PHOTO-CONFORMATIONAL TRANSITION CAUSES TEMPERATURE AND LIGHT EFFECTS DURING CHARGE RECOMBINATION IN REACTION CENTERS OF PHOTOSYNTHESIZING BACTERIA

K. V. Shaitan, I. V. Uporov, E. P. Lukashev,
A. A. Kononenko, and A. B. Rubin

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In the article, we develop a theory of photo-conformational (photo-phase) transitions in macromolecular structures. On this basis, we developed a new method of analyzing charge-transfer kinetics in reaction centers of photosynthesizing bacteria and distinguishing between kinetic effects themselves and effects connected with conformational mobility. It is shown that the slow relaxation kinetics are characterized by a minimum of two decay times with sharply differing temperature dependences.

A rethinking of the roles of various factors in the primary steps of photosynthesis is now in progress. It was previously assumed that temperature variations in the rate of electron transfer are almost entirely due to vibronic interactions [1-4], as in the case of redox reactions in solutions [5-6]. This point of view was supported by results from very satisfying interpretations of the sharp biphasic temperature dependence evident in the rate of cytochrome *c* oxidation by the photosynthetic reaction center (RC)* in cells of purple bacteria [7]. However, the conclusion reached from these data, that there is strong vibronic coupling during changes in the charge state in the RC pigment system, was not confirmed [8, 9]. Moreover, it is becoming increasingly apparent that regulation of electron transport in the RC is realized through microconformational movements of donor-acceptor groups and elements of the protein interior, in particular aromatic amino-acid residues [10-16]. Existing data on correlations of temperature and other dependences of rates of elementary processes in the RC with parameters characterizing protein dynamics [16-19] require more careful analysis.

The interrelationships between conformational fluctuations and electron transport are interesting in their own right. A change in conformation in response to an external influence, a photo-conformational transition, for example, might allow chemical processes to self-organize into complex structures and might make regulation of such processes possible [13, 20]. In this report, we will consider a mechanism for regulating charge recombination in the system photooxidized bacteriochlorophyll dimer-primary quinone acceptor of the RC in purple bacteria ($P^+Q_A^- \rightarrow PQ_A$). This is one of the simplest and clearest examples illustrating the physical significance of vibronic interaction and its use in integrating individual steps in functional processes and creating feedback loops in a bimolecular system.

As is well known, charge recombination takes place because of the rapid separation of charges in the RC and the formation of the $P^+Q_A^-$ ion-radical pair [21-27]; the kinetics of the recombination depend upon the conditions of the experiment. The appreciable sensitivity of recombination kinetics to the conditions of preliminary light adaptation is fundamental here. As pointed out for the first time in the report by Noks et al. [21] and later confirmed by Kleinfeld et al. [25], the effective rate of recombination for RCs cooled at saturating light intensities is close to the recombination rate at room temperature and is 3 times less than recombination rates for samples cooled in darkness.

*Abbreviations: RC) reaction center; CFC) conformational coordinate.

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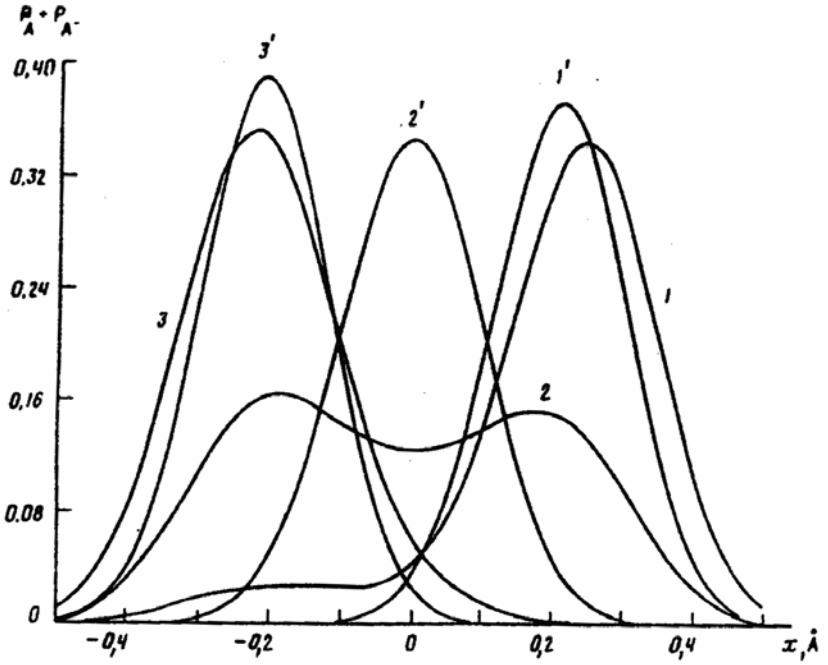

