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Self-regulation phenomenon of electron-conformational transitions in biological electron transfer under nonequilibrium conditions

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We present a theoretical description and experimental evidence for a nonlinear effect of repetitive stepwise alteration of photoexcitation intensity on quasi-stationary-state optical absorption changes of an electron transfer biomolecular system. This effect was studied for photosynthetic reaction centers, and analyzed with a model that takes into account correlative behavior of charges and slow structural motions of the macromolecule. Theoretically, photosynthetic reaction centers can be described with a conformational potential, the shape of which depends on the intensity of the photoexcitation. This is in agreement with previous studies on hysteretic behavior of reaction center absorbance, causing bifurcational appearance of a ‘‘light’’ conformational state that coexists with a ‘‘dark’’ conformational state within a particular range of exciting light intensity. The essential assumption that allows for observation of the effects reported here is the nonequilibrium character of macromolecule transitions between dark and light conformational states. Experimentally observed behavior of absorbance changes, caused by a sequence of photoexcitation intensity alternations, indicates unambiguously that the reaction center functions as a highly nonequilibrium, nonlinear dynamic system with a pronounced capability for regulation of the photoelectron flux by slow structural motions. This system could be considered as a molecular motor for pumping the charge separated state. [S1063-651X(99)08703-6]

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INTRODUCTION

The observation of new physical phenomena and effects was often related to the discovery of new classes of objects under physical investigation. A number of traditional objects of solid-state physics easily exemplifies this tendency: perfect, doped, or liquid crystals, glasses, etc. Biological systems, some of which have already become the objects of physical studies, may occupy a specific individual place in this rank. A pronounced example is charge transfer biomolecular systems. Among these systems is the pigment-protein complex—the photosynthetic reaction center (RC)—effectively performing charge separation of about 40 Å due to the energy of the primary donor P (bacteriochlorophyll dimer) photoexcitation ($P\xrightarrow{h\nu}P^*$)[1–4]. An isolated RC usually consists of three protein subunits containing specific molecules, or redox cofactors, which perform the function of an electron transfer (ET) chain for a photoexcited electron (see the schematic representation in Fig. 1). Structures of RCs from several species have been determined with high resolution x-ray analysis [5–7]. Spectroscopic studies of light-induced absorbance changes $\Delta A_{865}(I)$ in the bacteriochlorophyll dimer absorption band ($\lambda=865$ nm) provide an estimate of the fraction of RCs with separated charges at a given light intensity I (Refs. [1,4], and references therein). The main characteristic quantity that may be obtained from experiments in this case is:

$$\delta(I)=\frac{A_{865}(0)-A_{865}(I)}{A_{865}(0)}=-\frac{\Delta A_{865}(I)}{A_{865}(0)},\qquad 0\leq\delta\leq1. \tag{1}$$

$\delta(I)$ determines the probability of charge separation in a single RC under conditions of photoexcitation with light of intensity I.

The hysteretic behavior of the quantity $\delta(I)$under conditions of quasistationary variation of a light intensity I have been observed recently [8,9]. The authors showed that such a $\delta(I)$ dependence could be associated with dynamic self-organization taking place due to interaction between sepa-

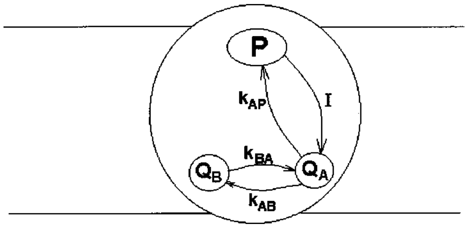

FIG. 1. Simplified schematic representation of the structure of the photosynthetic reaction center. The redox centers (cofactors) responsible for the charge separation and transfer across the membrane are shown: P is the bacteriochlorophyll dimer (the primary photoelectron donor), and Q_A and Q_B are the primary and secondary quinone acceptors. The short living intermediate acceptors are not shown in the scheme. I is the intensity of photoexcitation, measured in units of the number of charge separation events per second, and k_{AP}, k_{AB}, and k_{BA} are the rate constants of ET between redox cofactors (in s^{-1}). The direct charge recombination from Q_B to P is not shown because of the low probability of this process [11,12].

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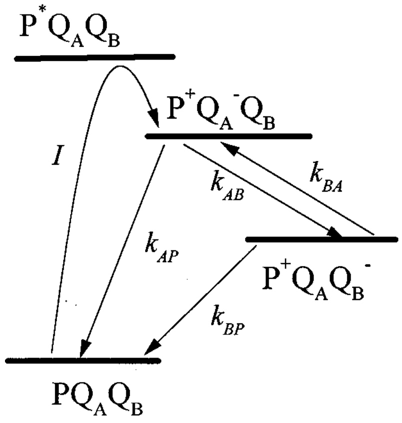

FIG. 2. Scheme of electronic levels for the photosynthetic RC. PQ_AQ_B and $P^*Q_AQ_B$ are the states with unexcited and photoexcited primary electron donors, respectively; $P^+Q_A^-Q_B$ and $P^+Q_AQ_B^-$ are the charge-separated states with an electron localized on the primary and secondary quinone acceptors, respectively. The short-living states with electron localization on intermediate acceptors are not shown. The arrows show the possible transitions of the electron between various states. For RC's from *Rb.sphaeroides* under normal conditions $k_{AP}\approx 10\ \mathrm{s}^{-1}$, $k_{BP}\leqslant 0.1\ \mathrm{s}^{-1}$, $k_{AB}\approx 10^4\ \mathrm{s}^{-1}$, and $k_{BA}\approx 10^3\ \mathrm{s}^{-1}$ [11,12]. The value of all rate constants are given for dark-adapted RC's.

rated charges and RC structure. The slow component of medium polarization, with a characteristic lifetime τ_x from seconds to tens of minutes, was assumed to play a decisive role in the observed effects. To account for the observed effects, a model was based on gradual rearrangements of the RC structure concurrent with consecutive elementary steps of RC turnover [8,9] (i.e., charge separation events resulting in electron localization on a secondary quinone acceptor Q_B followed by a charge recombination; see Fig. 2). The extent of the structural rearrangement depended on the frequency of the turnover, which is a function of quasistationary photoexcitation intensity *I*. The basis for this nontrivial behavior of the system is a backward action of rearranged structure on the rate of charge recombination. A violation of Le Chatelier's principle occurs as the result of this action. Each small fluctuation of the generalized structural variable *x* then becomes important due to the strong backward interaction in the system. A new equilibrium configuration of the system (conformational state) appears at a particular level of photoexcitation intensity. This state (the so-called ''light'' state) coexists with the initial ''dark'' conformational state within a particular range of exciting light intensity (bistability domain). A further increase of *I* caused a bifurcational vanishing of the dark conformational state of the RC. Experimental results describing the photoinduced bistability of RC absorbance were described in Refs. [8,9]. The theory of this effect was presented in Refs. [8,10].

