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Effects of mutual influence of photoinduced electron transitions and slow structural rearrangements in bacterial photosynthetic reaction centers

GRACHIK A. ABGARYAN[1], LEONID N. CHRISTOPHOROV[2], ALEXANDER O. GOUSHCHA[3,4], ALFRED R. HOLZWARTH[4], VALERY N. KHARKYANEN[3], PETER P. KNOX[5] and EUGENE A. LUKASHEV[5]
[1] *Erevan Medical University, Erevan, Armenia;* [2] *Bogoliubov Institute for Theoretical Physics NAS Ukraine, 14B Metrologichna Str., Kiev 252143 Ukraine;* [3] *Institute of Physics NAS Ukraine, Kiev, Ukraine;* [4] *Max-Planck Institut fur Strahlenchemie, Mülheim a.d. Ruhr, Germany;* [5] *Lomonosov Moscow State University, Moscow, Russia*

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Abstract. We describe the phenomenon of light-induced structural transformations in the reaction centers (RC) of photosynthetic bacteria which makes self-regulation of the RC charge separation efficiency possible. The nature of the effect is that the light-driven electron transfer (ET) between the RC redox-cofactors causes structural changes in the protein-cofactors system and this in turn affects the ET kinetics. If the electron-conformation interaction is strong enough, then such self-regulation gives birth to a new RC conformational state of enhanced charge separation efficiency.

We show experimental results of stationary and kinetic absorbance change characteristics under different photoexcitation conditions, indicating structural rearrangements on a rather long (minutes) time scale, mainly within the secondary acceptor binding pocket. To simplify the description, in constructing a theory of structure-function reorganization in the RC we employ the adiabatic approach. Final expressions enable us to make qualitative comparison with experimentally observed kinetics of the fast and slow stages of ‘free’ and ‘structurally controlled’ electron relaxation, respectively.

Key words: Non-linear dynamics, bistability, slow conformational mode, conformational state, light-induced changes, reaction center(s), *Rhodobacter sphaeroides*, electron transfer, adiabatic theory

Abbreviations: RC – reaction center; ET – electron transfer; Q_A – primary quinone acceptor; Q_B – secondary quinone acceptor; P – primary electron donor; cw – continuous wave

Introduction

Reaction centers (RC) of photosynthetic bacteria perform charge separation using the energy of absorbed light with the quantum yield close to 100% [1]. RC is a biomolecular complex that consists usually of three protein subunits and comprises a bacteriochlorophyll dimer (D) as an electron donor (often labelled also as P), two bacteriochlorophyll monomer molecules, two bacteriopheophytin molecules, nonheme iron ion and two quinone acceptors (the primary quinone acceptor Q_A and the secondary $-Q_B$) [2, 3]. The structure and properties under different illumination conditions have been elucidated for different RC species [2–5]. The light-induced conformational changes of RCs were observed in a number of studies [6–14]. In some of them [6, 7, 14] the possible physiological importance of these changes has been pointed out. The problem apparently is how the light-induced structural rearrangements of the RC affect the charge separation efficiency. It was shown in Refs. 15–18 that a self-consistent description of the electronic densities distribution among the redox co-factors and the slow structural rearrangements of RC predicts the formation of a new conformational state of RC, in line with our concept of dynamical self-organization in biomolecular systems [19, 20]. The latter implies certain nonlinear phenomena (characterized e.g. by hysteresis and bifurcations in the system transport properties) emerging due to the feedback between the flow of transferred charges and structural variables, see also [15–17, 21]. Experimental studies revealed the hysteresis behavior of the absorbance of RCs from *Rb. sphaeroides* with both (Q_A and Q_B) quinone acceptors active [15, 22, 23]. Two bifurcation points, one corresponding to the appearance of the new 'light' conformational state and another to the vanishing of the old 'dark' conformational state were found at the critical values of actinic light intensity $I_1^{cr} \approx 10^{10}$ quanta/(cm^2 s) and $I_2^{cr} \approx 5 * 10^{13}$ quanta/(cm^2 s) respectively ($2 * 10^{15}$ quanta/(cm^2 s) intensity corresponds to 1 s^{-1} photoexcitation rate [23]). Thus, bistability was detected experimentally and described theoretically within the interval of actinic light intensity $I_1^{cr} < I < I_2^{cr}$. What could be the role of this effect in the regulation of the efficiency of charge separation in intact photosynthetic RCs? To give the answer let us consider the expression for the absorbance changes in bacteriochlorophyll dimer absorption band ($\lambda = 865$ nm) for RCs with both quinone acceptors active. The time-dependent absorbance reads:

$$A_{865}(t) = c(t) * \varepsilon_{865} * l$$

where ε_{865} is the extinction coefficient, l is an optical path-length and $c(t)$ is the number of unexcited RCs per unit volume at instant t.

