtained indicate disinhibition in the given conditions also of other movements, which as a whole contribute to the formation of the active states of the reaction centres.

The notion of a spatial scale and the character of these movements in the dense protein matrix was given by the method of nuclear gamma-resonance spectroscopy on the chromatophores into the membrane iron-containing proteins of which was introduced the Mössbauer isotope ^{57}Fe [32, 33]. Figure 5 presents the temperature dependence of the Lamb–Mössbauer factor (f') in preparations of the chromatophores of *Rhodospirillum rubrum*. Usually in solid body matrices, the magnitude f', reflecting the probability of absorption of a γ-quantum by the ^{57}Fe nucleus without recoil, in the absence of a shift smoothly diminishes with rise in temperature. The mobility of the nucleus grows, and on absorption of a γ-quantum it shifts and part of the energy of the quantum passes into the kinetic energy of recoil of the nucleus. We found [31–34] that unlike the smooth course of the temperature dependence of $f'(T)$, which is actually observed in the region $T < 180$ K, at higher temperatures there is a relatively sharp fall in $f'(T)$ indicating a rise in the mobility of the Mössbauer nucleus of ^{57}Fe in its immediate environs in the protein matrix. The magnitude f' similarly changes with the degree of hydration [32]. In both cases, the temperature and hydration dependences of f' correlate with the changes in the functional activity of the samples of the chromatophores and the reaction centres. A similar correlation was also detected on investigation by the method of Rayleigh scatter of Mössbauer radiation which does not require the special introduction of isotopes and allows for the scatter of the γ-quanta in all atoms forming part of the preparation. The current theory of conformational dynamics of protein, into which we shall go below, relates the dependence of f' on temperature or hydration to change in the microviscosity of the local regions of the macromolecules containing the Mössbauer label or the viscosity parameters averaged over the whole membrane [35–37]. Sharp decrease in the factor f' on unfreezing is determined not by increase in the total amplitude of the movements of the nucleus but by decrease in microviscosity η on heating and as a result of this shortening of the time τ_s of the correlation of movement

$$\tau_s \sim \eta \sim e^{\varepsilon/kT}$$

where ε is the activational energy of the microviscosity of the flow.

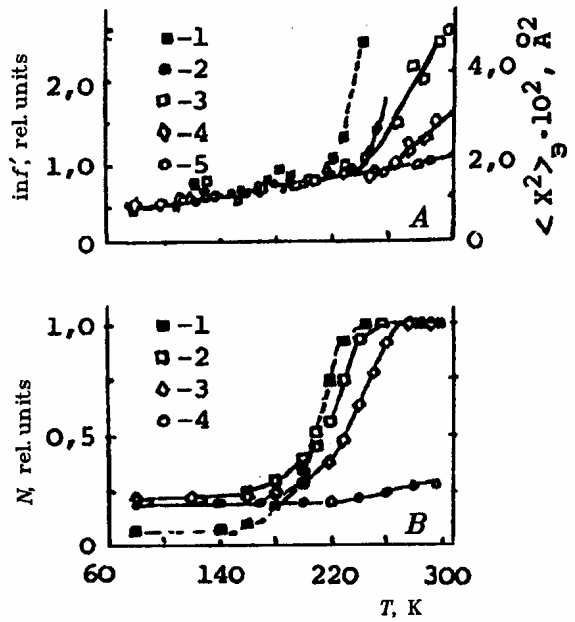

Fig. 5. Dependence on temperature of the probability of resonance absorption (f') of the Mössbauer labels of $^{57}FeCl_3$ introduced into the photosynthetic membranes (*A*). Efficiency N of electron transfer $Q_a^-Q_b \rightarrow Q_aQ_b^-$ in the preparations of the chromatophores of *Rsp. sphaeroides* of different degrees of hydration (*B*): *1*, water–glycerol suspension of chromatophores; *2–5*, preparations with different degrees of hydration; *A*, 65% (*2*); 30% (*3*); 10% (*4*); 3% (*5*); *B*, 30% (*2*); 10% (*3*); 3% (*4*).

