Environmental Control of Primary Photochemistry in a Mutant Bacterial Reaction Center

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The core structure of the photosynthetic reaction center is quasisymmetric with two potential pathways (called A and B) for transmembrane electron transfer. Both the pathway and products of light-induced charge separation depend on local electrostatic interactions and the nature of the excited states generated at early times in reaction centers isolated from *Rhodobacter sphaeroides*. Here transient absorbance measurements were recorded following specific excitation of the Q_y transitions of P (the special pair of bacteriochlorophylls), the monomer bacteriochlorophylls (B_A and B_B), or the bacteriopheophytins (H_A and H_B) as a function of both buffer pH and detergent in a reaction center mutant with the mutations L168 His to Glu and L170 Asn to Asp in the vicinity of P and B_B. At a low pH in any detergent, or at any pH in a nonionic detergent (Triton X-100), the photochemistry of this mutant is faster than, but similar to, wild type (i.e. electron transfer occurs along the A-side, 390 nm excitation is capable of producing short-lived B-side charge separation ($B_B^+H_B^-$) but no long-lived $B_B^+H_B^-$ is observed). Certain buffering conditions result in the stabilization of the B-side charge separated state $B_B^+H_B^-$, including high pH in the zwitterionic detergent LDAO, even following excitation with low energy photons (800 or 740 nm). The most striking result is that conditions giving rise to stable B-side charge separation result in a lack of A-side charge separation, even when P is directly excited. The mechanism that links $B_B^+H_B^-$ stabilization to this change in the photochemistry of P in the mutant is not understood, but clearly these two processes are linked and highly sensitive to the local electrostatic environment produced by buffering conditions (pH and detergent).

Introduction

The photosynthetic reaction center is responsible for the conversion of light energy into a transmembrane charge separation via a series of electron-transfer reactions between its redox-active cofactors. *Rhodobacter (Rb.) sphaeroides* is an anoxygenic purple non-sulfur bacterium whose reaction center is well characterized both functionally and structurally.[1] The *Rb. sphaeroides* reaction center consists of three protein subunits (L, M, and H) and ten cofactors.[2−4] The core of the reaction center is made up of the L and M subunits, which both contain five transmembrane helices arranged in a nearly C_2 symmetric fashion. The third protein subunit, H, lies predominantly on the cytoplasmic face of the intracytoplasmic membrane. Nine of the ten cofactors, a bacteriochlorophyll dimer (P composed of P_A and P_B), two accessory bacteriochlorophylls B_A and B_B, two bacteriopheophytins H_A and H_B, two quinones, Q_A and Q_B, and a non-heme iron (Fe), are also related by the C_2 symmetry. P is near the periplasmic face of the membrane and is flanked by B_A and B_B on each side. H_A and H_B are farther into the membrane followed by Q_A and Q_B with the iron atom between the quinones near the cytoplasmic side of the membrane. A carotenoid molecule, the tenth cofactor, is asymmetrically placed near B_B.

Upon direct excitation of any of the lowest excited singlet states of the cofactors, rapid energy transfer to P results in the formation of P*, and then electron transfer occurs with a time constant of a few picoseconds forming $P^+H_A^-$ presumably via $P^+B_A^-$. In about 200 ps, a subsequent electron transfer forms $P^+Q_A^-$ and then on longer time scales $P^+Q_B^-$ is formed. Under these excitation conditions, essentially no electron transfer from P to either B_B or H_B is observed despite the approximate C_2 symmetry relating the structure of the electron-transfer pathways on the two sides.[5−7] The quantum yield of A-side electron transfer in wild type under these conditions is nearly unity.[8] Since the elucidation of the structure two decades ago, much work has focused on understanding the ability of this pigment−protein complex to dictate the directionality of electron transfer.

