Self-Regulation Phenomena in Bacterial Reaction Centers. I. General Theory

Alexander O. Goushcha,*† Valery N. Kharkyanen,‡ Gary W. Scott,† and Alfred R. Holzwarth*
*Max-Planck-Institut für Strahlenchemie, Ruhr 45470, Germany; †Department of Chemistry, University of California at Riverside, Riverside, California 92521 USA; and ‡Institute for Physics, National Academy of Science–Ukraine, Kyiv 252028, Ukraine

ABSTRACT A model for light-induced charge separation in a donor-acceptor system of the reaction center of photosynthetic bacteria is described. This description is predicated on a self-regulation of the flow of photo-activated electrons due to self-consistent, slow structural rearrangements of the macromolecule. Effects of the interaction between the separated charges and the slow structural modes of the biomolecule may accumulate during multiple, sequential charge transfer events. This accumulation produces non-linear dynamic effects on system function, providing a regulation of the charge separation efficiency. For a biomolecule with a finite number of different charge-transfer states, the quasi-stationary populations of these states with a localized electron on different cofactors may deviate from a Lagmuir law dependence with actinic light intensity. Such deviations are predicted by the model to be due to light-induced structural changes. The theory of self-regulation developed here assumes that light-induced changes in the effective adiabatic potential occur along a slow structural coordinate. In this model, a "light-adapted" conformational state appears when bifurcation produces a new minimum in the adiabatic potential. In this state, the lifetime of the charge-separated state may be quite different from that of the "dark-adapted" conformation. The results predicted by this theory agree with previously obtained experimental results on photosynthetic reaction centers.

INTRODUCTION

Biological energy conversion and storage take place through elementary events of charge transport in biomolecules. Transient, localized charges interact with ionized, polarizable, or dipolar structural elements of the macromolecule to perturb cofactor and/or protein structural modes. These interactions couple localized electron states to nuclear degrees of freedom that may be reduced to a single (generalized) coordinate (see, e.g., Agmon and Hopfield, 1983). This coordinate may be either collective or localized, corresponding in the latter case to motion of specific structural groups. Characteristic relaxation times of structural motion may vary widely, facilitating either an adiabatic or a non-adiabatic elementary charge transfer event in the biomolecule (see Hoff and Deisenhofer, 1997, for a review). The present work focuses on effects that occur during multiple, sequential charge transfer events when structural relaxation is significantly slower than the charge transfer rate itself.

The relevance of slow structural dynamics to the function of biological charge transfer system function has been demonstrated many times. Photosynthetic reaction centers (RCs) exhibit a long-lived, structural relaxation for minutes after completion of electron transfer (ET) (Puchenkov et al., 1995; Kalman and Maroti, 1997). Numerous studies of bacteriorhodopsins indicate slow (tens of seconds) structural motions induced by a proton flux (Nagel et al., 1998; Sass et al., 1998). Long-lived structural modes are also important for the function of cytochrome oxidases (Einarsdottir et al., 1993) and ATPases (Noji et al., 1997). For such modes, transient, localized charges interact with structural elements of the biomolecule, and the effects of these interactions accumulate during successive events. Accumulated structural changes produce feedback on the charge transfer rate. Thus, slow conformational modes function as control modes to determine long-time biomolecule behavior (Haken, 1983). The action of a charged particle flux on slow conformational modes and structural feedback on charge transfer rate constants produce non-linear, self-regulation effects (Chinarov et al., 1992; Tributsch and Pohlmann, 1998; Goushcha et al., 1997a; Gushcha et al., 1994). These self-regulation processes should be quite important for the function of charge transfer biomolecules, modulating the charge-transfer rate. To describe these effects, we propose a self-consistent, adiabatic theory of charge transfer and structural motion. This theory develops a correlation of structural dynamics and electron transfer, ensuring a correct statistical description of electron-conformational dynamics in macromolecules by considering system diffusion along an effective adiabatic potential. This approach generalizes an adiabatic theory for a single ET event to the case of multiple, successive ET events, each of which induces small but long-lived structural changes that accumulate to influence subsequent events.

We develop this theory for photosynthetic reaction centers, but most of our results can be readily generalized to other macromolecular charge transfer systems. For an intact photosynthetic system, charge separation efficiency is de-

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Address reprint requests to Dr. Alexander O. Goushcha or Prof. Gary W. Scott, Dept. of Chemistry, University of California at Riverside, Riverside, CA 92521. Tel.: 909-787-4732; Fax: 909-787-4713; E-mail: goushcha@ucrac1.ucr.edu or garyscott@citrus.uer.edu.
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termined by the quantum yield of the primary charge separation event and the lifetime of the charge-separated state. The term "efficiency of charge separation" emphasizes that the average survival time of this state, as in isolated RCs, is the determining factor in intact systems, in which the quantum yield of the primary charge separation event is ≈1. (see Wraight and Clayton, 1973).

In this paper, we proceed as follows: 1) In the next (first) section, we develop a theory for a two-state charge-transfer system and for the more general case of a finite number of charge-transfer states. We show that the survival time of the charge-separated state reflects the light-induced structural changes of the system. 2) In the second section, we develop a general, kinetic description of the electron-conformational interaction in macromolecular systems. We show that slow, structural dynamics determine self-regulation effects in biomolecules. 3) In the third section, we analyze the dependence of stationary-state structural variable values with light intensity. 4) Finally, in the fourth section we apply the theory to photosynthetic RC recombination kinetics and quasi-stationary-state, light-induced effects.

SURVIVAL TIME OF THE CHARGE-SEPARATED STATE: DEPENDENCE UPON MACROMOLECULAR STRUCTURE

Consider first the average lifetime of the charge-separated state for a simple system consisting of a photodonor D and an acceptor A, both inserted into a suitable matrix. The scheme of electron transfers in this system may be described by

$$DA \underset{k_{rec}}{\overset{k_1=\eta I}{\rightleftharpoons}} D^+A^-, \tag{1}$$

in which $k_1 = \eta I$ is the first-order rate constant for photoinduced electron transfer from the light-absorbing photodonor D to the acceptor A. The rate of this process is proportional to the intensity of absorbed actinic light I, with a proportionality coefficient η; k_{rec} is the first-order rate constant for charge recombination.

Let $\rho(t, D)$ and $\rho(t, A)$ be the normalized populations of the states, DA and D^+A^-, respectively, at time t. Then these quantities satisfy simple coupled differential rate equations for a fixed structure of the system:

$$\begin{aligned} \frac{\partial \rho(t, D)}{\partial t} &= -\eta I \rho(t, D) + k_{rec}\rho(t, A); \\ \frac{\partial \rho(t, A)}{\partial t} &= \eta I \rho(t, D) - k_{rec}\rho(t, A); \end{aligned} \tag{2}$$

in which we will take $\eta = 1$. This substitution specifies the units of I as photoinduced charge separation events per unit time. The solution of Eq. 2 is:

$$\begin{aligned} \rho(t, D) &= 1 - \rho(t, A) \\ &= \rho_l(\infty, D) + [\rho(0, D) - \rho_l(\infty, D)]\exp(-\kappa t); \end{aligned} \tag{3}$$

in which

$$\kappa = I + k_{rec}; \tag{4}$$

$$\rho_l(\infty, D) \equiv \lim_{t\to\infty} \rho(t, D) = \frac{k_{rec}}{I + k_{rec}}; \tag{5}$$

and

$$\rho_l(\infty, A) \equiv 1 - \rho_l(\infty, D) = \frac{I}{I + k_{rec}}.$$

For a more general system with an arbitrary number of charge-transfer states, but only a single photodonor D, the quantity $\sigma(t) = 1 - \rho(t, D)$ defines the probability of charge separation at t. For a fixed, constant actinic light intensity I, $\sigma_l(\infty) \equiv 1 - \rho_l(\infty, D)$. For the case of a simple donor-acceptor pair (Eq. 1), we obtain:

$$\sigma_l(\infty) = \frac{I}{I + k_{rec}}, \tag{6}$$

corresponding to a Langmuir dissociation isotherm with a half-saturation intensity, k_{rec}.

The efficiency of charge separation under stationary-state illumination with a single photoactivated electron that transfers between a finite number of localized electron states is defined as the ratio of the stationary state probability of charge separation to the number of charge separation events per unit time

$$\tau_d = \frac{\sigma_l(\infty)}{\rho_l(\infty, D)I} \tag{7}$$

Here τ_d gives the average lifetime or "survival time" (Agmon and Hopfield, 1983) of separated charges relative to recombination. For the two-state system under consideration, $\tau_d = (k_{rec})^{-1}$. We show in the Appendix that, for the general case of a system with a finite number of localized electron states and a fixed structure, the value of τ_d, given by Eq. 7, depends only on structural organization and not upon the actinic light intensity. Moreover, τ_d can be measured by the system response $\sigma(t)$ to a short, saturating actinic flash or upon ceasation of continuous photoexcitation:

$$\tau_d = \int_0^\infty \sigma(t)dt. \tag{8}$$

Thus, τ_d equals the area under the recombination probability function. In the case of multiphasic relaxation this parameter is identical to the average lifetime of the charge-sepa-

rated pair. Approximating $\sigma(t)$ as $\sigma(t) = \Sigma_i A_i e^{-\gamma_i t}$, in which $\{\gamma_i\}$ are a set of relaxation rate constants, with corresponding weights, A_i, then it follows from Eq. 8 that $\tau_d = \Sigma_i (A_i/\gamma_i)$. Thus, τ_d is the time constant for some effective single-exponential relaxation process that gives the area under the relaxation kinetics curve equal to that of the real process. As a consequence, for the general case, using Eqs. 5 and 7, we obtain

$$\sigma_I(\infty) = \frac{I}{I + \tau_d^{-1}}. \quad (9)$$

Thus, the stationary state probability of photo-separated charges depends upon the actinic light intensity strictly in accordance with the Langmuir law, with a value $(\tau_d)^{-1}$ for the half-saturation intensity. This value is fixed at a fixed structure. Thus, any deviation of the experimental $\sigma_I(\infty)$ from a Langmuir curve implies that light-induced structural rearrangements occur and that τ_d depends on I.