Current work describes a nonlinear effect of self-regulation of the electronic-conformational transitions in RC's caused by strong electronic-conformational interactions in the system. It is shown below that the flexible macromolecular system performing charge separation and transfer may work as a molecular motor for a pumping of the charge separated state under nonequilibrium conditions. Also reported is the observation of a physical effect—a light-controlled change of stationary-state optical absorption under repetitive variation of the exciting light intensity.

THEORY

The scheme of photoinduced ET from the donor *P* to the final (secondary quinone) acceptor Q_B, followed by charge recombination in the RC (see Fig. 2), may be reduced to only two electronic states with electron localization either on the photodonor *P* or on the acceptor Q_B (see Refs. [11,12]):

$$P \underset{\tau_{BP}\approx k_{AP}^{-1}\exp\left(\dfrac{\Delta G_{AB}}{k_BT}\right)}{\overset{\tau_{PB}=I^{-1}}{\rightleftarrows}} B, \tag{2}$$

in which ΔG_{AB} is the free energy difference for an electron localized at Q_A and Q_B, and τ_{PB} is the average time of electronic transition to state B ($P^+Q_AQ_B^-$; see Fig. 2) determined by the photoexcitation intensity *I*. The time of the back transition of the electron from Q_B to P (the time of the electronic relaxation τ_{BP}) increases exponentially with an increase of the generalized structural variable value chosen in the dimensionless form [10]

$$x\equiv\frac{\Delta G_{AB}}{k_bT}. \tag{3}$$

Such a strong $\tau_{BP}(x)$dependence is due to the experimentally determined fact that the back reaction $P^+Q_AQ_B^- \rightarrow PQ_AQ_B$ occurs mainly via the state with electron localization on Q_A at normal conditions (see, e.g., Refs. [11,12]). Recent studies indicate a possibility for the value ΔG_{AB} to change by $\sim 6k_BT$, and even more under conditions of prolonged illumination of RC's [10]. This striking result has been attributed to light-induced bifurcation followed by the formation of a new conformational state in RC's. A drastic illumination-induced increase by more than two orders of magnitude of the electronic recombination time constant τ_{BP} has been reported recently for both the Q_B-inhibited [13] and Q_B-active RCs [14]. This fact has been attributed to a charge recombination from the charge-separated, light-adapted state $P^+(Q_AQ_B)^-$, which is characterized by a considerably changed structure in comparison to that of the dark-adapted state. The value k_{AP} was shown not to be influenced significantly by the exciting light of the normally used intensities ($\leqslant 100\ \mathrm{mW/cm}^2$) [14–17]. A large increase of the charge recombination time constant for the Q_B-inhibited RC's [13] was presumably caused by very high intensities of continuous wave exciting light used in that work. In the current work only lower intensities of exciting light are used; therefore, the light-induced changes of k_{AP} are not taken into account. Assume that a slow component *x* of the medium polarization is formed, mainly near the binding site of the secondary quinone acceptor Q_B [8,10,14]. This is supported by the results of x-ray studies of light-induced structural changes of the RC [7]. These studies revealed a large light-induced shift and rotation of the ubiquinone molecule in the Q_B binding pocket from its equilibrium position in the dark.

No significant light-induced structural changes have been observed in the Q_A binding pocket.

Choose a movement equation for x as the equation for the overdamped mode $(d^2x/dx^2 \approx 0)$ [8,10]:

$$q\frac{dx}{dt} = -\frac{\partial V_P(x)}{\partial x} - \left(\frac{\partial V_B(x)}{\partial x} - \frac{\partial V_P(x)}{\partial x}\right)\Theta(t) + \sqrt{2D}\zeta(t) = 0, \tag{4}$$

in which q is a generalized friction coefficient; $V_P(x)$ and $V_B(x)$ are the potential energies of the structural variable for the case of electron localization on P and Q_B, respectively; $\zeta(t)$ is the δ-correlated random process $[\overline{\zeta(t)\zeta(t+\tau)} = \delta(\tau)]$ that models thermal fluctuations of the structural variable; $\sqrt{2D}$ is an amplitude of these fluctuations; $D = k_BT/q$ is a generalized diffusion constant for the structural variable; and $\Theta(t)$ is a random function that describes the process of the photoelectron localization on Q_B in accord with scheme (2):

$$\Theta(t) = \begin{cases} 0 & \text{if the electron is localized on P} \\ 1 & \text{if the electron is localized on Q}_\text{B}. \end{cases} \tag{5}$$

The probabilities $n_P(t)$and $n_B(t)$ of electron localization on P and Q_B, respectively, are determined from the balance equations

$$\begin{aligned} \frac{dn_P(t)}{dt} &= -In_P(t) + k_{AP}\exp(-x)n_B(t), \\ \frac{dn_B(t)}{dt} &= -k_{AP}\exp(-x)n_B(t) + In_P(t), \end{aligned} \tag{6}$$

and the normalization condition is $n_P(t) + n_B(t) = 1$.

