Blue Light Drives B-Side Electron Transfer in Bacterial Photosynthetic Reaction Centers†

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Received July 27, 2001; Revised Manuscript Received October 11, 2001

ABSTRACT: The core of the photosynthetic reaction center from the purple non-sulfur bacterium *Rhodobacter sphaeroides* is a quasi-symmetric heterodimer, providing two potential pathways for transmembrane electron transfer. Past measurements have demonstrated that only one of the two pathways (the A-side) is used to any significant extent upon excitation with red or near-infrared light. Here, it is shown that excitation with blue light into the Soret band of the reaction center gives rise to electron transfer along the alternate or B-side pathway, resulting in a charge-separated state involving the anion of the B-side bacteriopheophytin. This electron transfer is much faster than normal A-side transfer, apparently occurring within a few hundred femtoseconds. At low temperatures, the B-side charge-separated state is stable for at least 1 ns, but at room temperature, the B-side bacteriopheophytin anion is short-lived, decaying within ∼15 ps. One possible physiological role for B-side electron transfer is photoprotection, rapidly quenching higher excited states of the reaction center.

The primary energy conversion process in photosynthesis takes place in the reaction center, an integral membrane, pigment−protein complex. The reaction center from purple non-sulfur bacteria has been studied in detail, in terms of both structure and function, resulting in a mechanistic picture of photosynthetic electron and proton transfer that forms the basis of our current understanding of biological solar energy transduction (Figure 1 and refs *1*−*6*). There remains, however, an enigmatic aspect of the reaction center structure that has never been completely understood. There is a striking rotational symmetry at the tertiary structural level that involves the two core reaction center subunits, L and M, and their associated cofactors (Figure 1A and refs *7*−*10*). As a result, there is not one but two potential electron transfer pathways in the reaction center made up of chemically identical cofactors (subscripts A and B in Figure 1B) in a quasi-symmetric protein environment. This structural symmetry is in marked contrast to the results of spectroscopic measurements of reaction center function which show a linear rather than a branched electron transfer pathway (Figure 1B and refs *1*, *2*, and *4*). Comparison of the bacterial reaction center to other photosynthetic reaction centers reveals that structural symmetry is common to all these systems and has been maintained for billions of years (*11*, *12*). This suggests that the symmetric cofactor arrangement serves some important, but as yet unidentified, function in the solar energy conversion process.

It is possible to distinguish between the two potential electron transfer pathways spectroscopically at the level of the bacteriopheophytin *a* molecules (H_A and H_B)[1] due to small differences in their Q_X transition energies (Figure 1C, inset). The anion state of the bacteriopheophytin is short-lived, however, and thus only directly observable via ultrafast spectroscopy. Femtosecond measurements of electron transfer in this system have generally been performed using excitation in the visible and near-infrared spectral regions, where the different cofactors have distinct absorbances (Figure 1C and refs *1*, *2*, and *4*). Under these conditions, charge separation occurs almost exclusively along the A-side electron transfer chain in wild-type reaction centers (Figure 1B). It was shown early on that it is possible to accumulate reduced B-side bacteriopheophytin photochemically (*13*, *14*). In more recent years, it has been possible to directly observe B-side charge separation under two different nonphysiological circumstances: by using mutagenesis to tip the thermodynamic or kinetic balance in favor of the B-side (*15*−*19*) or, alternatively, by exciting the reaction center with two photons during an ultrashort pulse of visible light, generating a higher excited or multiply excited state with sufficient energy to overcome the thermodynamic barriers to B-side electron transfer (*20*). Here, we show that a single higher-energy photon (390 nm) can result in B-side electron transfer under physiologically relevant conditions.

† This work was supported by the National Science Foundation (Grants MCB9817388 and BIR9512970). A.L.M.H. was supported by a graduate research training grant from the National Science Foundation (DGE-9553456).

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[1] Abbreviations: P, bacteriochlorophyll dimer; B_A and B_B, monomer bacteriochlorophyll cofactors on the A and B sides of the reaction center, respectively; H_A and H_B, bacteriopheophytin cofactors on the A and B sides of the reaction center, respectively; Q_A and Q_B, quinone cofactors on the A and B sides of the reaction center, respectively; LDAO, *N*,*N*-dimethyldodecylamine *N*-oxide; EDTA, ethylenediaminetetraacetic acid.

