FEBS 16727

FEBS Letters 383 (1996) 129–132

Femtosecond kinetics of electron transfer in the bacteriochlorophyll$_M$-modified reaction centers from *Rhodobacter sphaeroides* (R-26)

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Received 17 November 1995; revised version received 16 January 1996

Abstract Formation of the vibronic wavepacket by 90-fs excitation of the primary electron donor P in bacteriochlorophyll$_M$-modified reaction centers is shown to induce nuclear motions accompanied by (1) oscillation of the stimulated emission from excited primary electron donor P* and (2) wavepacket motions leading to electron transfer at 293 K from P* to bacteriochlorophyll (B_L) and then to bacteriopheophytin (H_L). The latter motions have low frequency (about 15 cm^{-1}) and are related to protein-nuclear motions which are along the reaction coordinate. When the wavepacket approaches the intersection of the reactant (P^*B_L) and product ($P^+B_L^-$) potential energy surfaces (~1.5 ps delay), about 60% of P* is converted to the $P^+B_L^-$ state. The $P^+H_L^-$ state formation is delayed by ~2 ps with respect to that of $P^+B_L^-$. It is suggested that the wavepacket is transferred to and moves also slowly on the $P^+B_L^-$ potential energy surface and approaches the intersection of the surfaces of $P^+B_L^-$ and $P^+H_L^-$ within ~2 ps (~8 cm^{-1}), indicating the electron transfer to H_L.

Key words: Femtosecond spectroscopy; Reaction center; Electron transfer

1. Introduction

Molecular dynamics simulations [1–3] have predicted that low-frequency (1–100 cm^{-1}) global protein motions play a key role in biochemical reactions. Femtosecond spectroscopy is a new tool for the investigation of light-induced reactions of chromophores accompanied by nuclear motions and electron transfer in pigment-protein complexes. Excitation of the pigments by fs pulses with broad spectral width creates simultaneous population of several vibronic levels with the formation of a superposition of corresponding wavefunctions. The formed wavepacket has semiclassical behavior of motion [4] on the potential energy surface of the excited state. It was shown [5] that these motions can be along the reaction coordinate and that the wavepacket can be transferred to the product potential energy surface. (For the wavepacket theory see [6].)

The electron transfer (ET) reaction in bacterial hexachromic reaction center protein (RC) occurs from the excited state of the bacteriochlorophyll special pair, primary electron donor P This reaction takes place within ~3 ps at 300 K and is accompanied by the reduction of bacteriopheophytin (H_L) probably via an intermediary electron acceptor, monomeric bacteriochlorophyll (B_L) (where L indicates chromophore location in the L protein subunit) (for a review see [7,8]). However, the participation of B_L in electron transfer process is still under debate [8–16].

Absorption and hole burning measurements at 2–60 K on bacterial RCs have revealed the progression of the enhanced ($S = 1.2$–2) frequency mode around 150 cm^{-1} (73, 110 and 148 cm^{-1}), which are accompanied by a ~30 cm^{-1} protein mode seen clearly in hole burning experiments on RCs with prereduced H_L [17]. The frequencies found in hole burning experiments in the range of 30–150 cm^{-1} were also established in Raman spectra [18].

An important contribution to the understanding of the primary step was made by a visualization of coherent nuclear motions in RCs by femtosecond spectroscopy in a wide range of temperatures (10 K–290 K) [19]. The presence of the phase coherence of low frequency motions on the ps time scale of electron transfer was demonstrated. The vibrational motions of the excited state reveal the fundamental frequencies around 15 cm^{-1}, and at 69, 92, 122 and 153 cm^{-1} in native *Rhodobacter sphaeroides* RCs [19] and at 15 and 77 cm^{-1} in DLL mutant of *R. capsulatus* [20]. An important finding would be to clarify what motion is along the ET coordinate.

The ps study of ET in borohydrate-treated RCs in which B_M (bacteriochlorophyll monomer located in M protein subunit) is modified and partially removed and the 800 nm absorption band is mostly attributed to B_L molecule has shown that the electron transfer among pigments has the same ps kinetics as for native RCs [21,22]. The bleaching of the H_L band is clearly delayed by a couple of ps with respect to that of the B_L band [22]. This finding was interpreted as an indication of a formation of the $P^+B_L^-$ state before the electron transfer to H_L. Femtosecond studies [10,23] provided some new information on the primary steps of electron transfer. It was found [23] that the step-wise electron transfer has time constants of 2.3, 0.9 and 200 ps for ET from P* to B_L, from B_L^- to H_L and from H_L^- to quinone (Q_A), respectively. The formation of a wavepacket moving along the ET coordinate is of interest for the study of sequential electron transfer.

