Molecular Dynamics Simulation of Electron Transfer in Proteins. Theory and Application to $Q_A \rightarrow Q_B$ Transfer in the Photosynthetic Reaction Center

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Electron transfer (ET) from the primary menaquinone $Q_A$ to the secondary ubiquinone $Q_B$, i.e., $Q_A Q_A^* \rightarrow Q_B Q_B^*$, in the photosynthetic reaction center of *Rhodopseudomonas viridis* has been simulated by using the method of molecular dynamics accounting for the classical motion of a protein's nuclear degrees of freedom, the redistribution of charge accompanying electron transfer being described quantum chemically. We outline the role of classical nuclear degrees of freedom in electron transfer, identifying the essential dynamic properties that should be determined from molecular dynamics simulations in order to characterize electron transfer. These quantities, all related to the energy difference $\Delta E(t) = E_B(t) - E_A(t)$ of virtual forward (electron tries to jump forward after ET) and backward (electron tries to jump backward after ET) electron transfer, and the respective variances of $\Delta E(t)$ and the average value of $\Delta E(t)$ before and after electron transfer, respectively, are as follows: the variance of $\Delta E(t)$ and the average value of $\Delta E(t)$ before and after electron transfer, respectively; the relaxation time of the energy–energy correlation function $\langle (\Delta E(t) - \langle \Delta E \rangle)(\Delta E(0) - \langle \Delta E \rangle) \rangle$ (120 fs); the time describing the relaxation of the energy distribution from an average value to an average value immediately after electron transfer (200 fs). The quantities in brackets are the respective simulation results. We determined also the free enthalpy difference of the transfer $Q_A Q_A^* \rightarrow Q_B Q_B^*$ ($-3.4$ kJ/mol). Our simulations indicate that the motion of the non-heme iron of the reaction center is not coupled to the $Q_B Q_B^*$ transfer. Interaction energies of $Q_B$ in different charge states with the protein environment have been calculated and reflect a stronger binding of $Q_A$ and $Q_B^*$ compared to that of $Q_B$.

1. Introduction

Photoreduction is one of the most important and most widely studied processes in living systems. The primary steps in photoreduction involve the absorption of light energy and its conversion into an electrochemical potential. In photosynthetic bacteria the step takes place in the so-called photosynthetic reaction center, a large protein-pigment complex, located in the cellular membrane. In the past such processes have been investigated in solvents, an area of inquiry that was very much influenced by the seminal work of Weller. For a long time photoinduced electron transfer in solvents was much better understood than that in photosynthetic proteins due to the many systems available for study and due to the seemingly simpler and better defined environment in which the transfer takes place. However, the availability of high-resolution X-ray structures of the reaction centers of the purple bacteria *Rhodopseudomonas viridis* and *Rhodopseudomonas sphaeroides* recently determined5,6 reversed the situation. Detailed reviews of the primary electron-transfer steps of photoreduction can be found in refs 7-9.

In particular, the available structures allow one to explore the mechanism of light energy conversion in these systems using the method of molecular dynamics simulation. Such simulations are considered today an important source of information on protein structure, function, and mechanism.4,4 These simulations can also provide an extremely detailed view of the electron-transfer process. Many questions addressed by the pioneers of photoinduced electron transfer in solution can now be answered, and details concerning the coupling between charge displacement and solvent motion can be uncovered.

The reaction center complex of *Rhodopseudomonas viridis* consists of four protein subunits, called cytochrome, L, M, and H. In addition, it contains 14 major cofactors: four heme groups are covalently attached to the cytochrome subunit, two closely associated bacteriochlorophyll b (BCMP, BCLP), forming the so-called special pair (SP), two accessory chlorophyll b (BCMA, BCLA), two bacteriopheophytins (BPM, BPL), one menaquinone ($Q_A$), one ubiquinone ($Q_B$), a carotenoid (NS1), and a non-heme iron ion (FE1). Some of these cofactors are shown in Figure 1. Absorption of light by the special pair leads to its first excited singlet state. This excitation initiates a sequence of electron-transfer reactions from the special pair to the bacteriopheophytin BPL, to the primary quinone $Q_A$, and finally to the secondary quinone $Q_B$. The secondary quinone actually receives two electrons through this pathway, and from the cytoplasmic side of the protein it receives two protons. $Q_B HS_2$ is therefore left in the reaction center.

The electron-transfer processes from the excited special pair to BPL, i.e., SP*BPL → SP*BPL−, and from BPL to the primary quinone $Q_A$, i.e., BPL−$Q_A$ → BPL$Q_A^*$, occur on a time scale shorter than a nanosecond, i.e., on a time scale that is accessible to computer simulations.9,10 Dynamics simulations of these processes have already been performed, leading to information about the structural response of the protein matrix to these electron-transfer steps, about the stabilization of the product state,11,12 about the temperature dependence of transfer reactions,13 and about the role of quantum tunneling processes.14,15

In this contribution we focus on the electron-transfer step $Q_A Q_A^* \rightarrow Q_B Q_B^*$. Many experiments regarding this transfer step have been carried out, and the function of the $Q_B$ binding site has been