Fig. 1. Shape of summated probability density function $[P_A(x) + P_A^-(x)]$ for the following values of parameters: 1) $k_0/k_r = 0.1$; $\tau = 20$ msec; 2) $k_0/k_r = 1$, $\tau = 20$ msec; 3) $k_0/k_r = 10$; $\tau = 20$ msec; 1') $k_0/k_r = 0.1$; $\tau = 1.14$ msec; 2') $k_0/k_r = 1$; $\tau = 1.14$ msec; 3') $k_0/k_r = 10$; $\tau = 1.14$ msec. Other parameters: $x_0(T = 300\ \text{K}) = 0.1$ Å, $\Delta = 0.25$ Å, $\beta = 0$, $k_r = 0.0207$ msec, $\alpha = 0.01$ msec^{-1} Å^{-1}.

This fact, together with the nonexponential character of recombination kinetics at intermediate temperatures and intensities of activating light undoubtedly indicate relaxation processes in the RC after rapid charge separation and a direct influence of this relaxation upon the recombination kinetics. Suppression of relaxation processes and inhibition of conformational mobility in the one and the same low-temperature region [28] also indicate a direct participation of conformational degrees of freedom in the recombination of $P^+Q_A^-$ and in the relaxation of the protein environment to a "light-adapted" state. From the point of view of physics, there are two important points here. When there is a change in the charge state of a donor-acceptor pair, there is a change in the balance of forces controlling the equilibrium conformation of the system adapted to dark. Because this balance is altered, the system will tend to move toward a new position of equilibrium. A change in light intensity will, in this case, change the effective probability of $P^+Q_A^-$ and, as a consequence, a change in the average conformation of the system will take place. We will therefore refer to this transition as photoconformational [20]. According to previously developed conceptions of dynamic organization of conformational degrees of freedom, based to a significant degree upon data from Mössbauer spectroscopy [12-13, 29-31] and results from computer experiments [32, 33], transition from one conformational state to another is realized through a mechanism of limited diffusion and can be described by equations of the Fokker—Planck type. A change in conformation under conditions of a microheterogeneous environment results in changes in the parameters determining the probability of electron tunnelling $Q_A^- \xrightarrow{e} P^+$ itself. At present, it is impossible to definitively say whether the primary role here is played by changes in distance, orientation, and parameters of the accepting modes or by interaction between π-bridges virtually participating in charge transfer. Assuming that these changes are relatively small in comparison with the average values involved, however, we can restrict ourselves to the first terms in expansions of the rates of these changes. We thus obtain a closed physical model for describing integration of the kinetics of the recombination and photo-conformational transition.

Note the difference between the conceptions we are developing here and variants of interpretation proposed previously [25-27]. Previously [22, 25], nonexponential recombination kinetics and effects of light adaptation of the RC were explained by the existence of a distribution of recombination rates k_r and by temperature variation of this distribution induced by the distribution of $P^+ \rightarrow Q_A^-$ with distance R in a pair; it was assumed that these distributions were different for states of RCs adapted to darkness and light respectively. The above-mentioned distributions were