If $\sigma(t)$ stands for the RC photoexcitation probability at instant t, then:

$$c(t) = c_0[1 - \sigma(t)] \tag{1}$$

where c_0 is the total number of RCs per unit volume. Hence,

$$\delta(t) \equiv -\frac{A_{865}(t) - A_{865}(0)}{A_{865}(0)} = \sigma(t). \tag{2}$$

It is easily shown that the stationary value of RC excitation probability for an arbitrary system with finite number of states is:

$$\sigma_I = \lim_{t\to\infty} \sigma(t) = \frac{I}{I + (\tau_d)^{-1}} \tag{3}$$

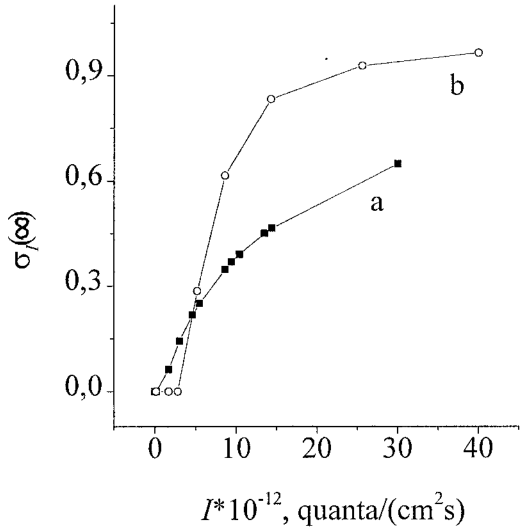

Figure 1. Optical absorbance changes $\sigma_I(\infty)$ upon variation of exciting light intensity I for RCs from *Rb. sphaeroides* (wild type) lacking the secondary quinone acceptor (curve *a*) and with native ubiquinone in the Q_B binding site (curve *b*). Experimental conditions: room temperature; buffer: 0.025% [LDAO], 10 mM concentration of *HCl-Tris*; pH = 8.0.

where I is the intensity of (actinic) light excitation, τ_d is the average lifetime of the separated charged relative to the recombination process. This important parameter together with the quantum yield of the primary processes of the charge separation characterizes the efficiency of the charge separation in intact systems.

The results of our measurements of $\sigma_I(\infty)$ with respect to I are presented in Figure 1. For the RCs without Q_B curve a shows a hyperbolic behaviour with the half-saturation constant $(\tau_d)^{-1} = k_{AD}$ (see Scheme (s1) below, restricted by the first-step excitation-recombination processes only). Since any internal rate constant is determined by the system structure, we may conclude that no significant light-induced structural change affecting the k_{AD} value has occurred at this transfer stage. However, Curve b for the RCs with Q_B shows a pronounced deviation from a hyperbola. According to (3), this clearly indicates the dependence of $(\tau_d)^{-1}$ (see below for its expression for the full scheme (s1)) on the exciting light intensity and thereby the movements, originated from light-induced charge redistribution, of the RC structural elements close to the Q_B binding site. These data motivate our further study of the influence of light-induced structural changes on τ_d, i.e. of the dependence $\tau_d(x)$ where x is a certain slow variable describing structural rearrangements.

Thus the problem consists in both the proper choice of the variable x for a specific RC model and the determination of the dependencies $\tau_d(x)$ and $x(t)$ for the stationary conditions of RC photo-activation. The theory developed below will be used as well for the quantitative description of the kinetics of RC electronic relaxation under variations of actinic light intensity.

Structural Self-Regulation in RC

In this section we proceed mainly theoretically, presenting, however, some key experimental observations.

As we suppose a pronounced temporal hierarchy in the (electron flow + structural motion) dynamics of RC, it is relevant to employ the adiabatic approach. This implies that a fast electronic subsystem creates an effective potential with fixed (slow) structural variables, and the motion of the latter proceeds in the field of this effective potential. The structural changes (changes of the nuclei positions) in turn cause an adiabatic redistribution of the electron density thus leading to the new self-consistent stationary electron-conformation states of the system.

The adiabatic theory was used in a number of studies (see e.g. [15–17, 19, 20]) for determination of the possible electronic-conformational states of biomolecular objects interacting with externally pumped fluxes of electrons or ions. We use the same approach and consider the RCs of photosynthetic bacteria under the conditions of their photo-activation. For isolated RC the electron transfer (ET) steps can be described by the following scheme (we omit here the short-lived intermediate electronic states):

$$\begin{tikzcd}
PQ_AQ_B \arrow[r, shift left, "k_{DA}"] & P^+Q_A^-Q_B \arrow[l, shift left, "k_{AD}"] \arrow[r, shift left, "k_{AB}"] & P^+Q_AQ_B^- \arrow[l, shift left, "k_{BA}"] \arrow[ll, dashed, bend left, "k_{BD}"]
\end{tikzcd} \tag{s1}$$

where k_{AD}, k_{AB}, k_{BA} and k_{BD} are the rate constants determining the probability per time unit of ET between corresponding co-factors. The constant k_{DA} reflects the sequence of fast processes of charge separation (which are not shown in the scheme) and is limited usually by the rate (intensity) I of photoexcitation of the primary donor D. Thus we assume that $k_{DA} \sim I$ with the proportionality coefficient equal to 1, i.e. $k_{DA} = I$ with I measured in 1/s, see previous section.