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The space between Qₐ and Qₜ in the photosynthetic reaction center is bridged by a non-heme iron (FEI in Figure 1), which is ligated by histidines of the L (L190, L230) and M (M217, M264) subunits and by one glutamic acid (M232). The function of the non-heme iron can be manifold. As a 3D transition element iron has a strong affinity to undergo complexation. Its ligation by histidines L190 and M217 leads to a bridge between Qₐ and Qₜ, which might enhance the tunneling efficiency of an electron between Qₐ and Qₜ. The small fluctuations of the iron ion, as observed in the simulations, are indicative of a relatively strongly ligated. The iron, in the case that it carries a net formal charge 2+, (the charge assumed in our simulation), would be involved in strong electrostatic interactions with Qₐ and Qₜ and the groups nearby. ESR studies have shown that both Qₐ and Qₜ interact with the iron atom, generating a highly distorted and broadened EPR signal. The species Qₐ·FeQₜ, which can be formed at low temperature, exhibits no detectable EPR signal, indicating a coupling of the two spins. After extraction of the iron, the transfer QₐQₜ → QₐQₜ in *Rhodopseudomonas sphaeroides* is slower by a factor of 2.327 and after removing the H-subunit the transfer is inhibited. An appropriate method to test the Q₆ binding site is experiments with herbicides that replace the quinone bound to the Q₆ site.29 Crystal structures of reaction centers with bound herbicides are known, and herbicide binding sites have been analyzed.29 The transfer Qₕ Qₐ → Qₘ Qₜ is much slower than the primary transfers SP₅→BPL → SP₅→BPL' and BPL'Q₄ → BPLQ₅+ and occurs on the time scale of microseconds to milliseconds. Therefore, molecular dynamics simulations of fluctuations and relaxation accompanying the QₐQₜ → Qₘ Qₜ transfer are beyond today's computing capacities.

We will adopt, therefore, the following procedures. In a first simulation (A), lasting 25 ps, we describe the state QₚQₜQₚ, i.e., the state before the transfer. In a second simulation (B), lasting 2 ps, the atomic partial charges of Q₆ and Qₚ are initially altered in accordance with the state QₕQₜ. The second simulation describes the response of the protein environment to the transfer QₕQₜ → QₚQₜ and the dielectric relaxation after the transfer. In a third simulation (C), lasting 20 ps, the equilibrium reached after the transfer is analyzed, e.g., average structure and atomic mobilities are determined. The coupling of the protein environment to the electron-transfer process is monitored through the energy ΔE(t) defined as the energy required for a virtual electron transfer, i.e., QₕQₜ → QₚQₜ in case of trajectory A and QₕQₜ → QₚQₜ in case of trajectory B.

We have also investigated in how far the non-heme iron is coupled to the QₐQₜQₜ → QₐQₜ transfer. For this purpose we have carried out the same simulation as in cases A and B, except that an iron with a small mass of 1 au had been assumed. We will refer to these simulations as A' and B'.

2. Method of Calculation

Our simulations are based on the X-ray structure of the photosynthetic reaction center of *Rhodopseudomonas viridis* at 2.3 Å resolution. Charge distributions for the special pair, the accesso-chlorophylls, and the bacteriopheophytins were taken from INDO calculations.30 The partial charges of the two quinones Qₕ and Qₚ and the semiquinones Qₚ· and Qₚ+ were calculated with the program package AMPAC29 using the AM1 Hamiltonian. The charge states for the amino acids are those for pH 7, assuming standard pK values, except for glutamate L104 which was protonated. Molecular dynamics simulations have been carried out with the program XPLOR43 using the CHARMM force field.42

To reduce the molecular dynamics simulations to a size that can be handled computationally, the stochastic boundary method43,44 as implemented in XPLOR has been applied. This method

(37) Deisenhofer, J.; Epp, Sining; Michel, H., manuscript in preparation.
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divides the simulated protein into three regions. A first inner region, a sphere centered at the non-heme iron and containing all the atoms within a radius of 26.5 Å, is described by conventional molecular dynamics. The chromophores involved in electron transfer (SP, BCLA, BPL, QA, and QB as well as BCMA and BPM) are contained in this region, together with 4207 atoms. In a second region, within a spherical shell around the first region with inner and outer radii of 26.5 and 29.0 Å, respectively, atomic motion is treated by Langevin dynamics, introducing thermal and frictional effects of a surrounding bath. This region contains 644 atoms. A third region, a spherical shell around the first two regions with inner and outer radii of 29.0 and 37.5 Å, respectively, was not simulated explicitly. Coulomb interactions of charged amino acids located in this region with atoms in the two inner regions were taken into account by representing each charged amino acid through a single, fixed point charge.

The first and second region together contained 5315 atoms, including 83 water molecules detected in the X-ray structure. Simulations have been carried out with an integration step size of 1 fs applying the SHAKE algorithm. Simulations with a "light" iron atom with a mass of 1 au were carried out with an integration step size of only 0.5 fs without application of SHAKE.

Dielectric fluctuations and relaxation accompanying electron transfer were monitored by calculating the expression \( \Delta E(t) = E_P(t) - E_R(t) \), where \( E_P(t) \) and \( E_R(t) \) denote the Coulomb energies of the reaction center with chromophore charge distributions according to the reactant and product state, respectively. Before the electron transfer, \( \Delta E(t) \) corresponds to the energy required to transfer an electron from the reactant to the product state when the protein motion corresponds to that of a charge distribution of the reactant state. After the electron transfer, \( -\Delta E(t) \) is the energy required for transfer of an electron from the product state to the reactant state when the protein motion corresponds to that of a charge distribution of the product state. We denote the mean value of \( \Delta E(t) \) before the transfer as \( \langle \Delta E \rangle_{Q_AQ_B} \), and the corresponding value after the transfer as \( \langle \Delta E \rangle_{Q_AQ_B} \). Data sampling took place in the following way: after equilibration, a 25-ps simulation (trajectory A) was run applying the charge distribution for the reactant state. In intervals of 10 fs the energy \( \Delta E(t) \) for a virtual transfer was determined as follows: for the momentaneous protein configuration the total Coulomb energy in the Q_AQ_B state, \( E_P(t) \), as well as in the state Q_AQ_B, \( E_R(t) \), were determined, and \( \Delta E(t) = E_P(t) - E_R(t) \) was calculated. Thus \( \Delta E(t) \) corresponds to the energy required for instantaneous electron transfer. The procedure described was adopted also for trajectories B and C.

