Molecular Dynamics Study of Early Picosecond Events in the Bacteriorhodopsin Photocycle: Dielectric Response, Vibrational Cooling and the J, K Intermediates

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ABSTRACT   Molecular dynamics simulations have been carried out to study the J_{625} and K_{590} intermediates of bacteriorhodopsin's (bRs) photocycle starting from a refined structure of bR_{568}. The coupling between the electronic states of retinal and the protein matrix is characterized by the energy difference $\Delta E(t)$ between the excited state and the ground state to which the protein contributes through the Coulomb interaction. Our simulations indicate that the J_{625} intermediate is related to a polarization of the protein matrix due to the brief (200 fs) change of retinal's charge distribution in going to the excited state and back to the ground state, and that the rise time of the K_{590} intermediate is determined by vibrational cooling of retinal.
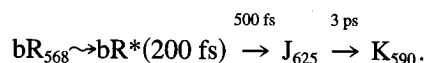
INTRODUCTION

Femtosecond solvation-dynamics studies of water, using a combination of ultrafast experimental fluorescence spectroscopy and nonequilibrium molecular dynamics simulations, have elucidated the relationship between solvent dynamics and the rates of chemical reactions in solution (Jimenez et al., 1994; Rosenthal et al., 1994). In comparison, little is understood about how protein dynamics affects biochemical reaction rates, although it is clear that protein dynamics plays a role in many important biological processes such as electron transfer in the photosynthetic reaction center and the photoisomerization of the retinal chromophore in bacteriorhodopsin. Application of our understanding of chemical reaction dynamics in solvents to biochemical reactions in proteins could be of enormous value.

In this regard it is quite fortunate that studies of solvation dynamics directly relate to a well studied biochemical reaction: the photoisomerization of retinal in the protein bacteriorhodopsin (bR). Bacteriorhodopsin naturally resides in the purple membrane of *Halobacterium halobium* and acts as a light-driven proton pump (Lozier et al., 1975). It contains a chromophore, retinal (see Figs. 1, 2), which rapidly (in 3 ps) photoisomerizes in situ from its *all-trans* to a 13-*cis* form and, thereafter, passes through several intermediate metastable states that are identified through their absorption spectra and kinetics. Fig. 1 displays bacteriorhodopsin's pump cycle schematically and presents the lifetimes of the various intermediates. For recent reviews of bR,

including its function and potential technological applications, see Mathies et al., 1991; Lanyi, 1992; Oesterhelt et al., 1992; Ebrey, 1993; Birge, 1995. Molecular dynamics studies of bR have been reviewed in Schulten et al., 1995.

Subpicosecond and high-resolution femtosecond spectroscopy provides the most detailed view of the first few intermediate states of in situ retinal during its photoisomerization. Upon absorption of a photon, ground state bR_{568} undergoes the following transitions

$$bR_{568} \overset{}{\rightsquigarrow} bR^*(200\ \text{fs}) \overset{500\ \text{fs}}{\longrightarrow} J_{625} \overset{3\ \text{ps}}{\longrightarrow} K_{590}.$$

bR* denotes the optically allowed singlet excited states, and J_{625} and K_{590} denote the first spectroscopically identifiable intermediates; the subscripts denote the wavelengths of the absorption maxima, in nanometers, of each species (Lozier et al., 1975); the time in parentheses gives the apparent lifetime of the excited state bR*; the times 500 fs and 3 ps are the rise times of the J_{625} and K_{590} intermediates, respectively. Retinal exists as an *all-trans* isomer in the bR_{568} ground state. The K_{590} state is readily trapped at low temperatures and contains retinal as a 13-*cis* isomer. Because of its universally short life time, the J_{625} state is more elusive than the K_{590} state. J_{625}, with an observed quantum yield of 0.64 ± 0.04 (Schneider et al., 1989; Govindjee et al., 1990; Tittor and Oesterhelt, 1990), starts to appear at about 200 fs when the excited state bR* begins to decay. J_{625} has a lifetime of 500 fs as observed through its absorption spectrum before the appearance of the K_{590} absorption. Raman spectroscopy suggests that the J_{625} state is a vibrationally excited form of K_{590} that thermally decays to its vibrational ground state, i.e., to K_{590}, in 3 ps (Doig et al., 1991). This interpretation of J_{625} concurs with femtosecond pump-probe experiments (Dobler et al., 1988; Mathies et al., 1988; Pollard et al., 1989; Doig et al., 1991), and seems reasonable, as discussed below, in comparison with experimental results on the related *cis*-stilbene isomerization in solution.
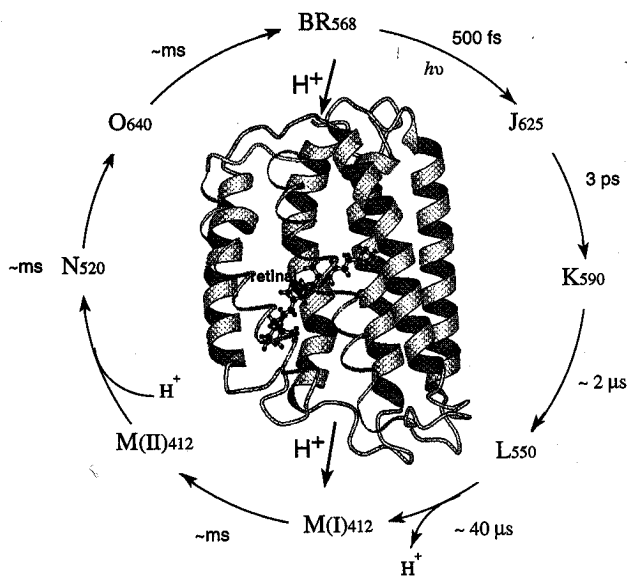
FIGURE 1 Bacteriorhodopsin and a widely adopted scheme of its photocycle. A photon is absorbed by bR_{568} in the primary photoreaction, and a proton is translocated from the cytoplasmic side (top) to the extracellular side (bottom) through the intermediates J_{625}, K_{590}, L_{550}, M_{412}, N_{520} and O_{640}. The intermediates are characterized through their absorption maxima of the retinal chromophore, i.e., the indices, e.g., 625, indicating the absorption maxima in nanometers. Retinal, embedded in the center of the protein, is strongly coupled electrostatically to the protein matrix.