10.1021/bi015612q CCC: $20.00
Published on Web 10/26/2001

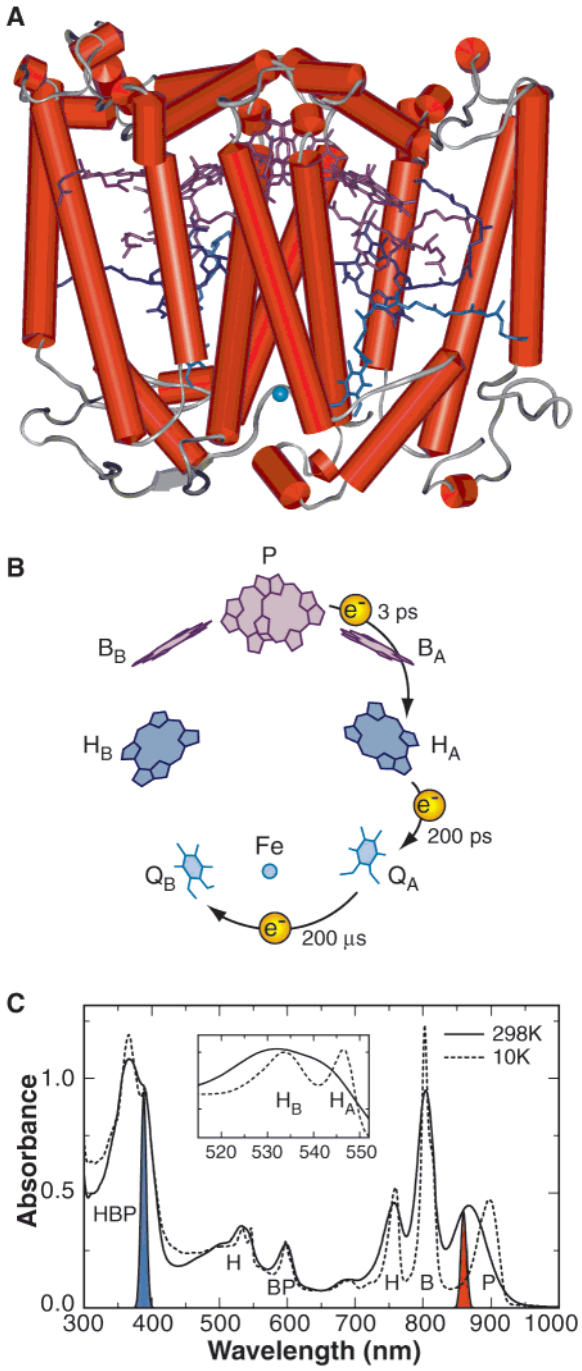

FIGURE 1: (A) Structure of the L and M subunits of the photosynthetic reaction center from *Rb. sphaeroides* and associated cofactors (from PDB entry 1PCR). (B) Cofactor arrangement, electron transfer pathway, and kinetics. P, bacteriochlorophyll dimer which serves as the initial electron donor; B, bacteriochlorophyll monomers; H, bacteriopheophytins; Q, ubiquinones. The A and B subscripts denote the two potential electron transfer branches. (C) Ground-state absorbance spectrum of the reaction center at 298 (—) and 10 K (- - -). The spectral profiles of the 100 fs pump pulses used to excite the reaction centers at 298 K are shown in blue (390 nm) and red (860 nm). The inset shows an expansion of the Q_X spectral region of the bacteriopheophytins.

MATERIALS AND METHODS

Procedures for the preparation of His-tagged *Rhodobacter sphaeroides* reaction centers expressed from a pRK-based plasmid have been described previously (*21*). These reaction centers are considered "wild-type" reaction centers in the studies reported below. R-26 reaction centers were prepared following the procedure of Feher and Okamura (*22*). For room-temperature measurements, reaction centers were suspended in 15 mM Tris-HCl (pH 8.0), 0.025% *N*,*N*-dimethyldodecylamine *N*-oxide (LDAO), 1 mM EDTA, and 0.1 mM *o*-phenanthroline. Samples were loaded in a spinning wheel with an optical path length of 2.5 mm. A final optical density of roughly 1.2 at 802 nm was used. For low-temperature measurements, quinones were removed from the reaction center samples as described previously (*23*) and the reaction centers were suspended in 15 mM Tris-HCl (pH 8.0), 0.025% LDAO, and 1 mM EDTA and then mixed with 67% (v/v) glycerol. The sample was placed between two glass plates with a rubber spacer of 1.2 mm, then attached to the coldfinger of a closed circulated helium displex (APD), and cooled to 10 K. The typical optical density of the sample was 1.5 in the sample holder at 800 nm and 10 K.

The femtosecond transient absorption spectrometer has been described previously (*24*). Laser pulses of 150 fs and 780 nm were generated from an amplified titanium sapphire amplifier which seeds a kilohertz optical amplifier system (CPA-1000, Clark-MXR). Part of the laser energy (~10%) was used to generate a white light continuum for the probe beams. The rest of the beam was either frequency doubled to create excitation at 390 nm or used to pump an optical parametric amplifier (IR-OPA, Clark-MXR) to generate excitation at 740 or 860 nm. Excitation at 806 nm was obtained directly from the fundamental output of the amplified laser. The excitation intensity was adjusted using a continuously variable neutral density filter. Transient absorption changes were measured from 460 to 760 nm and from 730 to 1030 nm using a dual-diode array detector (DDA, Princeton Instruments). The relative polarization of the excitation and probe beams was set to the magic angle.