3. Formal Description of the Coupling between Protein Motion and Electron Transfer

In this section we will outline how thermal fluctuations in \( \Delta E(t) \) control electron transfer. We will adapt for this purpose existing theories of electron transfer in solution. The starting point of our consideration is the fact that our molecular dynamics calculations treat the protein classically. Such a description suffices for low-frequency motions, but not for intramolecular high-frequency motions. Furthermore, the simulations do not account for intramolecular and Born energy type contributions to the redox energy. Hence, we can expect to describe by our simulations only a contribution to the electron-transfer rate that is due to coupling of the transfer process to the protein environment. To account for this limitation, we represent the electron-transfer rate formally through the following convolution integral:

\[
k_{tot} = \sum_{\epsilon} \epsilon k_{\epsilon}(\epsilon) S_{\Delta \epsilon}(\epsilon)
\]

This representation assumes that two classes of motions couple to the electron transfer, a class of quantum mechanical motions described by a line-shape function \( S_{\Delta \epsilon}(E) \) and a class of classical motions that give rise to the energy-transfer rate \( k_{\epsilon}(E) \). The two classes of motions exchange energy \( \epsilon \) and \( \Delta \epsilon \) during the transition, the sum of energies exchanged being zero since electron transfer is a thermal process, i.e., does not require external energy, except thermal energies exchanged with a heat bath, an exchange that is included in calculating \( S_{\Delta \epsilon}(E) \) and \( k_{\epsilon}(E) \).

In keeping with the character of molecular dynamics simulations we will focus on the properties of the classical contribution and assume that the quantum mechanical degrees of freedom give rise to an unspecified line-shape function \( S_{\Delta \epsilon}(E) \). However, for the sake of completeness we outline the properties of \( S_{\Delta \epsilon}(E) \) for the case that the quantum degrees of freedom are harmonic. The line-shape function \( S_{\Delta \epsilon}(E) \) is then the Franck-Condon and Boltzmann-weighted energy density of final states given by the convolution

\[
S_{\Delta \epsilon}(E) = \int dE_1 \int dE_2 \cdots \int dE_N \prod_{j=1}^N S_j(E_j) \delta (N \sum_{j=1}^N E_j - E - E_{redox-Born}) \]

Each of the line-shape functions \( S_j(E) \) describes a single degree of freedom and is

\[
S_j(E) = \frac{e^{-\frac{(E-E_0j)}{\hbar \omega_j}}}{\hbar \omega_j} \sum_{k=-\infty}^{\infty} \delta (k - \frac{E - E_0j}{\hbar \omega_j}) \frac{e^{-\frac{(E-E_0j)}{\hbar \omega}}}{\hbar \omega} (2s_j \sqrt{n_j(n_j + 1)})
\]

where \( s_j = 1/\sqrt{\sum_j} \) is the so-called reorganization energy in units of vibrational quanta and \( n_j \) is the average number of quanta thermally excited in the oscillator. The quantum mechanical line-shape function also accounts for the electronic and Born contributions to the redox energy \( E_{redox-Born} \).

We have presented the roles of quantum and classical degrees of freedom in (1) in an asymmetric way. In fact, in (1) the coupling of the classical degrees of freedom to electron transfer, in principle, is described exactly, whereas the quantum degrees of freedom enter through the final state density \( S_{\Delta \epsilon}(E) \) in a manner similar to their appearance in Fermi's golden rule. A description that applies the latter approximation to both classical and quantum mechanical degrees of freedom yields the transfer rate

\[
k_{tot} = \frac{2\pi V_0^2}{\hbar} \int d\epsilon S_\epsilon(\epsilon) S_{\Delta \epsilon}(\epsilon)
\]

where \( S_\epsilon(\epsilon) \) is a classical line-shape function and where \( V_0 \) describes the electronic coupling matrix element between reactant and product states, i.e., in the present case between Q_AQ_B and Q_AQ_B.

In order to describe, as required to apply (1), the coupling between the classical nuclear degrees of freedom and electron transfer exactly, a description is needed that expresses the corresponding coupling. Such coupling is provided by a model that describes the electronic quantum system by a two-state Hamiltonian:

\[
H_{el}(t) = \begin{pmatrix} \epsilon & V_{el} \\ V_{el} & \Delta E(t) \end{pmatrix}
\]

where \( \epsilon \) is the energy "exchanged" with the quantum mechanical nuclear and electronic degrees of freedom and where the energy difference \( \Delta E(t) \) describes the coupling and thermal motion of the classical degrees of freedom. The electron transfer can then be described by a 2 × 2 density matrix \( \rho \) that obeys the Liouville equation

\[
\partial \rho(t) = \frac{i}{\hbar} [H_{el}(t), \rho(t)] - [K, \rho(t)]_{++},
\]

\[
K = \begin{pmatrix} 0 & 1 \\ 0 & -1 \end{pmatrix}, \quad \rho(0) = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}
\]

Here we employed the notation \( [A,B]_+ = AB + BA \). \( s^{-1} \) measures the relaxation of the system in the product state Q_AQ_B, i.e.
essentially the rate of solvation of the state after electron transfer. $r_1(t)$ gives the probability of finding the system at time $t$ in the (initial) reactant state $Q_A Q_B$. Assuming for the sake of approximation a monoeponential decay of the initial state, the electron-transfer rate is approximately

$$k_1^{-1}(t) = \int_0^t dt \ r_1(t)$$

(7)

This transfer time has been determined in ref 31 for the primary and secondary electron transfers in the photosynthetic reaction center by solving eq 6.