What physical mechanisms may correlate macromolecular structural dynamics with photoactivated charge transfer along a cofactor chain? The following facts are relevant:

1. Electric fields produced by photo-induced separated charges at angstrom distances are calculated to be on the order of 10^7–10^8 V/cm, much higher than those that exist across biomembranes in vivo.
2. Protein subunits of biomacromolecules contain charged or polar groups with redox properties that depend upon the surrounding media.
3. Experiments show that characteristic time constants for structural relaxation in proteins range from nanoseconds to minutes.
4. Slight perturbations in the equilibrium positions of macromolecule structural elements may dramatically change the rates of electron transfer between cofactors.

Statements 1–3 require no additional discussion. Support for statement 4, although previously discussed, is now amplified. For a system with a finite number of localized electron states but with no interactions with its surroundings, electron motion should be completely coherent and may be described in terms of a non-equilibrium density matrix as periodic oscillations of the electronic populations of these states (Landau and Lifshitz, 1965). However, absolute coherence of photoelectron motion is destroyed by interaction with thermal oscillations of the nuclei with relaxation times of $\approx 10^{-13}$–10^{-11} s. This means that nondiagonal elements of the density matrix may be neglected for slow steps of charge separation. The non-adiabatic description given by Fermi's Golden Rule is appropriate for this type of donor-acceptor transition (Landau and Lifshitz, 1965). The theory of non-adiabatic transitions has been well-developed in solid state physics by Förster, Dexter, and Galanin (Förster, 1949; Dexter, 1953; Galanin, 1951). An appropriate description of elementary steps of electron transfer in chemical and biological systems was given by Levich and Dogonadze, 1959; Marcus, 1956; Marcus and Sutin, 1985; and Jortner, 1976. See also the review by Hoff and Deisenhofer, 1997. It was shown that, in the high temperature limit, the rate constant ω_{ij} of ET between the ith and jth cofactors depends exponentially on both the donor-acceptor distance R_{ij} and the value of $(\lambda_{ij} + \Delta G^{\circ}_{ij})^2/\lambda_{ij}$, in which λ_{ij} is the nuclear reorganization energy and ΔG°_{ij} is the standard Gibbs free energy difference between the donor and acceptor levels (Marcus, 1956; Marcus and Sutin, 1985). This means that either a change in the distance between cofactors on the order of ~1 Å or a change in the macromolecule structure such that $(\lambda_{ij} + \Delta G^{\circ}_{ij})^2/\lambda_{ij}$ changes by more than $k_B T$ may cause a significant difference in ω_{ij}.

The generalization of the Marcus expression for the rate constant of non-adiabatic ET in continuous media to the case of any solvent model shows that the rate constant for charge transfer may be expressed in terms of a function of the free energy difference between electron-localized donor and acceptor sites produced by a fluctuating polar medium (Tachiya, 1993). This approach leads to a Gaussian-like dependence of the charge transfer rate constant on the local electrostatic potential of the medium. Many current theories of charge transfer reactions in proteins are based on a similar evaluation of the probability distribution for a free energy difference ΔV_{ij} between product and reactant states (Warshel, 1982; Parson et al., 1998; Tachiya, 1993; Bandyopadhyay et al., 1999; Warshel and Parson, 1991; Webster et al., 1994). Such an approach not only provides for a correct molecular dynamic calculation of the potential surfaces of the reactant and product states, but also enables prediction of the influence of adiabatic structural motions. Molecular dynamic simulations show that photoinduced charge separation in photosynthetic reaction centers occurs in much shorter times than those required for the system to approach conformational equilibrium after the charge transfer step (Parson et al., 1998). Recent molecular dynamics studies also show that, for long-range electron transfer in proteins, cooperation between vibrational modes of the intervening medium and the transferring electron (inelastic ET) may significantly facilitate the ET reaction, even making it an activationless process (Daizadeh et al., 1997; Medvedev and Stuchebrukhov, 1997). The resulting analytical, modified Marcus expression for the ET rate constant, using a diabatic model of electron tunneling in fluctuating medium, shows that the activation energy may be significantly reduced due to inelastic interaction with phonons. This description is similar to the idea of adiabatic self-organized ET in an active medium (Tributsch and Pohlmann, 1998; Gushcha et al., 1994).

Adiabatic theories of particle transfer over a potential barrier lead to a Kramers-type dependence of the reaction rate constant, one that depends exponentially on the barrier height E_b (Kramers, 1940). Kramers' theory came from an assumption of a linear interaction between the particle and

its environment. Recent studies by Tributsch show that a nonlinearity with energy of frictional force dependence may result in a greatly increased probability of escape from a potential well (Tributsch and Pohlmann, 1998). In this description, the rate constant ω_{ij} depends exponentially on $(\alpha/2)(E_b - E_{LC})^2$, with α being a coupling coefficient with the medium and E_{LC} denoting the mean energy of exchange between the particle and medium during oscillation. This idea has been substantiated analytically for the problem of particle escape over a potential barrier in the case of strong interactions between the particle and structural modes of the surroundings (Čapek and Tributsch, 1999). The authors gave a description of uphill particle transfer for the simplest case. In this case, the coupling of the transferred particle to its surroundings was assumed to be mediated by only one specific mode, localized in the vicinity of the transferred particle. Exact calculations for more realistic cases of many interacting modes were not performed, but expected results for such calculations should be qualitatively the same, providing support for the phenomenological result obtained earlier (Tributsch and Pohlmann, 1998).

In this work we use a phenomenological description for modeling non-equilibrium structural effects that occur during sequential charge transfer through a protein. We take into account dependence of the rate constants ω_{ij} on biomolecule structure by coupling to different structural motion. In both the adiabatic and non-adiabatic descriptions, small changes in the values of parameters such as ΔG°_{ij}, λ_{ij}, E_b, E_{LC}, R_{ij}, ΔV_{ij}, which might be caused by electron transfer between cofactors, significantly affect the kinetics of the ET. Therefore, we assume that ET rate constants should be expressed as exponential functions of a structural parameter $X = X(x_1, x_2, x_3, \ldots, x_i, \ldots)$ that in turn depends on a complete set of structural variables $\{x_1, \ldots, x_N\}$, $\omega_{ij} \propto \exp(-X)$. The structural factor X is defined by either the adiabatic E_b, E_{LC}, ΔV_{ij}, ..., or non-adiabatic ΔG°_{ij}, λ_{ij}, R_{ij}, ΔV_{ij}, ..., parameters of the system. Thus, statement 4 above indicates that charge-conformational interaction, by which ET is coupled to structural dynamics, may significantly affect the main reaction rate. For the simplest two-state system (Eq. 1) the only kinetic parameter that depends upon macromolecular structure is the recombination rate constant, k_{rec}. Thus we write,

$$k_{rec}(X) = k^0_{rec} \exp(-X). \tag{10}$$

In this equation, the structural factor X is dimensionless, normalized with a scaling factor that depends on details of the particular system.

A complete set of structural variables $\{x_1, \ldots, x_N\}$ may be selected to span the coordinate space in many ways. Here we take the structural variables as a set of variables that are each distinguished by different relaxation times. The fastest variables ($\tau \leq 10^{-13}$ s) describe fast motion of single atoms and small groups, whereas the slowest variables, with relaxation times longer than a second, describe the global dynamics of macromolecular structural rearrangement.

Let us assume that there exist long-lived, light-induced structural rearrangements of the macromolecule. Because of their long relaxation times, these rearrangements can produce effects that accumulate from one single electron-transfer step to the next. Under stationary-state illumination conditions, accumulated structural changes produce a new, quasi-stable structure. The extent of structural changes depends only upon the illumination intensity. In particular, in the case of a large electron-conformational interaction, a "dark-adapted" conformational state may convert to a completely new conformational state under high-intensity illumination. Furthermore, this new "light-adapted" conformational state may coexist with the "dark-adapted" one over an intermediate range of illumination intensity. This result means that there is a photo-induced bistability of the macromolecular structure. Necessary conditions for realization of such an effect are a strong charge-conformational interaction and a long structural relaxation time relative to localized electron relaxation. The slow structural modes, represented in this theory as "slow, generalized coordinates" function as "control modes." These modes lead to a self-regulation of the photoexcited electron flux through the macromolecule, as recently demonstrated for photosynthetic RCs (Gushcha et al., 1994, Goushcha et al., 1997a,b). Below we develop a self-consistent, statistical theory of electronic-conformational transitions to describe such effects.