For a set of given values of the rate constants the kinetics of ET is described by a system of balance equations (see e.g. [15–17, 24]) that corresponds to Scheme (s1). The solution of such equations for the case of *Rb. sphaeroides* RC under normal physiological condition (when $k_{AB} + k_{BA} \gg k_{AD}, k_{BD}$; $k_{BD} \ll k_{BA}$, see Ref. [24, 25]) is given by

$$\begin{aligned}
&\rho(t, D) = 1 - \rho(t, A) - \rho(t, B), \\
&\rho(t, A) = \rho(0, A)\exp(-k_f t) + \rho_I(\infty, A)\Big(1 - \exp(-k_s t)\Big),
\end{aligned} \tag{4}$$

$$\rho(t, B) = -\rho(0, A)\exp(-k_f t) + \Big[\rho(0, A) + \rho(0, B) \\ -\rho_I(\infty, B)\Big]\exp(-k_s t) + \rho_I(\infty, B),$$

where $\rho(t, D)$, $\rho(t, A)$ and $\rho(t, B)$ are the populations of the co-factors D, Q_A and Q_B respectively; k_f and k_s are two apparent rate constants (fast and slow, respectively):

$$k_f \approx k_{AB} + I; \quad k_s = I + k_{AD}\frac{k_{BA}}{k_{AB} + k_{BA}} + k_{BD}; \\ \rho_I(\infty, A) = \frac{I\frac{k_{BA}}{k_{AB}}}{k_s}; \quad \rho_I(\infty, B) = \frac{I}{k_s}; \tag{4a}$$

From Equations (4) and (4a) it follows:

$$\sigma(t) = \rho(t, A) + \rho(t, B) = \sigma_I + [\sigma(0) - \sigma_I]\exp\left(-\frac{t}{\tau_{el}}\right), \tag{5}$$

where

$$\sigma_I = \frac{I}{I + \tau_d^{-1}}, \tag{6}$$

$$\tau_{el} = (k_s)^{-1} = [I + \tau_d^{-1}]^{-1} \tag{7}$$

and the lifetime τ_d of the charge separated pair (see Equation (3)) is defined by:

$$\tau_d = \left[k_{AD}\frac{k_{BA}}{k_{AB} + k_{BA}} + k_{BD}\right]^{-1}. \tag{8}$$

We have measured the recovery kinetics for RCs with and without Q_B -activity under different excitation conditions (Figure 2). As the square of the area under the curves equals the mean lifetime of the electron on the corresponding acceptor, we may conclude from Figure 2a that k_{AD} does not noticeably change on changing the excitation conditions. As for τ_d defined by Equation (8), from the data of Figure 2b it follows that τ_d increases up to 200 times concomitantly with increasing the RCs' exposure time to the actinic light of saturating intensity. Such a dramatic increase of the average lifetime of the charge-separated state is related closely to the light-induced structural rearrangements. The electron localization on the secondary quinone acceptor causes the slow structural changes of RC on the time scale of several (3–5) min, see below. These structural changes are presumably related to slow polarization of the Q_B-binding pocket. As a result, the free energy difference ΔG_{AB} for the electron localized either on Q_A or on Q_B changes (see the scheme in Figure 3) and we consider ΔG_{AB} as the appropriate physical parameter which is responsible for the light-induced changes of the τ_d value (see also Ref. [17–18]).

Next, we take into account that for the RCs from *Rb. sphaeroides* under physiologically normal conditions the following inequality is valid:

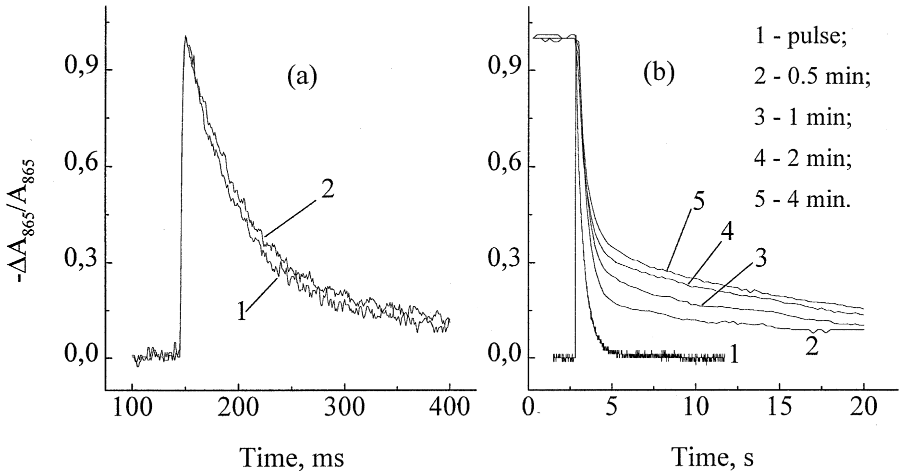

Figure 2. The primary donor recovery kinetics at 865 nm for RCs from *Rb. sphaeroides* (wild type) without ubiquinone in a Q_B binding site (a) and with reconstituted Q_B activity (b) following different excitation conditions. Curves in graph (a) were obtained for a pair of actinic flashes. The first flash in the pair corresponds to the dark-adapted (for more than an hour) sample (curve 1); the second flash was given 10s after the first (curve 2). The kinetics was the same as presented by curve 2 for the sample under background excitation and for the sample pre-illuminated with the light of close-to-saturating intensity. Curve 1 in graph (b) was measured after a short actinic flash, whereas curves 2–5 were obtained after pre-illumination of the sample with the strong actinic cw light during different time intervals indicated in the figure. Experimental conditions are the same as indicated in Figure 1 (for their details see [23]).