$\Delta E(t)$ introduced above is evaluated as the energy difference between the charge state, e.g., $Q_AQ_B$, for which a classical trajectory of the protein is evaluated, and a virtual charge state, e.g., $Q_AQ_B$. It is obvious that the properties of $\Delta E(t)$ influence the electron-transfer rate, an influence that would be described quantitatively by solving the evolution equation for the density matrix of the two-state system.

We now discuss which features of $\Delta E(t)$ are relevant in determining the transfer rate. For this purpose we assume momentarily for the purpose of argument that the classical degrees of freedom are also harmonic and that their influence can be accounted for by a line-shape function as in (4). In this case the line-shape function is given by the convolution

$$S_0(\Delta E) = \int dE_1 \int dE_2 \cdots \int dE_N \left[ \prod_{j=1}^N f_j S_j(E_j) \right] \delta(\sum_{j=1}^N E_j - \Delta E)$$

(8)

The single mode contributions are

$$S_j(E) \sim \frac{1}{\sqrt{2\pi \sigma_j q_j^2}} \exp \left( -\frac{(E_{0j} + \frac{1}{2} f_j q_j^2 - E_j)^2}{2f_j q_j^2 \sigma_j^2} \right)$$

(9)

where

$$\sigma_j = kT f_j$$

(10)

The line-shape function (8) can be identified with the normalized distribution of $\Delta E(t)$ values resulting from a molecular dynamics simulation. This distribution can be expressed by the single Gaussian

$$S_0(\Delta E) = (1/\sqrt{2\pi \Sigma}) \exp(-((\Delta E) - \Delta E)^2/\Sigma)$$

(11)

where

$$\Sigma = \sum_{j=1}^{N'} \frac{1}{2f_j q_j^2} \sigma_j \quad \langle \Delta E \rangle = \sum_{j=1}^{N'} (E_{0j} + \frac{1}{2} f_j q_j^2)$$

(12)

According to the central limit theorem, one can argue that even in the case where the individual classical degrees of freedom are not harmonic and, therefore, not accounted for by Gaussian-type line-shape functions (9), the convolution of many line-shape functions, irrespective of their exact form, should still yield a Gaussian like (11). For this property to hold, the line-shape functions $S_j(E)$ contributing in (8) must obey the Lindeberg condition, which requires that the wings of $S_j(E)$ decay rapidly enough. In fact, our simulations discussed below yield a distribution $S_0(\Delta E)$ that can be well represented by a Gaussian line shape.

The simulations presented below reveal that an electron transfer is accompanied by a change of the mean value ($\langle \Delta E \rangle$) from a value ($\langle \Delta E \rangle_{Q_AQ_B}$ before the transfer to a value ($\langle \Delta E \rangle_{Q_AQ_B}$ after the transfer. One can show that the overall effect of the classical degrees of freedom on $\Delta E(t)$ can be accounted for by effective energy potentials for reactant and product states that are consistent with a Gaussian line-shape function. The corresponding potential functions are exactly those assumed in the Marcus theory of electron transfer, namely, two displaced harmonic potentials with identical force constants:

$$E_R(q) = \frac{1}{2} f q^2, \quad E_P(q) = \frac{1}{2} f (q - \Delta)^2 + \Delta E_0$$

(13)

The three conditions (14)–(16) allow one to determine the potential surfaces (13) shown in Figure 2 from the quantities $\Sigma$, $\langle \Delta E \rangle_{Q_AQ_B}$, and $\langle \Delta E(t) \rangle_{Q_AQ_B}$ available through molecular dynamics simulations (see section 4.2). Actually, simulations yield slightly different values for the variance of the fluctuations of $\Delta E(t)$ before ($\Sigma_E$) and after ($\Sigma_{\Delta E}$) electron transfer. However, in the photosynthetic reaction center the variances do not depend strongly on the charge state of the chromophores, and hence a single value suffices to characterize the effective potential surfaces of the system. This feature is very fortunate since it implies that $\Delta E(q) = E_R(q) - E_P(q)$ is a monotonous (and linear) function of $q$, a feature exploited by the Marcus theory.

At this point it should be noted that the quantities $\Sigma$, $\langle \Delta E \rangle_{Q_AQ_B}$, and $\langle \Delta E(t) \rangle_{Q_AQ_B}$, and therefore also $f$, $\Delta$, and $E_0$, i.e., the potentials in Figure 2, can be found to vary with temperature. In fact, a significant temperature variation of these quantities has been observed in case of a simulation of the two transfers SP BPL $Q_A$ → SP $^+\mathbf{BPL}^{-}$ $Q_A$ → SP $^+\mathbf{BPL}^{-}$ $Q_A$. Actually, the coordinate $q$ used in depicting the potential curves $E_R(q)$ and $E_P(q)$ is only implicitly needed for a description of electron transfer. All that is explicitly needed for a quantum mechanical description of such transfer through the Hamiltonian (5) is knowledge of $\Delta E(t) = E_R(q(t)) - E_P(q(t))$, which results from the thermal motion of the protein, the explicit $q$ coordinate being irrelevant. If one assumes that the thermal motion is Brownian, one can seek a stochastic model that reproduces the line-shape function (11). Such a model is provided through the Fokker–Planck equation

$$\partial \rho(\Delta E,t) = D \partial_{\Delta E} S_0(\Delta E) \partial_{\Delta E} [S_0(\Delta E)]^{-1} \rho(\Delta E,t)$$