A KINETIC DESCRIPTION OF THE ELECTRON-CONFORMATIONAL INTERACTION IN A CHARGE-TRANSFER MACROMOLECULE

For the theoretical treatment of light-induced structural changes in a macromolecule undergoing photoinduced charge transfer and separation, we use a Langevin equation with two random forces to describe the mechanical motion of a flexible structure (Chandrasekhar, 1943):

$$\frac{d}{dt}\frac{\partial T(\dot{\mathbf{x}})}{\partial \dot{x}_i} = -\frac{\partial V_+(\mathbf{x})}{\partial x_i} - \frac{\partial R(\dot{\mathbf{x}})}{\partial \dot{x}_i} + \sqrt{2D_i}\,\vartheta_i(t) + F_i(t, \mathbf{x}), \tag{11}$$

in which $\mathbf{x} \equiv \{x_i\}$, $\dot{\mathbf{x}} \equiv \{\dot{x}_i\}$ are the sets of structural variables (degrees of freedom; $i = 1, 2, \ldots$) with rates of structural rearrangements; $T(\dot{\mathbf{x}})$ and $V_+(\mathbf{x})$ are the kinetic and potential energies for structural modes of the photoactivated macromolecule, respectively; and $R(\dot{\mathbf{x}})$ is a dissipative function of structural motion.

The last two terms on the right-hand side of Eq. 11 represent random forces. The first is a random force due to thermal motion. This force acts on the structural variable x_i, while the quantity $\vartheta_i(t)$ describes δ-correlated random processes with amplitudes $\sqrt{2D_i}$ to model thermal fluctuations

of the structural variables. The last term in Eq. 11 is a random force corresponding to interaction of a photoactivated electron with the macromolecular structure. Thus, $F_i(t, \mathbf{x})$ describes a discrete random process:

$$F_i(t, \mathbf{x}) \in [\hat{f}_n^i(\mathbf{x})], \quad n = D, A_1, A_2, \ldots \tag{12}$$

in which $f_n^i(\mathbf{x})$ is a force describing the interaction of a photoactivated electron, localized on cofactor n, with structural mode i. The probability of each component $f_n^i(\mathbf{x})$ is determined by the probability of electron localization on cofactor n at a fixed $\mathbf{x}$. These probabilities ($\rho(t, n|\mathbf{x})$) can be determined from the system of differential rate equations,

$$\frac{\partial \rho(t, n|\mathbf{x})}{\partial t} = \sum_m \{-\omega_{nm}(\mathbf{x})\rho_n(t, n|\mathbf{x}) + \omega_{mn}(\mathbf{x})\rho_m(t, m|\mathbf{x})\}. \tag{13}$$

These are the master equations for a random process (Eq. 12) (Horsthemke and Lefever, 1984). The quantity $\omega_{nm}(\mathbf{x})$ in Eq. 13 defines the rate constants of non-adiabatic transitions between the n and m cofactors at a fixed structure. We assume that the variables $\mathbf{x} = \{x_i\}$ represent overdamped conformational motions, a valid description for flexible structures like proteins. Although this assumption may be incorrect for high-frequency oscillations, these variables are thermally equilibrated and excluded from detailed consideration. From Eq. 11 and in accord with the results of Horsthemke and Lefever, 1984, for a coupled random process (Christophorov, 1995) we obtain the fundamental kinetic equation for the distribution function of both electron and structural variables of the macromolecule, $P(t; n, \mathbf{x})$,

$$\frac{\partial P(t; n, \mathbf{x})}{\partial t} = \hat{D}_n(\mathbf{x})P(t; n, \mathbf{x}) + \sum_m \{-\omega_{nm}(\mathbf{x})P(t; n, \mathbf{x}) + \omega_{mn}(\mathbf{x})P(t; m, \mathbf{x})\}, \tag{14}$$

in which

$$\hat{D}_n(\mathbf{x}) = \sum_i D_i \frac{\partial}{\partial x_i}\left[\frac{1}{k_B T}\frac{\partial V_n(\mathbf{x})}{\partial x_i} + \frac{\partial}{\partial x_i}\right], \tag{15}$$

$$-\frac{\partial V_n(\mathbf{x})}{\partial x_i} = -\frac{\partial V_+(\mathbf{x})}{\partial x_i} + f_n^i(\mathbf{x}), \tag{16}$$

and D_i is a diffusion constant corresponding to motion of the structural variables $\{x_i\}$ along the conformational potential surface $V_n(\mathbf{x})$ for electron localization on binding site n. This equation is general, but we further simplify it to reveal the role of control modes on macromolecule structural dynamics.

To simplify, we separate the variables $\{x_i\}$ into three groups, depending upon the relative magnitudes of the relaxation time constants τ_x and τ_{el} of the distribution function $P(t; n, \mathbf{x})$ over the structural and electron variables, respectively. Those variables $\mathbf{x}_{fast}$, for which $\tau_x \ll \tau_{el}$, belong to the first group. For the second group ($\mathbf{x}_{equal}$) the time constants are of the same order: $\tau_x \sim \tau_{el}$. The third group is characterized by slow structural motions ($\mathbf{x}_{slow}$) for which $\tau_x \gg \tau_{el}$. The two types of variables, $\mathbf{x}_{slow}$ and $\mathbf{x}_{equal}$, should be explicitly retained in a description of self-regulation effects for a system involving photoexcited electron transfer within a flexible structure. However, in the present treatment we retain only $\mathbf{x}_{slow}$, and ignore $\mathbf{x}_{equal}$. The fast variables, $\mathbf{x}_{fast}$, are not important for self-regulation effects, because these variables relax on much shorter time scales. We can integrate over these variables, using the substitution

$$P(t; n, \mathbf{x}) = \tilde{P}(t; n, \mathbf{x}_{slow})\frac{\exp\left(-\frac{V_n(\mathbf{x})}{k_B T}\right)}{Z_n^{fast}}, \qquad Z_n^{fast} = \int \exp\left(-\frac{V_n(\mathbf{x})}{k_B T}\right) d\mathbf{x}_{fast}, \tag{17}$$

in which $\tilde{P}(t; n, \mathbf{x}_{slow})$ is the distribution function for electron and slow structural variables.

Putting Eq. 17 into Eq. 14 and integrating over $\mathbf{x}_{fast}$, we obtain equations identical to Eq. 14 that are valid for time intervals $t \gg \tau_{fast}$. They may also be derived from Eq. 14 with a simple substitution,

$$\mathbf{x} \to \mathbf{x}_{slow}; \quad P \to \tilde{P}; \quad \omega_{nm} \to \tilde{\omega}_{nm}; \quad V_n \to \tilde{V}_n. \tag{18}$$

For ET rate constants between cofactors n and m, after such substitutions, we obtain:

$$\tilde{\omega}_{nm}(\mathbf{x}_{slow}) = \int \omega_{nm}(\mathbf{x}) \cdot \frac{\exp\left(-\frac{V_n(\mathbf{x})}{k_B T}\right)}{Z_n^{fast}} d\mathbf{x}_{fast}. \tag{19}$$

These transitions are non-adiabatic with respect to $\mathbf{x}_{fast}$, but adiabatic with respect to $\mathbf{x}_{slow}$.

The potential energy expression corresponding to slow structural variables can be easily obtained after substitution of Eq. 17 into Eq. 14 and integrating over $\mathbf{x}_{fast}$,

$$\tilde{V}_n(\mathbf{x}_{slow}) = -k_B T \ln \int \exp\left(-\frac{V_n(\mathbf{x})}{k_B T}\right) d\mathbf{x}_{fast}. \tag{20}$$

It is obvious that the quantity $\tilde{V}_n(\mathbf{x}_{slow}) \equiv G_n(\mathbf{x}_{slow})$, in which $G_n(\mathbf{x}_{slow})$ is a so-called quasi-free energy for an electronic state n, depends parametrically on the slow structural variables (Stratonovich, 1992; 1994). This means that $\tilde{V}_n(\mathbf{x}_{slow})$ represents the standard free energy for the electron state n with respect to the fast structural variables $\mathbf{x}_{fast}$, but it corresponds to the potential energy of electron state n with respect to the slow variables $\mathbf{x}_{slow}$.

We further proceed from Eq. 14, taking into account the substitutions (Eq. 18) and the actual role of the slow vari-

ables, $\mathbf{x}_{slow}$, only. Thus we can use an adiabatic approach that enables us to make the following simplification in Eq. 14. [Here we restrict consideration to the simpler case of ordinary slaving (Haken, 1983) in which fluctuations of the fast variable influence the evolution of the slow one only on average, without causing any noticeable fluctuations of the latter. The softer types of slaving will be discussed in a separate paper.]

$$P(t; n, \mathbf{x}) = \rho(t, n|\mathbf{x})P(t, \mathbf{x}), \quad \sum_{n} \rho(t, n|\mathbf{x}) = 1, \tag{21}$$

in which $\rho(t, n|\mathbf{x})$ are the relative probabilities to find an electron localized on cofactor n at fixed **x,** as determined from Eq. 13 using Eq. 18, and $P(t,\mathbf{x})$ is a distribution function for the slow structural variables. This function is defined by the expression

$$P(t, \mathbf{x}) = \sum_{n} P(t; n, \mathbf{x}). \tag{22}$$

Putting Eq. 21 into Eq. 14, we obtain an equation that describes the time evolution of this function,

$$\frac{\partial P(t, \mathbf{x})}{\partial t} = \sum_{i} D_i \frac{\partial}{\partial x_i}\left[-\frac{1}{k_B T} F^{I}_{ad-i}(\mathbf{x}) + \frac{\partial}{\partial x_i}\right] P(t, \mathbf{x}), \tag{23}$$

in which $F^{I}_{ad-i}(\mathbf{x})$ is a statistical quantity with dimensions of a force. This quantity describes the adiabatic action of the electron transfer upon the ith slow structural degree of freedom under conditions of slowly varying illumination intensity, I, ensuring that electronic relaxation processes are complete: $|\frac{\partial}{\partial t} \ln I(t)| \gg \tau_{el}$, and

$$F^{I}_{ad\cdot i}(\mathbf{x}) = -\sum_{n} \rho_I(\infty, n|\mathbf{x}) \frac{\partial V_n(\mathbf{x})}{\partial x_i}. \tag{24}$$

Equations 13, 23, and 24 provide the basis for self-regulation of a photoactivated electron flux by slow structural variables of a macromolecule.