The excitation intensity at 390 nm was kept at 2 μJ per pulse for all measurements reported here, and the excitation spot size was 1 mm in diameter. This corresponds to ~0.25 photon absorbed per reaction center each pulse. An excitation intensity dependence study was performed at 10 K using 390 nm excitation, in which excitation intensities of 0.25, 0.5, 1, 2, and 4 mW were used. The results (not shown) show that the amplitude ratio of 530 and 540 nm bands is independent of the excitation intensity over this range within experimental error. Thus, both A-side (involving H_A at 540 nm) and B-side (involving H_B at 530 nm) charge separation due to 390 nm excitation have the same intensity dependence, verifying that the observed B-side charge separation is due to single-photon excitation events (compare this result to previous studies of multiphoton excitation of reaction centers using 595 nm excitation resulting in B-side charge separation; see ref *20*).

Measurements using 390 nm excitation with stationary samples (for example, at low temperatures) resulted in more rapid degradation of the reaction centers than was observed using 860 nm excitation. Hence, for the low-temperature measurements using 390 nm excitation, measurements were never taken in any one part of the sample for more than 5 min. Under these conditions, no degradation was evident (the transient signals did not change over that time period), but only a limited number of transient spectra could be collected in any one run.

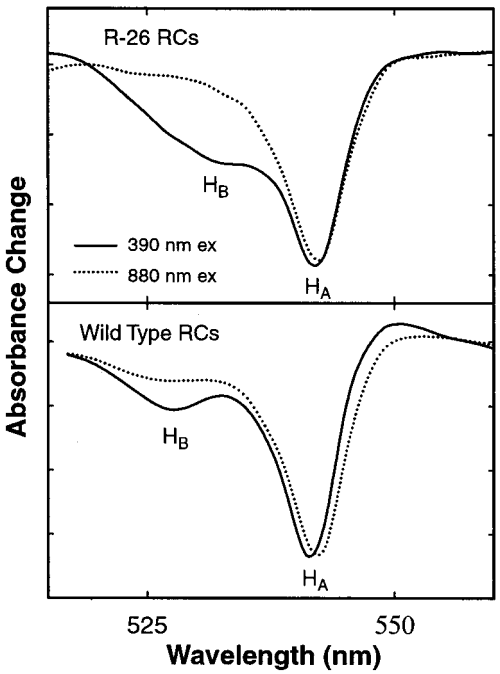

FIGURE 2: Comparison of the nondecaying amplitude spectra (the constant term in the exponential fit) resulting from a global fit of the time vs wavelength absorbance change surface of R-26 (carotenoid-less) and wild-type reaction centers at 10 K using 880 (···) and 390 nm (—) excitation.

Data analysis was carried out using locally written software (ASUFIT) developed under a MATLAB environment (Mathworks Inc., software available electronically upon request; *18*).

RESULTS AND DISCUSSION

Blue Light Initiates B-Side Charge Separation Forming H_B^-. The observation that the absorption of two visible wavelength photons during an ultrafast pulse can initiate B-side electron transfer in wild-type reaction centers (*20*) suggests the possibility that a single more energetic photon might do the same. Figure 2 compares the low-temperature (10 K) amplitude spectra (decay-associated spectra) of the nondecaying (constant) term in a global exponential fit of the wavelength-dependent absorbance change kinetics measured over a 1 ns time scale using 880 nm excitation with that using 390 nm excitation. All excitation was performed at sufficiently low intensities so that the observed absorbance changes were linear with excitation intensity and represented one-photon absorption events (see Materials and Methods for a more complete description). At this temperature, the two bacteriopheophytin Q_X transitions are clearly resolved (Figure 1C, inset). The 390 nm light excites the reaction center Soret band (the excitation pulse spectral profile is shown in blue in Figure 1C). This band is made up of transitions from all of the reaction center bacteriochlorophylls and bacteriopheophytins. The 390 nm excitation results in a significant amount of bleaching of the ground-state Q_X absorbance transition of H_B that persists on the nanosecond time scale (Figure 2). This effect is most pronounced in reaction centers from *Rb. sphaeroides* strain R-26, which lacks carotenoid, but is evident in reaction centers isolated from a wild-type background as well. Assignment of this state as a charge-separated state involving H_B^- is supported by the fact that excited singlet states of H_B are known to decay on a time scale of hundreds of femtoseconds due to energy transfer to neighboring cofactors (*25*−*29*). It has also previously been demonstrated that linear excitation of bacteriopheophytin at 750 or 760 nm or of bacteriochlorophyll at 595 nm in R-26 reaction center samples prepared in the same way at the same temperature does not result in long-lived bleaching of H_B, making it very unlikely that the nanosecond lifetime bleaching at 530 nm in Figure 2 represents a long-lived H_B excited state (*20*).