(17)

which describes the probability to observe an energy $\Delta E$ (for virtual electron transfer) at time $t$. One can prove that the probability distribution $\rho(\Delta E,t)$ asymptotically converges toward the line-shape function, i.e.
The Fokker–Planck equation (17) entails the constant $D$, which accounts for the random dynamics. For the Gaussian form of $S_{\alpha}(\Delta E)$ this quantity determines the energy–energy correlation function in a simple manner:

$$C_{\alpha\alpha}(t) = \frac{(\langle \Delta E(t_0 + t) - \langle \Delta E(0) \rangle \rangle)^2}{\langle \Delta E^2(0) \rangle} = e^{-2Dt/\Sigma^2}$$  \hspace{1cm} (19)

This correlation function can also be determined through a molecular dynamics simulation. One can then establish the stochastic model (17) by monitoring $\Delta E(t)$ in a molecular dynamics simulation, determine numerically the corresponding distribution function (11) and correlation function (19), and match the quantities $\Sigma \Delta E_0$, $D$. A stochastic model has considerable advantages over using in a quantum mechanical description $\Delta E(t)$ directly as obtained from a simulation. The first reason is that the simple formulation in terms of three parameters allows one to compare different electronic systems. The second reason is that the stochastic model considered allows one to give an expression for the electron-transfer rate $k_{\alpha}(\epsilon)$ in a simple and transparent form. This representation has been derived in ref 35. Employing the expression (7) the theory in $^{35}$ yields

$$k^{-1}(\epsilon) = \int_0^\infty dt \left( \int dE \exp \left[ \frac{\hbar}{\varepsilon} \left( \frac{\hbar}{\varepsilon} V_{el} E' - \frac{i \hbar}{\tau} \right) \right] \right) \times$$

$$\begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} \exp \left[ \frac{\hbar}{\varepsilon} \left( \frac{\hbar}{\varepsilon} V_{el} E' + \frac{i \hbar}{\tau} \right) \right] \right) I(E')$$  \hspace{1cm} (20)

where $I(E')$ represents the Kubo line-shape function

$$I(E') = \frac{1}{\pi} \text{Re} \left( \frac{1}{D \partial_{\Delta E} S_{\alpha}(\Delta E) \partial_{\Delta E} [S_{\alpha}(\Delta E)]^{-1}} + (i/\hbar)(\Delta E - E') \right)$$  \hspace{1cm} (21)

This expression is actually an approximation, accurate to third order in $V_{el}$, the representation conserving the trace of the density matrix and converging to the exact results in the limits of both slow and fast stochastic motion.

One can write (20) as

$$k^{-1}(\epsilon) = \int dE' \hat{k}^{-1}(\epsilon - E') I(E')$$  \hspace{1cm} (22)

where

$$\hat{k}^{-1}(\epsilon - E') = \int_0^\infty dt \left( \exp \left[ \frac{-\hbar}{\varepsilon} \left( \frac{\hbar}{\varepsilon} V_{el} E' - \frac{i \hbar}{\tau} \right) \right] \right) \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} \times$$

$$\exp \left[ \frac{-\hbar}{\varepsilon} \left( \frac{\hbar}{\varepsilon} V_{el} E' + \frac{i \hbar}{\tau} \right) \right] \right)_{11}$$  \hspace{1cm} (23)

is the electron-transfer time for a time-independent Hamiltonian, i.e., one with $\Delta E(t) = E'$. The description provided by (22) and (24) can be readily interpreted. The rate $k(\epsilon - E')$ is strongly peaked at $\epsilon = E'$ with a typical width of about $10^{-3}$ eV determined by either $V_{el}$ or $\hbar/\tau$, whichever is larger. However, the width of the Kubo line-shape function $I(E')$ is determined by the spectrum of the Fokker–Planck operator in (17) and by $\Sigma$, which leads to a bell-shaped dependence of $k(\epsilon)$ with a width of a few tenths of an electronvolt. This behavior, which is close to that described by the Marcus theory of electron transfer, has been discussed in more detail in ref 31. This reference also provides quantum mechanical calculations of transfer rates in the photosynthetic reaction center together with a detailed discussion of the $\epsilon$ dependence. Also an expression for the transfer rate, which is numerically more suitable than eq 22, is introduced. Such $\epsilon$ dependence implies that the intramolecular redox energy is not required to be tuned exactly to an optimal value, but rather that changes of the redox energies of the chromophores engaged in the electron transfer can be accommodated. The recent findings by Kirmayer et al. $^{47}$ that the chromophore BPL (bacteriochlorophyll) in the photosynthetically reaction center of Rh. sphaeroides can be exchanged for a bacteriochlorophyll with a change in redox energy of about 0.2 eV, however, with a minor concomitant change in the electron-transfer rate, attests to our conclusion.

In our analysis of molecular dynamics simulations below we will focus on the quantities that are relevant in the context of the electron-transfer theory outlined. We will discuss the behavior of $\Delta E(t)$ and of the corresponding distribution $S_{\alpha}(\Delta E)$ as well as consider the energy–energy correlation function.