The lack of B-side charge separation in wild type reaction centers with P as the electron donor is predominantly the result of the energetic arrangement of charge-separated states in the system. Holten and co-workers have performed a number of studies of electron-transfer directionality using mutants in which the primary electron acceptor, H_A, has been replaced with a bacteriochlorophyll making small changes in the ground-state absorbance band of H_B (530 nm) easier to detect. The addition of mutations to modify the local environments of B_A and B_B has resulted in charge separation from P* forming the state $P^+H_B^-$ with a quantum yield as high as 30%.[9−12] Mutants have also been constructed that have modified the B-side such that

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its initial charge-separated state is greatly stabilized, but with the A-side left intact. In mutants that have B_B replaced with a bacteriopheophytin or in which the B_B ligand is replaced with glutamic acid, about 35% B-side electron transfer has been observed.[13,14]

None of these mutants speak directly to the role of the B-side pigments in wild-type reaction centers. It has been known for some years that it is possible to generate B-side charge separation in wild-type reaction centers in which H_A has been reduced by continuous illumination in the presence of an external reductant.[15,16] More recently, two-photon excitation with 600 nm light has been shown to result in B-side transfer,[17] and 390 nm excitation results in rapid bleaching at 530 nm due to H_B^- formation that decays within 15 ps at room temperature and is stable for at least a nanosecond at low temperature.[18] The B-side charge-separated state resulting from 390 nm excitation utilizes the accessory bacteriochlorophyll as the cation, forming $B_B^+H_B^-$. Linear (one photon) excitation at other wavelengths (including direct excitation of the lowest excited singlet states of the monomer bacteriochlorophylls and bacteriopheophytins) has not been shown to create any stable H_B^- in wild-type reaction centers.[19]

The mutant used in this work, L168HE/L170ND, has two potentially negatively charged residues introduced near P and the monomer bacteriochlorophylls. (In the mutant designation "L168HE", the first letter refers to the protein subunit, the number is the amino acid residue in the respective subunit, and the two letters following the number are the amino acid as it appears in the native strain followed by its mutation.) At pH 8 and above, the P/P^+ midpoint potential of L168HE/L170ND is 125 ± 5 mV below wild type,[20] and the rate of primary electron transfer is about 40% faster than wild type in this mutant due to the change in charge separation energetics that accompanies the change in P/P^+ midpoint potential.[7] The P/P^+ midpoint potential is pH dependent in this mutant.[21] These mutations also likely affect the redox properties of the nearby monomer bacteriochlorophylls, though direct measurement of these midpoint potentials is not possible. The mutation L168HE additionally may remove a hydrogen bond from the ring A acetyl group of the A side bacteriochlorophyll of P (P_A).[22−24]

In the mutant L168HE/L170ND, a high yield of B-side charge separation ($B_B^+H_B^-$) lasting for hundreds of picoseconds was found using 390 nm light. It is also possible to form a mixture of A-side ($P^+H_A^-$) and B-side ($B_B^+H_B^-$) charge separation by using 800 or 740 nm light at pH 8.0 in this mutant. Additionally, it is possible to switch between almost exclusively long-lived A-side or B-side charge separation in this mutant, using 390 nm excitation, by changing the buffer pH (low pH favors A-side, high pH favors B-side).[21] Because the B-side charge-separated state in this mutant is similar to that observed in wild type upon 390 nm excitation ($B_B^+H_B^-$), but is stable for hundreds of picoseconds at room temperature, it is a useful model for studying the mechanism of this type of B-side charge separation reaction.

Another interesting aspect of the L168HE/L170ND mutant is that at high pH where $B_B^+H_B^-$ is essentially the only charge separated state formed, there is no obvious photochemistry involving P, even though both energy transfer kinetics and redox potentials would suggest that such photochemistry should be observed.[21] Apparently, P itself is also substantially affected by the change in electrostatic environment that accompanies the pH shift, but the nature of this change is not understood.