Assume that the system can be characterized by a single slow structural degree of freedom: the generalized configurational coordinate x. Then Eq. 23 may be rewritten as:

$$\frac{\partial P(t, x)}{\partial t} = D \frac{\partial}{\partial x}\left[\frac{1}{k_B T} \frac{\partial V^{I}_{ad}(x)}{\partial x} + \frac{\partial}{\partial x}\right] \cdot P(t, x), \tag{25}$$

in which the adiabatic potential of the system, $V^{I}_{ad}(x)$, at fixed light intensity I is determined from Eqs. 16 and 24 with an uncertainty $C(I)$

$$V^{I}_{ad}(x) = V_{+}(x) - \sum_{n} \int_{x_0}^{x} f_n(\varsigma)\rho I(\infty, n|\varsigma) d\varsigma + C(I). \tag{26}$$

Note that the subscript "*ad*" in the expression V^{I}_{ad} means "adiabatic," not to be confused with the free energy difference ΔV_{AD} between the donor and acceptor levels. $V_{+}(x)$ has its minimum at x_0. $f_n(\varsigma)$ has the same meaning as the force introduced in Eq. 12 for $i = 1$. Calculation of $C(I)$ will be discussed elsewhere.

The quantity $V^{I}_{ad}(x)$ serves as the effective adiabatic potential for the slow structural mode. This potential determines the average value of x over the electron distribution function. This potential is of a statistical nature, depending upon a stationary-state distribution of localized electron populations at a fixed structure. This structure is determined and controlled by the illumination intensity, I. For times $t > \tau_{xslow}$, stationary-state conditions are reached. The corresponding stationary-state distribution function can be written as

$$P_I(\infty, x) = Z^{-1} \exp\left(-\frac{V^{I}_{ad}(x)}{k_B T}\right);$$
$$Z = \int dx \cdot \exp\left(-\frac{V^{I}_{ad}(x)}{k_B T}\right). \tag{27}$$

The minima and maxima of this function, x_{ext}, define the stationary states of the macromolecule at a fixed I. Thus, the effective adiabatic potential $V^{I}_{ad}(x)$ for the open non-equilibrium system described by Eq. 1 is the analog of a standard Gibbs free energy, G°, which determines the probability to find a closed system in a particular equilibrium state with given free energy. The stationary states defined by x_{ext} in this open system are the analog of the equilibrium states in a closed system, and the values of x_{ext} can be determined from

$$\left.\frac{\partial V^{I}_{ad}(x)}{\partial x}\right|_{x=x_{ext}} = \sum_{n} \rho_I(\infty; n|x) \left.\frac{\partial V_n(x)}{\partial x}\right|_{x=x_{ext}} = 0. \tag{28}$$

Those states that correspond to potential minima define the *conformational coordinates* of the system. The functions $\rho_I(t, n|x)$, $P(t, x)$ as well as their stationary values $\rho_I(\infty, n|x)$, $P_I(\infty, x)$, and the $x_{ext}(I)$ depend on the structure of the system and determine each experimentally measured quantity $q(n, x)$ by averaging. Averaging over the electron variables,

$$\bar{q}(t) \equiv \langle q(n, x)\rangle_{el} = \sum_{n} \rho(t, n|x) \cdot q(n, x). \tag{29}$$

When averaged over both the electron variables and generalized configurational coordinates,

$$\bar{\bar{q}}(t) \equiv \langle\langle q(n, x)\rangle\rangle_{el, x} = \int dx \cdot P(t, x)\bar{q}(t, x). \tag{30}$$

Before proceeding to the next section we should comment on our use of a single structural variable (Eq. 25). In practice, the application of any theory to a particular biomolecular system often requires a decrease in the system

dimension to a few generalized structural coordinates or even to a single coordinate. Of course the system configuration is determined by tens of thousands of physical variables, and the configuration of one system is a point in the multi-dimensional configurational space. As shown above, the fast structural variables relax to quasi-equilibrium values and fluctuate about them. These quasi-equilibrium values themselves continue adiabatically to follow changes in the slow variables until at long-time the system dynamics may be determined by a small number of slow variables or even a single slowest variable. The dynamics of such slow variables is determined by the potential profile of the quasi-free energy (see Eq. 20). Such a hierarchy is characteristic for the relaxation of complex systems with a large number of variables. It has been called "the slaving principle" (Haken, 1983). Thus it is reasonable to assume that at long times, the slow structural rearrangement of a biomolecule may be described by a small number of variables. For a description of phase transitions in systems with a large number of variables, consideration is often restricted to a single "control mode" or "order parameter." Similarly, the description of chemical reactions in complex molecular systems may also be described with a single "reaction coordinate." For example, long-time relaxation processes in biopolymers are often described by bounded diffusion of initial multi-dimensional distribution function along a particular trajectory of the potential surface (see, e.g., Agmon and Hopfield, 1983; Rubin et al., 1990; Gudowska-Nowak, 1994; Frauenfelder et al., 1991, 1999).

Thus, we restrict present considerations to a one-dimensional model of slow structural rearrangements induced by a photoinduced charge separation in biomolecules. We treat x as a global configurational coordinate that describes slow photoinduced structural changes. In this case, the structural factor $X(x)$ introduced above may also be identified as a configurational coordinate because as it is well known that the dimension of generalized variables does not affect the calculation of trajectories and free energies (Goldstein, 1980). Consequently, we modify Eq. 10 making the substitution $x \rightarrow X$, assuming that the configurational coordinate x is monotonic in configurational space because system energy decreases during relaxation, and slow system diffusion is determined by the trajectory. Note that x does not describe arbitrary structural rearrangements, but only those responsible for slow structural relaxation to a new potential minimum in configurational space. Finally, we obtain:

$$k_{rec}(x) = k^0_{rec} \exp(-x), \tag{31}$$

an expression that will be used in subsequent sections. It is important to note that the adiabatic potential for the structural factor X has its minimum at $X_{min} = X(x_{min})$; therefore the proposed substitution of variables leads to the equivalent consideration in our phenomenological model.

STABLE STATES OF THE TWO-STATE SYSTEM: A CONFORMATIONAL APPROACH

To determine the light intensity dependence of macromolecule stationary states from Eq. 28 we select, as an example, a harmonic potential with effective elastic constant χ, $V_+(x) = \chi(x^2/2)$, and, hence an effective adiabatic potential, $V^1_{ad}(x)$. Such a potential represents Gaussian fluctuations of x around equilibrium (see, e.g., Zusman, 1980). The quantities $f_n(x)$ ($n = D, A$) are defined as additional stochastic forces that act on the configurational coordinate when an electron is localized on cofactors D and A, respectively. We assume that these forces are constant, but not equal to each other. That is, $f_D(x) = f_D = \chi * x_D$ is a force acting on the structure when an electron is localized on donor D; and $f_A(x) = f_A = \chi * x_A$ is a force acting on the structure when an electron is localized on acceptor A. In general, $x_A \neq x_D$. The quantity $\xi = |x_A - x_D|$ characterizes the electron-conformational interaction of the system. It is proportional to the additional force acting on the configurational coordinate in a charge-separated donor-acceptor pair. We assume here that the force constant χ is the same for the charge-neutral and charge-separated states, although this need not necessarily be true (i.e., in general $\chi_D \neq \chi_A$). Furthermore, we showed in our recent paper (Goushcha et al., 1999) that for photosynthetic bacterial reaction centers the probe potential $V_+(x)$ is probably not harmonic with different curvatures in the charge-neutral (PQ_AQ_B) and in the charge-separated ($P^+Q_AQ_B^-$) states. Parson and coworkers arrived at a similar conclusion in their molecular dynamic studies of the ET reaction $P^*BH \rightarrow P^+B^-H$. They showed that the force constant for the P^+B^-H state is larger than that it is for the P^*BH state (Parson et al., 1998). In the current work, we explore qualitatively the conditions for non-equilibrium structural transitions and the emergence of new conformational states. For this treatment, the fact that $\chi_D \neq \chi_A$ is not essential, and we assume that $\chi_D = \chi_A \equiv \chi$ to obtain analytical solutions.

Using expressions for light-dependent, stationary-state electron populations (Eq. 5), the recombination rate constant dependence on x (Eq. 31), and stochastic forces (Eqs. 12, 16, and $f_{A,D}(x)$), the equation defining the stationary states of the system (Eq. 28) becomes

$$x_{ext} = x_D + (x_A - x_D)\frac{I}{I + k^0_{rec}\exp(-x_{ext})}. \tag{32}$$

This equation is valid for macromolecular ET systems that can be accurately described as two-state systems (Eq. 1), explicitly indicating the dependence of x_{ext} on I.