As a result of these studies a one-dimensional model has been proposed (Mathies et al., 1988; Doig et al., 1991) that attempts to describe the photodynamics of retinal, depicted in Fig. 3. Upon absorption of a photon, the electronically excited retinal coherently moves along the bR* potential energy surface, crossing nonadiabatically in 200 fs to the ground state, where it remains vibrationally "hot" for 500 fs and then decays either back to the initial state bR_{568} or, within 3 ps, to the product state K_{590}. This model does not explicitly include the protein in the reaction coordinate. It is quite peculiar that this model ignores the protein motion considering the similarities between solution dynamics studies and the bR photocycle.

The primary events in the photocycle of bR resemble the photoisomerization of cis-stilbene and other activationless photoisomerization reactions that have been studied extensively during the past 10 years using both ultrafast spec-

FIGURE 2 Retinal chromophore in an all-trans configuration; shown are the labels of some key atoms as well as the charge shifts experienced by retinal upon photoexcitation (excited state charges minus ground state charges, in units of electron charge).

troscopy and molecular dynamics simulations (Frederick et al., 1991; Vachev et al., 1993; Rosenthal et al., 1994; Jimenez et al., 1994). In cis-stilbene the photoexcited electronic state starts decaying in solution within 250 fs, a vibrationally hot photoproduct forms, and the final product thermally relaxes together with the solvent on a picosecond time scale (Waldeck, 1991; Sension et al., 1992, 1993; Todd and Fleming, 1993). This series of events resembles the primary picosecond events in bR. Although the photoisomerization of cis-stilbene is essentially barrierless, the solvent may slow down the isomerization via a friction mediated by the electrostatic and steric interactions between the solvent and solute. Likewise, the dynamics of bR may either drive or inhibit the retinal isomerization. In general, it is found that when a solute is photoexcited, solvents such as water or methanol respond on two different time scales (Rosenthal et al., 1994; Jimenez et al., 1994). During the first 100 fs the solvent heats up; the thermal energy is conducted mostly through the vibrational motions of hydrogens. After this initial, inertial response, thermal decay occurs on a picosecond time scale. The solution responds on the same time scale that the cis-stilbene excited state decays, which suggests that the intervibrational relaxation within the chromophore may compete with the solvent response in driving the photodynamics.

Despite the obvious structural differences between a solvent and a protein, the similarities between the photoisomerization of retinal in bR and cis-stilbene in solution suggest that protein dynamics may affect biochemical reaction rates in a similar way to how solution affects chemical rates. Because the molecular dynamics simulations and ultrafast experiments agree remarkably well in case of solvent photoprocesses (Frederick et al., 1991; Vachev et al., 1993; Rosenthal et al., 1994; Jimenez et al., 1994), it is most reasonable to use these techniques to study reactions in proteins and, in particular, in the femtosecond response of bR to the photoisomerization of retinal. Previous molecular dynamics simulations examined the photoisomerization of retinal in bR, however, none have examined the inertial response of the protein and its relationship to solution dynamics. Here we would like to suggest how electrostatic and vibrational coupling between retinal and bR contribute to the absorption spectrum and lifetimes of the retinal excited state and of the J_{625} or K_{590} intermediates, how the protein dynamics may accelerate the decay of the bR* excited state during the first few hundred femtoseconds of the photoisomerization, and how ultrafast experiments might be designed to probe the inertial response and collective nuclear motion in bR.