In the present work RCs in which B_M is modified and does not interfere with the bleachings of the B_L were used for fs study. It was found that low-frequency oscillation (about 15 cm^{-1}) related to protein nuclear motions is along the reaction coordinate. When the wavepacket formed by fs excitation approaches for the first time (~1.5 ps) the intersection of the reactant potential energy surface and the product surface ~60% of the excited state is converted to the $P^+B_L^-$ state. The $P^+H_L^-$ state formation is delayed by ~2 ps with respect to that of $P^+B_L^-$ probably due to the fact that the wavepacket is transferred to the $P^+B_L^-$ potential surface and the electron transfer from $P^+B_L^-$ to $P^+H_L^-$ takes place when the wavepacket approaches an intersection of surfaces of these states.

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Abbreviations: DA, light-minus-dark absorbance changes ; P, primary electron donor; B and H, bacteriochlorophyll and bacteriopheophytin monomers, respectively, located in L or M protein subunits; ET, electron transfer; RC, reaction center; S, Pekar-Huang-Rhys factor

SSDI S0014-5793(96)00113-5

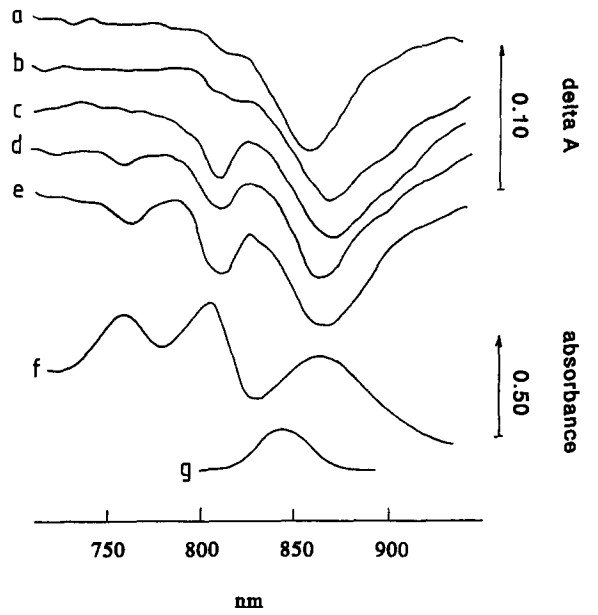

Fig. 1. (a–e) Difference (light-dark) spectra of borohydrate-modified *Rhodobacter sphaeroides* (R-26) RCs excited by 90-fs pulses centered at 845 nm and measured at different delays as follows: (a) 0.2 ps; (b) 0.9 ps; (c) 2.2 ps; (d) 4.5 ps; (e) 19 ps. (f) Absorbance spectrum of the sample measured by the same fs setup. (g) Spectrum of the fs excitation pulses.

2. Materials and methods

RCs from *R. sphaeroides* (R-26) were isolated as described earlier [21]. Borohydrate treatment to modify a bacteriochlorophyll monomer located in the M subunit was performed as described earlier [21]. When the A_{800}/A_{860} ratio reached 1.4–1.5 RCs were dialyzed against 100 mM Tris-HCl (pH 8.0) buffer containing 0.1% lauryldimethylamine *N*-oxide and were purified by using DEAE-cellulose chromatography. The quantum yield of photooxidation of P on the ms time scale was close to 100%. 2 mM sodium ascorbate was added to keep P in the neutral state before the arrival of each pump pulse (1 Hz repetition rate). The concentration of the sample was adjusted to an optical density of 0.5 at 860 nm and 293 K (optical path length of 1 mm).

The femtosecond spectrometer described earlier [24] consisted of a cpm dye laser (~50 fs pulse duration) with Rhodamine 590 (Exciton) jet pumped by a cw argon laser (Spectra Physics) and DODCI (Exciton) jet. Femtosecond pulses were amplified in a 6 pass dye jet amplifier and in a dye cell both pumped by 10 ns laser pulses at 538 nm. The amplified pulses were focused in a water cell ($H_2O + D_2O$) to produce a fs continuum. Its near-IR part (around 840 nm) was amplified in a Styryl 9M (LDS 821, Exciton) cell. The spectrally filtered emission centered at 845 nm was used as excitation and a small fraction (~4%) of the whole NIR emission — as measured and reference pulses. The measured and exciting pulses propagated through delay lines and the sample. Then the measured and reference pulses were passed through a polychromator and directed by the three-lens system to photodiode array of OMA-2 (PARC, Princeton). Cross-correlation function of the exciting and measuring pulses showed ~90 fs pulse duration. A spectral width of excitation pulse at 845 nm was 450 cm^{-1}. The measuring pulse was 90% depolarized. The relative position of the zero time delay within a 700–950 nm range differed by less than 100 fs.