When the temperature is increased to 295 K, the spectral signature of H_B^- is still evident, but the B-side charge-separated state is shorter-lived. Figure 3A shows a series of room-temperature transient absorbance change spectra initiated by excitation of wild-type *Rb. sphaeroides* reaction centers with a 150 fs pulse of 390 nm light (—) and compares them to absorbance change spectra due to 860 nm excitation (- - -). The bacteriopheophytin ground-state absorbance peak in the 510−550 nm region is flat-topped at room temperature, with the long wavelength side being made up primarily of the Q_X transition of H_A, peaking near 540 nm, and the short wavelength side being primarily the Q_X transition of H_B, peaking near 530 nm (Figure 1C, inset). As shown in Figure 3A (—), both the 530 nm side (the H_B side) and the 540 nm side (the H_A side) of the bacteriopheophytin Q_X band become bleached within the time resolution of the apparatus upon 390 nm excitation. There is also instantaneous bleaching at both 595 nm (not shown) and 800 nm, but not at 860 nm, suggesting that one or both of the monomer bacteriochlorophylls are also excited at this wavelength, but not P. A broad absorbance increase centered at 630 nm also appears very early in the time course. By comparison, 860 nm excitation (dotted lines in Figure 3) results in the initial formation of P* which involves bleaching of the 860 and 595 nm bands of P as well as stimulated emission in the 900 nm region. There are no distinct absorbance changes at early times (other than a general broad absorbance increase) in the 530−540 nm region or in the 630 nm region using 860 nm excitation.

During the first 0.3 ps using 390 nm excitation, both the bleaching of the H_B ground state and the absorbance increase at 630 nm become more prominent and the extent of bleaching of the H_A ground state at 540 nm diminishes, at least in relative terms. Between 0.3 and 0.8 ps, most of the bleaching in the ground-state Q_X band of the bacteriopheophytins is due to H_B at 530 nm. The 630 nm absorbance increase that appears with the H_B bleaching has a shape and peak position very similar to that of the anion absorption band that has been measured previously by selective photochemical reduction of H_B in *Rb. sphaeroides* reaction centers (*13*). Recent ultrafast measurements in reaction center mutants of *Rb. capsulatus* that undergo B-side electron transfer have also shown an anion peak in the same region, though shifted to ∼640 nm (*17*, *19*).

The rapid bleaching at 530 nm and absorbance increase near 630 nm suggest that within a few hundred femtoseconds charge separation occurs involving H_B^-. However, excited states of reaction center cofactors also show ground-state bleaching and broad absorbance increases in the visible part of the spectrum. It is therefore useful to compare the early time transient absorbance spectra using 390 nm excitation, with transient absorbance spectra obtained after directly exciting the lower-energy transitions of the bacteriochloro-