For this specific example, $x_{ext}(I)$ (Eq. 32) at specific values of ξ was determined in our recent work (Goushcha et al., 1999). A small, monotonic, light-induced increase in $x_{ext}(I)$ was obtained for the case of a weak interaction, $\xi = (x_A - x_D) \leq 4$. The concomitant increase in the lifetime of the charge separated state, τ_d, obtained using Eq. 32 is

shown in Fig. 1. The smooth, light-induced increase of τ_d for curves 1 and 2 is due to deformation of the "dark" conformational state. This effect may be described as "self-regulation" of the photoactivated electron transfer rate due to a slow structural deformation, reflecting macromolecule structural changes from an electron-conformational interaction modulated by the photoinduced electron flow.

More complex behavior of $x_{ext}(I)$ occurs in the case of a strong charge-conformational interaction $\xi > 4$. For illumination intensities, $I_{II}^{cr} > I > I_{I}^{cr}$, in which

$$I_{I,II}^{cr} = k_{rec}^{0} \frac{x_{I,II}^{cr} - x_D}{x_A - x_{I,II}^{cr}} \exp(-x_{I,II}^{cr}) \tag{33}$$

and

$$x_{I,II}^{cr} = \frac{x_A + x_D}{2} \pm \sqrt{\left(\frac{x_A - x_D}{2}\right)^2 - (x_A - x_D)},$$

three values of the extrema $x_{ext}^{(i)}(I)$; $i = 1, 2, 3$ are obtained. Two branches, $x_{ext}^{(1)}(I)$ and $x_{ext}^{(3)}(I)$, give minima adiabatic potential, corresponding to stable structural states of the system, while a third, $x_{ext}^{(2)}(I)$, gives a maximum for an unstable state. The parameter k_{rec}^{0} slightly perturbs these dependencies, shifting them to a higher light intensity with an increase in k_{rec}^{0}. Experimentally, one can only observe stable branches of these dependencies. This means that experimentally measured system parameters may reveal discontinuities at particular illumination intensities. The survival time, τ_d, of a charge-separated state, in the case of a strong charge-conformational interaction, for the branch $x_{ext}^{(1)}(I)$ is significantly shorter than this time for one belonging to the branch $x_{ext}^{(3)}(I)$. (Compare *curves 3* or *4* with *curve 1,* Fig. 1). In fact, following the discussion in the first section,

$$\frac{\tau_d[x_{ext}^{(3)}(I)]}{\tau_d[x_{ext}^{(1)}(I)]} = \exp(x_{ext}^{(3)}(I) - x_{ext}^{(1)}(I)), \tag{34}$$

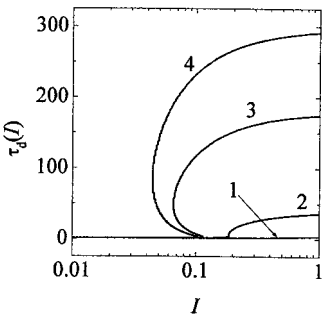

FIGURE 1 Dependence of the survival time τ_d of the charge-separated state on illumination intensity I for various values of the electron-conformational interaction parameter $\xi = x_A - x_D$: 1) $\xi = 2$; 2) $\xi = 4$; 3) $\xi = 5.2$; 4) $\xi = 6$. The curves were obtained for the following set of parameters: $x_D = 2$; $k_{rec}^{0} = 10$.

and $\xi = x_A - x_D$ may be determined from experiment as

$$\xi = \ln \frac{\tau_d(I \to \infty)}{\tau_d(I \to 0)}. \tag{35}$$

The appearance of a new, light-induced stable structural state for $\xi > 4$ and the coexistence of this state with the initial stable state represents a non-equilibrium phase transition of the "monostability-bistability" type (Haken, 1983, Stratonovich, 1994).

The problem of thermodynamic stability of stationary states at coordinates $x_{ext}^{(1)}(I)$ and $x_{ext}^{(3)}(I)$ and the related problem of thermal fluctuations in the configurational coordinate around stationary-state values can be solved using a distribution function over x of the form $P(t, x) = P_D(t, x) + P_A(t, x)$ (see Eq. 22). An evolution equation for this function is described by Eq. 23, where using Eq. 26, the statistical potential $V_{ad}^{I}(x)$ (Goushcha et al., 1997a) is given by

$$V_{ad}^{I}(x) = \frac{\chi}{2}\left[(x - x_D)^2 - 2(x_A - x_D)\left(\ln \frac{I\exp(x) + k_{rec}^{0}}{I\exp(x_D) + k_{rec}^{0}}\right) + \left(\frac{I(x_A - x_D)}{I + k_{rec}^{0}\exp(-x_D)}\right)^2\right]. \tag{36}$$

Previously, we analyzed this expression for many values of the electron-conformational interaction parameter, ξ (Goushcha et al., 1997a 1999). A second, light-induced potential minimum may appear for $\xi > 4$, a case corresponding to a distribution function $P_{eq}^{I}(\infty, x)$ with two maxima (Fig. 2 *A*). For $\xi < 4$, the light-induced deformation of the adiabatic potential causes a deformation of the distribution function with only a shift in the distribution maximum toward larger values of the conformational coordinate (Fig. 2 *B*). The evolution of the distribution function with light intensity for the case of a weak interaction has been described in the literature. See, e.g., studies of the $P^+Q_A^- \rightarrow PQ_A$ reaction in photosynthetic bacterial RCs (Shaitan et al., 1991; Uporov and Shaitan, 1990).

The abscissas of the potential $V_{ad}^{I}(x)$ extrema determine stationary values x_{ext} of the slow configurational coordinate as a function of ξ (see Eq. 35). The values $x_{ext}^{(1)}(I)$ and $x_{ext}^{(3)}(I)$ correspond to adiabatic potential minima, whereas $x_{ext}^{(2)}(I)$ corresponds to a maximum. The minima determine the *conformational states* of the macromolecule at steady-state illumination intensity I. The thermodynamic stability of these conformational states is determined by both the depth of the potential minima and the height of the barrier between them.

Often the ensemble properties of macromolecules may be satisfactorily described by the most probable behavior of these macromolecules near potential minima. For this description, we use a *conformational approach* and introduce

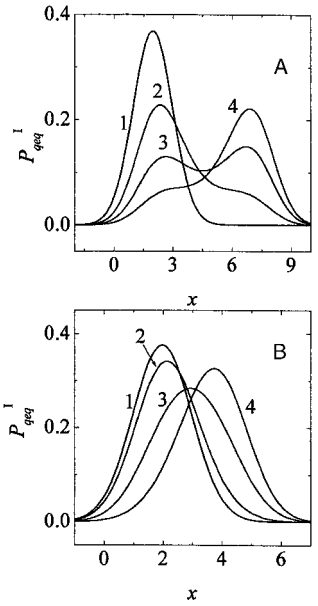

FIGURE 2 Quasi-equilibrium distribution functions calculated for $\xi = 2$ (*A*) and $\xi = 5.3$ (*B*) for the following values of *I*. *a, curve 1:* $I = 0$; *curve 2:* $I = 0.1$; *curve 3:* $I = 0.5$; *curve 4:* $I = 2.0$; *b, curve 1:* $I = 0$; *curve 2:* $I = 0.07$; *curve 3:* $I = 0.1$; *curve 4:* $I = 0.13$; $x_D = 2$; $k_{rec}^0 = 10$.

the function

$$\nu_\alpha(t) = \int_{G_a} dx \cdot P(t, x - x_\alpha), \tag{37}$$

in which we integrate over all configurations of the coordinate $x \in G_a$ near a potential minimum α at the point x_α (van Kampen, 1992). This function, ν_α, determines the population at the minimum α of the adiabatic potential.

States with an x such that $x \in G_a$ are treated as the same conformational state, α. The probability of realizing different conformational states is determined by the forward and reverse transition rates over potential barrier (Kramers, 1940; van Kampen, 1992). These probabilities are obtained from Eq. 25 using Eq. 37. Using this conformational approach, the average value of an observable $q(t)$ can be written as

$$\bar{\bar{q}}(t) = \sum_\alpha \nu_\alpha(t)\bar{q}(t, x_\alpha), \tag{38}$$

in which $\bar{q}(t, x_\alpha)$ is the average value over electron variables at t in conformational state α.

For a system undergoing photoinduced charge separation and described by a double-minimum adiabatic potential, we introduce two distinct conformational states, "light" and "dark," denoted as l and d, respectively. Providing that the barrier height between the two minima is sufficiently high, then equilibration near the minima may occur without thermally activated transitions between the minima. In this case, the non-equilibrium distribution function for x, corresponding to the evolution Eq. 25, may be written as

$$P(t, x) = \begin{cases} \nu_d P_d(t, x), & \text{when } x < x_{ext}^{(2)}(I); \\ \nu_l P_l(t, x), & \text{when } x > x_{ext}^{(2)}(I); \end{cases} \tag{39}$$

in which ν_d and ν_l ($= 1 - \nu_d$) are the integrated populations of the "dark" and "light" conformational states, respectively (see Eq. 37). These quantities depend on the illumination intensity and its prior variation. Calculation of these quantities was previously discussed (Goushcha et al., 1997a) and is not repeated here. The distribution functions, $P_d(t, x)$ and $P_l(t, x)$, equal $P(t, x)$ for the intervals, $\lfloor -\infty, x_{ext}^{(2)}(I) \rfloor$ and $\lfloor x_{ext}^{(2)}(I), \infty \rfloor$, respectively, and are determined by Eq. 25.