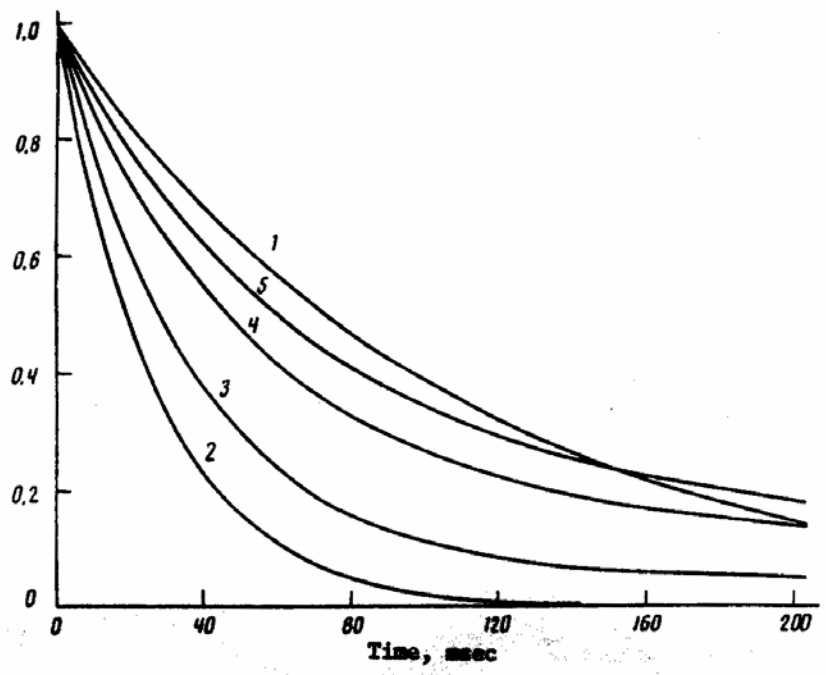

Fig. 2. Kinetic curves of dark recombination of $P^+—Q_A^-$ under various conditions. 1) at temperature of 300 K; 2) after chilling of RC preparation in dark to 80 K; 3-5) evolution of kinetic curves during chilling of RC preparation to 80 K in background light of the following intensities: $1.2 \cdot 10^{-2}$ W·m^{-2}, $7 \cdot 10^{-2}$ W·m^{-2}, and $2 \cdot 10^{-2}$ W·m^{-2}, respectively. Preparation of RC from bacterium *Rhodopseudomonas sphaeroides* was resuspended in 70% glycerine and contained 10^{-2} M *o*-phenanthroline.

considered static, and the dynamics of their evolution as a consequence of conformational relaxation were not considered. In another group of reports [26-27], the connection between conformational changes and recombination kinetics was discussed. Here, conformational changes were introduced in a two-level approximation. Let us emphasize that the nature of the temperature effects remains an open question within the framework of these models, since there is no real possibility of separating temperature dependences into recombination rates proper k_r and rates of conformational relaxation. The dynamic approach presented below suggests a regrouping of kinetic data in which these effects can be separated out.

Let us consider transfer from a donor to acceptor, with allowance made for conformational lability of the carriers. Conformational mobility can be taken into consideration by introducing the conformational coordinate (CFC) x. This coordinate can describe the relative positions or relative orientations of a donor and acceptor or a group surrounding a donor and acceptor. We will introduce into the discussion two functions $P_A(x, t)$ and $P_A^-(x, t)$, which are the probability densities of two random events: the neutral and charged states of the acceptor at a CFC value x. Changes in these functions can occur due to two types of processes: charge transfer and movement along the CFC. These processes may be interrelated: in some regions of conformational space, charge transfer may be impeded, while in some others it may be facilitated. The temporal evolution of the functions introduced can be described with the aid of a Fokker—Planck system of equations:

$$\frac{\partial P_A}{\partial t} = D_A \frac{\partial}{\partial x}\left(\frac{\partial P_A}{\partial x} + \frac{1}{kT} P_A \frac{\partial U_A}{\partial x}\right) - k_0(x) P_A + k_r(x) P_A^-,$$

$$\frac{\partial P_A^-}{\partial t} = D_A^- \frac{\partial}{\partial x}\left(\frac{\partial P_A^-}{\partial x} + \frac{1}{kT} P_A^- \frac{\partial U_A^-}{\partial x}\right) + k_0(x) P_A^- - k_r(x) P_A^-. \qquad (1)$$

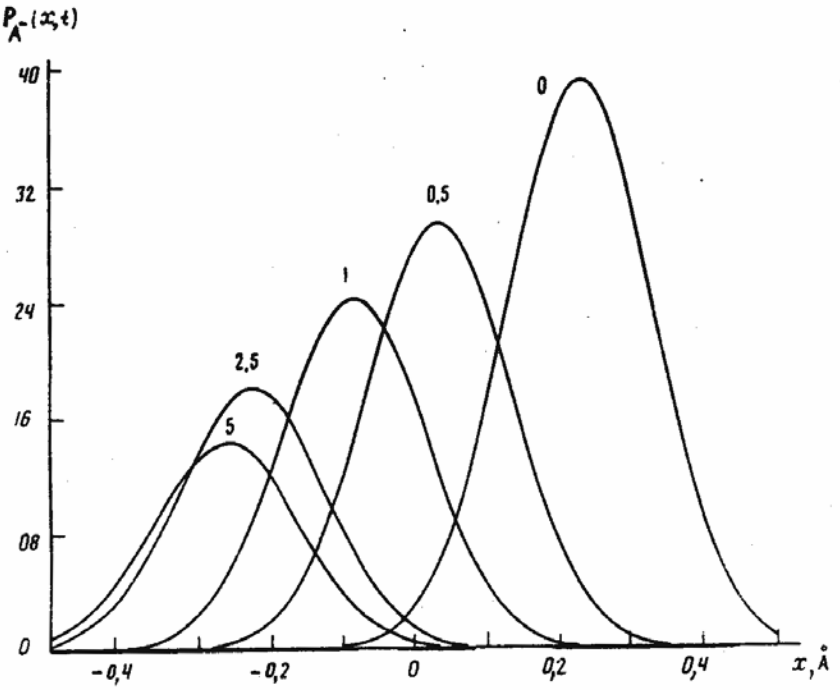

Fig. 3. Evolution of the function $P_{A^-}(x, t)$ in response to brief flash of light. Numbers above maxima of curves correspond to time elapsed since light flash, in units of $\tau = 20$ msec. Remaining parameters: $x_0 = 0.1$ Å, $\beta = 0$, $k_0 = 0$, $k_r = 0.0207$ msec^{-1}, $\alpha = 0.064$ msec^{-1}Å^{-1}.