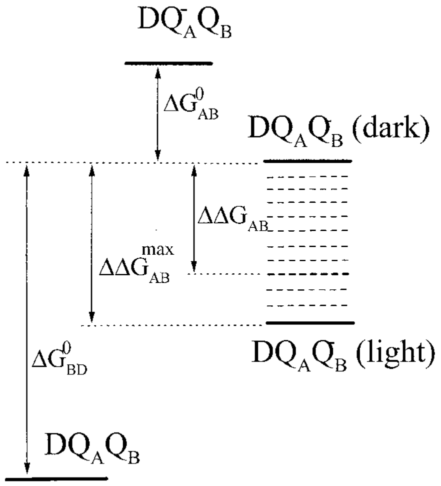

Figure 3. Schematic representation of RC electronic levels. ΔG^0_{BD} and ΔG^0_{AB} are the differences of electron Gibbs energies for the dark-adapted state, $\Delta\Delta G^{\max}_{AB}$ is the maximum possible change of the free energy difference, ΔG^0_{AB}, for the sample adapted for a long time to a strong actinic light. $\Delta\Delta G_{AB}$ corresponds to the ΔG^0_{AB} change at the intermediate value of I.

$$k_{AD}\frac{k_{BA}}{k_{AB}+k_{BA}} \gg k_{BD}, \tag{9}$$

reflecting the fact that for the electron localized on, Q_B, the probability of the indirect recombination pathway (via Q_A), is much higher as compared to the direct pathway [24, 25]. As the rate constant k_{AD} only slightly changes under conditions of prolonged illumination, the considerable light-induced changes of the τ_d value can be ascribed primarily to the variation of both k_{AB} and k_{BA}. Thus we can choose the slow (control) structural variable in the form:

$$x = \frac{\Delta G_{AB}}{k_B T}, \tag{10}$$

where k_B is the Boltzmann constant and the value ΔG_{AB} changes from ΔG^0_{AB} for the dark-adapted sample to $\Delta G^0_{AB} + \Delta\Delta G^{\max}_{AB}$ for the samples adapted for a long time to a strong actinic light (see Figure 3). The maximum light-induced shift of the free energy difference can be easily estimated as $\Delta\Delta G^{\max}_{AB} \approx 5.3 k_B T \approx 0.13$ eV, since $\ln 200 \approx 5.3$. Taking into account Equations (8) and (9) we can rewrite τ_d in the form:

$$\tau_d(x) = k^{-1}_{AD}(\exp(x) + 1), \tag{11}$$

which manifests the sought x-dependence of τ_d, causing the abovementioned non-hyperbolic saturation effect shown in Figure 1.

Note also that inequality (9) holds for various values of x because

$$k_{BD} \sim \exp\left[-\frac{(\lambda_{BD}-\Delta G_{BD})^2}{4\lambda_{BD}k_B T}\right]; \quad \Delta G_{BD} = \Delta G^0_{BD} - x \cdot k_B T, \tag{12}$$

where we adopt the Marcus-type description of ET rate constants [26], λ_{BD} is the reorganization energy, and we assumed that $\lambda_{BD} > \Delta G_{BD}$, which is valid for the RCs from *Rb. sphaeroides*, wt [27].

The co-factor electronic populations reach their stationary values at the fixed adiabatic variable x during the time interval

$$\tau_{el}(x) = \left[I + k^{-1}_{AD}(\exp(x) + 1)\right]^{-1}. \tag{13}$$

It is easy to show that the value $\tau_{el}(x)$ is always less or equal to ca. 1 s except perhaps the case when $I \approx 0$ and x is very large. It means that the condition of adiabaticity:

$$\tau_x \gg \tau_{el} \tag{14}$$

is fulfilled for all $I > 0$ (here τ_x is a characteristic relaxation time for the structural variable). We consider the slow structural variable as overdamped, and within the adiabatic formulation of the problem the motion equation is:

$$q\frac{dx}{dt} = -\frac{\partial V_0(x)}{\partial x} + \sum_{i=D,A,B} f_i n_i(x, I) + \sqrt{2D}\xi(t), \tag{15}$$

where $V_0(x)$ is the ground state potential energy of the structural variable x (without photoexcitation of the RC); q is a friction coefficient; f_D, f_A and f_B are the random forces that act on the structural variable when the electron is localized on D, Q_A, and Q_B, respectively; $n_i(x, I)$ stands for stationary populations $\rho_I(\infty, i)$. The last term in Equation (15) determines a random stochastic δ-correlated force originating from the thermal movement of the molecules. This force causes diffusion of the structural variable with the diffusion constant D along the potential adiabatic surface of regular forces. As it can be easily seen, the stationary values of the co-factor electronic populations may be written as follows:

$$\begin{aligned} n_A(x, I) &= \frac{I}{I \cdot (1 + \exp(x)) + k_{AD}}; \\ n_B(x, I) &= \frac{I \exp(x)}{I \cdot (1 + \exp(x)) + k_{AD}}; \\ n_D(x, I) &= \frac{k_{AD}}{I \cdot (1 + \exp(x)) + k_{AD}}. \end{aligned} \tag{16}$$