4. Results and Discussion

1. Structure of the Reaction Center with $Q_{\alpha}^-$. Starting from the X-ray structure the chosen segment of the reaction center has been equilibrated at 300 K for 15 ps. The charge distribution assumed represents a reaction center with a neutral special pair and a primary menaquinone with a charge of $-1$. A neutral special pair has been chosen because the time needed for neutralization of SP$^+$ is $\tau = 0.2 \mu s$, $^{32}$ which is shorter than the time, $\tau \approx 200 \mu s$, for the transfer $Q_A^-Q_B^- \rightarrow Q_A^-Q_{\alpha}^-$. $^{47}$ Following the equilibration a 25-ps trajectory A has been calculated. The results of this simulation will be presented now.

We first investigated the average structure resulting for trajectory A. We found that the X-ray structure was largely preserved; the mean deviation between our average structure and the X-ray structure, represented in the form of a root-mean-square (rms) value, is 2.0 Å for all the atoms and 1.1 Å for all the C atoms. These values are, however, larger than those found in previous simulations for neutral chromophores $^{11}$ with corresponding rms values of 1.06 and 0.63 Å, respectively. The structural differences are believed to be mainly due to the different charge distribution since the X-ray structure corresponds to chromophores in the neutral state. Differences to the calculation reported in ref 11 could also appear due to the selection of another segment of the reaction center in the present simulation.

An interesting feature of the average structure of run A is a shortening of the distance between $Q_A$ and FE1 by about 0.4 Å relative to the distance found in the X-ray structure. The closer distance should be due to the electrostatic interaction between the positively charged FE1 ($+2$) and $Q_A^-$. Since the electron transfer between $Q_A^-Q_B$ and $Q_{\alpha}^-Q_B$ involves electron tunneling and, therefore, would be strongly affected by the edge-to-edge distance between $Q_A$ and $Q_B$, a closer FE1–Q$_A$ distance and, simultaneously, a closer Q$_A^-$Q$_B$ distance may prepare the reaction center for the following electron transfer $Q_A^-Q_B^- \rightarrow Q_{\alpha}^-Q_B^-$. In this context it may be interesting to examine the fluctuations of the distance between $Q_A^-$ and $Q_B$. The center-to-center distance observed during a trajectory of 25 ps is shown in Figure 3. The distance shows smaller fluctuations with sporadic large deviations from the mean distance of more than ±0.6 Å. On a longer time scale even larger displacements may occur. Therefore, these fluctuations could strongly influence the electronic matrix element between $Q_A^-$ and $Q_B$. Consequently, the rate-limiting step for the electron transfer $Q_A^-Q_B^- \rightarrow Q_{\alpha}^-Q_B^-$ could be an appropriate fluctuation of the edge-to-edge distance or other structural rearrangements of the groups, e.g., of the non-heme iron and of the histidines L190 and M217, bridging the gap between $Q_A$ and $Q_B$.

We have determined also the atomic mobilities of trajectory A. These mobilities are presented in Figure 4. The values are quite different from those previously determined for reaction centers with neutral chromophores and for reaction centers with

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chromophores in the SP⁺BPL⁻QₐQₐ state. The special pair in the SP BPLQₐQₐ state is much more flexible than in simulations involving a neutral Qₐ, i.e., SP BPLQₐQₐ and SP⁺BPL⁻QₐQₐ states, whereas the quinones are remarkably stiff. The latter observation seems to contradict the assumption made above that structural fluctuations involving the Qₐ⁻Qₐ edge-to-edge distance could be central to the transfer Qₐ⁻Qₐ → QₐQₐ. These mobilities, however, represent dynamical properties only on a short time scale of 20 ps, and the few large deviations have only a minor influence of the overall rms value.

2. Simulation of Electron Transfer to Qₐ. As explained in section 3 the electron transfer in a protein is controlled to an important degree by fluctuations of ΔE(t) due to classical motion of the nuclear degrees of freedom. In Figure 5a we present these fluctuations resulting from our simulations (see Method of Calculation) for the final 1 ps of trajectory A. The fluctuations of ΔE(t) were monitored during the entire 20-ps time period of trajectory A. The resulting distribution of ΔE values is shown in Figure 6a. This distribution can be matched well to a Gaussian distribution as expected from the central limit theorem (see section 3) and assumed by the Marcus theory. The corresponding parameters are ΣQₐQₐ = 6.9 kcal/mol and ΔE)QₐQₐ = 2.06 kcal/mol. We have also analyzed for trajectory A the decay of the energy–energy correlation function CΔE(t) defined in (19). The correlation function is presented in Figure 7. Shown is also a match to an exponential exp(-t/τ) from which a decay time τ of 120 fs is obtained.

As outlined above, the quinones in the reaction center were recharged at the end of trajectory A corresponding to a Qₐ⁻Qₐ → QₐQₐ⁻ transfer, and trajectory B started. The ΔE(t) values resulting from this trajectory during a period of 1 ps, immediately
after the electron transfer, are also presented in Figure 5. One observes clearly that $\Delta E(t)$ before electron transfer (recharging of quinones) fluctuates around an average value of about 20 kcal/mol, exhibits a response to the transfer through a very rapid relaxation process completed within about 200 fs, and resumes then fluctuations around a new average value of about -23 kcal/mol.