3. Results

Fig. 1 shows the difference (light−dark) absorbance spectra in the range of 700–950 nm for modified RCs at different delays after excitation by 90-fs pulses at 845 nm. The absorbance changes in the P band at 865 nm were about 13% of the sample absorbance at 865 nm. At 0.2 ps delay (curve a) the spectrum of ΔA reflects the excited state P* and is characterized by a bleaching of the P band around 850 nm and by a small bleaching at 813 nm which reflects the second excitonic component of the P transition. At the latter delay (curves b–e) the P band bleaching is shifted to 865 nm due to dynamic hole burning [24]; a new bleaching centered at 805 nm (B_L band) is developed and at 2.2 ps (curve c) approaches ~60% of maximal bleaching (curve e). At 2.2 ps delay no marked bleaching around 760 nm (H_L band) is observed. After 3 ps delay the bleaching at 760 nm is developed and at 4.5 ps (curve d) approaches ~70% of maximal bleaching (curve e).

For kinetic measurements around the H_L band (760 nm), B_L band (805 nm) and P band (865 nm), the amplitudes (ΔA) of the corresponding spectral troughs on the broad background were measured as suggested in [22]. Fig. 2 shows the kinetics of the bleaching troughs at 760 and 805 nm and kinetics at 905 nm (stimulated emission from P*) normalized to the bleaching trough at 865 nm. The bleaching of the P band is instantaneous with the excitation pulse (not shown). The first part of the bleaching of the B_L band is delayed by ~1.5 ps with respect to that of the P band probably reflecting the formation of the $P^+B_L^-$ state. After 2.5 ps delay some relaxation of the B_L band is observed. Then the bleaching is gradually developed up to delay of 19 ps. The first part of the bleaching of the H_L band is delayed by ~3.5 ps with respect to that of the P band and ~2 ps to that of the B_L band. After 5 ps delay the H_L band bleaching is gradually increased up to a delay of 19 ps. This bleaching is accompanied by the additional bleaching of the B_L band. This behavior might be related to the relaxation of the medium around B_L caused by formation of the $P^+H_L^-$ state. The 905 nm stimulated emission kinetics are roughly described by 2.8 ps exponential curve (not shown) in agreement with earlier measurements [26]. However, in agreement with [19,20] it has clear oscillatory behavior with a period of 2.4 ps (frequency of ~14 cm^{-1}). This frequency range was registered in hole burning [17] and Raman [18] spectra.

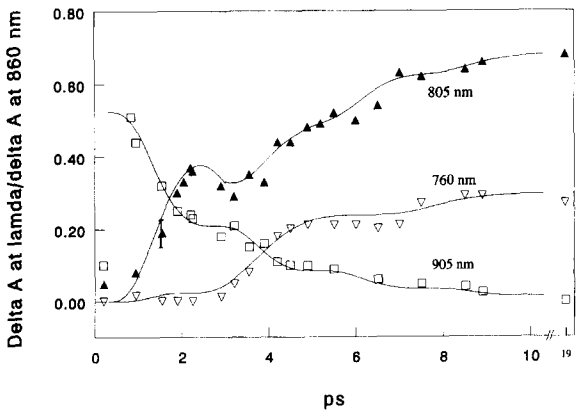

Fig. 2. Kinetics of ΔA at 905 nm (stimulated emission from P*) and the bleaching troughs around 805 nm (B_L band) and 760 nm (H_L band) normalized to the bleaching troughs at 860 nm (for determination of the spectral troughs see [13]). Solid curves are the modeling functions based on the simulation of wavepacket motions on the potential surfaces of the P*, $P^+B_L^-$ and $P^+H_L^-$ states (see Fig. 3 and text for details). Wave packets were constructed as a superposition of several vibronic wavefunctions as shown in [4,24].