The joint solution of Eqs. (4) and (6) provides a self-consistent description of the stochastic behavior of the electronic and structural variables. It is a coupled random process with the mutual distribution function $P_t(\alpha,x)$, $\alpha = P$and B, that satisfies equations

$$\begin{aligned} \frac{\partial P_t(P,x)}{\partial t} &= D\frac{\partial}{\partial x}\left[\frac{1}{k_BT}\frac{\partial V_P}{\partial x}P_t(P,x) + \frac{\partial P_t(P,x)}{\partial x}\right] \\ &\quad - IP_t(P,x) + k_{AP}\exp(-x)P_t(B,x), \\ \frac{\partial P_t(B,x)}{\partial t} &= D\frac{\partial}{\partial x}\left[\frac{1}{k_BT}\frac{\partial V_B}{\partial x}P_t(B,x) + \frac{\partial P_t(B,x)}{\partial x}\right] \\ &\quad + IP_t(P,x) - k_{AP}\exp(-x)P_t(B,x). \end{aligned} \tag{7}$$

The method of passage from Eqs. (4) and (6) to Eq. (7) is given in Ref. [18] only for the case when the rate constants of the transitions do not depend on the variable x (the backward action is absent), and the term $\sqrt{2D}\xi(t)$ in Eq. (4) is absent. Generalization of this method for the case of x-dependent rate constants was made in Ref. [19]. An approach based on a consideration of Eqs. (7) as the initial equations is possible as well, but such an approach would be more formal and would not help to describe clearly the physical meaning of the phenomenon studied.

The stationary populations of states with an electron localization on P and Q_B can be obtained from Eqs. (6):

$$\begin{aligned} n_P(\infty) &= \frac{k_{AD}\exp(-x)}{I + k_{AD}\exp(-x)}, \\ n_B(\infty) &= \frac{I}{I + k_{AD}\exp(-x)}. \end{aligned} \tag{8}$$

These populations may be reached through a monoexponential process with the characteristic time constant $\tau_\text{el} = (I + k_{AD}\exp(-x))^{-1}$. This value does not exceed 1 s for all possible sets of RC parameters that correspond to real experimental conditions due to either the term $k_{AP}\exp(-x)$ under low excitation intensity or the term I at high intensity (Refs. [1,4,11], and references therein). The only exclusion is when the system is in a dark relaxation state, and is characterized by a large value of the structural parameter x. This case is not studied in the current work. Therefore, for current consideration, the correlation $\tau_x \gg \tau_\text{el}$ is fulfilled and the solution of Eq. (7) can be found in the adiabatic approximation

$$P_t(\alpha,x) = n_a(t)P_t(x,I), \quad \alpha = P,B, \tag{9}$$

in which the distribution function of the structural variable $P_t(x,I)$ satisfies the equation

$$\frac{\partial P_t(x,I)}{\partial t} = D\frac{\partial}{\partial x}\left[\frac{1}{k_BT}\frac{\partial V_\text{eff}(x,I)}{\partial x}P_t(x,I) + \frac{\partial P_t(x,I)}{\partial x}\right]. \tag{10}$$

$V_\text{eff}(x,I)$ is an adiabatic potential for the structural variable corresponding to the photoexcitation intensity I. This potential is of statistic origin, and can be defined from the equation

$$\frac{\partial V_\text{eff}(x,I)}{\partial x} = \frac{\partial V_P(x)}{\partial x}n_P(\infty) + \frac{\partial V_B(x)}{\partial x}n_B(\infty). \tag{11}$$

An application of the adiabatic approximation (9) corresponds to a substitution of the function $\Theta(t)$in Eq. (4) by averaging over the time value $n_B(t) = \overline{\Theta(t)}$. The population $n_B(t)$ is a time-dependent function, so averaging is made over the time interval $\tau_x > \tau_0 \gg \tau_\text{el}$. The physical meaning of such a procedure is obvious: the slow structural variable is not sensitive to single jumps of the photoelectron between the states P and B, but susceptible only to their average populations or even to the average charge distribution corresponding to multiple successive ET events in the system.

Exact solutions of Eqs. (4) and (6) have been obtained for the stationary case in the absence of thermal fluctuations [19]. Analysis of the limiting transition case $\tau_x \gg \tau_\text{el}$, given in the cited work, indicated the correctness of the adiabatic approximation (9). The influence of fast variable fluctuations on the slow variable may give first order small terms to the effective potential $V_\text{eff}(x,I)$ and the diffusion constant D. A separate work will show that these terms can be neglected for the current case. Thus, for the sake of simplicity, do not take small terms of this type into account. For the RC's stationary distribution function over the structural variable x at a fixed value of the excitation intensity I a solution is obtained:

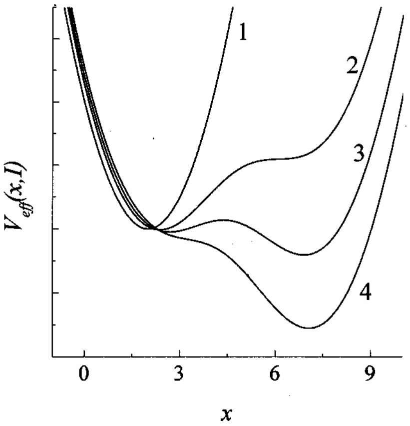

FIG. 3. Dependence of the effective adiabatic potential $V_{\mathrm{eff}}(x,I)$ from photoexcitation intensity I calculated from Eq. (10). The curves correspond to the following values of light intensity I: (1) 0, (2) 0.07 s^{-1}, (3) 0.1 s^{-1}, and (4) 0.13 s^{-1}. The value $I=1\ \mathrm{s}^{-1}$ corresponds to the light intensity value 3×10^{15} quanta/cm^2 s) (see Ref. [22]). The probe potentials $V_P(x)$ and $V_B(x)$are taken as parabolic shaped. The values of parameters used in calculations are $k_{AP}=10\ \mathrm{s}^{-1}$and $x_P=2$; $\Delta x=5.3$.

$$P_S(x,I)=Z^{-1}\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right),$$
$$Z=\int_{-\infty}^{\infty}\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right)dx. \qquad (12)$$