METHODS

The structure of bR employed in our simulations is the one reported in Humphrey et al., 1994. This structure showed a very small (1.8 Å) Root Mean Squared (RMS) deviation of C_α backbone atoms from the model structure of Henderson et al., 1990, from which it had been derived. The protonation states of titratable groups were standard, except for Asp-96 and
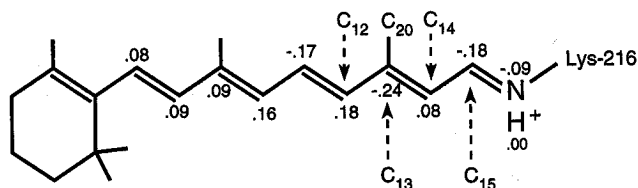
Asp-115, which were assumed to be protonated according to observations reported in Gerwert et al., 1989 and Engelhard et al., 1990.

The parameters and charges used for bR are those of the CHARMm parameter file parmallh3x.pro and the CHARMm partial charge file topallh6x.pro (Brooks et al., 1983), except for retinal. Water molecules inside bR were described by a TIP3P potential (Jorgensen et al., 1983) with the CHARMm parameter file param19.sol. The charges for the ground and excited states of retinal were taken from semiempirical calculations as in Zhou et al., 1993, except for changes to fit the explicit hydrogen model, i.e., the explicit hydrogen atoms added to the retinal backbone were given partial charges of 0.03, with the corresponding heavy atom charges reduced by 0.03.

The shift of retinal's partial atomic charges between its excited and its ground state are shown in Fig. 2. We incorporated the formation of the retinal excited state by instantaneously changing both the retinal charge distribution and the potential energy governing the torsional motion of the 13–14 bond. In the ground state the torsional angle is governed by the potential depicted by a dashed line in Fig. 4, a and b. The parameters used for this potential are those also employed in Humphrey et al., 1994. To model the photoexcitation of bR_{568} this potential was replaced by the potential shown as a solid line in Fig. 4 a for a period of 200 fs, the assumed lifetime of the excited state. After this period the potential depicted by a solid line in Fig. 4 b was applied; this potential describes the crossing to the ground state. The parameters used for the latter two potentials were the same as those used in Humphrey et al., 1995. However, in the present case the surface crossing potential depicted in Fig. 4 b was actually left in place for the entire remaining simulation period; the rational for this choice was that the potential is actually very similar to the ground state surface near the 13-cis minimum of the 13–14 bond torsion, that continuous application of the potential prevents back-reaction to bR_{568}, and that a switch of the potential surface would constitute a significant perturbation that would influence the key observables of our study, i.e., the temperature of retinal as measured by the kinetic energy of its atoms and the energy gap function $\Delta E(t)$ introduced below. The model of the excited state dynamics employed here is discussed in Schulten et al., 1995.

All simulations and analyses described in this paper were carried out using the program X-PLOR (Brünger, 1992). bR was modeled in vacuum at a starting temperature of 300 K. A cut-off distance of 8 Å for nonbonded interactions was used, and the dielectric constant ε was assumed to be 1. An explicit hydrogen bonding term in the energy function was employed. The time step of the simulations was chosen as 0.5 fs. No coupling to a heat bath was applied during the simulation, i.e., a microcanonical ensemble was assumed.

Note that by changing the excited state energy function and charge distribution instantaneously, the dipole moment of the chromophore changes instantaneously as well, hence, suddenly perturbing the internal electrostatic environment of the protein. Similar nonequilibrium molecular dynamics techniques have been applied to study the solution response to the photoexcitation of a pigment in solvent (Vachev et al., 1993; Rosenthal et al., 1994).

We monitored the dielectric fluctuation and relaxation of the protein matrix accompanying the charge redistribution of retinal because of photoexcitation by calculating the energy difference, $\Delta E(t)$, between the ground state (after charge redistribution, index gr) and the excited state (after charge redistribution, index ex) contributed by interaction between the retinal chromophore and the protein matrix. Because of the motion of the protein, represented by a multi-dimensional configuration vector $\vec{q}(t)$, the energy difference

$$\Delta E(t) = E_{ex}[\vec{q}(t)] - E_{gr}[\vec{q}(t)] \qquad (1)$$

between the two states is fluctuating in time. When the protein motion is simulated with a chromophore charge distribution of the ground state retinal, $\Delta E(t)$ corresponds to the energy required to instantaneously promote the chromophore from its electronic ground state to its electronic excited state without changing the nuclear conformation; i.e., $\Delta E(t)$ approximates the changes in the absorption maximum associated with the protein motion. $\Delta E(t)$ is determined by evaluating the energies $E_{ex}[\vec{q}(t)]$ and $E_{gr}[\vec{q}(t)]$ for a simple trajectory or by averaging over a set of trajectories $\vec{q}(t)$.

RESULTS

This section provides the molecular dynamics simulation results for the dielectric fluctuation and thermal relaxation during the initial 5 ps after initiation of the excited state dynamics of retinal as described in Methods. We examined each response of the protein in detail, evaluating $\Delta E(t)$ and the thermal relaxation of retinal.