We have analyzed the fluctuations of $\Delta E(t)$ after electron transfer during the 20-ps time period of trajectory C. The distribution of $\Delta E$ values are presented in Figure 6b. Like for the distribution in Figure 6a, a Gaussian can be matched well, the parameters being $\sigma_{Q_0Q^-} = 8.8$ kcal/mol and $\langle \Delta E \rangle_{Q_0Q^-} = -22.8$ kcal/mol. It is of particular interest to notice that the values $\sigma_{Q_0Q^-}$ and $\sigma_{Q_0Q^+}$ differ only by 1.9 kcal/mol, i.e., by 20%, which is sufficiently close to allow the assumption of identical (same f values) harmonic potentials $E_{\text{g}}(q)$ and $E_{\text{g}}(q)$ in the Marcus type presentation of the line-shape function $S_g(\Delta E)$ as outlined in section 3.

According to the fluctuation–dissipation theorem a relationship between equilibrium correlation functions and response functions exists. Applied to the correlation function $C_{\Delta E \Delta E}(t)$ the theorem states

$$C_{\Delta E \Delta E}(t) = R_{\Delta E \Delta E}(t), \quad t \geq 0$$

$$E = [S_g(\Delta E)]^{-1}S_g(\Delta E)$$

where the differential operator $\delta / \partial / \partial \Delta E$ in the framework of the Fokker–Planck equation (17) accounts for a perturbation for which $R_{\Delta E \Delta E}(t)$ describes the response. Adding $\delta$ to the Fokker–Planck operator $D \delta / \partial / \partial \Delta E S_g(\Delta E) \delta_{Q_0}[S_g(\Delta E)]^{-1}$ reveals that $\delta$ corresponds to a time-independent displacement of the equilibrium value ($\Delta E$), i.e., to the same situation that arises through electron transfer as shown in Figure 5. Hence, one expects that the decay times of $C_{\Delta E \Delta E}(t)$ in Figure 7 are the same as the decay of $R_{\Delta E \Delta E}(t)$ corresponding to Figure 5. In fact, an inspection of Figures 5a and 7 shows that the relaxation phenomena depicted are governed by nearly identical relaxation times. The difference in relaxation times assigned are most likely due to the poor statistics of the simulation data and due to the fact that the equivalence stated by the fluctuation–dissipation holds only in the limit of linear response theory, i.e., for small perturbations; the perturbation due to a charge displacement between $Q_A$ and $Q_B$ might not be small.

The relaxation process after electron transfer corresponds to a solvation of the charged chromophores by the protein matrix. Previous simulations have shown that the contributions to this relaxation due to the long range of the Coulomb interactions cannot be assigned to specific groups but rather are distributed between backbone, side chains, and chromophores. To elucidate a possible effect of the motion of the photosynthetic reaction center's non-heme iron on the electron transfer, simulations with a light "iron" of a mass of 1 au have been performed (trajectories A' and B'). The corresponding behavior of $\Delta E(t)$, i.e., fluctuations and relaxation, of $\Delta E(t)$ over a 1.5-ps time period are also presented in Figure 5. One observes that the behavior is similar to that of trajectory A, B: average values before and after transfer and amplitudes of fluctuation are similar for trajectories A, A', B'. In particular, the extreme isotopic replacement does not affect the fast response of the system after electron transfer. We can conclude, therefore, that the function of the iron ion is mainly of electrostatic (attraction of $Q_A^-$ and of $Q_B^-$) and electronic nature (d orbitals involved in electron transfer).

We want to demonstrate now that the energy values $\langle \Delta E \rangle_{Q_0Q^-}$ and $\langle \Delta E \rangle_{Q_0Q^+}$ are consistent with available experimental data on the free enthalpy differences between states $Q_AQ_B$ and $Q_AQ^+_B$. However, a difficulty in making a direct comparison between our

simulations and the observations arises because we presently describe only one of the contributing factors in (1), namely, $k_{\text{et}}(t)$, and miss the other factor $S_{\text{qm}}(t)$ which, in particular, accounts for the intramolecular redox energies and the Born energies. Since in the following we will consider only the differences in energy between states $Q_AQ_B$ and $Q_AQ^+_B$ and since the Born energy for both states should be nearly the same, the Born energy contributions are actually immaterial, but the redox energy difference is still required. This energy difference contributes to the term $\Delta E_0$ of the potentials in Figure 2.

We start from relationships (15) and (16), from which we can infer

$$f^2 = \langle \Delta E \rangle_{Q_0Q^-} - \langle \Delta E \rangle_{Q_0Q^+}$$

$$\Delta E_0 = \langle \Delta E \rangle_{Q_0Q^-} + \langle \Delta E \rangle_{Q_0Q^+}$$

The corresponding value of 46.3 kcal/mol for $f^2$ is significantly larger than that determined from simulations of the transfer steps of $\text{SP}^+\text{BPL}^- \rightarrow \text{SP}^+\text{BPL}^-$ or $\text{BPL}^+Q_A \rightarrow \text{BPL}^+Q_A^-$, the values found for the latter being 8.51 and 20.0 kcal/mol, respectively. The value of $\Delta E_0 = -1.2$ kcal/mol. Both values obtained for (26) and (27) should be rescaled by the inverse of the high-frequency contribution to the dielectric constant $\varepsilon_\infty$ in the protein interior. Assuming $\varepsilon_\infty = 2$, we obtain $f^2 = 23.2$ kcal/mol and $\Delta E_0 = -0.6$ kcal/mol. The latter value needs to be corrected for the redox energy difference of $Q_A$ and $Q_B$, which according to ref 23 in DMF measures -2.8 kcal/mol. This results in an overall free enthalpy difference of -3.4 kcal/mol, which is in agreement with measured $\Delta H$ values for $\text{Rhodopsseudomonas spheroides}$ lying between -2.0 and -7.4 kcal/mol. The point of intersection $q# = \langle \Delta E \rangle(q)$ and $E(q)$ in Figure 5 within the approximate assumptions of the Marcus theory defines the activation energy for the process $Q_A^-Q_B^+ \rightarrow Q_AQ^-B_-$ according to $\Delta E^# = E(q#)$. One obtains thereby the well-known expression

$$\Delta E^# = \frac{f^2/2 + \Delta E_0}{f^2}$$

Using the rescaled and redox energy corrected values for these quantities, one obtains $\Delta E^# = 1.9$ kcal/mol. This low value certainly is favorable for the transfer process; however, it might be at variance with the fact that the electron transfer $Q_A^-Q_B^+ \rightarrow Q_AQ^-B_-$ is quite slow. Since the time scale of our simulations is in the range of picoseconds and since proton uptake by the protein would occur on a longer time scale and is not considered in the calculation, comparison of our data with experimental results is difficult. A change in free energy connected with the transfer