Consider the simplest case of harmonic initial probe potentials $V_P(x)$ and $V_B(x)$. They determine the shape of effective adiabatic potential $V_{\mathrm{eff}}(x,I)$ in accord with Eq. (11). Then, for the case of electron localization on P, the potential minimum corresponds to $x=x_P$, whereas for the case of electron localization on Q_B, the global minimum of the potential shifts to $x=x_B$. Figure 3 shows the dependence of the adiabatic potential upon the structural variable x at the value of the parameter $\Delta x=x_B-x_P=5.3$. This value is close to the estimate from experiments on hysteretic behavior of RC's [10]. The critical value of this parameter, above which the bistability appears in the system, was found to be equal to 4 [8,10]. From the condition $\partial V_{\mathrm{eff}}(x,I)/\partial x=0$, one can find the extrema x_s of the adiabatic potential as a function of photoexcitation intensity. Taking into account Eqs. (8) and (11), this condition gives the dependence $I(x_S)$:

$$I=-k_{AP}\exp(-x_S)\frac{\partial V_P(x_S)/\partial x_S}{\partial V_B(x_S)/\partial x_S}, \qquad (13)$$

in which x_S are the stationary values of the structural variable corresponding to the extrema of the adiabatic potential $V_{\mathrm{eff}}(x,I)$. The reciprocal dependence $x_S(I)$ is shown in Fig. 4 for harmonic potentials $V_P(x)$ and $V_B(x)$ with equal curvature, and for the value $\Delta x=x_B-x_P=5.3$. The set of minima of the conformational potential $V_{\mathrm{eff}}(x,I)$ that correspond to the branches $x_s^{(1)}(I)$ and $x_S^{(3)}(I)$ of the dependence depicted in Fig. 4 should be considered as that defining the conformational coordinates, whereas correlation (13) is the condition for their value determination. The intermediate branch $x_S^{(2)}(I)$ of the stationary states of the structural variable corresponds to the sets of maxima of the adiabatic potential $V_{\mathrm{eff}}(x,I)$. The position of the maximum, i.e., the value $x_S^{(2)}$, changes considerably under the increase of excitation light intensity from $x_{cr}^{I}\approx x_P$ to $x_{cr}^{II}\approx x_B$. The positions of the minima barely change under I variation, and the corresponding values are $x_S^{(1)}(I)\approx x_P$ and $x_S^{(3)}(I)\approx x_B$ for the dark and light states, respectively. The equilibration of the distribution function near the adiabatic potential minima (corresponding to the dark and light conformational states) occurs on a time scale of minutes [8,10,14]. This means that the conformational transition through the potential barrier (where $x=x_S^{(2)}$) could occur on a time scale of several hours; thus it can be considered forbidden. In this case the total distribution function over the whole range of x variation is no longer equilibrium . Thus we can write

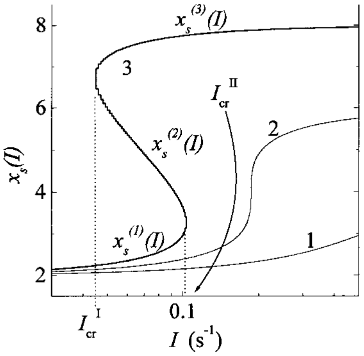

FIG. 4. Dependence of the stationary-state values $x_s(I)$ of the structural variable from photoexcitation intensity I calculated from Eq. (12) at various values of the parameter $\Delta x=x_B-x_P$, which characterizes the magnitude of an electron-conformational interaction: $\Delta x=1.5$(1), $\Delta x=4$ (2), and $\Delta x=5.3$(3). $I_{\mathrm{cr}}^{\mathrm{I}}$ and $I_{\mathrm{cr}}^{\mathrm{II}}$ are the values of photoexcitation intensity corresponding to appearance of ''light'' and disappearance of ''dark'' conformational states, respectively. The curves are calculated for the following values of the parameters: $k_{AP}=10\ \mathrm{s}^{-1}$ and $x_P=2$.

$$P(x,I)=\begin{cases} v_dP_{\mathrm{dark}}(x,I), & x<x_S^{(2)}(I)\\ v_lP_{\mathrm{light}}(x,I), & x>x_S^{(2)}(I),\end{cases} \qquad (14)$$

in which the quasiequilibrium distribution functions over the configurations of the ''light'' and ''dark'' conformational states are

$$P_{\mathrm{dark}}(x,I)=\frac{1}{Z_d}\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right),$$
$$Z_d=\int_{-\infty}^{x_S^{(2)}(I)}dx\,\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right),$$
$$P_{\mathrm{light}}(x,I)=\frac{1}{Z_l}\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right), \qquad (15)$$
$$Z_l=\int_{x_S^{(2)}(I)}^{\infty}dx\,\exp\left(-\frac{V_{\mathrm{eff}}(x,I)}{k_BT}\right),$$

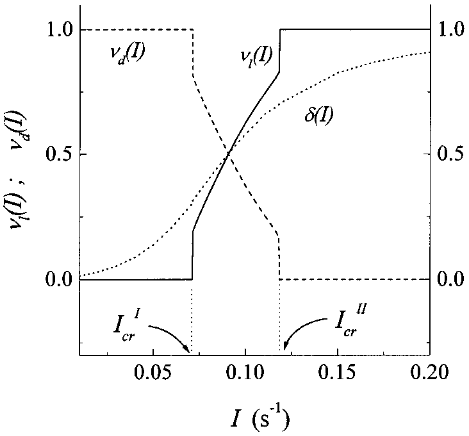

FIG. 5. Dependencies of the population of the "light" conformational state $v_l(I)$ (solid line) and the "dark" conformational state $v_d(I)$ (dashed line) from the intensity of the stationary-state photoexcitation I, which was reached after its stepwise variation from zero. The behavior of the experimentally measured quantity $\delta(I)$ under the same conditions calculated from Eq. (15) is shown by the dotted line. The values of parameters are $k_{AP}=10\ \mathrm{s}^{-1}$ and $x_P=2$; $\Delta x=5.3$.

and the quantities $v_d=Z_d/Z$ and $v_l=Z_l/Z$ define the integral populations of the dark and light conformational states, respectively.