Though it has been possible to generate B-side electron transfer as $P^+B_B^-$, $P^+H_B^-$, or $B_B^+H_B^-$, either by altering excitation conditions and/or through mutagenesis, there is currently no known role of any of these B-side states in vivo. Given the relatively minor alterations that result in the generation of these states (typically one or two changed amino acids), it seems possible that other factors, not present in isolated reaction center preparations, may affect the environment of the reaction center in ways that alter the pathway of charge separation. This gives rise to the question of just how sensitive the electron-transfer pathway is to the environment. The L168HE/L170ND mutant described above is an excellent test system for studying environmental effects on formation of the state $B_B^+H_B^-$, as well as changes in the nature of P, because $B_B^+H_B^-$ is stabilized in the mutant and easily recorded, and both its formation and the photochemical activity of P are known to be sensitive to pH and excitation wavelength. This report explores these issues in the L168HE/L170ND mutant as a function of the interplay between excitation wavelength, pH, and the identity of the detergent.

Materials and Methods

L168HE/L170ND reaction centers were isolated as previously described from the corresponding histidine-tagged mutant strain of *Rhodobacter sphaeroides*[7,20] and suspended in one of the following buffers depending on the desired pH: (1) 15 mM Tris-HCl, 0.025% LDAO, 1 mM EDTA, 150 mM NaCl pH 8.0 (referred to as TLE8.0) or pH 7.2 (referred to as TLE7.2); (2) 15 mM Tris-HCl, 0.05% Triton X-100, 1 mM EDTA, 150 mM NaCl pH 8.0 (TTE8.0); and (3) 15 mM CHES, 1 mM EDTA, 150 mM NaCl pH 9.5 with either 0.025% LDAO or 0.05% Triton X-100 (CLE9.5 or CTE9.5, respectively).

Transient Absorbance Spectroscopy. The apparatus used for transient absorption spectroscopy with subpicosecond time resolution has been described previously.[25] Experiments were performed in a pulse/probe beam configuration. For each of the excitation wavelengths used, 850, 800, or 740 nm, the pulse energy was between 1.5 and 2 μJ and the repetition rate was 1 kHz. The diameter of the beam was 1 mm and its spectral full-width-at-half-maximum was 5 nm with a temporal duration of $\sim$150 fs. The probe beam was polarized at the magic angle (54.7°) with respect to the excitation pulse. Measurements were taken at room temperature. 1,10-orthophenanthroline (1 mM) or terbutryn (50 uM) was added to block Q_A to Q_B transfer for some of the experiments, and the sample was then placed in a spinning wheel with an optical path length of 2.5 mm. For other experiments, sodium dithionite was added to reduce Q_A and the sample was placed in a stirred 2 mm path length cuvette. All samples were used at $OD_{800} \sim 1$. Difference transient absorbance spectra were recorded from 450 to 1025 nm (two sets of data were taken from 450 to 780 nm and from 750 to 1025 nm) with 110 steps between -1 and 10 ps and an additional 60 steps of 10 ps, extending the time scale out to 610 ps. Spectral dispersion at early times was corrected empirically,[26] and data analysis of the transient absorbance surfaces (time vs wavelength) was performed as previously described by global fitting to a sum of either 2 (P excitation, data at pH 8.0 previously reported[7]) or 3 (B or H excitation) exponential decay terms and a constant.[27] Excitation of B or H results in additional kinetic complexity, necessitating an additional fitting component. This is due to both energy transfer and alternate electron-transfer pathways when these cofactors are excited, as will be described below.

Electron Paramagnetic Resonance Spectroscopy. Electron Paramagnetic Resonance (EPR) data were obtained in L168HE/L170ND reaction centers in CLE9.5 at ambient temperature on