Let us choose the potential $V_0(x)$ as parabolic, $V_0(x) = \frac{1}{2}kx^2$, where k is an elastic constant. Then the condition for the sum of all regular forces acting on the structural variable x to be equal to zero is given by:

$$-kx + \sum_i f_i n_i(x, I) = 0. \tag{17}$$

Taking into account that $\frac{n_A}{n_B} = \exp(-x) \ll 1$ we can neglect n_A in comparison with n_B. Then we obtain the equation for the stationary values x_{st} of the structural variable:

$$x_{st} = x_D + (x_B - x_D)\frac{I \exp(x_{st})}{I[1 + \exp(x_{st})] + k_{AD}}, \tag{18}$$

where $x_D = \frac{f_D}{k}$ and $x_B = \frac{f_B}{k}$ are the equilibrium values of the structural variable under the condition of permanent localization of the electron on co-factor D and B, respectively (for the sake of simplicity we here consider the elasticity constant k to be the same for different conformational states of the system).

Equation (18) has been introduced for the first time in Refs. 15–17 and then refined in [21, 28] where an exact solution of the problem of the feedback with the two-level random system was found. Figure 4 shows the dependence $x_{st}(I)$ at different values of the parameter $\xi = x_B - x_D$. It is easy to see that for $\xi \geq 4$ the dependence is characterized by the bistability region with the width 2Δ:

$$\frac{x_B + x_D}{2} + \Delta \geq x \geq \frac{x_B + x_D}{2} - \Delta, \tag{19}$$

where

$$\Delta = \sqrt{\frac{\xi^2}{4} - \xi}. \tag{19a}$$

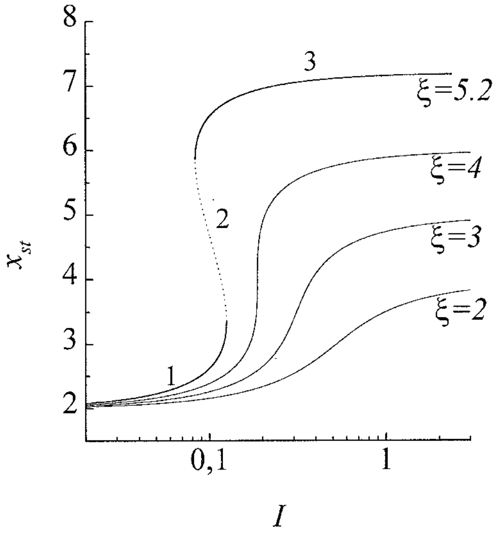

Figure 4. Dependencies of the stationary values x_{st} of the structural variable upon the light intensity I at a number of values of the parameter $\xi = x_B - x_D$. The parts 1 and 3 of the upper curve correspond to the stable states (shown with the solid line). The part 2 of the same curve corresponds to the unstable state of the system (see the text). $x_D = 2$; $k_{AD} = 10$.

Thus the two bifurcation points correspond to the following values of the structural variable and light intensity:

$$x_{I,II} = \frac{x_D + x_B}{2} \pm \Delta;$$
$$I_{I,II} = \frac{k_{AD}}{\exp(x_{I,II})\frac{x_B - x_{I,II}}{x_{I,II} - x_D} - 1}. \tag{20}$$

As it follows from Equation (15) the lower (solid bold curve 1 in Figure 4) and the upper (solid bold curve 3 in Figure 4) branches of the dependence $x_{st}(I)$ correspond to stable states with the very smoothly changing value of the structural variable, whereas curve 2 in Figure 4 corresponds to an unstable state of a system. For the stable stationary states of RC we can write:

$$x_{st}^{(1)}(I) \approx x_D; \quad x_{st}^{(3)}(I) \approx x_B. \tag{21}$$

For the unstable RC states:

$$x_{st}^{(2)} = \frac{x_I - x_{II}}{I_I - I_{II}} I + \frac{I_I x_{II} - I_{II} x_I}{I_I - I_{II}} \approx \frac{-\Delta}{I_{II}} I + x_I, \tag{22}$$