For short times and high temperatures $\tau_s << 10^{-7}$ s, the nucleus is mobile and on absorption of a quantum has time to shift appreciably in the time of the excited state (10^{-7} s) and, therefore, the factor f' of absorption without recoil is very small. As against this, at low temperatures when viscosity is high and $\tau_s >> 10^{-7}$ s, no appreciable shifts of the nucleus have time to occur in the time of the excited state and $f' \sim 1$. In the region $\tau_s \simeq 10^{-7}$ there also occurs a break in the curve $f'(T)$ caused by the structural phasic transition in the protein–membrane complex. The amplitudes of the shifts of the protein fragments contributing to its intramolecular mobility amount to $\geq 0.5-1.5$ Å. This is of fundamental importance since such large ($>>0.1$ Å) shifts in a solid medium of protein require the formation of fluctuational cavities and the movements themselves must be of a stochastic character.

Below we shall consider these problems in more detail. Apparently, in the course of diffusional movements the relatively mobile secondary quinone Q_b may directly approach Q_a forming with it a contact conformation. Humidity and temperature influence the conformational degrees of freedom and, thereby, the possibility of formation of the contact state, while the rate of the elementary act of the electron transition in this state depends very weakly on these parameters. However, the electron transition, as such, changes the balance of forces in the contact confirmation and, thereby, gives rise to a relaxational change in the conformational state.

Thus, the dynamic changes in the structure of the protein complexes are necessary to create their reactive states in the energy-transforming membranes of photosynthesizing organisms. There is every reason to believe that this conclusion also applies to other biological membranes.

CONFORMATIONAL DYNAMICS OF THE PROTEIN OF THE REACTION CENTRES

The close correlation between the functional activity of the proteins of the reaction centres and their internal conformational mobility with the amplitudes of the movements of the groups far exceeding the amplitudes of the valent vibrations is exceptionally important for the formation of fundamental ideas on the physical mechanisms of the functioning of biomacromolecules. In a solid protein matrix, mobility in the conformational degrees of freedom of the biopolymers is of a stochastic character and is realized as restricted diffusion of the fragments and groups of macromolecules [35–54]. The diffusional processes in solid structured media (biopolymers) are of quite a complex nature, closely associated with the correlations of fluctuations and call for special study. Let us take a closer look at this situation. As is known, low vibrations of the atoms in the molecules and crystals with the amplitudes $x_a \sim 0.01-0.1$ Å may be quite well described within the harmonic approximation (Fig. 6). The corresponding elastic constants for the valent vibrations lie in the range $K \sim 10^5-10^6$ dynes/cm and the characteristic frequencies $\omega \sim 10^{14}-10^{13}$ s^{-1}. The effects of anharmonism account for the intermode interaction leading to rapid vibratory relaxation with the characteristic times $\tau \sim 10^{-12}$ s. However, the harmonic approximation is not at all suitable for describing conformational movements in biomacromolecules with amplitudes $x_a > 0.1$ Å detected in the proteins of the reaction centre (Fig. 5). The density of the protein globule is comparable with that of fluids and organic crystals. Therefore, the shifts here by a value exceeding 0.1 Å as in the case of fluids require the formation of a fluctuational cavity or "hole" [54] characterized by the free activational energy ΔG_s^*. It may be shown that this situation is equivalent to random migrations over a set of fluctuating potential wells separated from each other by barriers [36, 37, 41] (Fig. 6). In other words, the conformational movements in protein are limited in amplitude as in solids but require activational energy as in the case of diffusion in a liquid. The dynamics of the conformational mobility is described by equations of the Fokker–Planck type

$$\frac{\partial P(x,t)}{\partial t} = \frac{\partial}{\partial x} D(x) \left[\frac{\partial P}{\partial x} + \frac{1}{RT} P \frac{\partial U(x)}{\partial x} \right], \tag{1}$$

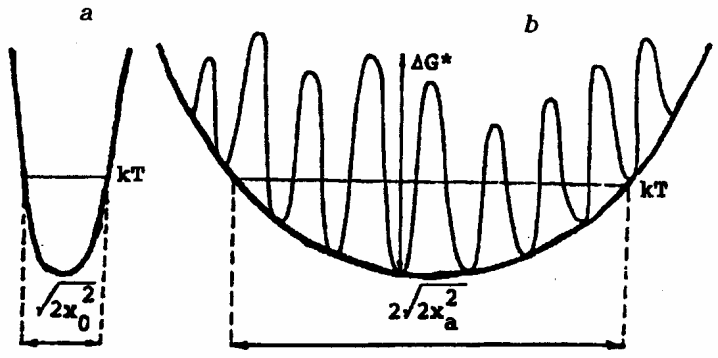

Fig. 6. Characteristic quasi-one-dimensional profile of the potential energy: (*a*) for high frequency (valent) vibrations; (*b*) for conformational movements. In the local minima high-frequency vibrations occur practically the same as in case *a*. The energy fluctuations lead to migration from one local minimum to another and ultimately to restricted diffusion in the whole classically accessible region. The local minima and the heights of the barriers ΔG^*, generally speaking, are not fixed and fluctuate through interactions with other degrees of freedom.