(52) Wright, C. A. Personal communication.
TABLE I: Interaction of $Q_B$ and $Q_B^+$ with the Protein Matrix

<table>
<thead>
<tr>
<th>residue</th>
<th>before transfer</th>
<th>after transfer</th>
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*Only residues showing an electrostatic or van der Waals interaction in either the $Q_B$ or $Q_B^+$ state with an absolute value of the interaction energy larger than 2.0 kcal/mol are given.

TABLE II: Interaction of $Q_B$ in the States $Q_B^-$ and $Q_B^+$ with the Protein Matrix

<table>
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<tr>
<th>residue</th>
<th>state $Q_B^-$</th>
<th>state $Q_B^+$</th>
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<tbody>
<tr>
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<td>van der Waals</td>
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<td>TRP M23</td>
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</table>

*Only residues showing an electrostatic or van der Waals interaction in one of the two states with an absolute value of the interaction energy larger than 2.0 kcal/mol are shown.

After the electron transfer, hydrogen bonding between $Q_B^-$ and the residues L190 (histidine), L224 (isoleucine), and L225 (glycine) or in electrostatic interaction with L223 (serine) and the positive iron.
Electron Transfer in Proteins

likely the most important aspect of our investigation is the conceptual framework that we have provided for the analysis of molecular dynamics calculations in terms of properties relevant for electron-transfer reactions. Not surprisingly—since molecular dynamics allows only a description of classical motion—the framework is very close to the Marcus theory which deals with the coupling of low-frequency, i.e., classical, solvent motion to electron transfer. However, rather than assuming that the Marcus description necessarily holds, we have shown using classical line-shape functions following Hopfield's treatment that the energy for virtual electron transfer $\Delta E(t)$ in a protein matches perfectly the mold of the Marcus theory with the advantage that one has complete control over all aspects of the theory, i.e., all quantities entering the description can be obtained from simulations and, therefore, can be well understood. The most surprising aspect of our analysis may be that the so-called solvent coordinate appears in the theory only in a very formal way, to describe the distribution of $\Delta E(t)$ values, but is eliminated when actual properties such as electron transfer rates are described. We find it very pleasing that the phenomenon of electron transfer in this context reproduces very nicely the fluctuation–dissipation theorem of nonequilibrium statistical mechanics in that the relaxation time of the correlation function $C_{\Delta E\Delta E}(t)$ is identical with the solvent relaxation time after the transfer, described by the response function $R_{\Delta E\Delta E}(t)$. The description presented allows one also to express the factor of the electron-transfer rate controlled by coupling to the classical degrees of freedom in terms of a Kubo line-shape function that governs the coupling between fluctuations of $\Delta E(t)$ and a quantum mechanical process. We hope that we have provided a consistent and useful framework, well founded in the previous theories, within which the coupling of classical degrees of freedom to quantum mechanical electron transfer can be analyzed by molecular dynamics simulations.

We may finally comment on our findings regarding the interaction of $Q_b$ in the states $Q_AQ_b, Q_AQ_b^-, Q_AQ_b^-, Q_AQ_b^-$, and $Q_AQ_b^-$ with the protein matrix. These quinone states show different binding strengths which are mainly due to electrostatic interactions with amino acids and the non-heme iron at the binding site. The investigations need, however, to be further improved by accounting for exact atomic charges and for polarization effects.

Acknowledgments. K.S. thanks A. Wellner for numerous long and enjoyable discussions regarding the nature of the coupling between electron transfer and solvent motion. The question discussed remained on his minds until he saw a chance with this work to finally address them. We thank J. Deisenhofer for kindly providing us with the X-ray structure of Rhodopseudomonas viridis and A. Brünger for making upgraded versions of the program XPLOR available to us. Discussions with H. Treutlein and C. A. Wraight were very helpful and stimulating. M.N. gratefully acknowledges financial support by the Kanton Zürich, Switzerland. The research was carried out in the Center for Parallel Computation in Molecular Dynamics funded by the National Institute of Health. Computing time was granted by the National Center of Supercomputer Applications supported by the National Science Foundation.

5. Summary

We have investigated in this paper the slowest electron-transfer reaction in the photosynthetic reaction center protein complex, $Q_AQ_b \rightarrow Q_AQ_b^-$ in which an electron charge is shifted by almost 20 Å. The time scale of the reaction, micro- to milliseconds, does not permit a simulation, but rather we studied structural and dynamical properties before and after the transfer, the response of the protein environment to an enforced transfer (relaxation time and energy change), and the coupling of the transfer to classical protein motion and its influence on the tuning of redox energies of $Q_A$ and $Q_b$. We also studied in detail the interactions of the reaction intermediates $Q_b, Q_b^-$, and $Q_b^{2-}$ with the binding site.