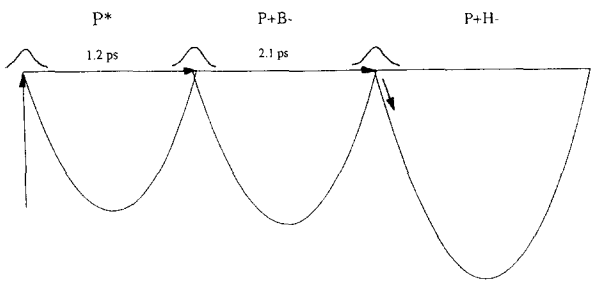

Fig. 3. Scheme of the formation of wavepacket on the P* potential surface due to the fs excitation. This wavepacket moves on the surface and is transferred to the product ($P^+B_{H^-}$) surface at the intersection of two potential surfaces. The wavepacket transfered to the $P^+B_{H^-}$ surface continues the motion on this surface and approaches the intersection between the $P^+B_{H^-}$ and $P^+H_{L^-}$ surfaces where an electron is transfered from B_{L^-} to H_L. The numbers 1.2 ps and 2.1 ps show half period of oscillations (14 and 8 cm^{-1}, respectively) of the wavepackets on the P* and $P^+B_{H^-}$ potential surfaces, respectively, corresponding to the times of the wavepacket approach of the surfaces intersections.

4. Discussion

We propose an electron transfer model for RCs based on the assumption that due to 90 fs excitation of P the formation occurs of a low frequency ($\sim$14 cm^{-1}) wavepacket which moves with a period of 2.4 ps. This motion is probably a protein motion along the ET coordinate (see Fig. 3). After the half period of the wavepacket motion ($\sim$1.2 ps) on the potential surface of P^*B_L state the wavepacket encounters the intersection of P^*B_L and $P^+B_{L^-}$ potential surfaces. As a result a partial electron transfer occurs from P* to B_L accompanied by a decrease of the stimulated emission by a factor 2.5 (Fig. 2). In Fig. 3 the modeling functions based on the motion of a wavepacket constructed as a superposition of several vibronic wavefunctions [4,24] are shown by solid curves. At 2.5 ps delay in 40% of RCs the wavepacket is returned to the P^*B_L surface and 60% is transferred to the $P^+B_{L^-}$ surface. At this delay the B_L band bleaching is $\sim$40% of the P band bleaching. This means that the difference extinction coefficient ($\Delta\varepsilon$) for active B_L at 805 nm is $\sim$0.67 of the P band at 863 nm. Taking into account the value of $\Delta\varepsilon$ for P at 860 nm equal to 108 mM^{-1} cm^{-1} [27], one obtains a $\Delta\varepsilon$ for B_L of 72 mM^{-1} cm^{-1}. This value is close to that for bacteriochlorophyll in solution [28].

It is also suggested that the transferred wavepacket continues the motion with a period of 4.2 ps ($\sim$8 cm^{-1}) on the $P^+B_{L^-}$ surface approaching the intersection with the $P^+H_{L^-}$ surface at a half period of motion (2.1 ps). At the intersection of the $P^+B_{L^-}$ and $P^+H_{L^-}$ surfaces the wavepacket motion produces about 65% of the maximal bleaching of the H_L band (Fig. 2). This means that almost all produced B_{L^-} is converted to H_{L^-} at this delay. After the electron transfer from B_{L^-} to H_L the B_L band should relax to the original state. This is not the case since this band is bleached at further delay showing only a small relaxation at 3 ps (Fig. 2). We assume that this behavior is due to the protein relaxation around B_L molecule (induced by a formation of the $P^+H_{L^-}$ dipole) with two time constants of 0.83 ps and 3.2 ps producing the bleaching of B_L molecule.

The fs data presented in Figs. 1 and 2 in the range from $\sim$2 ps to 19 ps delays are very similar to the ps data described earlier [22] for modified RCs and support the assumption that the electron transfers from P* to B_L and from B_{L^-} to H_L are separated in time and sequential. In an earlier paper [22] it was shown that the H_L band at 545 nm is partially ($\sim$20%) bleached even at $\sim$2 ps delay. However, there is an indication [29] that some contribution from B_L to the 545 nm transition can be observed.

The energy position of $P^+B_{L^-}$ state was clearly shown [25] to be below that of P* by $\sim$200 cm^{-1} in RCs with prereduced quinones. In open RCs this number is also negative and close to 450 cm^{-1} [23]. This rules out the possibility for superexange mechanism of the electron transfer proposed by some authors (see [30,31]).

Acknowledgements: We thank Prof. V.P. Skulachev, Prof. A. Hoff, Dr. V.A. Drachev and Dr. D.P. Krindach for help and stimulating discussion, and Dr. A.V. Sharkov for help in construction of fs spectrometer. Support by the Russian Fundamental Research Foundation, ISF (MUM 000 and MUM300), NWO and INTAS is gratefully acknowledged.

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