The first terms on the right-hand sides of equations (1) describe variations of P_A and P_{A^-} due to diffusion movement along the CFC in a potential field given by functions U_A and U_{A^-} respectively. The potential energies of the acceptor in the neutral and charged states are different, as pointed out previously. These differences at least should show up in the equilibrium values of CFC in the neutral and charged states of the acceptor. We used the following forms of U_A and U_{A^-} in subsequent computations:

$$U_A = \frac{1}{2}K(x-\Delta)^2; U_A = \frac{1}{2}K(x+\Delta)^2. \tag{2}$$

Representation of the potential energy by means of a parabola is entirely valid near the minimum. Having chosen potential functions in form (2), we can neglect possible variation in the elastic constants K, assume that only the equilibrium CFC of the acceptor changes when there is a change in the charge state, and introduce the conventions $x_{min} = \Delta$ in the neutral state and $x_{min} = -\Delta$ in the charged state Q_{A^-}. D_A and D_{A^-} are diffusion coefficients for the conformational modes of the acceptor and can be assumed to equal one another.

The second and third terms on the right-hand sides of expressions (1) are terms proportional to $k_0(x)$ and $k_r(x)$ and describe variations of probability density functions due to charge-transfer processes. The function $k_0(x)$ is the rate of charge transfer to the acceptor at a given value of the conformational coordinate x and $k_r(x)$ is the rate constant of recombination with the acceptor under the same conditions. For reactions of charge photo-transfer, the rate $k_0(x)$ is proportional to the intensity of the excitatory light and can vary over a wide range. By assuming that variations of conformational variables are relatively small, we can expand the functions $k_0(x)$ and $k_r(x)$ into series in terms of x and restrict ourselves to linear terms:

$$k_0(x) = k_0 + \beta x;\ k_r(x) = k_r + \alpha x. \tag{3}$$

Of course, this approximation is invalid in regions of conformational space where expression (3) becomes negative. It is necessary to keep in mind, however, that formulas (3) are actually used for small x and small values of the coefficients α and β. In the following discussion, the coefficients α and β will satisfy the condition of positivity (3) in a significant region of conformational space.