Dielectric response to the initial excitation

The initial photoexcitation of bR_{568} (at $t = 0$) induced a sudden change in the dipole moment of the chromophore and, consequently, the protein responded on a characteristic timescale of 100 fs, rearranging its nuclear coordinates to accommodate the new electrostatic forces.

Fig. 5 a shows $\Delta E(t)$ during 25 ps of molecular dynamics simulation of bR_{568}. $\Delta E(t)$ fluctuated strongly. The distribution of $\Delta E(t)$ matches a normal distribution

$$P(\Delta E) \approx \frac{1}{\sqrt{2\pi}\,\sigma} \exp\left[-\frac{(\Delta E - \langle \Delta E \rangle)^2}{2\sigma^2} \right] \qquad (2)$$

where the average of $\Delta E(t)$, $\langle \Delta E \rangle$, is 11.65 Kcal/mol, and the variance σ is 2.47 Kcal/mol. A similar distribution was obtained in the molecular dynamics simulation of the photosynthetic reaction center (Nonella and Schulten, 1991; Schulten and Tesch, 1991).

The normalized autocorrelation function of $\Delta E(t)$, defined as

$$C_{\Delta E, \Delta E}(t) = \frac{\langle (\Delta E(t) - \langle \Delta E \rangle)(\Delta E(0) - \langle \Delta E \rangle) \rangle}{\langle (\Delta E(0) - \langle \Delta E \rangle)^2 \rangle}, \qquad (3)$$

has been evaluated using a 100-ps trajectory and determining the average as a time average, rather than an ensemble average. The reader may consult Xu and Schulten, 1995, for details of the calculation. The resulting correlation function
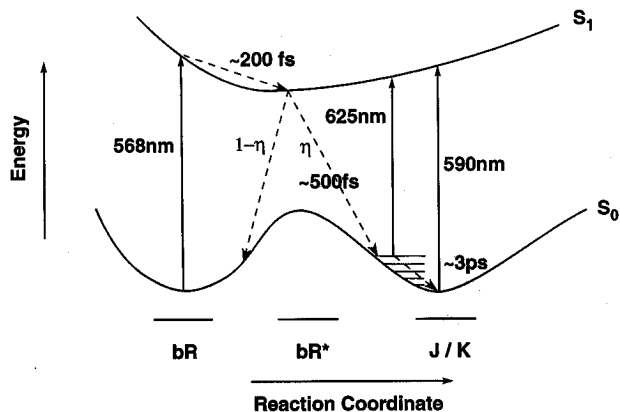

FIGURE 3 Scheme for the ground-state and the excited-state potential surfaces versus the reaction coordinate.

FIGURE 4   Potentials for the torsional angle
of the 13–14 bond of retinal; (a) the excited-
state potential S_1 (solid line) and the ground
state potential surface S_0 (broken line); (b) sur-
face crossing energy potential (solid line) su-
perimposed on the ground state potential sur-
face S_0 and the excited-state potential S_1
(broken lines).

(a)   **(b)**
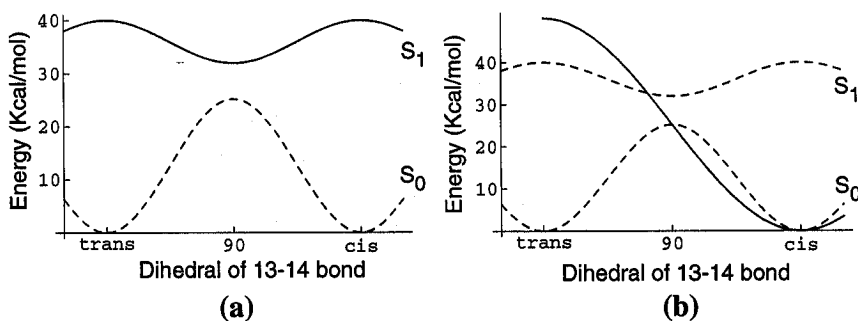
is presented in Fig. 6. The short time behavior of $C_{\Delta E, \Delta E}(t)$
can be matched to an exponential decay

$$C_{\Delta E, \Delta E}(t) \approx e^{-t/\tau_0}, \quad \tau_0 = 73.8 \text{ fs}. \tag{4}$$

However, as is evident from Fig. 6, the match is rather poor.
The correlation function $C_{\Delta E, \Delta E}(t)$ has a long-time oscilla-
tory behavior, which is similar to that reported in Schulten
and Tesch, 1991. The time scales of the decay and of the
oscillations of $C_{\Delta E, \Delta E}(t)$ are similar to those observed in the
inertial response of solvents to photoexcitation of solutes
(Jimenez et al., 1994; Rosenthal et al., 1994).

Dielectric relaxation and dipole echo

We consider first the case that retinal in bR actually does
not undergo a photoisomerization. In this case, the photo-
excitation of retinal is accounted for solely through a change
of its partial atomic charges at $t = 0$ and resaturation of the
ground state charges at $t = \tau = 200$ fs. The episode of a
changed dipole moment induces in the protein a dielectric
response. This response involves a polarization and depo-
larization of the protein matrix with a relaxation time of
about 100 fs. The polarization of the protein matrix and,
hence, the dipole echo, communicated through the Coulomb
interaction, affects the energy gap $\Delta E(t)$ of retinal.