In the case of an instantaneous increase of exciting light intensity from zero, when $V_{\mathrm{eff}}(x,0)=V_P(x)$and $P_S(x,0)=Z^{-1}\exp(-V_P(x)/k_BT)$ up to the value $I\in[I_{\mathrm{cr}}^{I},I_{\mathrm{cr}}^{II}]$, such a sharp stepwise variation of I results in cutting off part of the population $\Delta v_d=\int_{x_S^{(2)}}^{\infty}P_S(x,0)dx$ from the initial population of the dark conformational state that was equal to $v_1(0)=1$ at $I=0$. As a consequence of this stepwise increase from $I=0$ to $I\in[I_{cr}^{I},I_{cr}^{II}]$, the populations of the dark and light conformational states becomes equal to $v_d(I)=1-\Delta v_d(I)$ and $v_l(I)=1-v_d(I)=\Delta v_d(I)$, respectively.

The calculated dependencies $v_d(I)$and $v_l(I)$ for the RC's are shown in Fig. 5. The jumps of values v_d and v_l are related to the bifurcational character of both the appearance of the light conformational state at $I=I_{\mathrm{cr}}^{I}$ and the disappearance of the dark conformational state at $I=I_{\mathrm{cr}}^{\mathrm{II}}$. These jumps could not be observed for experimentally measured quantities, because the depths of the potential valleys that appear or disappear bifurcationally near the critical values $I_{\mathrm{cr}}^{\mathrm{I}}$ and $I_{\mathrm{cr}}^{\mathrm{I}}$ approach zero, and the statistical weight of these states in the measured quantity is negligible. Indeed, the experimentally measured quantity $\delta(I)$ [Eq. (1)] includes the averaging over the RC's distribution function $P(x,I)$. Taking into account Eq. (14), we obtain

$$\delta(I)=\int_{-\infty}^{\infty}\frac{I}{I+k_{AP}\exp(-x)}P(x,I)dx$$
$$=v_d(I)\,\delta_{\mathrm{dark}}(I)+v_l(I)\,\delta_{\mathrm{light}}(I),\qquad(16)$$

in which

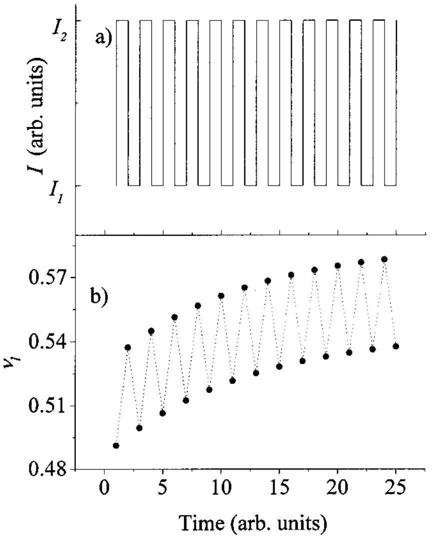

FIG. 6. (a) Protocol of an exciting light intensity alteration between two stationary-state levels $I_1=0.09\ \mathrm{s}^{-1}$ and $I_2=0.1\ \mathrm{s}^{-1}$. (b) Changes of the value $v_l(I)$ calculated from Eqs. (18)–(22) for the values of parameters $\Delta x=5.3$ and $k_{AD}=10\ \mathrm{s}^{-1}$, and under the condition of instantaneous alteration of the exciting light intensity, in accordance with the protocol shown in panel (a). Within the bistability domain, $\delta(I)\approx v_l(I)$; see Eq. (27).

$$\delta_{\mathrm{dark}}(I)=\int_{-\infty}^{x_S^{(2)}(I)}\frac{I}{I+k_{AP}\exp(-x)}P_{\mathrm{dark}}(x,I)dx,$$
$$\qquad(17)$$
$$\delta_{\mathrm{light}}(I)=\int_{x_S^{(2)}(I)}^{\infty}\frac{I}{I+k_{AP}\exp(-x)}P_{\mathrm{light}}(x,I)dx.$$

The dependence of $\delta(I)$, calculated in accord with Eqs. (16) and (17), is shown in Fig. 5 by the dotted line. The shape of the curve is qualitatively the same as that obtained in earlier experiments [8,9].

The functions $v_d(I)$ and $v_l(I)$ are not unambiguously determined by exciting light intensity functions; they depend on the protocol of the $I(t)$ variation. These functions are functionally dependent quantities of $I(t)$.

In a sequence of stepwise alterations of exciting light intensity from some particular value I_1 to a different value I_2 and back, applied in equal time intervals, let $I_{1,2}\in[I_{cr}^{I},I_{cr}^{II}]$ and $I_2>I_1$ [Fig. 6(a)]. The population v_d of the dark conformational state decreases by a factor $q<1$ concurrently with each step of a sharp stepwise increase of light intensity from I_1 to I_2. Similarly, the population v_l of the light conformational state decreases by a factor $p<1$ concurrently with each decreasing step of the light intensity from the value I_2 to I_1:

$$\text{when }I_1\xrightarrow{\text{increase}}I_2,\quad v_d^{(n)}(I_2)=qv_d^{(n)}(I_1),$$
$$\qquad(18)$$
$$\text{when }I_1\xleftarrow{\text{decrease}}I_2,\quad v_l^{(n+1)}(I_1)=pv_d^{(n)}(I_2).$$

The successive periods of exciting light alteration are distinguished with the superscript n. The reduction factors q and p

depend only on the values I_1 and I_2 of the exciting light intensity, and are determined by the expressions

$$q=q(I_1,I_2)=\frac{Z_1(I_2)}{Z_1(I_1)},$$
$$p=p(I_1,I_2)=\frac{Z_2(I_1)}{Z_2(I_2)}, \tag{19}$$

in which

$$Z_{1,2}=\int_{-\infty}^{x_S^{(2)}(I)} dx\, \exp\left(-\frac{V_{\mathrm{eff}}(x,I_{1,2})}{k_B T}\right). \tag{20}$$