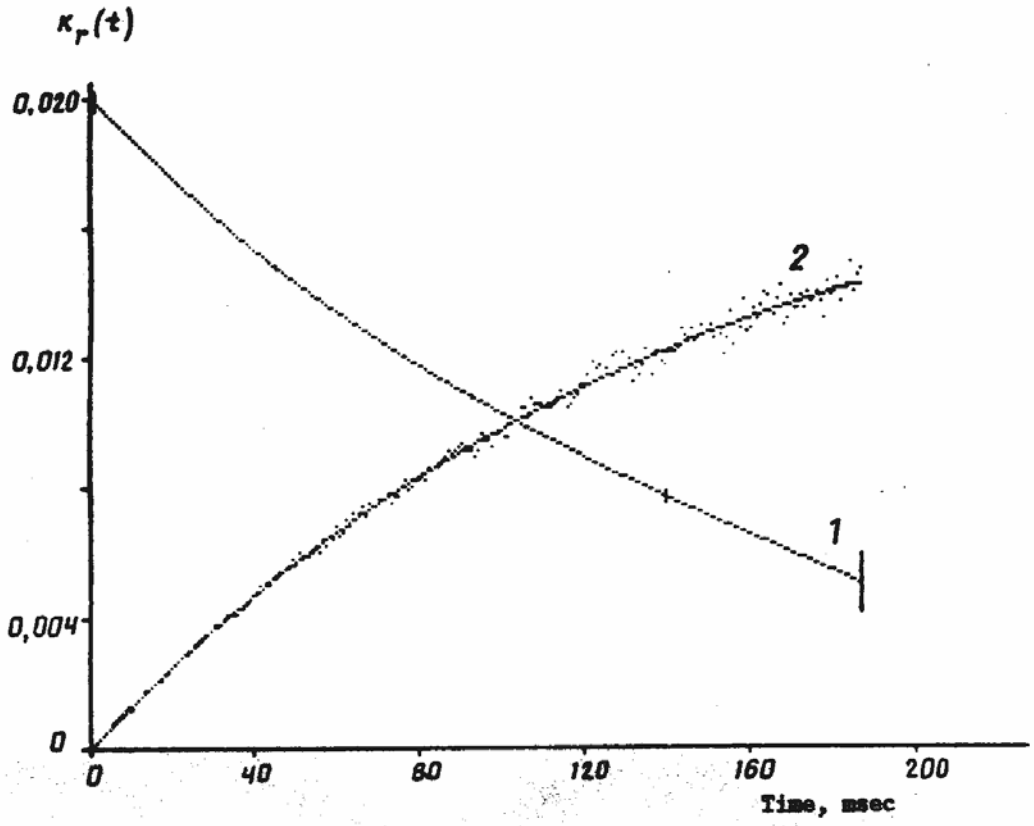

Fig. 4. Typical dependence of the instantaneous recombination constant $k_r(t)$ upon time (1); confidence intervals are indicated by lines. (2) Natural logarithm of the corresponding kinetics; i.e., $\ln P_A^-(t)$ for a sample frozen in light to 100 K.

In addition to specifying the functions U_A, U_A^-, $k_0(x)$, and $k_r(x)$, in a clear form, it is also necessary to add boundary and initial conditions to system (1). The logical requirements for integrability of the sum of the functions P_A and P_A^- emerge as boundary conditions. the integral of this sum in the investigated region of conformational space equals one. The initial values of $P_A(x, 0)$ and $P_A^-(x, 0)$ are given in accordance with the situation under analysis.

The methods for solving system of equations (1) with functions of the type (2) and (3) were discussed in detail previously [20]. We will briefly discuss the types of stationary solutions for system (1). An equilibrium between the neutral and charged forms of the acceptor is established for a constant action of light upon the system; this equilibrium is dependent upon the light intensity. During intense illumination, the equilibrium shifts toward the charged form and occupies a region of conformational space of size $\sim(kT/K)^{1/2}$ corresponding to the charged acceptor, i.e., $P_A \leq 1$ and $P_A^-(x) \sim 1$ at $x \sim -\Delta$. In the absence of illumination, the reverse situation occurs: the acceptor is in the neutral state and the most probable value of the conformational coordinate equals Δ. The average value of the coordinate of the acceptor, irrespective of its charge state, smoothly varies when the intensity of incident light is increased from $x \sim \Delta$ to $x = -\Delta$ (Fig. 1). We interpret this behavior of the system as a photo-conformational transition.

During rapid cooling, the stationary distribution obtained ($P_A + P_A^-$) is fixed; but, because of the finite rate of cooling and the increase $\tau = kT/D$ when T is lowered, some change in the distribution function occurs. As the analysis, shows, however, the change in the average coordinate for the stationary distribution function is not very large (Fig. 1a, b) and a state with kinetic characteristics similar to the original is trapped. By varying the intensity of illumination of the samples of RC, we can thus vary the parameter k_0/k_r over a wide range and trap specific conformational states during cooling. The series of kinetic measurements carried out (Fig. 2) (see below) confirms the conception of a photo-conformational transition at the acceptor portion of the RC. An increase in the intensity of the adapting light creates a state of the system in which, even at low temperatures, the recombination kinetics practically coincide with the kinetics of the process at $T = 300$ K. In other words, there is a chance of a conformational transition at room temperature in response to a saturating flash of light and formation of Q_A^-. At low temperatures, conformational mobility is inhibited, causing the difference in kinetics shown in Fig. 2 (curves 1 and 2). When cooled