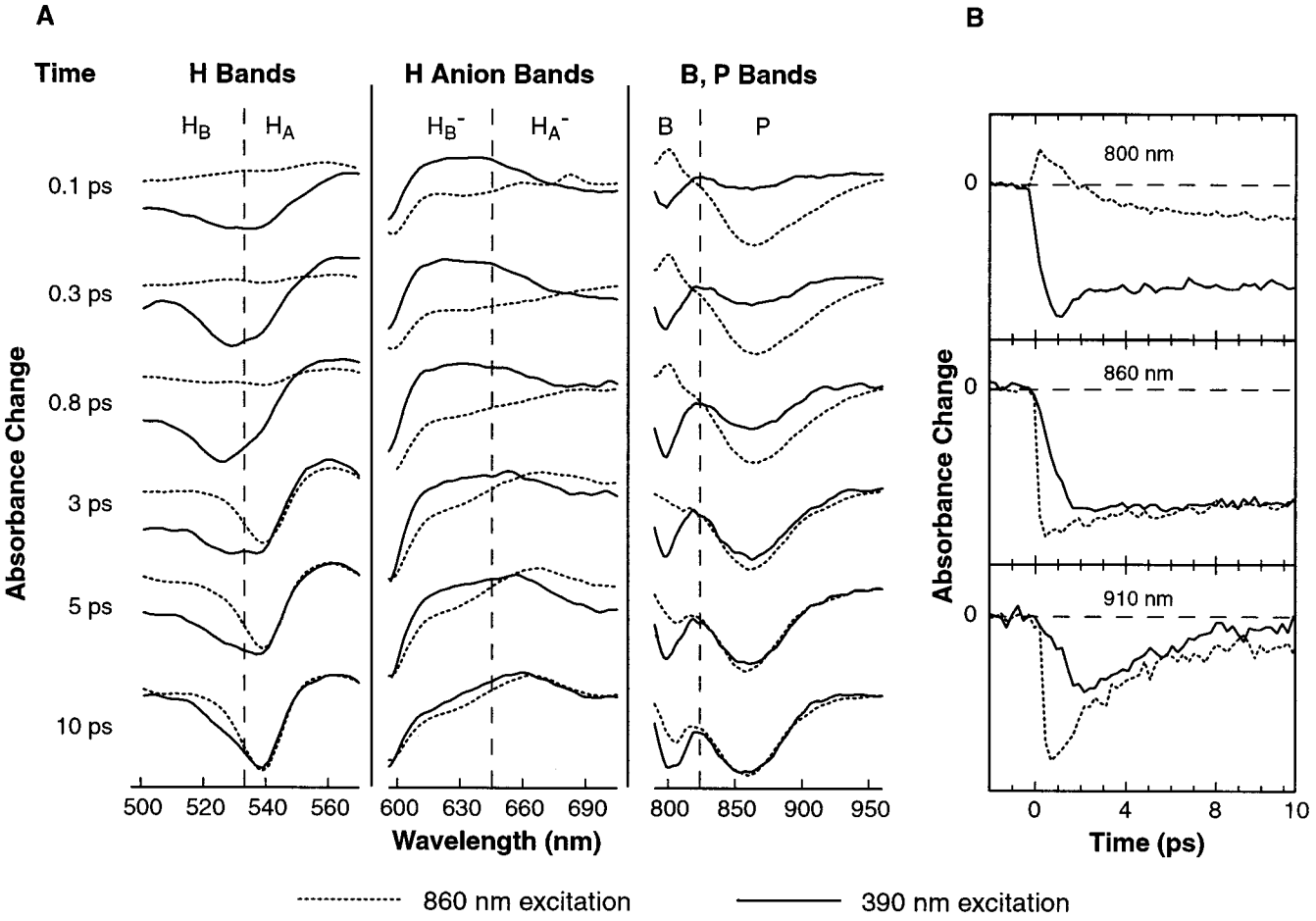

FIGURE 3: (A) Time-resolved absorbance change spectra of wild-type reaction centers from *Rb. sphaeroides* at room temperature using two excitation wavelengths: 390 (—) and 860 nm (- - -). Time, from 0 to 10 ps, is shown along the far left side. Spectra using 390 and 860 nm excitation are normalized at the 10 ps time point in each case. The same scale factor is used at the other times. (B) Time-dependent changes in the absorbance spectra using 390 (—) and 860 nm (- - -) excitation at three different detection wavelengths: 800 nm (ground-state absorbance band of the monomer bacteriochlorophylls, B_A and B_B), 860 nm (ground-state absorbance band of P), and 910 nm (the stimulated emission from P). Time points are spaced by 250 fs. The data sets using 390 and 860 nm excitation were normalized at 860 nm and 10 ps.

phylls and bacteriopheophytins in their Q_Y transitions. In this way, it is possible to gauge the involvement of bacteriopheophytin and bacteriochlorophyll excited states in the difference spectra obtained using 390 nm excitation. Such a comparison is shown in Figure 4. For each excitation wavelength, two spectra are shown, the absorbance change spectrum present 0.3 ps after excitation and the absorbance change spectrum observed after 17 ps. These spectra are reconstructed from multiexponential fits of the whole time versus wavelength absorbance change surface in the 500−720 nm region in such a way that effects of dispersion are removed (see the legend of Figure 4).

The two most similar 0.3 ps spectra arise from direct excitation of B (806 nm, Figure 4C) and of P (860 nm, Figure 4D). In both cases, there is an overall broad excited-state absorbance increase throughout the spectrum that becomes gradually larger toward the red end of the spectrum. In addition, there is a bleaching of the 595 nm band due to ground-state bleaching of the reaction center bacteriochlorophylls (B and/or P) which have Q_X transitions in this region.

Excitation at 740 nm (Figure 4B) results in bleaching of the ground-state Q_X bands of H_B (530 nm), H_A (540 nm), and the bacteriochlorophyll Q_X bands (595 nm). Ground-state bleaching of the bacteriochlorophyll Q_X bands is observed because energy transfer from H to B is so rapid that much of the bacteriopheophytin excited state has already transferred its energy to the monomer bacteriochlorophyll and to some extent to P by 0.3 ps (*25*−*29*). There is again a broad absorbance increase that slopes upward toward the red, and becomes nearly flat above 625 nm.

Excitation at 390 nm results in a 0.3 ps difference spectrum that is distinctly different from that at any of the other wavelengths (Figure 4A). Bleachings in both the bacteriochlorophyll and bacteriopheophytin Q_X spectral regions are observed, with the bacteriopheophytin bleaching centered close to 530 nm as would be expected for the involvement of H_B. This relatively equal bleaching of the bacteriopheophytin and bacteriochlorophyll Q_X bands at 0.3 ps is similar to that observed in the $P^+H_A^-$ state at 17 ps and is what one would expect for a charge-separated state. In addition, the substantial absorbance increase near 630 nm using 390 nm excitation that is associated with H_B^- formation (see above) is much greater than absorbance change features seen in this region of the spectrum upon formation of bacteriopheophytin or bacteriochlorophyll excited states using the other excitation wavelengths (Figure 4B−D). Thus, the 0.3 ps spectrum formed upon 390 nm excitation does not appear to be a simple excited state or mixture of excited states of the reaction center cofactors and instead shows the characteristics of a charge-separated state involving H_B^-.