where $D(x)=D_0 e^{-\varepsilon(x)/kT}$ is the coefficient of diffusion over the conformational degree of freedom x inversely proportional to the microviscosity of protein. The dependence of the diffusional coefficient on the conformational coordinate effectively allows for the microheterogeneity of the intraprotein medium and the interaction of the given conformational degree of freedom x with other conformational modes [42]. $U(x)$ is the conformational potential, $P(x, t)$ is the probability density of finding the system in the conformation x at the moment of time t. For constant D and for a sufficiently wide class of potentials $U(x)$, the dependences of the mean square shift on time are determined by the formula

$$<[\Delta x(t)]^2> \simeq x_a^2(1 - e^{-t/\tau_s}) . \quad (2)$$

where τ_s is the relaxation time of the given degree of freedom x.

Figure 7*a* schematically shows the dependences of the mean square shift on time in different systems. Figure 7*b* presents the experimental dependence $\langle[\Delta x(t)]^2\rangle$ for iron ions rigidly bound to the amino acid residues of HSA [55, 56] obtained by the method of Mössbauer Fourier spectroscopy recently developed in [40, 57].

The conformational lability of the biomacromolecules is very important in terms of regulation of functional activity at the level of the elementary act of chemical reactions [58–63]. Change in the electron state of individual groups through, for example, electron transfer $I_1 \overset{e}{\rightleftharpoons} I_2$ alters the very fine balance of interactions in the biopolymer and as a consequence changes the surface of the conformational energy $U_1(x) \rightarrow U_2(x)$ and the geometry of the equilibrium configuration of the macromolecule (Fig. 8). Thus, change in the chemical (charge) state of the group usually causes passage to another surface and then the process of conformational relaxation to a new equilibrium. The situation is complicated by the fact that, in conditions of a microheterogeneous medium, the rate constants of the chemical reactions may significantly depend on the conformation of certain portions of the macromolecule. An additional correlation appears here between the conformational mobility and functional activity.

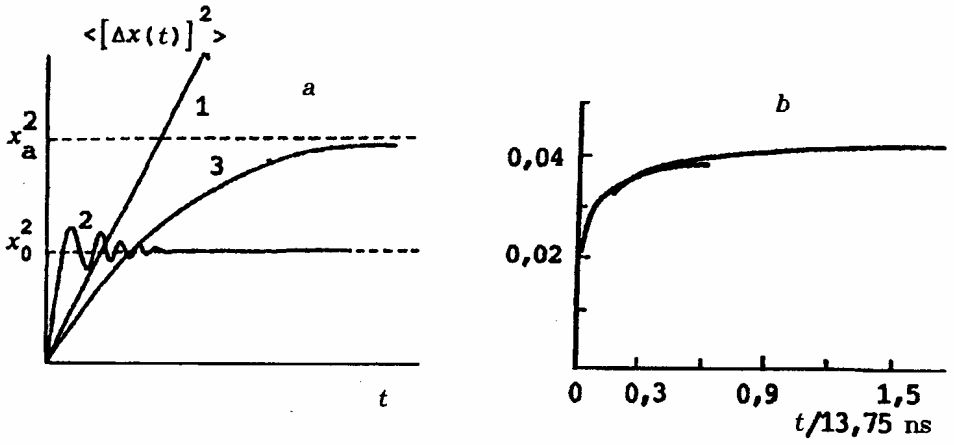

Fig. 7. Characteristic dependences of the mean squares of the shifts on time (*a*) for: *1*, diffusion in a liquid; *2*, high-frequency vibrations (Fig. 6*a*); *3*, restricted diffusion conformational movements (Fig. 6*b*). Dependences of mean squares of the shifts reconstructed from the Mössbauer spectra (*b*) [55]. The zero position on the ordinate axis corresponds to 0.03 Å^2 (fast processes with $t<1$ ns). Portion of the time dependence to $t\simeq 20$ ns.