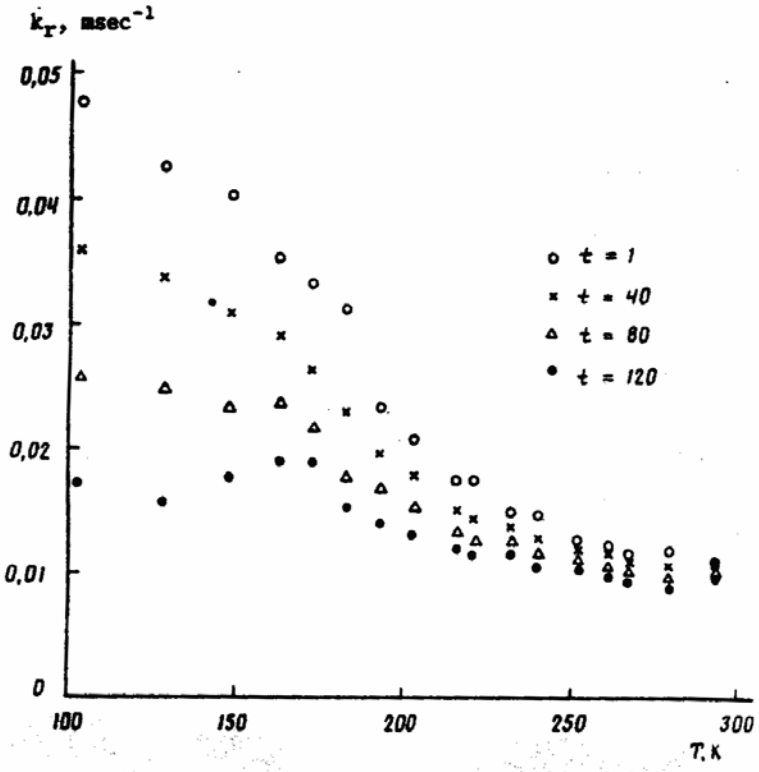

Fig. 5. Values of instantaneous recombination constant $k_r(t)$ at various times after flash and at different temperatures.

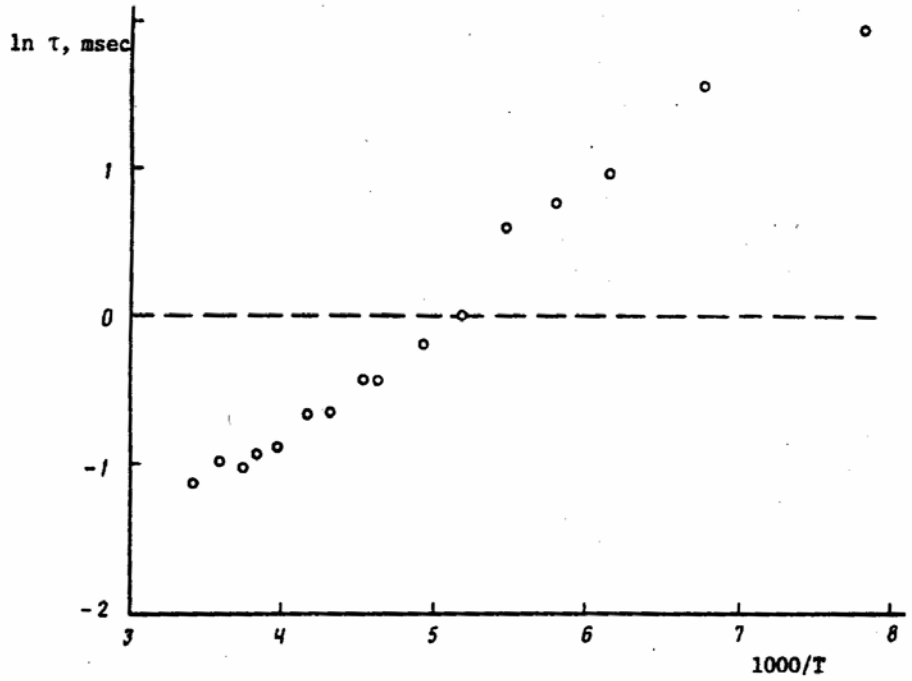

Fig. 6. Rapid conformational relaxation time in Arrhenius coordinates. Energy of activation E_a = 1.4 kcal/mole.

in the presence of light and when subjected to constantly increasing intensity of illumination, however, the system is trapped in states ever closer to the equilibrium position of Q_A^-; this is apparent in the successive changes in the kinetic curves shown in Fig. 2 (curves 3-5).

Let us note yet another interesting feature of the stationary charge state on the acceptor. It turns out that the precise value for the most probable CFC of the acceptor in the charged state for system (1) does not, generally speaking, coincide with the position of the minimum of potential U_A^-; it equals:

$$\bar{x}_{A^-} = -\Delta - \alpha x_0^2 \tau, \tag{4}$$

i.e., a shift of the most probable CFC takes place relative to the minimum of potential U_A^-. This shift is of a dynamic nature and is due to the heterogeneity of the conformational space with regard to rate of charge recombination. Since recombination of charge and acceptor actually proceeds more slowly in regions of conformational space with $x < 0$ at positive values of the parameter α than in regions $x > 0$, the probability of the acceptor remaining in the charge state in the region $x < 0$ is higher. A shift in the average position of the reduced form of the acceptor takes place because of this mechanism.