Fig. 7 shows how the energy gap $\Delta E(t)$ responds when
the excited state charge distribution is applied. Fig. 7 a
represents a single trajectory, where the fluctuations are too
large to reveal any systematic response. Averaging of $\Delta E(t)$
over 30 trajectories with different initial conditions (veloc-
ities), exhibits an "echo" at about $t = 500$ fs, which rides on
top of the response of $\Delta E(t)$ characterized by $C_{\Delta E, \Delta E}(t)$ (see
Nonella and Schulten, 1991). It is important to realize that

the dipolar response, presented in Fig. 7, arises for bR$_{568}$ in
the case of photoexcitation without an isomerization reac-
tion. Such a system has recently been realized through
reconstitution of bacteriorhodopsin with a retinal analogue
incapable of all-trans → 13-cis isomerization (Delaney et
al., 1995).

It is of interest and obvious relevance to investigate the
dipolar relaxation of the protein matrix as experienced
through $\Delta E(t)$ in the case that retinal undergoes a photoi-
somerization upon light excitation. For this purpose we
applied both the excited state charge distribution and the
modified excited state torsional potential and crossing po-
tential, and determined the resulting energy gap $\Delta E(t)$. The
result is shown in Fig. 8, which gives $\Delta E(t)$ averaged over
50 trajectories. One can clearly discern again a dipolar
relaxation together with an echo at about 450 fs. This echo
coincides with the rise of the J$_{625}$ intermediate in bR$_{568}$'s
photoinduced proton pump cycle depicted in Fig. 1. After t
> 200 fs, the energy gap $\Delta E(t)$ in Fig. 8 can be matched, as
shown in Fig. 9, to an exponential decay

$$\Delta E(t') \approx E_1 + E_2 e^{-t'/\tau_J}, \quad \tau_J = 603.8 \text{ fs}, \tag{5}$$

where $t' = t - 200$ fs, $E_1 = 3.5$ Kcal/mol, and $E_2 = 5.8$
Kcal/mol. It is interesting to note that oscillatory fluctua-
tions remain around the exponential decay even after aver-
aging over 50 trajectories.

It is curious that the dipolar response in bR, after photo-
excitation of retinal, occurs on the timescale of the J$_{625}$
intermediate. We also note that the strength of the dipole
echo, as reflected by $\Delta E(t)$, agrees approximately with the
spectral shift of the J$_{625}$ intermediate. In the case of Fig. 7,
i.e., without applying isomerization force fields, the depth
of the echo at $t = 500$ fs, relative to the asymptotic value at

FIGURE 5   (a) Energy gap $\Delta E(t)$ for bR$_{568}$; (b)
distribution of $\Delta E(t)$ values. The dots represent the
distribution sampled from (a); the line shows a
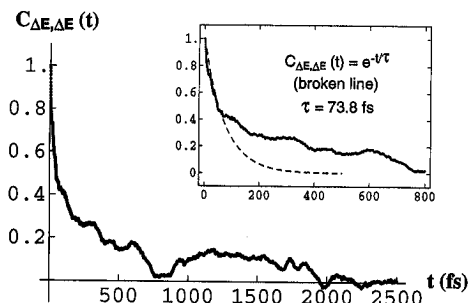least squares fit to distribution (2).
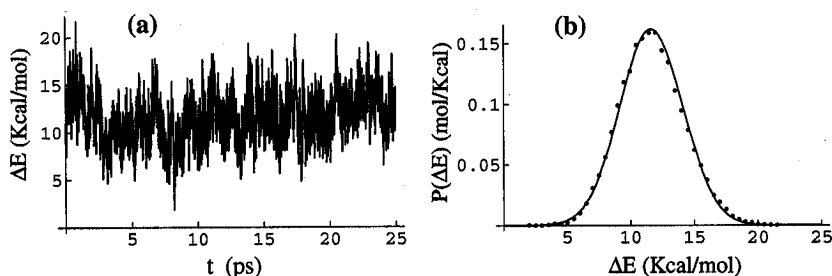
FIGURE 6 Normalized autocorrelation function of $\Delta E(t)$ as defined in Eq. (3) evaluated from 100-ps trajectory of bR_{568}. The broken line in the insert shows a least squares fit to an exponential decay (see Eq. 4) for small t.
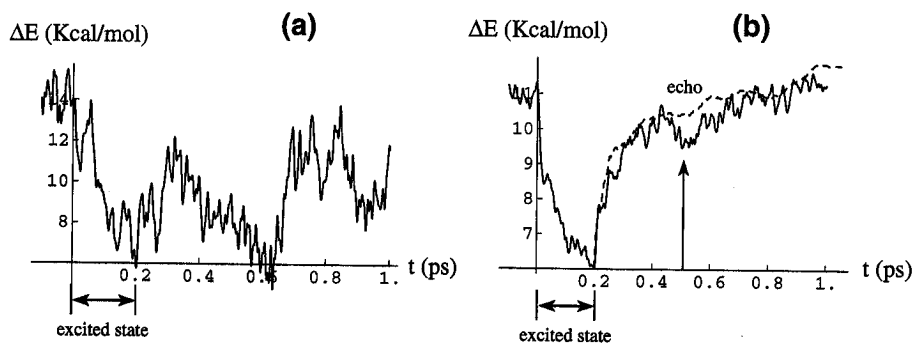
long t, is 2.5 Kcal/mol, an energy that corresponds to a 32-nm red shift of K_{590}. The red shift of J_{625}, relative to K_{590}, is 35 nm. It should be noted, however, that ΔE accounts for the spectral shift of in situ retinal in bacteriorhodopsin only in first order perturbation theory; local electric fields can actually alter the electronic structure of ground and excited state retinal and, thereby, contribute to the spectral shift in a fashion not reflected in first order perturbation theory. Such contribution has actually been predicted in recent *ab initio* quantum chemical calculations of the spectrum of bacteriorhodospin (Logunov and Schulten, 1995) and should enhance the effects discussed above.