The theoretical expression for an experimentally measured quantity, $q(t)$, at steady-state illumination intensity I is

$$\bar{\bar{q}}(\infty) = \nu_d\langle q(x)\rangle_d + \nu_l\langle q(x)\rangle_l. \tag{40}$$

In Eq. 40, averaging is done over all values of x in the "dark" state (subscript "*d*") and in the "light" state (subscript "*l*"), respectively. This expression is valid outside the bistability domain if one recognizes that $\nu_d = 1$, $\nu_l = 0$, and $x_{ext}^{(2)} = \infty$ at $I < I_I^{cr}$; whereas $\nu_d = 0$, $\nu_l = 1$, and $x_{ext}^{(2)} = -\infty$ at $I > I_{II}^{cr}$. In the conformational approach Eq. 40 can be simplified to the more convenient form,

$$\bar{\bar{q}}(\infty) = \nu_d \cdot q(x_{ext}^{(1)}(I)) + \nu_l \cdot q(x_{ext}^{(3)}(I)). \tag{41}$$

In the final section of this paper, we apply Eqs. 40 and 41 to a brief analysis of illumination-dependent absorbance changes in photosynthetic RCs. We also present other recent experimental observations that provide support for the idea of self-regulation phenomena in photosynthetic RCs.

APPLICATION TO BACTERIAL REACTION CENTERS

Photosynthetic reaction centers have been among the most comprehensively studied biological systems over the last 30 years (Hoff and Deisenhofer, 1997, and references therein). Many researchers have discussed light-induced conformational transitions in RCs (see, e.g., Graige et al., 1998; McMahon et al., 1998; Kalman and Maroti, 1997; Kleinfeld et al., 1984b; Shaitan et al., 1991). The charge recombination rate constant in RCs depends on structural coordinates such as the donor-acceptor distance (Kleinfeld et al., 1984b; Shaitan et al., 1991). These authors experimentally determined, in effect, the distribution function for the generalized conformational coordinate both in the dark and under illumination by quenching structural relaxation at cryogenic temperatures. They obtained a light-induced increase in the donor-acceptor distance of ~1 Å. Recent EPR studies of RCs show that light-induced conformational changes are not simple relative translations of the donor and primary quinone acceptor (Q_A) molecules, but that they are more

likely rearrangements of the protein structure (Zech et al., 1997). More recent x-ray studies of RCs from *Rb. sphaeroides* showed a large, ~5 Å, light-induced translation of the secondary quinone acceptor from its location in the dark-adapted system accompanied by a 180° rotation about the isoprene axis (Stowell et al., 1997). These authors also reported light-induced changes in the protein structure that affect the protonation of amino acid residues. Recent molecular dynamic calculations demonstrated the existence of two distinctly different binding sites for the neutral secondary quinone Q_B and semiquinone anion Q_B^- (Grafton and Wheeler, 1999). The authors showed for the first time that the protonation of ASP L213 should occur prior to occupation by Q_B^- of its stable (quasi-equilibrium) site, ~5 Å distant from the site which is at equilibrium for neutral quinone in the dark. Such motion of an ubi-semiquinone Q_B^- from the non-equilibrium position that is characteristic for the dark-adapted structure to a quasi-equilibrium position that is stable for the light-adapted structure indicates the importance of non-equilibrium structural transitions in RCs. These observations explain previously reported light-induced changes in the transient absorption spectrum of *Rb. sphaeroides* RCs (Kleinfeld et al., 1984b), but the physical phenomena responsible for these new conformations remained unexplained. Using the theoretical approach described above, we now elucidate reasonable mechanisms that lead to these structural changes in RCs.

The relationship between light-induced absorbance changes and the average survival time of the charge-separated state

Upon photoexcitation of RCs from purple bacteria *Rhodobacter (Rb.) sphaeroides,* a large absorbance decrease is observed in the 865-nm absorption band for the primary donor bacteriochlorophyll dimer (See Clayton, 1965). The absorbance $A_{865}(t)$ depends on the illumination intensity through $\sigma(t)$,

$$A_{865}(t) = (1 - \upsilon)A_{865}(0) + \upsilon A_{865}(0)(1 - \sigma(t)) \quad (42)$$

in which $\upsilon < 1$ is the fraction of total absorbance at 865 nm that is due to reduced (not photooxidized) photoelectron donor P and $A_{865}(0)$ is the steady-state absorbance in the absence of photoactivation.

From Eq. 42 one can easily determine the relationship between the theoretical quantity, $\sigma_I(t)$ (see first section) and experimentally measured values of $\delta(t) = -\{[A_{865}(t) - A_{865}(0)]/[A_{865}(0)]\}$. For stationary-state conditions,

$$\delta_I \equiv \lim_{t\to\infty} \delta(t) = \nu\sigma_I(\infty) = \upsilon \frac{I}{I + \tau_d^{-1}}. \quad (43)$$

For steady-state illumination, a more convenient form is

$$\delta_I' = \frac{\delta_I}{\upsilon - \delta_I} = -\frac{A_{865}(I) - A_{865}(0)}{A_{865}(I) + (1 - \nu)A_{865}(0)} \equiv \tau_d I. \quad (44)$$

The experimental dependence of absorbance changes at 865 nm with steady-state illumination intensity is shown in Fig. 3. In this figure, curve 1 corresponds to RCs from *Rb. sphaeroides* with inhibited ET from the primary Q_A to the secondary Q_B quinone. We call these RCs "Q_B-lacking," whereas RCs with allowed ET between quinone acceptors are called "Q_B-containing" RCs. [The isolation procedure for these RCs and the details of the experimental setup are described elsewhere (Zakharova et al., 1981; Mueller et al., 1991; Goushcha et al., 1997b)]. The value $\upsilon = 0.9$ was estimated as the ratio of the amplitude of absorbance change at saturating light intensity to the steady-state absorbance at $I = 0$. One expects a linear relationship of δ_I' vs. I for a system with fixed structure obeying a Langmuir law. The slope of this type of plot yields the survival time of the charge-separated state for the RCs. For Q_B-containing RCs, a simple plot deviates from a Langmuir law (*curve 2* in Fig. 3). As Eq. 44 indicates, this deviation occurs because τ_d depends on I due to light-induced structural rearrangements of the RCs.

The conclusions drawn above are valid for a system in which both 1) a single photoactivated electron transfers between a finite number of electronic states, and 2) charge trapping by exogenous acceptors is negligible. The first condition is verified for the RCs by the absence of cytochrome *c* or any other exogenous electron donors that might rapidly reduce the bacteriochlorophyll dimer P^+ after initial photoactivation in double-flash experiments. Thus there are no states with doubly reduced quinone acceptors in these experiments. The second condition was verified in recent studies, which showed that large changes in the ET and charge recombination kinetics of RCs upon prolonged illumination are not related to the loss of photochemical activity of the RCs, but rather are due to the formation of new

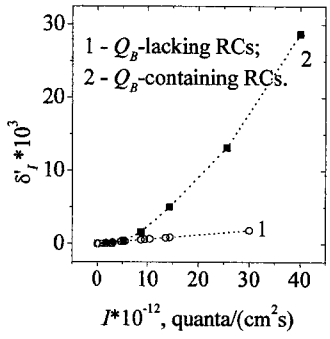

FIGURE 3 Experimentally measured optical absorbance changes, δ_I', as a function of illumination intensity for Q_B-lacking (*curve 1, open circles*) and Q_B-containing (*curve 2, solid squares*) RCs from *Rb. sphaeroides,* wt. The RC concentration was ~1 μM. A_{280}/A_{802} = 1.3 ± 0.5. Buffer conditions: 10 mM HCl-Tris (pH = 8.0), ambient temperature (T = 20°C), 0.025%. LDAO. The samples where thoroughly degassed before experiments by multiple freeze-thaw-pump cycles at 77 K and the pressure 10^{-6} torr. Q_B-lacking RCs were prepared by addition of a 20 μM of *o*-phenanthroline solution to a 1 μM solution of RCs.

"light-induced" conformations of the RCs (Kalman and Maroti, 1997).

The experimentally measured quantity $\delta(t)$ can be calculated with the formalism developed above for the distribution function. Consider a homogeneous sample of isolated RCs at concentration C and optical pathlength l that absorbs with an "ideal" theoretical absorbance $A'_{865} = \varepsilon'_{865}\, l\, C * \delta_{DA}$, in which $\delta_{DA} = 1$ for $A = D$ and $= 0$, otherwise. Taking an ensemble average over the distribution of RC electronic and conformational states yields the following result:

$$\bar{\bar{A}}_{865}(t) = \langle\langle A'_{865}(t, x)\rangle\rangle_{el,x} = \varepsilon_{865} l\, C \int dx P(t, x)\rho(t; D|x). \tag{45}$$

Hence, the calculated experimental quantity $\delta(t)$ is given by

$$\delta(t) = \int dx \sigma(t, x) P(t, x), \tag{46}$$

in which, for the simplest case of a reduced, two-level RC model, the quantity $\sigma(t, x) = 1 - \rho(t, D|x)$, and $\rho(t, D|x)$ is defined by Eq. 3.