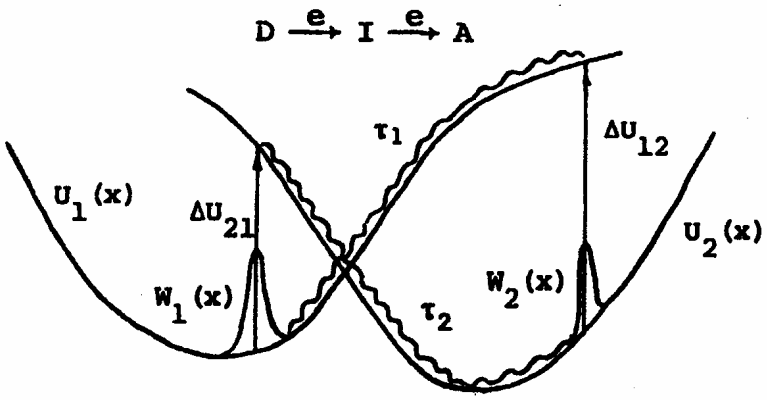

Fig. 8. Functioning of the molecular "machine" by electron transfer from D to A. Change in the electron state of group 1 leads to a corresponding change in the conformational potential. Undulating arrows show conformational relaxation with the characteristic times τ_1 and τ_2. $W_1(x)$ and $W_2(x)$ are the dependences of the reaction rates $I \rightarrow I^-$ and respectively $I^- \rightarrow I$ on conformation. The differences in the conformational energies at the points of the transition ΔU_{12} and ΔU_{21} are compensated by a corresponding fall in the thermal effects of the reactions.

In the simplest case when there are reversible changes in the chemical state of the group I coupled with conformational changes over the coordinate x, the electron-conformational interaction in this portion may be described by a set of linking equations of the Fokker–Planck type with extra terms describing the chemical reactions [59]:

$$\frac{\partial P_1(x,t)}{\partial t} = \frac{\partial}{\partial x} D_1 \left[\frac{\partial P_1}{\partial x} + \frac{1}{kT} P_1 \frac{\partial U_1}{\partial x} \right] - k_{12}(x) P_1 + k_{21}(x) P_2$$

$$\frac{\partial P_2(x,t)}{\partial t} = \frac{\partial}{\partial x} D_2 \left[\frac{\partial P_2}{\partial x} + \frac{1}{kT} P_2 \frac{\partial U_2}{\partial x} \right] + k_{12}(x) P_1 - k_{21}(x) P_2 \qquad (3)$$

Here $D_{1,2}$ and $U_{1,2}$ are diffusional coefficients and conformational potentials relating to different chemical states of the group I, k_{12} and k_{21} are the rate constants of the chemical conversions depending on the conformation of the system. This system was analysed for differing assumptions of the form of the functions $D(x)$, $U(x)$ and $k(x)$ [41, 45, 47, 59–63].

We checked the ideas developed in experiments on preparations, reaction centres, where we studied the temperature dependence of the process of back electron transfer between P^+ and Q_a^- ($P^+ \leftarrow Q_a^-$). Passage of an electron to the secondary acceptor Q_b was blocked by *o*-phenanthroline. The experiments were conducted in conditions when the preparation was frozen in the dark, then illuminated followed by measurement of the kinetics of recombination of $P^+ \leftarrow Q_a^-$ after switching off the light. In another series of experiments, the sample was frozen to the same temperatures but with simultaneous illumination. Figure 9 shows that in the first case of "dark" cooling, the rate of recombination grows as the temperature falls due to preferential population in the dark of the contact states Q_a favourably positioned for the recombination $P^+ \leftarrow Q_a^-$. However, on "light" cooling, the situation fundamentally changes. The rate of recombination slows by a factor of 3–4 and does not depend on temperature over the whole range. Evidently, change in the charge state of the complex on illumination of $PQ_a \rightarrow P^+Q_a^-$ causes the system to pass to the stressed-conformational state, which then relaxes to a new equilibrium. In the course of this movement, fall in temperature fixes the