Thermal relaxation

The question arises then what determines the decay of the J_{625} state or, what is the same, the rise of the K_{590} intermediate. As pointed out above, Raman scattering data suggest that the relevant timescale of 3 ps is related to the relaxation of a vibrationally excited retinal generated through the photoexcitation (Doig et al., 1991). This proposition can be tested readily by molecular dynamics simulations through monitoring the temperature of retinal and the residue Lys-216 determined through the kinetic energy according to

$$T(t) = \frac{2}{3k_B N} \sum_{j=1}^{N} \frac{1}{2} m_j v_j^2(t). \quad (6)$$

where N is the number of atoms of retinal and Lys-216, and where the summation runs over all atoms j of retinal and Lys-216. Figs. 9 and 10 present the temperature $T(t)$ of the chromophore (plus Lys-216) as a function of simulation time. $T(t)$ exhibits strong fluctuations in the case that the quantity is based on a single trajectory. However, averaging over 50 trajectories reveals a time-dependent temperature $T(t)$, which exhibits two processes: the first process represents the rise of the temperature with a timescale of 500 fs reflecting the formation of a vibrationally hot J_{625} state; the second process governs the decay of the vibrational excitation at $t > 500$ fs, which can be fit to an exponential relaxation

$$T(\tilde{t}) \approx T_0 + T_1 \exp(-\tilde{t}/\tau_K), \quad (7)$$

where $\tilde{t} = t - 500$ fs, $T_0 = 300$ K, $T_1 = 48.8$ K, and $\tau_K = 2.83$ ps. This timescale obviously agrees well with the expectations due to the experimental data in Doig et al., 1991.

DISCUSSION

We performed molecular dynamics simulations to model the initial femtosecond and picosecond events in the photocycle of bacteriorhodopsin. The simulations provide insight into the J_{625} and K_{590} intermediates, particularly their lifetimes: the rise of the J_{625} state is due to both vibrational excitation as well as a polarization of the protein matrix induced by the excited state charge shift in retinal; the decay of the J_{625} state and the rise of the K_{590} state reflect simply vibrational relaxation of retinal. The molecular dynamics simulations also indicate that the protein responds to the chromophore photoisomerization rapidly and collectively.

The molecular dynamics simulations reproduce the experimental time scales for the thermal decay of the J_{625} and K_{590} intermediates and lend support to the notion that J_{625} is a vibrationally excited form of the thermally relaxed K_{590} isomer. The product of the retinal photoexcitation displays two different decay processes in the molecular dynamics simulation. The first, which occurs on the 500 fs time scale, is mediated by the electrostatic interactions between the vibrationally hot photoproduct and the protein environment. This process resembles the dielectric friction observed in

FIGURE 7 (a) The energy gap function $\Delta E(t)$ for bR after photoexcitation in the case that no photoisomerization is induced (see text). The excited state charge distribution is employed during the time interval $0 < t < 200$ fs. (b) Same as (a) except averaged over 30 trajectories. The broken line shows a comparison with $\lambda_1 + \lambda_2 C_{\Delta E, \Delta E}(t)$, where λ_1 and λ_2 have been determined through a least squares fit.