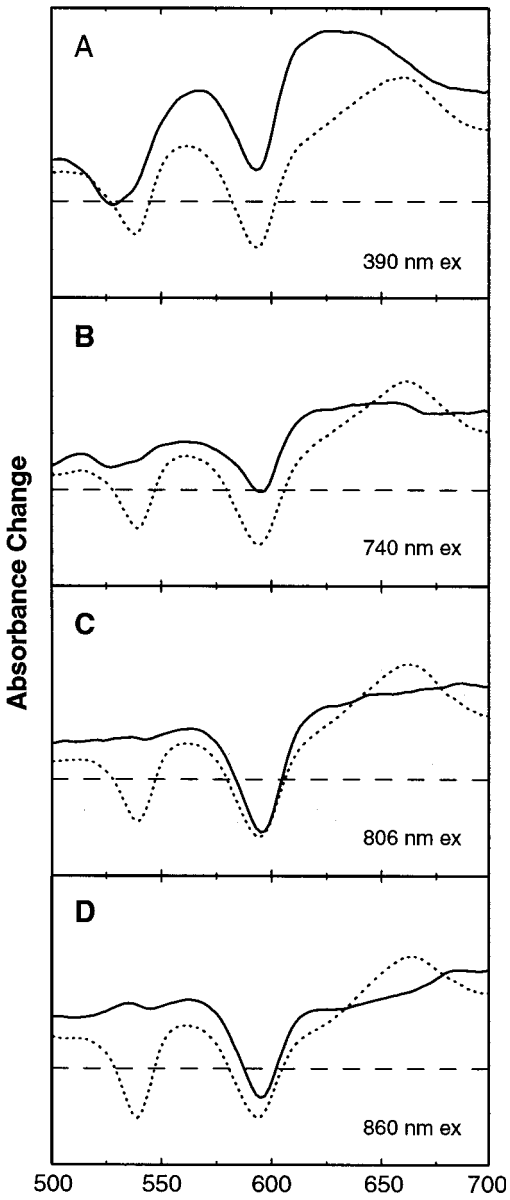

FIGURE 4: Room-temperature time-resolved absorbance change spectra at 0.3 (—) and 17 ps (- - -) using the indicated excitation wavelengths. The straight dashed line in each panel represents zero absorbance change. Spectra were obtained by fitting the time vs wavelength absorbance change surfaces between 0 and 800 ps and between 500 and 720 nm with a sum of three exponential terms and a constant and then plotting the reconstituted absorbance change spectra from the fit at 0.3 and 17 ps in each panel. The fit included corrections for dispersion (measured using CS_2 birefringence). By performing a dispersion correction in conjunction with a fit, we could generate a more accurate depiction of the 0.3 ps spectral changes than is possible looking at the raw data (dispersion correction of raw data, which is discrete, is more problematic): (A) 390 nm excitation (Soret band), (B) 740 nm excitation (mostly H_B with some H_A), (C) 806 nm excitation (mostly B_B with some B_A), and (D) 860 nm excitation (P).

It is interesting that though H_A is apparently excited at 390 nm (in Figure 3A, at 0.1 ps using 390 nm excitation there is bleaching at both 530 and 540 nm), there is no evidence for formation of H_A^- at early times as there is for H_B^-. The H_A ground-state bleaching at 540 nm becomes less pronounced, or at least overshadowed, by the 530 nm bleaching during the first 0.3−0.8 ps, and little or no absorbance increase near 660 nm, normally associated with H_A^- (*4*), is observed during this time (Figure 3). It is not possible to directly observe the fate of the excited state(s) of H_A formed upon 390 nm excitation, but it may well decay via rapid energy transfer to other cofactors, as does the lowest excited singlet state of H_A (*25*−*29*).

In recent years, there have been reports of some direct electron transfer occurring from excited states of the bacteriopheophytins and monomer bacteriochlorophylls on very rapid time scales in wild-type reaction centers (see, for example, refs *25*, *27*, *28*, and *30*−*33*). However, there is considerable controversy at present about what states are involved and what the yield of these reactions is. In any case, there is no direct evidence for B-side electron transfer involving H_B when the lowest excited singlet states of the reaction center bacteriopheophytins and bacteriochlorophylls are directly excited at low intensities (*20*) in contrast to the situation described above for 390 nm excitation.

Identity of the Initial Cation Associated with B-Side Charge Separation. It is argued above that H_B^- is formed within a few hundred femtoseconds after 390 nm excitation of reaction centers, but what is the electron donor for this charge separation? Given the bleaching at 595 nm associated with this state, it would presumably have to involve one or more of the bacteriochlorophyll molecules in the reaction center. An 800 nm absorbance decrease immediately follows 390 nm excitation (Figure 3). By comparison, the extent of bleaching of P at 860 nm remains low initially, not reaching a maximum until well after 1 ps (Figure 3B). The P bleaching that does grow in on the 1−2 ps time scale is accompanied by stimulated emission from P* in the 900−920 nm region (Figure 3B), implying that much of the P bleaching observed at this time is due to delayed formation of P*. Thus, P^+ seems to be an unlikely candidate for the initial electron donor upon 390 nm excitation as P ground-state bleaching forms too slowly and appears to have substantial excited-state character initially. Given the proximity of B_B to H_B, and the rapid bleaching of the monomer bacteriochlorophyll band at 800 nm, a more likely possibility for an initial charge-separated state is $B_B^+H_B^-$, though the evidence for this is still indirect. B^+H^- on one side of the reaction center or the other has been considered as at least a possible product of the direct excitation of the bacteriopheophytins and the monomer bacteriochlorophylls in wild-type reaction centers (*25*, *27*, *28*, *30*−*33*), but there does not appear to be detectable B-side electron transfer using these excitation wavelengths in the wild type, under linear (one photon) excitation conditions (*20*).