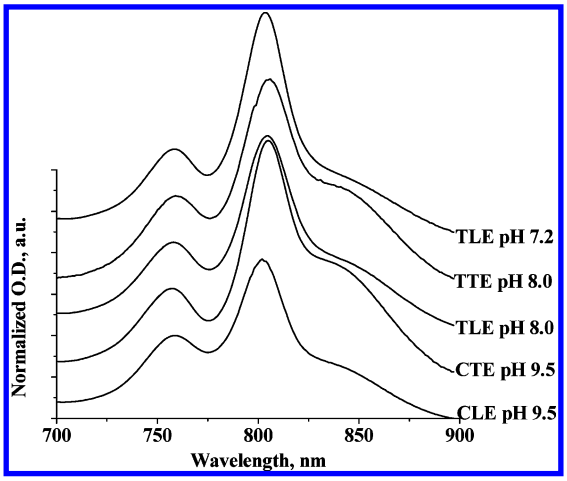

Figure 1. The ground-state absorbance spectra of L168HE/L170ND reaction centers in different buffers normalized to the Q_y transition of H at 760 nm. The spectra are offset for clarity.

a Bruker E580 CW/pulsed spectrometer, equipped with a standard TE_{102} rectangular cavity. Samples were contained in a quartz flat cell during the measurement, with the flat cell rotated to minimize microwave losses in the aqueous samples. EPR instrument parameters included the following: microwave frequency = 9.745 GHz; magnetic field modulation frequency = 100 kHz; modulation amplitude = 3.0 G; center field = 3470 G; sweep width = 100 G; microwave power = 10 mW; sweep time = 42 s; and *x*-axis resolution = 1024 points. Measurements were obtained in the dark.

Results

Ground-State Spectra of the L168HE/L170ND Mutant in Different Buffers. Detergent and buffer effects on the ground state 860 nm P absorbance band position and shape have previously been observed in wild type and mutant reaction centers.[28−33] Figure 1 shows the spectrum of the L168HE/L170ND mutant reaction centers in the buffers TLE7.2, TLE8.0, TTE8.0, CLE9.5, and CTE9.5 (buffers defined in the Methods section). The spectra have been normalized to the Q_y transition of H at 760 nm because the mutation is thought to have the least effect on the bacteriopheophytin spectra. The buffers TLE7.2, TLE8.0, and CLE9.5 contain the zwitterionic detergent LDAO. The buffers TTE8.0 and CTE9.5 contain the uncharged detergent Triton X-100. Under all conditions, the mutant has a blue-shifted P ground-state band compared to wild type (about 15 nm giving rise to a maximum near 845 nm). In buffers containing LDAO, there is also a substantial change in the shape of the P band compared either to wild type or to the mutant in Triton X-100. The band becomes broader and the optical density decreases at the maximum relative to either the 800 or 760 nm bands. The spectral changes seen under the various conditions are reversible if the detergent is exchanged.

Charge Separation Pathway Is Dictated by pH and Detergent. It is possible to distinguish between A- and B-side electron transfer by performing transient absorbance measurements in the visible region of the spectrum and monitoring the Q_x bacteriopheophytin transitions. In the ground-state spectrum of *Rb. sphaeriodes* reaction centers at 10 K, the Q_x absorption bands for the bacteriopheophytins (H_A and H_B) are resolved. The transition centered at 530 nm has been assigned to H_B and that centered at 540 nm to H_A.[34] At room temperature, the transitions broaden and are overlapped, but the two sides of the spectral band are still distinct enough to detect significant involvement of H_B in the photochemistry. These differences are used to discriminate between charge-transfer pathways following optical excitation of the reaction center.