FIGURE 8 (a) The energy gap function $\Delta E(t)$ for bR after photoexcitation in the case that an *all trans* → *13-cis* photoisomerization has been induced (see text). The energy gap function has been averaged over 50 trajectories. The excited state charge distribution and the isomerization force field are applied for $0 < t < 200$ fs. (b) Same as (a) except for a stretched timescale to reveal details at $t \approx 420$ fs, i.e., the time at which a dipole echo is expected.
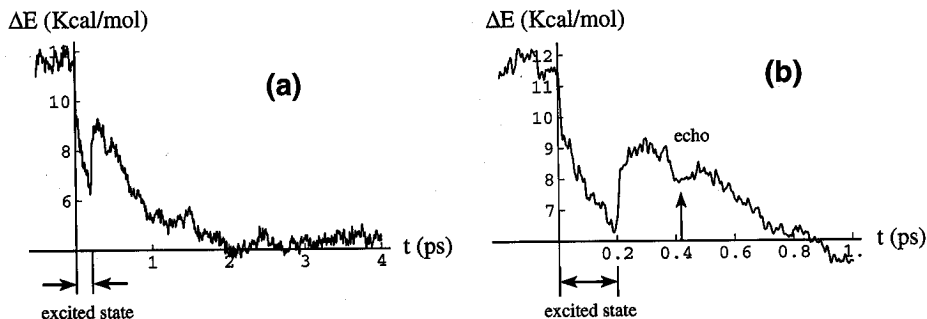
the photoisomerization of *cis*-stilbene. The second decay process occurs with a time constant of 3 ps and represents the thermal decay of the photoproduct to the K_{590} intermediate. The results are consistent with other simulations and experiments, in particular, with the simulation of cooling of a protein that yielded a realistic heat diffusion coefficient (Tesch and Schulten, 1990).

With confidence gained from the fact that the simulations reproduce the kinetic time scales, we turned to examining the response of the protein to a sudden change in the charge distribution of the retinal chromophore due to the absorption of a photon. The protein environment responds on a timescale of 100 fs, which is similar to that found both in solution (Vachev et al., 1993; Rosenthal et al., 1994) and in other proteins. For instance, the 100 fs timescale also characterizes the dielectric response of the protein for electron transfer in photosynthetic reaction centers (Nonella and Schulten, 1991; Schulten and Tesch, 1991). It seems that this timescale is universal for the response of a protein to an instant charge shift of an electronic state, i.e., for the coupling between the quantum mechanical degrees of freedom and the classical protein environment.

The response of bR resembles the inertial response of a solution to photoexcitation of a solute, which has been studied extensively using both molecular dynamics simulations and ultrafast fluorescence spectroscopy. This is quite remarkable because the structure of solution and protein are quite different. Solution molecular dynamics studies indicate that the initial solvent response arises from vibrational motion of the hydrogens (in solvents such as water and
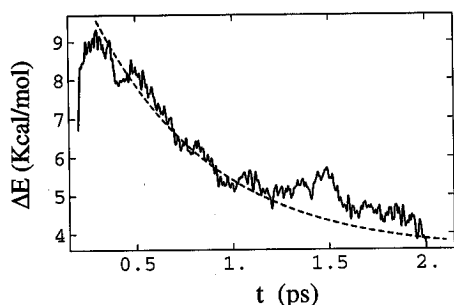
methanol) and can occur on time scales as short as 40 fs. The protein responds somewhat slower, but still shows multiple decay processes.

Since liquids are composed of small, disconnected molecules, whereas proteins are composed of an extended, covalently linked polymer system, how close of a similarity of the dielectric relaxation of liquids and proteins can one expect? The virtual temperature independence of the dielectric relaxation rate in the photosynthetic reaction center, as observed in simulations (Schulten and Tesch, 1991), implies that electrical interactions distort the protein conformation only minimally without crossing of barriers of activation; from this one might expect weak dielectric relaxation. However, the existence of significant low order charge multipoles in protein subvolumes of, e.g., 8 Å diameter, implies strong long range interactions such that many small contributions, nevertheless, lead to a significant dielectric relaxation. This view of dielectric relaxation is strongly supported by the evaluation of electron transfer rates in Xu and Schulten, 1992 and 1994, which shows that key features of electron transfer as observed in photosynthetic reaction centers can be reconciled with a coupling of the process to many modes of the protein rather than with strong coupling to few degrees of freedom as stipulated by earlier theories. In view of the importance of long range electron transfer for
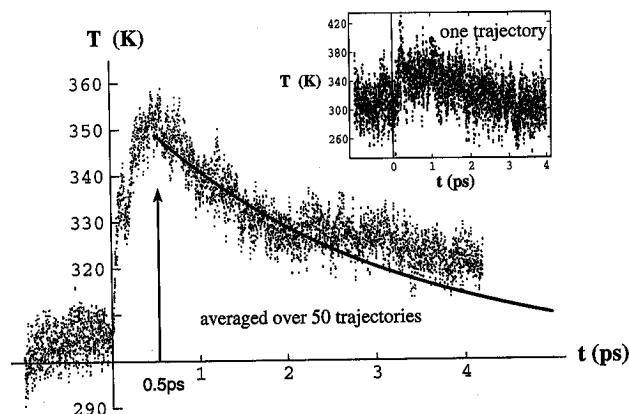
FIGURE 9 $\Delta E(t)$ (*solid line*) and a least square fit (*broken line*) to an exponential relaxation (see Eq. 5) for $\Delta E(t)$ after the excited state.