These expressions describe the change in populations of dark and light RC conformational states on multiple repetitive alterations of exciting light intensity between the values I_1 and I_2 within the bistability domain. The initial conditions for such changes are given by $I(t=0)=I_1$ and $v_d^{(1)}(I_1)=Z_1(I_1)/Z$. Thus, after $n+1$ periods of reaching of the light intensity $I=I_1$, we obtain

$$v_d^{n+1}(I_1)=(1-p)+pq v_d^{(n)}(I_1), \tag{21}$$

which is in fact the recurrent relationship for v_d. From Eq. (21) it follows that

$$v_d^N(I_1)=(1-p)\frac{1-(pq)^{N-1}}{1-pq}+(pq)^{N-1}v_d^{(1)}(I_1). \tag{22}$$

The quantities $v_d^N(I_1)$ and $v_d^N(I_2)$ have their asymptotic constant values at $N\to\infty$:

$$v_d^{\infty}(I_1)=\frac{1-p}{1-pq},\quad v_d^{\infty}(I_2)=\frac{1-p}{1-pq}q, \tag{23}$$

which only depend on the photoexcitation intensities I_1 and I_2, not the initial population $v_d^{(1)}(I_1)$. The population behavior of the light conformational state is then given by [Fig. 6(b)]

$$v_l^{(n)}(I_\alpha)=1-v_d^{(n)}(I_\alpha),\quad \alpha=1,2. \tag{24}$$

If the light intensity values I_1 and I_2 fall inside the interval

$$k_{AP}\exp(-x_P)\gg I_2>I_1\gg k_{AP}\exp(-x_B), \tag{25}$$

then, in accord with Eq. (17), we obtain

$$\delta_{\mathrm{light}}(I)\approx 1,\quad \delta_{\mathrm{dark}}(I)\approx 0. \tag{26}$$

These relationships become more exact when both I_1 and I_2 values approach the center of the bistability domain. As a result of I being deeply inside the bistability domain, Eq. (16) gives the approximate relationship

$$\delta(I)\approx v_l(I). \tag{27}$$

Therefore, the experimentally measured value $\delta(I)$ should reveal the dependence, as shown in Fig. 6(b).

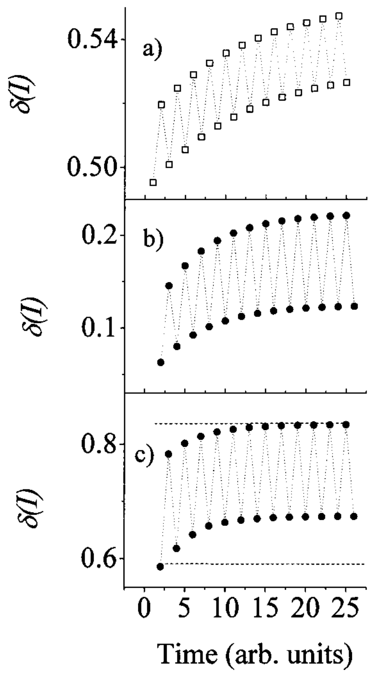

FIG. 7. (a) Dependence of the experimentally measured quantity $\delta(I)$ with an alteration of photoexcitation intensity between two stationary-state levels $I_1=0.09\ \mathrm{s}^{-1}$ and $I_2=0.1\ \mathrm{s}^{-1}$ in accordance with the protocol shown in Fig. 6(a), and calculated for identical parabolic-shaped probe potentials $V_P(x)$ and $V_B(x)$. Panels (b) and (c) show $\delta(I)$behavior with an alteration of the photoexcitation intensity between the following levels: $I_1=0.005\ \mathrm{s}^{-1}$, $I_2=0.015\ \mathrm{s}^{-1}$ [panel (b)], and $I_1=0.025\ \mathrm{s}^{-1}$, $I_2=0.055\ \mathrm{s}^{-1}$ [panel (c)]. Dependencies in panels (b) and (c) are calculated for the Morse-shaped "dark" probe potential $V_P(x)$ and parabolic-shaped "light" probe potential $V_B(x)$. The values of the parameters are $x_B-x_P=5.3$ and $k_{AD}=10\ \mathrm{s}^{-1}$ for each dependence.

The correlation of Eq. (25) is automatically fulfilled in the model because values of the exciting light intensity that correspond to the bifurcation points are given by [Eq. (13)]

$$I_{\mathrm{cr}}^{\mathrm{I}}\approx k_{AP}\exp(-x_B)\frac{x_B-x_P}{x_B-x_{cr}^{I}},$$
$$I_{\mathrm{cr}}^{\mathrm{II}}\approx k_{AP}\exp(-x_P)\frac{x_{\mathrm{cr}}^{II}-x_P}{x_B-x_P}. \tag{28}$$

This means that for $I_{1,2}\in[I_{\mathrm{cr}}^{\mathrm{I}},I_{\mathrm{cr}}^{\mathrm{II}}]$, the nonequalities (25) are always fulfilled.

In the calculated dependence of v_l with time, the peaks and minima reveal a slightly converging behavior [Fig. 6(b)]. A detailed analysis shows that this dependence may also follow a parallel increase. This behavior depends strongly on the shape of the probe potentials $V_P(x)$ and $V_B(x)$. The sequence of peaks and minima for the dependencies $v_l(I)$ and $\delta(I)$always reveal nondiverging behavior for harmonic probe potentials and for light-intensity alteration within the entire bistability domain [Figs. 6(b) and 7(a)]. This behavior also occurs for the case of different curvatures of the dark $V_P(x)$ and light $V_B(x)$ probe harmonic potentials. In contrast, the anharmonic probe potentials may readily produce diverging behavior in the sequence of peaks and minima in $\delta(I)$. For example, for the case of the Morse-shaped dark probe potential $V_P(x)$ and the harmonic light probe potential $V_B(x)$, a pronounced diverging behavior in $\delta(I)$ is obtained at the low-light-intensity interval within bistability domain [Fig. 7(b)]. The diverging behavior changes to a converging one when both the lower I_1 and the upper I_2 values of the exciting light intensity are closer to the high-light-intensity bifurcation point $I_{\mathrm{cr}}^{\mathrm{II}}$ [see Fig. 7(c)]. Consequently, the analysis of $\delta(I)$ behavior upon repetitive alteration of photoexcitation intensity within the bistability domain may provide extremely valuable information about the shape of the system conformational potential as well as about the degree of the probe potential anharmonicity. In addition, such intriguing properties of the charge transfer macromolecule cannot be obtained using conventional theories of charge-conformational interaction, in which the *a priori* defined number of conformational states determines the macromolecule properties. These models assume a light-independent shape of the effective conformational potential. Nonequilibrium conditions for forward and back transitions of molecules between different conformational states cannot be created in such models. Such conditions, which are a prerequisite for the effects reported above, can be achieved with the light-driven conformational potential only. The theoretical requirements for such an effect are the nonlinear dynamic regimes of a functioning macromolecular charge transfer system.