or, even simpler,

$$x_{st}^{(2)} \approx -\Delta I \exp(x_B) + x_B. \tag{23}$$

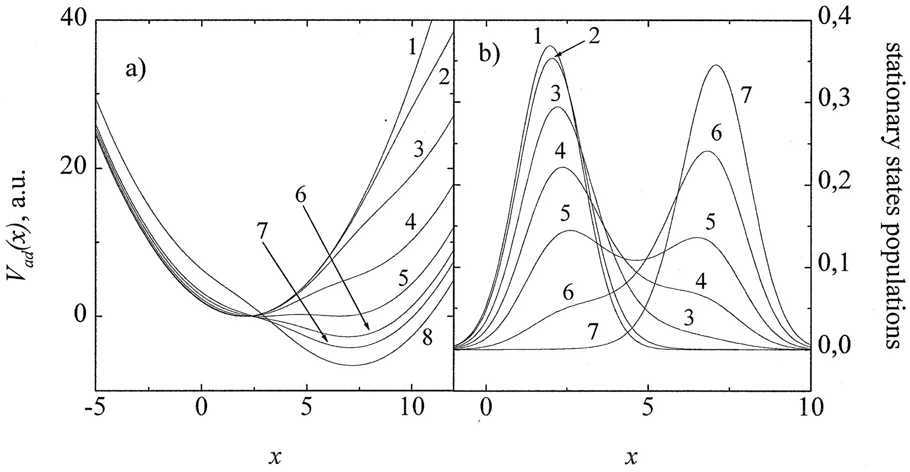

Figure 5. The adiabatic potentials (graph a)) and corresponding populations of the stationary states (graph b)) calculated for the RCs from *Rb. sphaeroides* at different values of actinic light intensity I with the following set of parameters: $\xi = 5.2$; $x_D = 2$; $k_{AD} = 10$; $k = 0.5$. The values of I are: graph a): $1 - 0$; $2 - 5 * 10^{-4}$; $3 - 5 * 10^{-3}$; $4 - 3 * 10^{-2}$; $5 - 10^{-1}$; $6 - 2 * 10^{-1}$; $7 - 3 * 10^{-1}$; $8 - 1$; graph b): $1 - 0$; $2 - 10^{-2}$; $3 - 3 * 10^{-2}$; $4 - 10^{-1}$; $5 - 2 * 10^{-1}$; $6 - 3 * 10^{-1}$; $7 - 1$. The actinic light intensity $I = 1$ corresponds to $2 * 10^{15}$ quanta/(cm^2 s).

The adiabatic potential which corresponds to Equation (15) can be easily obtained from (24):

$$\frac{\partial V_{ad}(x)}{\partial x} = \frac{\partial V_0(x)}{\partial x} - \sum_{i=D,A,B} f_i n_i(x, I), \tag{24}$$

which for the case under consideration leads to:

$$V_{ad} = \frac{k(x - x_D)^2}{2} - k(x_B - x_D) \ln \frac{[1 + \exp(x)]I + k_{AD}}{[1 + \exp(x_D)]I + k_{AD}} + C(I), \tag{25}$$

where

$$C(I) = \frac{k}{2} \left[\frac{(x_B - x_D) I \exp(x_D)}{I(1 + \exp(x_D)) + k_{AD}} \right]^2$$

is an integration constant which is chosen from the condition: $V_{ad}(x = x_0,\ I = 0) = 0$.

Figure 5 shows the development of both the adiabatic potential (graph a) and the population of the stationary states (graph b) at the chosen value of parameter $\xi = 5.2$ upon variation of the light intensity I. The relative thermodynamic population of the stationary states inverts from 1 for the ‘dark’ state under low values of I ($I \leq I_I$) to 1 for the ‘light’ state at $I \geq I_{II}$ (see Figure 5). Experiment shows, however, that the equilibrium between the ‘dark’ and ‘light’ states of RC within the

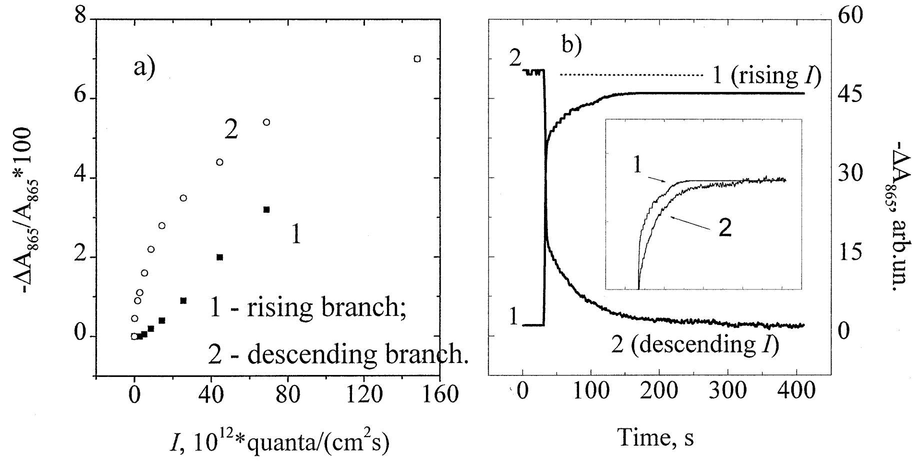

Figure 6. (a) Dependence of the optical absorbance changes δ of R-26 Q_B-containing RCs upon the prehistory of illumination intensity (1 – rising branch, 2 – descending branch, see [23] for more experimental details). (b) Dynamics of reaching a quasi-equilibrium following stepwise increase (curve 1) and stepwise decrease (curve 2) of the exciting light intensity within the interval $10^{12}-10^{14}$ quanta/(cm^2 s) for RCs from *Rb. sphaeroides* with reconstituted Q_B activity. The difference of the amplitudes reflects the hysteresis in the system. *Insert*: the same curves put together to demonstrate the difference in the kinetics. Experimental conditions are as those indicated in Figure 1.

bistability domain is hardly observed, see Figure 6a and [15, 17, 22, 23] (we mean here that the thermal transitions between two minimums of the adiabatic potential within the bistability domain are negligible). So, we rewrite Equation (15) in the form:

$$q\frac{dx}{dt} = -\frac{\partial V^I_{ad}(x)}{\partial x} + \sqrt{2D}\xi(t), \tag{26}$$

assuming that

$$V^I_{ad}(x) \approx \begin{cases} \dfrac{k_D(x-x_D)^2}{2}, & x \sim x_D; \\ \dfrac{k_B(x-x_B)^2}{2}, & x \sim x_B. \end{cases} \tag{27}$$