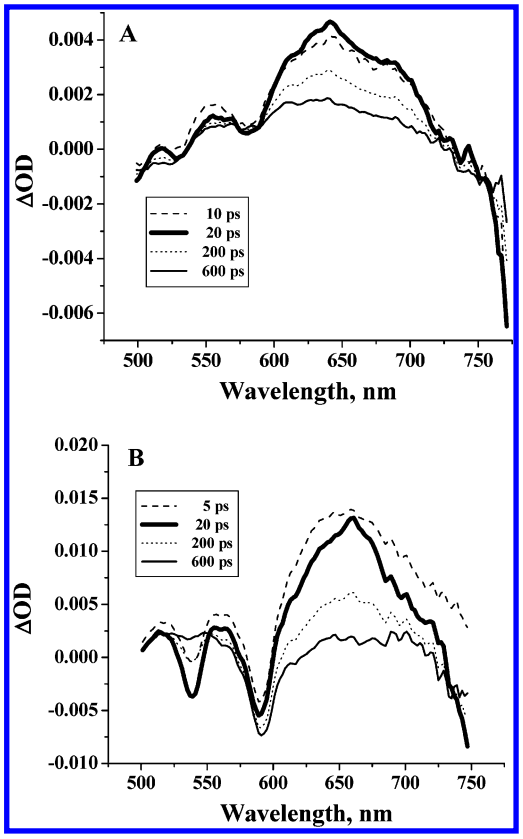

Figure 2. Time-resolved spectra of L168HE/L170ND reaction centers in a spinning wheel suspended in (A) CLE9.5 with 1,10-orthophenanthroline and (B) CTE9.5 with terbutryn following excitation with 800 nm light. Data have been corrected for dispersion.

Previous work has shown that excitation of L168HE/L170ND reaction centers with 850 nm light in TLE8.0 results in only A-side electron transfer.[7] Excitation with 390 nm light in CLE9.5 and TLE7.2 has been shown to result in predominantly B-side and A-side charge separation, respectively, at 600 ps.[21] With 390 nm excitation at pH 7.2, just as in wild type,[18] there is B-side charge separation at early times, but this decays leaving only A-side charge-separated states on long times. In CLE9.5 little or no evidence has been found for A-side electron transfer at any time with 390 nm excitation.[21]

Figure 2 shows transient difference absorbance spectra in the Q_x spectral region using 800 nm excitation for L168/L170ND mutant reaction centers in CLE9.5 and CTE9.5. Data were reconstructed from a global analysis by fitting the time vs wavelength absorbance change surface to three exponentials and a constant because it was possible to correct for dispersion effects[26] more accurately in the fit at early times than to correct the raw data. Reaction centers in CLE9.5 display bleaching of the ground-state Q_x transition of H_B near 530 nm that is accompanied by an anion band near 630 nm (Figure 2A). There is also a bleaching of the Q_x transition of bacteriochlorophyll at ~585 nm that presumably corresponds to B_B bleaching. This is confirmed in the Q_y spectral region where B_B bleaching is also observed at 800 nm (Figure 3B below and ref 21). These spectral features decay with a time constant of about 200 ps, as observed in wild type. Very similar results were also found with 740 nm excitation in CLE9.5 (data not shown).