EXPERIMENT

For experimental studies, RC's from the photosynthetic purple bacteria *Rhodobacter (Rb.) sphaeroides* were used. RC's were isolated following the procedure described in Ref. [20]. The details of the sample preparation are presented elsewhere [21]. After isolation, the sample contained 80% RC's with a Q_B site occupied with native ubiquinone (measured by the amplitude of a ~1-s component in the primary donor recovery kinetics following a single saturating actinic flash). No reconstitution of the secondary quinone Q_B activity procedure was applied. The ratio of protein absorbance to bacteriochlorophyll absorbance was $A_{280}/A_{800}=1.3$. This ratio characterizes the purity of the sample. A 1-cm path-length cuvette was used for the experiments. The sample had a steady-state absorbance $A_{865}=0.2$. The sample was thoroughly degassed before experiments began, by multiple freeze-thaw-pump cycles down to 77 K and 10^{-6} mbar. This procedure allowed us to avoid the nonfunctional oxidation of semiquinones by oxygen or any other gases under prolonged illumination of the RC's. All experiments were then conducted at ambient temperature on a home-made optical setup described previously [9].

The absorbance changes at $\lambda_{\max}=865$ nm (the maximum of the primary photoelectron donor absorption band) were measured under various illuminating conditions. This absorption band bleaches with photoexcitation of RC's, providing a measure of the number of RC's in the charge separated state $P^+Q_AQ_B^-$ (Refs. [1–4], and references therein). The incident testing light was set at $I_{\mathrm{test}}=10^{12}$ quanta/cm^2 s). A PEM-90 (HINDS Instruments) photoelastic modulator ($2f=100$ KHz) was positioned between two crossed Glan polarizers, and used as a high-frequency testing beam chopper. After passing through the sample, the testing light was monitored with a Hamamatsu S1336-5BQ photodiode connected to an EG&G lock-in analyzer. Light from a halogen lamp, filtered with an IR heat filter and interference filter ($\lambda_{\max}=850$ nm, $\Delta\lambda\approx 60$ nm), was mounted at 90° relative to the measuring beam, and was used as background excitation. The intensity of the exciting light was controlled either by smooth variation of the incandescent lamp current or by an instantaneous change of the calibrated neutral density filters.

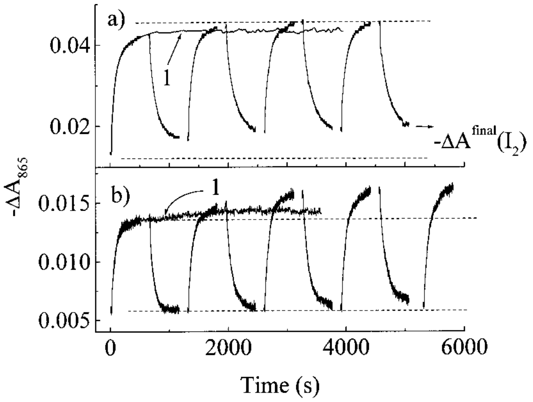

FIG. 8. Absorbance changes at $\lambda=865$ nm for RC's from *Rb. sphaeroides* with repetitive alterations of exciting light intensity between the levels $I_1=1.5$ μW/cm^2, $I_2=3.6$ μW/cm^2 [panel (b)], and $I_2=3.6$ μW/cm^2, $I_3=12.8$ μW/cm^2 [panel (a)]. Curve 1 in both graphs shows the behavior of ΔA_{865} given that a single step is applied to increase the photoexcitation intensity between the lower and higher levels (see the text for more details).

The sample, which had been initially dark adapted for more than 1 h was then subjected to a continuous wave photoexcitation of smoothly increasing intensity. After the exciting light intensity I_1 had been reached, this intensity was kept constant for a period of time $\Delta t \geq 10$ min. This was needed to complete electronic relaxation of RC's. The exciting light intensity was then stepwise increased to the value I_2. The next stage of light intensity variation was a stepwise decrease from I_2 down to I_1, with a waiting time interval of approximately 10 min between changes. Such a sequence of I alterations was applied repetitively following the protocol shown in Fig. 6(a). After each step variation of I, the proper time was taken until the quasi-stationary-state level of $A_{865}(I)$ was achieved.

Values I_1 and I_2 of the excitation light intensity were chosen so that they fell inside the bistability domain for *Rb. sphaeroides* RC's [8,10]. Figure 8 represents the results obtained for $I_1=1.5$ μW/cm^2, $I_2=3.6$ μW/cm^2 [panel (b)], and $I_2=3.6$ μW/cm^2, $I_3=12.8$ μW/cm^2, [panel (a)]. The pronounced change of ΔA_{865} concurrent with the repetitive variation of I is in complete agreement with the theory developed above (Fig. 7). There was no detectable change of A_{865} on the long time scale $t>600$ s when a single increasing step of I increases from I_1 up to I_2 [curve 1 in Fig. 8(b)] or from I_2 up to I_3 [curve 1 in Fig. 8(a)] was applied.

After reaching the final value $-\Delta A^{\mathrm{final}}(I_2)$ of absorbance change [Fig. 8(a)], the photoexcitation intensity was decreased in a stepwise manner to $I_1=1.5$ μW/cm^2. The relaxation kinetics measured at 865 nm showed an almost complete recovery of the primary donor absorbance to the value $-\Delta A^{\mathrm{in}}(I_1)$, that is characteristic for the light intensity

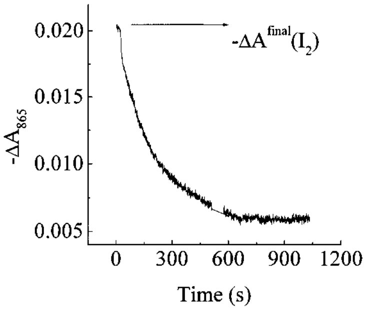

FIG. 9. Primary donor recovery kinetics measured after an abrupt stepwise decrease of the exciting light intensity from $I_2 = 3.6$ μW/cm^2 to $I_1 = 1.5$ μW/cm^2 (see the text for more details). The break in the experimental curve corresponds to the interruption of the data collection.