Equation 46 may be used to model both transient absorption kinetics and quasi-stationary state effects for RCs. The calculated, light-induced, stationary-state absorption changes of RCs from Eq. 46 reveal nonlinear behavior of $\delta(\infty)$ and were described theoretically elsewhere (Gushcha et al., 1994; Goushcha et al., 1997a). The parameters x_D and x_A, discussed above, have a straightforward physical interpretation for RCs: they are generalized configurational coordinates for permanent localization of the photoelectron either on the donor D, as in thoroughly dark-adapted RCs, or on a quinone acceptor, Q_B or Q_A, for thoroughly light-adapted RCs, respectively.

Recombination rate constants as exponential functions of conformational coordinates

Another remarkable property of RC dynamics is the dependence of primary donor recombination kinetics upon illumination conditions (Kleinfeld et al., 1984b; Shaitan et al., 1991; Kalman and Maroti, 1997). For *Rb. sphaeroides* RCs that lack a secondary quinone acceptor Q_B, the charge recombination rate constant k_{rec} (see Eq. 1) equals the recombination rate constant $k_{AP} = 10\ s^{-1}$ for the radical pair $D^+Q_A^-$ (Parson and Ke, 1982; Kleinfeld et al., 1984b). For Q_B-containing *Rb. sphaeroides* RCs, electron transfer may be described by a two-level electronic scheme under physiological conditions (Kleinfeld et al., 1984a; Labahn et al., 1994). This situation is also exactly like the one of Eq. 1 with an effective recombination rate constant,

$$k_{rec} \approx k_{AP}\frac{k_{BA}}{k_{AB}} = k_{AP}\exp\left(\frac{\Delta G_{AB}}{k_B T}\right), \tag{47}$$

in which k_{AB} and k_{BA} are the forward and reverse electron transfer rate constants between the primary and the secondary quinone acceptors, respectively, and ΔG_{AB} is the free energy difference between the states $P^+Q_A^-Q_B$ and $P^+Q_AQ_B^-$. Note that ΔG_{AB} for the non-equilibrium case described in this work is the difference in the quasi-free energies $G_{P^+Q_AQ_B}(x)$ and $G_{P^+Q_A^-Q_B}(x)$. This difference equals the conventional standard free energy difference ΔG°_{AB} when RCs reach their equilibrium stable state in the dark (when $x \equiv x_D$).

The parameters k_{AP} and $\Delta G_{AB}/k_B T$ may both be important in modeling the correlation of photoelectron transfer with RC structure. As discussed in the first section, both parameters determine the survival time of the charge-separated state

$$(\tau_d)^{-1} = k_{AP} \quad \text{for } Q_B\text{-lacking RCs;} \tag{48}$$

and

$$(\tau_d)^{-1} = k_{AP}\exp\left(\frac{\Delta G_{AB}}{k_B T}\right) \quad \text{for } Q_B\text{-containing RCs.}$$

Our experiments demonstrate that one may need only consider one of these two parameters.

Fig. 4 *A* shows the primary donor absorbance recovery kinetics, measured at $\lambda = 865$ nm, for RCs from *Rb. sphaeroides* (wild type, wt) with inhibited $Q_A^- \rightarrow Q_B$ electron transfer. In this case, the recombination kinetics are determined by k_{AP}. Trace 1 of Fig. 4 *A* was obtained after flash-activation of a dark-adapted sample, while trace 2 was measured after switching off 2 min of background illumination that pre-illuminated the RCs ($\lambda = 700$–900 nm and $I^{exp} = 2\ mW/cm^2$). The values k_{AP} observed were 13 s^{-1} and 9 s^{-1} for the dark-adapted sample and the pre-illuminated sample, respectively. Such small but detectable differences in k_{AP} may well be due to light-induced structural changes in the RCs (Kleinfeld et al., 1984b; Shaitan et al., 1991).

Fig. 4 *B* shows the primary donor recovery kinetics for RCs from *Rb. sphaeroides* (wt) that are active in $Q_A^- \rightarrow Q_B$ electron transfer. We used isolated RCs with ~75% occupation of the Q_B site with native ubiquinone, without any reconstitution of Q_B activity. The sample was thoroughly degassed before experiments by multiple freeze-thaw-pump cycles down to 77 K and 10^{-6} torr. This procedure allowed us to avoid photo-oxidation of semiquinones by oxygen or other oxidants during illumination. Trace 1 in Fig. 4 *B* corresponds to flash-activated kinetics of a thoroughly dark-adapted sample. Trace 2 was measured after switching off 2 min of pre-illumination of the sample with saturating actinic light $I^{exp} = 2\ mW/cm^2$ at $\lambda = 700$–900 nm. Curve 1 in Fig. 4 *B* may be modeled with a recombination half-lifetime of ~1 s, whereas curve 2 reveals a decay half-lifetime of ~200 s. Such a drastic increase in the average survival time of the charge-separated state for a pre-illuminated sample cannot

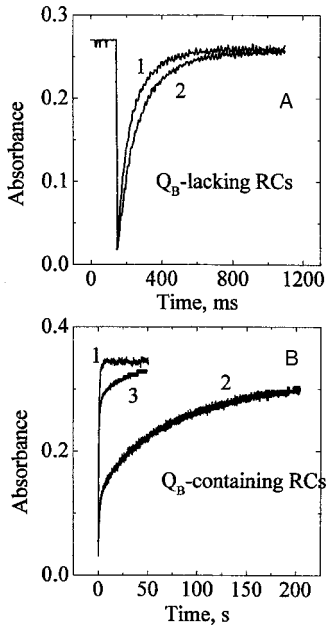

FIGURE 4 Primary donor recovery kinetics for RCs from *Rb. sphaeroides,* wt under different illumination conditions. (*A*) Q_B-lacking RCs: *curve 1* gives the absorbance change kinetics for a sample that was dark adapted and then flash-activated for 2 ms. *Curve 2* gives these kinetics following the cessation of 2 min of saturating intensity illumination. (*B*) Q_B-containing RCs: curves 1 and 2 are the kinetics for the same conditions as in (*A*). *Curve 3* was obtained as the response to a short, saturating flash (2 ms) applied 12 min after turning off 2 min of saturating intensity illumination to the sample. The buffer conditions were the same as used for the experiments shown in Fig. 3.

be explained by a light-induced decrease in the rate constant k_{AP}, which changes only slightly for similar pre-illumination conditions (see Fig. 4 *A*). Consequently, this increase in the recombination time constant for a Q_B-containing sample may reflect a light-induced increase in the value $|\Delta G_{AB}|/k_BT$.

In these experiments we assume a closed system with a fixed number of localized electron states and an absence of exogenous donors or acceptors. These assumptions are supported by the following observations. Several minutes after cessation of prolonged illumination, the RCs had undergone complete electron relaxation. Then we applied a saturating illumination flash. In cases of both Q_B-lacking and Q_B-containing RCs, the bleaching amplitude caused by this flash was the same as for a dark-adapted sample. Thus, the native activity of the pre-illuminated RCs was restored following charge recombination. However, the recovery kinetics of the primary donor, in response to this illumination flash, were quite different from those of a dark-adapted sample. These kinetics (not shown in Fig. 4 *A*) were nearly the same as those obtained for a 2-min, pre-illuminated sample in the case of Q_B-lacking RCs. For Q_B-containing RCs, the kinetics were intermediate between those of the thoroughly dark-adapted and of the pre-illuminated sample (*trace 3* in Fig. 4 *B*). This means that a significant fraction (15–20% for Q_B-containing RCs) of the RCs remain trapped in a second, light-induced conformation, one with a relaxation time $\geq$10 min for Q_B-containing RCs. A similar observation was reported by Kalman and Maroti (1997) who applied much higher intensities of actinic illumination to Q_B-lacking RCs to saturate the electronic state Q_A^-. This difference in experimental conditions may explain why our results for Q_B-lacking RCs differ from those of Kalman and Maroti, as well as why our results for Q_B-containing RCs are similar to theirs, even though they worked with Q_B-lacking RCs.

As noted above, the effects reported here cannot be explained by formation of states like $P^+Q_AQ_B$ with the electron having been captured by exogenous acceptors. Nevertheless, we do not completely exclude formation of such states and their influence on the dynamics of the system. This very interesting problem requires special attention, but was not studied in the present work. Additionally, sample preparation protocols involving treatment with detergents play a role in the effects observed. These results are not reported here either, but we plan to report them in subsequent work.