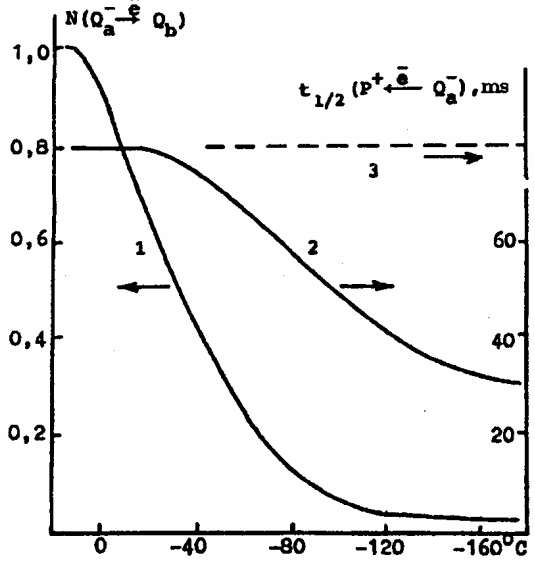

Fig. 9. Dependence on temperature of the efficiency of electron transfer $Q_a^-Q_b \rightarrow Q_aQ_b^-$ (*1*); the recombination time of $P^+ \leftarrow Q_a$ (*2*) in reaction centre preparations frozen in the dark and also the recombination time $P^+ \leftarrow Q_a$ in reaction centre preparations frozen in the light.

system in this or that point of the conformational coordinate. Naturally, the rate of subsequent recombination had to depend on the regime of cooling and the intensity of the illumination on cooling as has been observed in experiments. It was found, for example, that the kinetic curves of recombination of the samples at room temperatures and cooled in weak light to 80 K greatly differ from each other. However, if cooling is carried out in the presence of strong background illumination (2×10^3 W.m^{-2}) these differences virtually disappear. In other words, at room temperature in response to a saturating flash and reduction of Q_a, the corresponding conformational transition has time to occur. On cooling in the dark, the difference in the kinetics is a consequence of the fact that the conformational mobility is inhibited. However, on cooling in strong light, the system again conformationally "revives" and is fixed in a position close to the equilibrium position at room temperature $(P^+Q_a^-)_{relax}$. Thus, by changing the intensity of illumination and the regime of cooling of the reaction centre samples, we may fix on freezing certain conformational states which the system successively reaches during relaxation.

Solution of the set of equations (3) [61] with the corresponding dependence of the rate of recombination on the conformation showed that after the light flash the evolution of the initial dark distribution over the conformations of the system with an uncharged acceptor occurs as follows. Immediately after the flash, there is rapid reduction of the primary acceptor $Q_a \rightarrow Q_a^-$. The initial distribution of the system over the conformations $P_{Q_a^-}(x, t)$ at the first moment of time remains the same, i.e. $P_{Q_a}(x, 0)$. However, this distribution of $P_{Q_a}(x, 0)$ is not equilibrium for the surface of the conformational energy ($U_{Q_a^-}(x)$) of the complex with distributed acceptor (Fig. 9). As a result, there is conformational relaxation with shift of the distribution maximum towards a new minimum of the potential energy, with simultaneous recombination of the charges $P^+ \leftarrow Q_a^-$ with a rate constant $k_r = k_{21}(x)$ depending on the conformational coordinate x.

If in a time much shorter than the time of charge recombination $1/k_r$ there is conformational relaxation of the complex with the charged acceptor Q_a^-, a value of the rate of recombination will be observed corresponding to the minimum of the potential $U_{Q_a^-}(x)$. However, if the relaxation time is very long as compared with $1/k_r$, the system is inhibited and the effective value of the rate of charge recombination $P^+ \leftarrow Q_a^-$ will correspond to the initial conformational position of the uncharged acceptor. Intermediate situations are also possible. In [62–63] the authors devised an algorithm of analysis of the kinetics of such photoconformational transitions. It allows one to determine the values of the rates of electron transfer in the course of recombination as a function of the depth of the conformational transition performed which is determined by the time elapsing after the initial charge separation (Fig. 10).

Figure 10 shows that ultraslow relaxation in the system occurs even at $T \sim 100$ K. These slow low temperature relaxational processes apparently do not require activational enthalpy but occur over very intricate trajectories in the configurational space of the biomacromolecule and are characterized by high activational entropy [42, 64, 65]. With rise in the temperature, mobility grows and the conformational relaxation time shortens, and at the same time the system manages to pass to a conformational state with a smaller value of the recombination constant, i.e. to perform a "deep" conformational transition.

Situations of sharp inhibition of conformational mobility of the system below a certain temperature threshold are also possible. This occurs on oxidation of high potential cytochrome C_h where the value of the effect sharply diminishes with no change in the oxidation rate [60, 61].