For the average over the fluctuations $\xi(t)$ values of $x(t)$ we obtain from Equation (26):

$$\bar{x}(t) = \begin{cases} x_d(t) = x_D - [x(0) - x_D]\exp\left(-\dfrac{1}{\tau^D_x}\right) & x(0) < x^{(2)}_s; \\ x_l(t) = x_B - [x(0) - x_B]\exp\left(-\dfrac{t}{\tau^B_x}\right) & x(0) > x^{(2)}_s; \end{cases} \tag{28}$$

$$\tau^j_x = \frac{k_j}{q}, \quad j = D, B,$$

where k_j are the elasticity constants for the potential of the 'dark' state (k_D) and of the 'light' state (k_B) (note that these constants are different in general, though for simplicity we set them equal each other, see Equation (18)).

The variation of the intensity I of an actinic light influences weakly the values of the coordinates of the stationary states $x_{st}^{(1)}$ and $x_{st}^{(3)}$ that correspond to the minima of the adiabatic potential. However, the influence of I on the position of the maximum of the adiabatic potential is strong enough (see Equations (21), (22)). Thus, with I increasing, the coordinate of the potential maximum $x_{st}^{(2)}$ gets shifted towards the 'dark' conformational coordinate $x_{st}^{(1)}$ that results in decreasing the population of the 'dark' state of RC. The method of calculation of populations ν_l and ν_d of two different minima of the adiabatic potential has been elaborated and described in the recent work [17]. Here we note only that it allows the probabilities $\nu_d(t)$ and $\nu_l(t) = 1-\nu_d(t)$ of realization of the 'dark' and the 'light' RC states to be calculated. These quantities depend on both the value of the actinic light intensity and the pre-history of the sample irradiation. Then for an arbitrary function $q_i(x)$ that depends on both the electronic (i) and structural (x) variables and has the meaning of an experimentally measured quantity, the average over the electronic variables value may be written as follows:

$$\langle q_i(x)\rangle_{el} = \sum_i q_i(x) n_i(t,x). \tag{29}$$

This quantity reaches its stationary value on the time scale $t \geq \tau_{el}$:

$$\langle q_i(x)\rangle_{el} = \sum_i q_i(x) n_i(I,x) \tag{30}$$

where the values of $n_i(I,x)$ are given by Equation (16). After averaging the quantities (30) over the structural variable x and using the approximation (27) we finally obtain:

$$\Big\langle \langle q_i(x)\rangle_{el} \Big\rangle_x \approx \nu_d(t)\langle q_i(\bar{x}_d(t))\rangle_{el} + \nu_l(t)\langle q_i(\bar{x}_l(t))\rangle_{el}, \tag{31}$$

which is the expression for the experimentally measured stationary values. For example, to obtain the absorbance changes expression we should substitute $1-\Delta_{iD}$ for $q_i(x)$, where Δ_{iD} stands for Kronecker's symbol. Then, according to (29–31), $\delta(t) \cong \nu_d(t)\sigma_I(x_d(t)) + \nu_l(t)\sigma_I(x_l(t))$ which is employed in the next section.

Discussion and Conclusion

Consideration of self-consistent behavior of electronic and structural variables of RC shows that the slow structural rearrangements of RC which are launched by the electron localization on Q_B are stored after the multiple turnover events. This causes the shift of the stationary value of the conformational coordinate in the 'light' state to the higher value as against that for the 'dark' conformational state of RC. In accord with Equation (11) this leads to an exponential increase of the average lifetime $\tau_d(x)$ of the charge separated state. One can consider this as a non-linear effect of self-regulation of the electron flux which results in an increase of the efficiency of the charge separation process in RC. A more dramatic situation takes place when a new light-induced state of RC appears. This corresponds to the emergence of the second minimum of the system adiabatic potential at large values of the structural variable when $I = I_I^{cr}$. As it can be seen from Figure 4, the stationary values of the structural variable are almost independent of the intensity of stationary actinic light I in both the ‘dark’ and ‘light’ states of the system. Then we can write:

$$\frac{\tau_d[x^{(3)}(I)]}{\tau_d[x^{(1)}(I)]} \approx \exp(x_B - x_D). \tag{32}$$

In accord with experimental results, this ratio varies ca. 200 times, depending on the illumination conditions. This corresponds to $x_B - x_D \approx 5.3$, or light-induced changes of the free energy difference ΔG_{AB} by ca. 0.13 eV. Such a large change of ΔG_{AB} can be related to the (slow) component of Q_B-site polarization that takes place after the electron localization on Q_B. The pronounced light-induced structural changes within the Q_B binding pocket (discovered recently by X-ray structural analysis, see Ref. [14] where the authors describe the light-induced shift by 5 Å with simultaneous 180° propeller twisting of the ubiquinone from its equilibrium position in the dark) support our conclusion. The transition of the system to the new light-induced conformational state becomes thermodynamically favorable (see Figure 5) at the light intensities $I \geq \frac{I_I^{cr}+I_{II}^{cr}}{2}$, though its probability remains rather low due to the barrier between the two states. The transition takes place at $I > I_{II}^{cr}$ (at $I \approx 10^{14} - 10^{15}$ quanta/cm^2 s) and may be realized completely during the time interval 10–15 min (see also below). The essential rearrangement within the Q_B binding pocket causes a strong increase of the charge separation efficiency. The high efficiency remains preserved even under decrease of the actinic light intensity down to $I \approx 10^{13}$ quanta/cm^2 s (see Figure 4), ensuring thus the stable regime of RC functioning in a wide interval of the actinic light intensity. Thus, the bistability domain may provide both the stabilization of the system in its ‘dark’ or ‘light’ state and the switching of the RC between these two states.