$I = I_1$ (Fig. 9). This also indicates the irrelevance of the observed effects to any nonfunctional traps of the charges. All experimental results presented here support the explanation of slow relaxation kinetics shown in Fig. 9 within the model of adiabatic relaxation in the double well conformational potential. A detailed analysis of the RC's equilibration kinetics in response to a stepwise variation of the light intensity will be presented in a forthcoming paper. The effects reported above were repeatable many times with the same sample, until the degradation of the sample became obvious in 5–7 days.

DISCUSSION AND CONCLUSION

The striking experimental results described above could not be explained with conventional linear theories or by some artifacts arising from trapping of charges by exogenous acceptors to electrons that could accumulate during a lengthy period of RC illumination. Assume, for example, that such exogenous acceptors of electrons are really effective, and that they ensure an accumulation of redox states $P^+(Q_AQ_B)$during the lengthy period of RC's photoexcitation with light of intensity I_2, producing a bleaching of the corresponding absorption band. A sequence of stepwise repetitive alterations of exciting light intensity between the levels I_2 and I_1 $(I_2 > I_1)$also produces an alteration in the sample optical density. The possible accumulation of reaction centers in the redox state $P^+(Q_AQ_B)$in this case can never produce an absorption band bleaching larger than that obtained during prolonged excitation of RC's with a light of intensity I_2. Similarly, one may abandon all possible explanations based on the influence of artifacts such as trapping charges by exogenous donors or acceptors. Instead, the experiment shows the prime importance of repetitive ''up'' and ''down'' alterations of the excitation light intensity in the effect of increasing steady-state absorbance changes ΔA_{865}. The highly nonequilibrium conditions, which are created by such alterations of *I*, should be of prime importance in a treatment of the above experimental results. These experimental results may be well described using the ideas of nonlinear dynamic behavior of RC's under nonequilibrium conditions, in accord with developed theory. It was shown previously that a prerequisite for nonlinear dynamic effects in Q_B-active RC's is fulfilled due to a sufficiently strong electron-conformational interaction [8–10]. The estimate of the parameter Δx, that characterizes the magnitude of this interaction, gave $\Delta x \geqslant 5.3$ [10]. This corresponds to a light-induced increase in the Gibbs free energy difference ΔG_{AB} by more than 130 meV [compare Eq. (3)]. Such a large increase, which is controlled by the correlative behavior of transferring charges and structure, seem to be ubiquitous for biological charge transfer systems. A number of studies showed that the free energy actually drops with time for an electron on a variety of intermediate acceptors of the RC, throughout its survival on this acceptor [23–25]. Structural changes that occur in response to ET in Q_B-inhibited RC's decrease the free energy gap between the charge neutral state PQ_A and the charge separated state $P^+Q_A^-$ by 120 meV [25], which is very close to the value estimated previously [10] and used in the current work for Q_B-active RC's.

The behavior of optical density changes for RC's turned out to be dependent on both the initial I_1 and final I_2 light-intensity levels, as well as on the light intensity interval $\Delta I = (I_1 - I_2)$ width. For an alteration of light intensity close to the low-intensity bifurcation point, a pattern of divergent behavior of peaks and minima sequences was measured, as shown in Fig. 8(b). For higher values of I_1 and I_2, the corresponding dependence of optical density changes revealed a converging behavior [see Fig. 8(a)]. A comparison with the corresponding theoretical dependencies of $\delta(I)$ [Figs. 7(b) and 7(c)] indicates the essential anharmonicity of the probe conformational potential of the RC, at least for the ''dark'' conformational state PQ_AQ_B.

The experimentally observed effects of ΔA_{865} changes that were obtained under conditions of *I* variation were found to have a wider excitation intensity range than that predicted theoretically. This means that more comprehensive studies must be carried out to obtain more accurate parameters for theoretical modeling (no fitting procedure was applied in the studies; all parameters for the calculations were taken from experiments). The determination of the proper shape of the effective conformational potential seems to be one of the most important problems for future studies.

It is shown in this work that the experimentally observed change of optical density at $\lambda = 865$ nm (the maximum of the primary donor absorption band) can be explained within the idea of self-regulation of electronic-conformational transitions in photosynthetic RC's. One of the main features of this system is a forbidden direct thermally activated transition from the dark conformational state over a potential barrier to the light conformational state. This transition may nevertheless take place due to a fast light-induced shift of the barrier maximum that separates the two different conformational states. This allows the system to be described as a strongly nonequilibrium one, in which the intensity of the photoexciting light stands for the control parameter. The results obtained provides a clear example of the synergetic behavior in a system on the macromolecular level.

Based on the presented results, it can be concluded that the biomolecular complex of RC's not only provide a high efficiency of charge separation due to the absorbed energy of the photoexcitation, but also undergo a light-induced structural rearrangement, thus ensuring the stabilization of the electron-hole pair with respect to the recombination process. The features of such rearrangements are described by the effective adiabatic potential for a generalized structural variable that is constructed from both the dark $V_P(x)$ and light $V_B(x)$ potentials of the system, and is controlled by the intensity of the photoexcitation. The good qualitative agreement between experimental and theoretical results suggest that additional investigations into the shape of the initial potentials $V_P(x)$ and $V_B(x)$ are necessary to achieve a better quantitative match.

In conclusion, the current work describes an increase in stationary-state optical absorbance changes caused by repetitive alterations of exciting light intensity within particular intervals. It provides evidence supporting the characterization of the RC as an essentially nonlinear dynamic system. Furthermore, the above results prove that the RC functions like a molecular motor for pumping a charge-separated state. This agrees with the idea of the relevance of dynamic self-organization effects, proposed for the first time by Haken [26] and Prigogine [27], to the high efficiency of a functioning biological charge transfer system [8–10,28–30].

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