Figure 11 presents the temperature dependences of the oxidation rate of high potential cytochrome C_h and the amplitudes of change in absorption corresponding to the number of active cytochrome molecules. It will be seen that in approximately the same temperature interval, the amplitude of the signal and the share of elastic Rayleigh scatter of Mössbauer radiation for an unchanged rate constant of electron transfer ($C_h \rightarrow P$) change in a threshold manner. Evidently, the process of oxidation of C_h is conformationally controllable. This may occur, for example, through formation of the π-bridge on movement of one of the aromatic amino acid residues (tyrosine) [66, 67]. If the position of this bridge depends on the value of the generalized conformational coordinate, then a most favourable situation exists for its

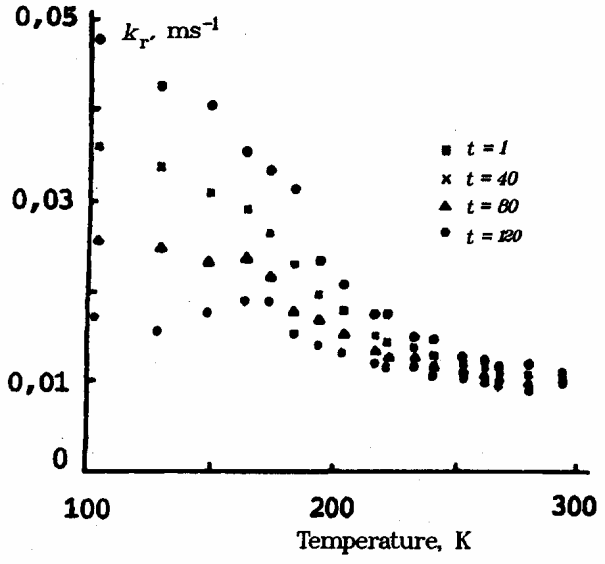

Fig. 10. Values of the instant recombination constant $K_r(t)$ at different times after the flash and for different temperatures.

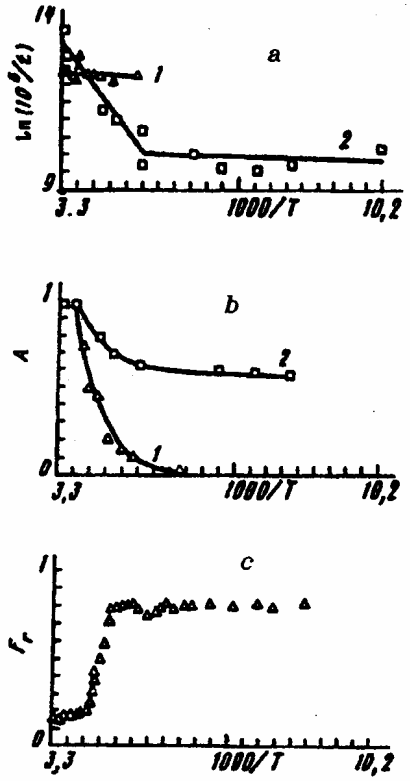

Fig. 11. Dependence on temperature of the rate constant of oxidation of cytochrome C (*a*) and change corresponding to complete oxidation (recorded from passage to a plateau) of absorption (*b*) in the reaction centres. *1*, $E_h = +300$ mV (oxidation of high potential cytochrome); *2*, $E_h = -20$ to -60 mV (oxidation of low potential cytochrome); (*c*) dependence on temperature of the fraction of elastic Rayleigh scatter of Mössbauer radiation (F_r) in the membrane preparations of *E. shaposhnikovii*.

formation and hence for electron transfer to P^+. Analysis of equations of type (3) showed that this occurs for an irregular, random character of the dependences of the diffusional coefficients on the conformation of the system or for a sufficiently wide distribution of the heights of the potential barriers (Fig. 6) overcome by the particular protein group on conformational relaxation [33, 37]. The reason for this irregularity in the final analysis consists in the very complex character of the hypersurface of the conformational energy of the biopolymers [41, 64].

MATHEMATICAL MODELS OF ELECTRON TRANSFER

The processes of electron transport in photosynthesis constitute a single multilevel system characterized by a hierarchy of times and rate constants and the features of the structural organization of its constituent elements (mobile carriers and macromolecular complexes) in the photosynthetic membranes. The intensity and duration of light exposure (from 10^{-12} s to hours) act here as the controlling parameter, as may be readily checked in experimental conditions. All this makes the system of photosynthetic electron transport one of the few

biological systems for which it is possible to construct optimal mathematical models, identify the parameters and check experimentally on their match with the real object of modelling.