The behavior of the nonstationary functions $P_A(x, t)$ and $P_A^-(x, t)$ after a light is switched on can be divided into two stages. During the first stage, which lasts for a time $t < \tau$ (τ is the characteristic conformational relaxation time), the bell-shapes of functions P_A and P_A^- emerge; then, for $t > \tau$, the bell-shapes of the functions P_A and P_A^- are steadily maintained during the process of evolution (except for changes in amplitudes and positions of maxima). It is especially interesting to see how a brief intense flash of light affects the system. This situation is common in experiments using pulse spectrophotometry of photosynthesizing objects. Prior to the flash of light, there are no acceptors in the charge dorm if the system is in the dark, i.e.,:

$$P_{A^-}(x, 0) = 0; P_A(x, 0) \sim \exp\{-U_A/kT\}. \tag{5}$$

When there is a fairly intense but brief flash of light, complete charge transfer to the acceptor takes place during the time of light action; however, conformational equilibrium is not established in the system during the time of flash action. Thus, the following distribution for Q_A and Q_A^- is established when the action of an intense flash of light ($k_0\tau^* \gg 1$) is completed:

$$P_A(x, \tau^*) \simeq 0; P_{A^-}(x, \tau^*) \simeq P_A(x, 0). \tag{6}$$

Since the distribution function for the charged acceptor is in disequilibrium here relative to the corresponding surface of potential energy $U_A^-(x)$, conformational relaxation takes place. This process is also accompanied by charge recombination. The evolution of the function $P_A^-(x, t)$ for the case under consideration is shown in Fig. 3. The abscissa of the maximum of this function is displaced according to the pattern:

$$\xi(t) = -\Delta - \alpha x_0^2 \tau + (2\Delta + \alpha x_0^2 \tau) e^{-t/\tau}. \tag{7}$$

Because of charge recombination, the neutral form of the acceptor appears over the course of time and the amplitude of the function $P_A(x, t)$ localized in the region $x = \Delta$ increases. More detailed analysis shows that the probability of the acceptor existing in the charged form can be computed in this case using a formula given in a report by Uporov and Shaitan [20]:

$$P_{A^-}(t) = \int P_{A^-}(x, t)dx = \exp\left\{-\int_0 k_r(\xi(t))dt\right\}. \tag{8}$$

From formula (8) the kinetics of charge recombination should be nonexponential if the rate constant is a function of the CFC. Formula (8) constitutes an exact result in the case of linear dependence $k_r(x)$; however, numerical analysis indicates that kinetics of the type in (8) will occur in cases when $k_r(x)$ depends upon x in a fairly arbitrary fashion over times longer than the conformational relaxation time. Formula (8) permits a new approach to the analysis of recombination kinetics. Of importance here is the possibility of determining rate constants of recombination at various moments in time and thus determining the heterogeneity of the conformational space with respect to charge transfer.

Using the proposed model, we worked through data on the kinetics of the reduction of photooxidized bacteriochlorophyll (P) by primary quinone Q_A under conditions when direct electron transfer in the system of acceptors $Q_A^-Q_B \rightarrow Q_AQ_B^-$ was blocked by *ortho*-phenanthroline. Preparations of RC were obtained as described previously [34]. Photoinduced changes in absorption by P at 870 nm were recorded on a computerized single-beam

differential pulse spectrophotometer described previously [35] and equipped with a cryostat allowing the temperature of the sample to be varied from 80 to 300 K. A ISSh-100-3M xenon flash lamp (energy of flash 9 mJ, duration at half-height of profile equalled 10 μsec, spectral composition 400-600 nm) was used for photoexcitation. A KGM-300 heat lamp (white light, maximum intensity 200 $W \cdot m^{-2}$) was used as a constant light source for light adaptation.

The kinetic curves obtained for recombination of the ion-radical $P^+Q_A^- \rightarrow PQ_A$ naturally allowed us to determine the probability of QA existing in the reduced state. We took the logarithms of the experimental values and then carried out regularization of the experimental dependence by approximation using polynomials. Following this, instantaneous rate constants of recombination $k_r(t)$ were calculated:

$$k_r(t) = -\partial / \partial t \ln P_A(t). \tag{9}$$

The results of the processing are presented in Fig. 4. Curve 2 was obtained by taking the logarithm of the experimental kinetic curve and subsequently approximating it with a fourth-degree polynomial. Curve 1 corresponds to the instantaneous value of the rate constant. Vertical bars correspond to confidence intervals determined by linear regression.