In contrast to CLE9.5 (LDAO, high pH) reaction centers,

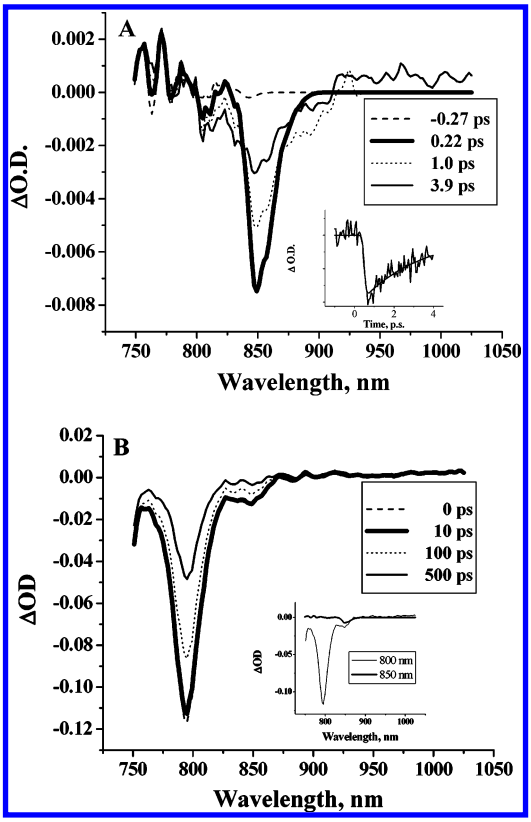

Figure 3. Time-resolved spectra for L168HE/L170ND reaction centers in CLE9.5 in the Q_y region following excitation at (A) 850 or (B) 800 nm. Data have been corrected for dispersion. The data were acquired on the same sample in a spinning wheel with 1,10-orthophenanthroline, first the 850 nm excitation data, then the 800 nm excitation data. The inset in panel A is a fit of the decay of P (lifetime 3.5 ps) and the inset in panel B is an overlay of <1 ps traces (point of maximal bleaching) to show the dramatic difference in relative scale between absorbance changes with 800 and 850 nm excitation.

reaction centers in CTE9.5 (Triton X-100, high pH) give rise primarily to A-side photochemistry on the many picosecond time scale, using 800 nm excitation (Figure 2B). At early times (subpicosecond), there is a rise at 630 nm, suggestive of the anion of H_B^-, but very little bleaching is observed at 525 nm, making it unclear whether there is an extremely short-lived B-side charge separated state formed. In any case, after several picoseconds, the state $P^+H_A^-$ is formed and this progresses to $P^+Q_A^-$ in 230 ps (compared to 200 ps in wild type reaction centers). At low pH (7.2), the only stable charge separation observed is on the A-side regardless of the detergent (data not shown). Excitation with 390 nm at pH 7.2 in TLE has been discussed previously and results in transient B-side electron transfer that decays in 10−15 ps.[21]

The Primary Donor, P, Does Not Function under Conditions That Promote B-Side Charge Separation Stability. Previous work has shown that there is essentially no A-side electron transfer at high pH in LDAO containing buffer (CLE9.5). This is unexpected and warrants additional exploration. A comparison between 800 (monomer bacteriochlorophyll) and 850 nm (P) excitation of the L168HE/L170ND mutant reaction centers in CLE9.5 is shown in the Q_y spectral region in Figure 3. Excitation at 850 nm resulted in only very small absorbance changes that decay substantially over the first few picoseconds (Figure 3A), suggesting that these changes are not due to normal charge separation (which lives much longer). This is striking as there is substantial absorbance at 850 nm in this sample and thus P is clearly absorbing light. In fact, there is more ground-state P absorbance relative to B than in the low pH sample (Figure 1), yet the low pH sample gives rise to ample excited and charge separated states upon direct excitation of P.[21]

With use of 800 nm excitation of reaction centers in CLE9.5, there is limited bleaching at 850 nm (Figure 3B). The size of the bleaching at 850 nm is an order of magnitude less than that observed at 800 nm under these excitation conditions. This is shown in the inset of Figure 3B where the transient absorbance change data at the point of maximum bleaching (<1 ps) is compared by using the two different excitation wavelengths on the same sample. The 800 nm bleaching that is formed upon 800 nm excitation slowly decays over hundreds of picoseconds, presumably due to the rapid formation and then decay of long-lived $B_B^+H_B^-$. Evidence that the state formed is $B_B^+H_B^-$ has been presented above.

EPR Spectra. The lack of A-side electron transfer in L168HE/L170ND reaction centers at a high pH and in LDAO, even upon direct excitation of P, coupled with the low maximum optical density of the P band under the same conditions, gave rise to the possibility that P was partially oxidized in CLE9.5 buffer. To determine if there was a population of P that is oxidized in the dark under these conditions, EPR spectra were obtained (data not shown). No substantial radical signal was found, though in a small population of the sample (<4%), a weak signal with a *g* value of 2.0040 was observed indicative of a bacteriopheophytin or tyrosine radical[35,36] or a quinone uncoupled to iron; however, this was not considered further.