In early models of electron transfer, equations of kinetics were used based on the law of mass action, postulating the independence of the redox states of the donor and acceptor components of the electron transport chain. This, however, could be true only for mobile carriers. The macromolecular complexes of the carrier molecules must be regarded as a single system where electron transfer in any region changes the state of the whole complex. Therefore, on electron transfer, the passages between the states of the complex as a whole must be considered. The system of corresponding differential equations includes the dependences on time of the probabilities of the states of the whole complex, solving which one may find how the states of the individual carriers forming it change [67, 68]. These equations are linear; however, their number rapidly grows with increase in the number of carriers and, accordingly, the states characterizing the behaviour of the complex. Identification of the models includes choice of the structure of the model and determination of its parameters by the best matching experimental data on the kinetics of electron transfer. This requires the construction of models of fragments of the electron transport chain and its individual parts for which there are reliable kinetic data. Models allowing for the possibility of two-electron reduction of the secondary quinone have been constructed for the reaction centre preparations of *Rhodobacterium sphaeroides*.

To identify the parameters, we used the kinetic curves of the photo-induced changes in absorption reflecting the redox conversions of P and the quinone acceptors. The conditions of ambiguity of identification of the parameters have been formulated only for linear systems to which the isolated bacterial reactions centres and PS I complexes belong. In the case of more complex non-linear systems, additional criteria of the dependence of the kinetic characteristics on the regime of illumination and the action of inhibitors were adopted. Identification was based on minimization of the sum of the weighted squares of the deviations of the theoretical values of the variables from the experimental. The problem of the search for the minimum of the functional was solved by the Pauem, Hook-Jeeves direct search method.

Identification of the model showed that the values of the rate constants of electron transfer in the recombination reaction $P^+ \leftarrow Q_a^-$ are not the same in the dark or in the light and differ by at least one order. This result corresponds to the above-outlined ideas on the different conformational states of the reaction centres formed in the dark or in the light on functioning of the electron transport chain. The cause of such a major difference in the values of the "dark" and "light" constants cannot be merely due to change in the Coulomb interactions and overlap of the electron wave functions with change in the redox states of the carriers but is linked with the process of reorganization of the protein of the reaction centre [69–72]. The model was analysed having regard to the dependence of the recombination constant $P^+ \leftarrow Q_a^-$ on the coefficient of conformational diffusion (D) and the difference in the distances (ΔR) between the components of the donor–acceptor pair in the dark and light conformations

$$\bar{k} = D/a\,\Delta R \exp(-E/kT) ,$$

where a is the width of the energy well and E is the activational energy. As a result, we obtained the value of the characteristic time of completion of the total conformational rearrangements in the reaction centre protein ($\tau \lesssim 10^2$ s). This result is also of interest in that it shows the possibilities of current methods of mathematical modelling from theoretical determination of the parameters of the object used in the equations but cannot be found directly in experiments. Thus, identification of the parameters of mathematical models may

become a serious instrument in the study of the real properties of photosynthetic electron transport. It turns out [68–76] that effective values of the rate constants of electron transfer in the light and in the dark also differ by 1–2 orders for other types of reaction centres of PS I and PS II. The greatest differences have been established for the rate constants of recombination with the photo-oxidized pigment of ion-radical products in the reduced part of the reaction centre chain.

From the results of identification, it follows that change in the external conditions (redox potential of the medium, humidity, viscosity) has little influence on the parameters of transfer within the complexes. Thus, regardless of the conditions, the rate constant of electron transfer $C_h \rightarrow P^+$ is 3×10^6 s^{-1}. However, the rate constants of transfer involving mobile carriers closely (within 2–3 orders) depend on the conditions of the medium. At the level of PS I such a portion is the stage of formation of the complex of the donor component of plastocyanin with the reaction centre protein of PS I, at the level of PS II the interaction with the water-decomposing system in the donor part and with the secondary quinone in the acceptor part of the electron transport chain. Evidently, regulation of the electron transport chain of photosynthesis by the whole plant cell operates through the effects in the "inlet" and "outlet" portions of the electron transport chain, where transport is effected by the mobile carriers.

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