One of the reasons why it has been difficult to produce a clear spectrum of the B-side charge-separated state upon 390 nm excitation is that the early time B-side photochemistry is apparently occurring in parallel with excited-state energy transfer and photochemistry on the A-side. This has made it difficult to use kinetic modeling to determine the difference spectrum of the initial B-side charge-separated state using the 390 nm excitation data alone. However, it does appear to be possible to use the kinetic differences between A-side and B-side electron transfer to generate a double-difference spectrum of the B-side charge-separated state present at 10 ps, a point where 860 nm excitation has resulted in almost purely $P^+H_A^-$, while there is still a small amount of B-side charge-separated state remaining using 390 nm excitation

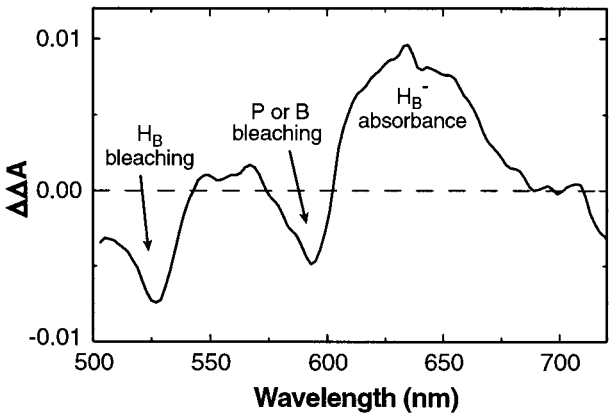

FIGURE 5: Room-temperature difference−difference absorbance spectrum obtained by subtracting the transient absorbance spectrum determined using 860 nm excitation at 10 ps from that using 390 nm excitation at 10 ps. Normalization was performed in such a way that the contributions from $P^+H_A^-$ in the final spectrum were removed, leaving contributions from the state involving H_B^-. The *y*-axis units are arbitrary.

(Figure 3A). By normalization of the 860 and 390 nm difference spectra so that the amount of H_A bleaching is equal between them and then subtraction of the two spectra, a 10 ps double-difference spectrum of the B-side charge-separated state results without interference from excited states or $P^+H_A^-$. This is shown in Figure 5. (Note that the normalization used in Figure 5 is different from that in Figure 3. In Figure 3, the spectra were normalized to the amount of long-lived P^+ that formed. Thus, Figure 5 is not just the difference in the 10 ps spectra of Figure 3 using 390 and 860 nm excitation.) Figure 5 shows three clear spectral features in the double-difference spectrum. There are absorbance decreases at 527 and 593 nm corresponding to the ground-state bleaching of H_B and either P or B. There is also an absorbance increase at 630 nm corresponding to H_B^-.

The double-difference spectrum at 10 ps in Figure 5 could be that of either $P^+H_B^-$ or $B_B^+H_B^-$. It is possible that the initial charge-separated state, suggested to be $B_B^+H_B^-$ as described above, evolves into $P^+H_B^-$ on the picosecond time scale. However, $P^+H_B^-$ has been observed previously at room temperature in mutants that undergo B-side electron transfer and has been reported to have a nanosecond lifetime (*17*, *19*). While it could be that these mutations affect the energetics in such a way that $P^+H_B^-$ is more stable, and thus longer-lived, the simplest conclusion is that the B-side charge-separated state generated by 390 nm excitation never involves formation of $P^+H_B^-$ and remains in the $B_B^+H_B^-$ state during the lifetime of H_B^-.

Spectral Evolution and Decay of the B-Side Charge-Separated State. Over a period of ~15 ps, the state involving H_B^- that was formed by 390 nm excitation disappears. It is not clear what the fate of this charge-separated state is, but there are several possibilities. First, this decay is apparently temperature-dependent (the H_B^--containing state in Figure 2 is long-lived at low temperatures). Thus, whatever the mechanism of decay, there is presumably an activated state involved. It is interesting that on roughly the same time scale during which H_B^- decays, $P^+H_A^-$ is formed (Figure 3A). The appearance of H_A^- can be seen both in the increase in the level of bleaching of the 540 nm H_A band and in the appearance of the anion band at 660 nm associated with H_A^- (in Figure 3A, the spectra from 390 nm excitation evolve over time to more and more resemble the spectra obtained from direct 860 nm excitation of P) (*4*, *34*). Coincident with these changes, the bleaching of the 530 nm H_B ground-state band recovers and the absorbance due to H_B^- at 630 nm decays. Thus, one possibility is that $B_B^+H_B^-$ decays via some activated intermediate (perhaps an excited state like P*) to form $P^+H_A^-$. However, at this point it seems equally likely that recombination of $B_B^+H_B^-$ via some activated state results in the ground state. Note that in the reaction centers used for this study, no Q_B was present, and thus, electron transfer from H_B to Q_B was not possible.