It is relevant to consider the kinetics of RC electronic relaxation under variation of the intensity of stationary actinic light. All the conclusions made above concerning the fixed intensity of actinic light I are obviously valid for the adiabatic variation of I, i.e. when

$$\frac{d\ln I}{dt} \ll \tau_{el}^{-1}. \tag{33}$$

The situation is different when the intensity of the stationary actinic light changes sharply from $I = I_i$ to $I = I_f$. Then in accord with Equation (31) and taking into account Equations (2), (15), we should apply the expression for $\delta(t)$ which describes the phase of ‘free’ electronic relaxation (see the fast component of electronic relaxation in Figure 6b):

$$\delta(t) = \nu_d(I_i)\left\{\sigma_{I_f}(x_D) + \left[\sigma_{I_i}(x_D) - \sigma_{I_f}(x_D)\right]\exp\left(-\frac{t}{\tau_{el}(x_D)}\right)\right\} + \\ +\nu_l(I_i)\left\{\sigma_{I_f}(x_B) + \left[\sigma_{I_i}(x_B) - \sigma_{I_f}(x_B)\right]\exp\left(-\frac{t}{\tau_{el}(x_B)}\right)\right\}, \tag{34}$$

so that at $t > \tau_{el}$:

$$\delta(t) = \nu_d(I_i)\sigma_{I_f}(x_D) + \nu_l(I_i)\sigma_{I_f}(x_B), \tag{35}$$

where, obviously, $\sigma_I(x_B) \gg \sigma_I(x_D)$, i.e. the efficiency of the charge separation in the ‘light’ conformational state is much higher than in the ‘dark’ conformational state.

On the second, slow phase of the electronic relaxation (time scale of minutes, Figure 6b), the system behavior is controlled by the redistribution of the electron density inside each of two valleys of adiabatic potential. This is a kind of ‘controlled’ electronic relaxation related to the change of the position $x_{st}^{(2)}(I)$ of the potential barrier that causes the further redistribution of RCs between the ‘dark’ and the ‘light’ states (the corresponding new populations of the potential valleys are $\nu_d(I_f)$ and $\nu_l(I_f)$. Below is the quantitative description of the slow phase of electronic relaxation.

a) the case $I_f > I_i$.
Then $\nu_d(I_i) - \nu_d(I_f) = \nu_l(I_f) - \nu_l(I_i) > 0$ and for the time $t > \tau_{el}$ an increase of $\delta(t)$ is described by the expression:

$$\delta_+(t) \approx \nu_d(I_f)\sigma_{I_f}(x_D) + \nu_l(I_i)\sigma_{I_f}(x_B) + [\nu_d(I_i) - \nu_d(I_f)]\sigma_{I_f}(\bar{x}_l(t)) \tag{36}$$

where:

$$\bar{x}_l(t) = x_B + (x_D - x_B)\exp\left(-\frac{t}{\tau_x^B}\right). \tag{36a}$$

b) the case $I_f < I_i$.
Then $\nu_d(I_f) - \nu_d(I_i) = \nu_l(I_i) - \nu_l(I_f) > 0$ and the quantity $\delta(t)$ decreases in accord with:

$$\delta_-(t) \approx \nu_d(I_i)\sigma_{I_f}(x_D) + \nu_l(I_f)\sigma_{I_f}(x_B) + [\nu_l(I_i) - \nu_l(I_f)]\sigma_{I_f}(\bar{x}_d(t)) \tag{37}$$

where:

$$\bar{x}_d(t) = x_D + (x_B - x_D)\exp\left(-\frac{t}{\tau_x^D}\right). \tag{37a}$$

As it can be easily seen, the experimentally observed electronic relaxation kinetics (see e.g. Figure 6b) proves the adiabatic character of the ET events with respect to the slow structural rearrangements of RC in qualitative agreement with our conclusions. Detailed processing of such data could make it possible to determine the specific parameters of the adiabatic potential responsible for self-consistent behavior of the electronic-conformational system of RC, etc. This as well as more rigorous stochastic theory beyond the first order adiabatic approximation will be presented elsewhere.

Finally, we note that similar effects of long-time RC protein relaxation observed in the study of proton binding kinetics and leading to two distinct conformations of RC were recently reported [29], so that, as should be expected, the protonatable groups can play an important control role in our predictions.

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Address for correspondence: L.N. Christophorov, Bogoliubov Institute for Theoretical Physics NAS Ukraine, 14B Metrologichna Str., Kiev 252143 Ukraine.
E-mail: lchrist@bitp.kiev.ua