From the comparison of Eq. 47 with Eq. 31 we may assume that for the case of bacterial photosynthetic reaction centers the parameter $|\Delta G_{AB}|/k_BT$ may be identified with the slow generalized configurational coordinate x. This coordinate reflects slow, light-induced structural rearrangements in Q_B-containing RCs during system relaxation to its quasi-equilibrium, charge-separated state $P^+\ Q_AQ_B^-$. During relaxation, the system moves along a single trajectory in configurational space with a concomitant decrease in free energy, which remains a single-valued function of x. As already noted, this free energy should be considered as a quasi-free energy with respect to slow relaxation of medium polarization (Stratonovich, 1992, 1994). The quantity ΔG_{AB} is a single-valued function of the configurational coordinate during system relaxation along a chosen trajectory in configurational space. It should be considered as a quasi-free energy difference for the system, which does not yet reach thermodynamic equilibrium and is still subject to illumination. Hence we define

$$\frac{|\Delta G_{AB}|}{k_BT} = x, \tag{49}$$

emphasizing thus the dependence of the quasi-free energy difference ΔG_{AB} on the slow structural variables that govern system relaxation toward equilibrium in a charge-separated or a charge-neutral state. Assuming neglect of the relatively small, light-induced changes in the rate constant k_{AP} we estimate from Eq. 35 the light-induced changes in x as

$$\Delta x = \ln\frac{200}{1} \approx 5.3. \tag{50}$$

Therefore, the free energy difference $|\Delta G_{AB}|$ increases by $\geq$130 meV as a result of structural changes caused by multiple successive turnovers of the RC. Note that one may expect even larger light-induced changes in the standard free energy difference, $\Delta\Delta G^{\circ}_{AB}$, because the illumination conditions used in our experiments are not necessarily sufficiently intense or prolonged to satisfy RC equilibration near the potential minimum x_A. Thus, the generalized configurational coordinate, analogous to the perpendicular coordinate in the Agmon-Hopfield model, Eq. 49, is suitable for analysis of the correlation of structural dynamics and electron states in Q_B-containing RCs. Several recent papers have shown that slow structural dynamics play an important role in ultrafast charge separation steps in RCs, providing evidence for their adiabatic character and an illumination-controlled drop of the acceptor free energy (Holzwarth and Mueller, 1996; Lin et al., 1996). An analysis shows that the structural changes in response to ET in Q_B-lacking RCs cause a decrease in the free energy gap between donor (DQ_A) and acceptor ($D^+Q_A^-$) states of 120 meV (McMahon et al., 1998), comparable to the above result. Such a decrease in the free energy may be common in many biological charge transfer systems.

CONCLUSION

In the current paper we have developed a formalism for describing the evolution of open, non-equilibrium biomolecular systems with photoinduced charge separation. Upon steady-state illumination, such a system may have several stationary states, the number and relative thermodynamic stability of which depends on the illumination intensity. The thermodynamic stability of such an open system is determined by Eq. 27, in which the system effective adiabatic potential V^I_{ad} is used instead of the standard free energy G° to describe equilibrium. Note that the fundamental Gibbs relationship $P(G^{\circ}) = Z^{-1} \exp(-G^{\circ}/k_BT)$ that defines the probability of finding the system with a particular free energy G° is valid only for a system in thermodynamic equilibrium with an absence of external forces, which is not true in this case. Therefore, Eq. 27, which defines the probability of finding a non-equilibrium system in configuration x, can be considered as a generalization of the Gibbs relationship for the case of non-equilibrium biomolecular systems with photoinduced charge separation.

The general kinetic formalism developed in this work describes non-equilibrium changes in macromolecular structure caused by light-induced redistribution of charge density among redox co-factors, both under steady-state and non-stationary-state illumination intensities. Such structural changes may have relaxation times of up to minutes due to slow structural modes to provide adaptation of the macromolecule for prolonged illumination. This adaptation results in a continuous deformation of the initial "dark" conformational state, for the case of weak electron-conformational interaction, $\xi = |x_A - x_D| < 4$, and results in a continuous shift of the adiabatic potential minimum from x_A to x_D following an increase in the illumination intensity. For the case of a strong electron-conformational interaction, $\xi = |x_A - x_D| \geq 4$, the initial "dark" conformational state shifts only slightly with an I increase until the light intensity reaches a critical value I^{cr}_I. At this illumination level a new "light-adapted" conformational state of biomolecule appears. The minimum for this new conformational state also does not shift significantly in x with a further increase in I. However, the relative free energies of the two conformational states and the shape of their potential profiles change considerably with I variation. Such a behavior is an example of a first-order non-equilibrium phase transition (Haken, 1983) in biomolecular systems.

The second, "light-adapted" conformational state has a much longer survival time for the charge-separated state relative to the "dark-adapted" conformational state. This situation was shown experimentally for the case of photosynthetic RCs. Functionally important, light-induced structural rearrangements in RCs were discussed originally in 1984 by Kleinfeld et al. Because slow structural motion provides a structural "memory" effect, the "light-adapted" conformational state may remain unrelaxed for a long time, even during a significant decrease in the illumination intensity.

The theory of non-equilibrium charged particle flow through a flexible macromolecular structure developed in this work may be relevant for a description of many nonlinear effects that have been observed in other charge transfer, biomolecular systems. A strong correlation between the proton flux (concentration) and macromolecular conformation in bacteriorhodopsin may be explained within nonlinear dynamic theory (Hong, 1999; Brown et al., 1997; Sass et al., 1998). If the macromolecular system can be characterized by two generalized conformational coordinates that function as control parameters, we predict that not only mono and bistable functioning regimes exist, as described here, but sustained or damped oscillations may additionally occur. The period of these oscillations could be much longer than the characteristic time for a single charge-transfer step. One example might be the oscillations of dye luminescence and pH observed recently in bacteriorhodopsins for different levels of optical excitation (Tributsch and Bogomolni, 1994; Birge, 1994). For a system that includes three or more conformational coordinates or control modes, more complicated, non-periodic regimes, such as dynamic chaos, might occur.

We have described properties of charge-conformational systems of biomolecules that are responsible for their functional behavior. Electron transfer enzymes, such as cytochrome *c* oxidase and cytochrome *c* peroxidase, proton pumping systems like bacteriorhodopsin and ATPases, and the ion channels of biomembranes, for which efficiency of function is likely determined by many structural control

parameters, may be additional examples. Finally, the *mechanics* of slow structural changes that accumulate by the action of multiple, elementary events likely play a significant role in the function of a variety of enzymes, not necessarily only those involving charge transfer.

APPENDIX

We denote $\bar{\rho}(t) = \Sigma_n \rho(t; n)|n\rangle$, in which $|n\rangle$, $n = 1, \ldots N$ is a system of normalized orthogonal vectors satisfying the condition $\langle n|m\rangle = \delta_{nm}$. Then Eq. 2 for $\rho(t; n)$, in the case of an arbitrary but finite number of electronic states, may be written as follows:

$$\frac{\partial \rho(t)}{\partial t} = \hat{L}\bar{\rho}(t), \tag{A1}$$

in which the operator $\hat{L}$ is

$$\hat{L} = \hat{L}_0 - I*[|D\rangle\langle D| - |A\rangle\langle D|]*\rho(t, D), \tag{A2}$$

and $|D\rangle$ and $|A\rangle$ are normalized orthogonal vectors corresponding to states with an electron on the donor P or on the acceptor A, respectively. We introduced terms containing $I*\rho(t, D)$ in the last term of the right-hand side of Eq. A2.

Let ρ_0 be a solution of the equation,

$$\frac{\partial \bar{\rho}_0(t)}{\partial t} = \hat{L}_0\bar{\rho}_0(t). \tag{A3}$$

It is easy to show, after substitution, that the formal solution of Eq. A1 is

$$\bar{\rho}(t) = \bar{\rho}_0(t) - I\int_0^t d\tau[\bar{\rho}_0^{\mathrm{D}}(t-\tau) - \bar{\rho}_0^{\mathrm{A}}{}_{(t-\tau)}]\rho(\tau, D), \tag{A4}$$

in which

$$\bar{\rho}_0^{\mathrm{D}}(0) \equiv |D\rangle, \quad \bar{\rho}_0^{\mathrm{A}}(0) \equiv |A\rangle. \tag{A5}$$

Using the Laplace transformation $\tilde{\rho}(s) = \int_0^\infty \exp(-st)\rho(t)dt$ on Eq. A4 and multiplying it from the left side by $\langle D|$ we obtain

$$\tilde{\rho}(s; D) = \tilde{\rho}_0(s; D) - I[\tilde{\rho}_0^{\mathrm{D}}(s; D) - \tilde{\rho}_0^{\mathrm{A}}(s; D)]\tilde{\rho}(s; D), \tag{A6}$$

from which we observe

$$\tilde{\rho}(s; D) = \frac{\tilde{\rho}_{0(s; D)}}{1 + I[\tilde{\rho}_0^{\mathrm{D}}(s; D) - \tilde{\rho}_0^{\mathrm{A}}(s; D)]}. \tag{A7}$$

Obviously $\tilde{\rho}_0^{\mathrm{D}}(s; D) = 1/s$. Using the known correlation (Korn and Korn, 1968): $\lim_{s\to 0} s\tilde{f}(s) = \lim_{t\to\infty} f(t)$ and taking into account that $(1/s^2) - (\tilde{\rho}_0^{\mathrm{A}}(s; D)/s)$ defines the Laplace-transform of $\int_0^t[1 - \rho_0^{\mathrm{A}}(t', D)]dt'$, we find from Eq. A7 that

$$\rho_{\mathrm{I}}(\infty, D) = \frac{1}{1 + I\int_0^\infty \sigma_0^{\mathrm{A}}(t)dt}. \tag{A8}$$

Hence, in accord with the definition of the first section,

$$\sigma_{\mathrm{I}}(\infty) \equiv 1 - \rho_{\mathrm{I}}(\infty, D) = \frac{I}{I + \left(\int_0^\infty \sigma_0^{\mathrm{A}}(t)dt\right)^{-1}}. \tag{A9}$$

Then, in agreement with Eq. 7 the survival time of the charge-separated state that characterizes the efficiency of stationary state charge separation in intact systems is

$$\tau_{\mathrm{d}} = \int_0^\infty \sigma^{\mathrm{A}}(t)dt, \tag{A10}$$

and does not depend on the intensity of illumination.

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