Summary of the Mechanism of B-Side Electron Transfer Initiated by 390 nm Excitation. The 390 nm excitation of wild-type reaction centers generates an excited state that gives rise to a substantial amount of B-side charge separation involving H_B^- within a few hundred femtoseconds. Both the early time spectral evolution (P is not involved at early times in the photochemistry) and comparison to previous work ($P^+H_B^-$ is thought to have a nanosecond lifetime; *17*, *19*) imply that this initial B-side charge-separated state does not involve P^+. Thus, the most likely identity for the initial B-side electron transfer product is $B_B^+H_B^-$.

The B-side bacteriopheophytin anion disappears over a time period of ~15 ps at room temperature, but remains for at least 1 ns at low temperatures. The temperature dependence demonstrates that some step in the decay of the B-side charge-separated state is activated, but little else about this process is known. It is also not at all clear why excitation at 390 nm apparently does not excite P directly at all. Presumably, all of the reaction center bacteriochlorophylls and bacteriopheophytins have transitions in the Soret region. More complete studies of blue excitation and B-side electron transfer as a function of temperature, using other excitation wavelengths in the blue and green regions of the spectrum and using various mutations affecting reaction center energetics, are in progress to sort out these issues.

Possible Physiological Roles of B-side Electron Transfer. Now that it is possible to observe B-side electron transfer under physiological conditions using wild-type reaction centers, what role might it serve in bacterial photosynthesis? The reaction centers used in this study were prepared in such a way that Q_B is missing, and thus, one might think that if Q_B were present, electron transfer could continue from H_B to Q_B. Experiments designed to test this are in progress using mutants in which Q_A has been removed via mutagenesis to unambiguously detect direct Q_B reduction. However, it is unlikely that electron transfer from H_B to Q_B would be able to compete effectively with the rapid decay of the H_B^- state at room temperature. On the A-side, electron transfer between H_A and Q_A takes place with a time constant of ~200 ps, and the distances and orientations are similar on the B-side (Figure 1B).

If the B-side electron transfer is not there to provide an alternate reduction path for Q_B, then what is its purpose? Even if additional experiments show that there can be direct transfer to Q_B, why invent an electron transfer mechanism for blue light so fundamentally different from those for visible and near-infrared light? One possibility is some form of blue or UV light photoprotection. As described in Materials and Methods, reaction centers that remained in the 390 nm beam for extended periods of time degraded more

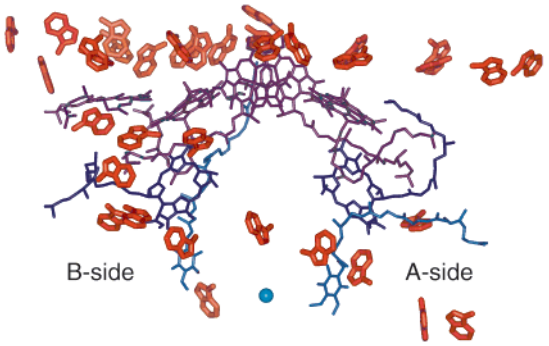

FIGURE 6: Arrangement of tryptophan residues (red) in the photosynthetic reaction center of *Rb. sphaeroides* (from PDB entry 1PCR).

rapidly than when exposed to the 860 nm beam. Similar effects are seen with porphyrin systems which can be excited for extended periods in the visible region (*35*), but apparently degrade rapidly upon excitation with femtosecond pulses into the Soret band (unpublished results). This presumably is because the higher excited states are more likely to lead to events such as ionization and radical formation. It may be advantageous for the photosynthetic apparatus to remove these higher-energy states as rapidly as possible. Extremely rapid charge separation along the B-branch directly from higher excited states could serve this function, at least for some of the reaction center cofactors.

Further, the Soret band of the reaction center cofactors overlaps significantly with the spectrum of fluorescence from reaction center tryptophans, and the excited state of tryptophan in the reaction center is known to be strongly quenched by the reaction center cofactors (approximately half of the tryptophan excited states in the reaction center are quenched within 70 ps; see ref *36*). Thus, B-side electron transfer could serve as a sink for both higher-energy excited states that originate from direct blue light absorption by the reaction center cofactors and from UV light absorption by the protein followed by energy transfer to the bacteriochlorophyll and bacteriopheophytin Soret band. Indeed, there is a concentration of tryptophan molecules near the B-side cofactors in the reaction center structure (Figure 6 and PDB entry 1PCR), suggesting that accepting energy from the network of tryptophan residues could be one role of the B-side. Further study of both the reaction center's sensitivity to blue and/or UV excitation and the effects of blue and/or UV light and of B-side mutations on cell growth and the viability of the photosynthetic apparatus are in progress.

ACKNOWLEDGMENT

This is publication 500 from the Center for the Study of Early Events in Photosynthesis, Arizona State University.

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BI015612Q