Figure 5 shows the results of processing the recombination kinetics at different temperatures. Four series of points correspond to initial values of recombination rate constants at different moments in time. The approach developed above allows us to directly examine the process of conformational relaxation. From the graphs presented, it is evident that the recombination constant at high temperatures is independent of time; i.e., conformational relaxation takes place over a substantially shorter time than the characteristic charge recombination time in the initial conformational state ($k_r^{-1} \sim$ 80 msec). At low temperatures $T < 200$ K, conformational relaxation slows down and the kinetics of the photo-conformational transitions are superimposed upon the recombination kinetics. It is evident from Fig. 5 that unfreezing of the conformational dynamics takes place in a temperature region ~200 K. A similar conclusion was previously reached from an analysis of data from Mössbauer spectroscopy [17]. This suggests a cooperative character for the motion of the acceptor and its environment.

An analysis of Fig. 5 leads us to the following conclusions. The total change in the rate of recombination, due both to the increase in temperature from 100 to 300 K and conformational relaxation over a time exceeding the minimum time for the recording system $t \sim 1$ msec, lies within an interval from 0.048 to 0.01 $msec^{-1}$. Almost complete conformational relaxation over the entire temperature interval is known to take place here over a time of ~120 msec. The amplitude of the temperature dependence k_r (120 msec) from 100 to 300 K indicates that the maximum change in rate of recombination of the relaxed state falls within a range of 0.02-0.01 $msec^{-1}$. We know that this is precisely the temperature dependence of k_r for the relaxes state because of the virtual coincidence of values for the rate k_r (120 msec) at 100 K and the rate of recombination of the state adapted to light at the same temperature. Let us consider further changes in $k_r(t)$ at fixed temperatures which reflect the depth of conformational transition at corresponding moments in time. At $T \gtrsim 250$ K, virtually complete relaxation takes place over a time $\tau \sim 1$ msec. In the region $T <$ 200 K, contributions with characteristic times of ~80 msec become significant; at $T < 220$ K, contributions of processes with characteristic times of 40 msec become significant.

Existence of a clear temporal dependence $k_r(t)$, even at 100 K, practically rules out interpretation of the topmost curve in Fig. 5 as a temperature dependence of $k_r(t)$ in an unrelaxed state. Thus, the data in Fig. 5 suggests that the intrinsic temperature variation in the recombination rate of $Q_A^- \rightarrow P^+$ in the range of 100-300 K odes not exceed 25% of the total change in this value for any conformations. Consequently, the observed temperature and light effects on the kinetics of this process are primarily due to a conformational transition (see also [10, 26, 27]). This mechanism for regulating the kinetics of intraprotein processes is apparently not unique and is of profound significance. The use of donor—acceptor pairs with reaction rates independent of temperature under otherwise equivalent conditions allows regulation of the process only as a result of variation of macromolecular structure and changes in the immediate environment during confirmational movements. A goal-directed change in kinetic parameters can be obtained in this fashion, and a chain of sequential conversions can be coordinated. It is especially interesting that coordination of the rates of biochemical processes can be attained without substitution of reagents (the choices of which are limited), using instead changes in structure and microenvironment, which, in the final analysis, are controlled by the amino-acid sequence selected over the course of evolution [36].

A number of physical problems concerning the dynamics of microheterogeneous structured environments arise in connection with the problem under consideration. From formulas (3), (7), and (8) it follows that the distance in Fig.

5 along the vertical from the highest level, 0.048 msec, to the intersection with the corresponding curve is proportional to the change in the conformational coordinate x over the corresponding time at a given temperature. The fairly complex recombination kinetics is most interesting. Thus, processes with characteristic times $\tau \lesssim 1$ msec make contributions to conformational relaxation which are significant and sharply increasing with increases in temperature (Fig. 5, topmost curve).

The temperature dependence of the rapid relaxation can be determined in a monoexponential approximation (Fig. 6). It is evident from Fig. 5 that, in addition to rapid processes, processes with times of $\tau \sim 100$ msec make significant contribution to relaxation at low temperatures. The contribution of these processes at $T > 200$ K is insignificant. It is very significant that processes with times of ~100 msec take place at $T \sim 100$ K, allowing almost complete relaxation of the state Q_A^- at low temperatures. The existence of at least tow relaxation processes with substantially differing temperature dependences is reminiscent of the α- and β-relaxation which takes place in many glasses [37]. At the same time, it is necessary to note that the rates of temperature-related changes in relaxation time are much slower than in typical glasses because of the mixture of organic molecules and that identification with α- and β-relaxation would be premature. Great heterogeneity is undoubtedly a factor making the corresponding temperature changes smoother. The very clear division of the relaxation process into two components may be a consequence of substantial differences between structural elements participating in the given process, differences between the ubiquinone molecules themselves, for example, and, on the other hand, small groups of molecules (water for example). Apparently, substantial differences in interactions between hydrophilic and hydrophobic elements may also emerge as an additional factor involved in the observed effects.

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