FIGURE 10   Temperature of retinal together with Lys-216, $T(t)$, (*data points*) for one trajectory and an average over 50 trajectories. Shown is also a least squares fit (*solid line*) to an exponential decay (see Eq. 7) for $T(t)$ at $t > 500$ fs. The excited state exists during $0 < t < 200$ fs.

protein dielectric relaxation, it is unfortunate that the need of extensive ensemble averages forced us here to assume Coulomb forces with a cutoff, otherwise the calculations would not have been feasible.

It is interesting to note that the dielectric response of the protein occurs on the same timescale as has been suggested for the coherent motion of the excited state vibrational wave packet of retinal in femtosecond pump-probe experiments, because the standard interpretation of these experiments explicitly ignores the protein motion. It is generally presumed that the ultrafast pump pulse (6 fs in width) excites a coherent vibration in the chromophore and, subsequently, the protein motions remain vibrationally frozen during the photoisomerization. The present simulations suggest, however, that the reaction coordinate for the photodynamics should at least implicitly include the collective dielectric response of the protein as well. It is further suggested that the coherent motion of the protein may, in fact, drive the true retinal excited state toward the lower energy ground state potential energy surface (see Fig. 3). As the isomerization reaction proceeds, the protein drives the excited state potential energy surface toward the ground state, enhancing the radiationless decay of the excited state. In this sense the collective motion of the protein becomes part of the reaction coordinate for the in situ reaction.

We model the onset and decay of the retinal excited state by instantaneously changing the charge distribution of the chromophore. This sudden perturbation causes the protein to respond collectively, producing an electrostatic echo that transiently red shifts the bR* electronic excited state. The echo appears on a timescale 2τ, where τ is the lifetime of the excited state. Here, τ is 200 fs. The echo indicates a short-time, collective, coherent motion in the protein and most likely would not exist at significantly longer time scales because the coherence would dephase.

Our findings lend credence to the suggestion that the J_{625} state derives its spectral shift to some extent from a dielectric response of the protein matrix. This can also explain why it has been impossible so far to stabilize the J_{625} state: the state does not constitute a metastable situation that could be stabilized, e.g., at low temperature, but rather constitutes a vibrationally hot state of bR with a strong, but only intermediate dipolar polarization.

It would be most interesting to examine the response of bR with the same experimental techniques employed in studies of the inertial response of solution to photoexcitation of a pigment; however, it would be suitable for this purpose to reconstitute bR with a retinal analogue that does not isomerize to isolate the protein response from the isomerization reaction was done in Delaney et al., 1995.

The dielectric relaxation (echo) should manifest itself experimentally as a transient red-shifted spectroscopic event occurring at time of $\sim 2\tau$ after an impulsive excitation of the chromophore for a duration τ, i.e., on the same time scale as the rise time of the J intermediate. It is important that the probe molecule be de-excited impulsively to see the strong response of the protein. In standard pump-probe experiments, for example, the excited state of the target molecule decays exponentially and, therefore, the surrounding protein would respond in kind to the slow decay of the excited state charge distribution and most likely not exhibit an echo. Experiments do exist, however, for probing molecules impulsively, such as impulsive Raman scattering and femtosecond pump-dump experiments; perhaps such a technique could be employed to observe the echo in the protein response.

Although these simulations ignore the instantaneous electronic polarization of the protein, we still argue that the experiments should reveal our suggestions, especially because molecular dynamics simulations of solution reproduce experiments so well. It is unclear how the neglected electronic polarization affects the short-time dynamics of the dielectric response of a protein, although it might be expected that the effects observed here could be partially damped when the electrons in the protein instantaneously reorganize themselves because of sudden changes in charge distribution of the chromophore. Additionally, it is noted that the retinal chromophore is surrounded in bR by a number of presumably highly polarizable tyrosine groups. Nevertheless, nonequilibrium molecular dynamics studies of solvents provide an accurate representation of the decay lifetimes observed in ultrafast fluorescence experiments. These results lend confidence to our suggestions as well.

Clearly the effects of the protein environment play a role in the early spectroscopic events of bR and, no doubt, in other proteins. Hopefully, future ultrafast experiments will attempt to probe the short-time dielectric response of proteins, attempting to separate the effects of simple electrostatic and dynamics in biological chemistry. It is most interesting that the inertial response of the protein is so similar to that of solution. Indeed, it may turn out that protein dynamics and solution dynamics play analogous roles in enhancing chemical reaction rates. Upon submission of this paper we learned that such experiments have already been undertaken (S. Boxer, private communication), and that, indeed, bR does exhibit a short-time (~100 fs) relaxation comprising a substantial fraction of the solvation.

Finally, future theoretical studies of the bR photoisomerization reaction should include a combination of *ab initio* electronic structure methods to provide the electronic states of retinal and molecular dynamics simulations to incorporate the protein environment. Only with these combined techniques will theory successfully elucidate the initial primary subpicosecond events in the photodynamics of bR.

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