Discussion

Photosynthetic reaction centers have maintained a quasi-C_2 symmetry in their pigment cofactor arrangement across kingdoms and species. Despite the remarkable conservation of the two electron-transfer pathways (A and B) and decades of research, the physiological significance of this arrangement remains elusive. In reaction centers isolated from *Rb. sphaeroides*, electron transfer along the A-side cofactors is readily observed with near unity yield under most conditions. Direct excitation of P in wild-type reaction centers does not result in detectable electron transfer along the B-side pathway. One successful method of generating moderate amounts of B-side electron transfer upon P excitation has been mutagenesis designed to stabilize $P^+B_B^-$.[9−11,13] It is also possible to observe B-side electron transfer from higher excited states of wild-type reaction centers generated either via 2-photon absorption[17] or via excitation in the blue (near 390 nm).[18] The mechanism of the B-side electron transfer observed upon blue light excitation is different from that observed upon direct excitation of P when the state $P^+B_B^-$ has been stabilized by mutagenesis. In wild-type reaction centers, instead of forming $P^+B_B^-$, the state $B_B^+H_B^-$ is initially formed, and then decays via an activated pathway on the picosecond time scale at room temperature and much more slowly at low temperature.[18]

The *Rb. sphaeroides* mutant L168HE/L170ND used in the current work forms a $B_B^+H_B^-$ state that is long-lived (hundreds of picoseconds) even at room temperature. In addition, the product of electron transfer in this mutant is pH dependent, undergoing predominantly A-side transfer at low pH and predominantly B-side charge separation ($B_B^+H_B^-$) at high pH, following 390 nm excitation.[21]

The Formation and Decay of the State $B_B^+H_B^-$. B-side charge separation in wild type reaction centers upon 390 nm excitation has been shown to be extremely rapid.[18] In the mutant reported here, it can occur by using excitation wavelengths of

800, 740, and 390 nm; however, it does not occur from the lowest excited state of P formed with 850 nm excitation. The spectral differences between the states formed with various excitation wavelengths at pH 9.5 are not significant after several picoseconds (data not shown), nor are these states spectrally distinct from the $B_B^+H_B^-$ state formed with blue light in wild type.[18] Thus there is no obvious effect of the L168HE/L170ND mutations on the pathway or rate of formation of $B_B^+H_B^-$, but clearly these mutations have greatly lengthened its decay time.

The slow decay of $B_B^+H_B^-$ in the L168HE/L170ND mutant is presumably due to changes in the energetics of the intermediate states involved. In L168HE/L170ND, the two mutations are known to lower the oxidation potential of P substantially (80 mV in the case of L168HE alone and nearly 130 mV in the case of the double mutant L168HE/L170ND at pH 8.0). Undoubtedly there are effects on the energetics of the other cofactors, especially B_B in the case of L168HE. Theoretical predictions (ref 37 and personal communication, M.Gunner) on the electrostatic field in wild type predict that the effect due to the introduction of a negative charge at L168 on the midpoint potential of B_B/B_B^+ is more than half that predicted for the effect on the midpoint potential of P/P^+. Given that the midpoint potential of P decreases by about 80 mV in the L168HE mutant, one might expect a stabilization of B_B^+ relative to B_B by ~40 mV due to the mutation. The effects on the midpoint potentials of other cofactors are predicted to be smaller (10% to 20% relative to the effect on the P/P^+ midpoint potential); H_B and B_A could theoretically become harder to reduce on the order of ~10 mV. However, it is not possible to experimentally assess the changes on any of the cofactors except P. No predictions were made regarding L170 in Gunner's work. In wild type, there is an activation barrier to the decay of $B_B^+H_B^-$.[18] Thus it seems likely that the free energy of the state $B_B^+H_B^-$ has been substantially lowered in the mutant L168HE/L170ND creating a greater activation barrier for its decay than was observed in wild type.[18]

The Lack of A-Side Electron Transfer under Conditions When $B_B^+H_B^-$ Is Formed. A completely unexpected observation concerning the L168HE/L170ND mutant was that the same conditions (pH, detergent) that stabilize the state $B_B^+H_B^-$ essentially eliminate A-side electron transfer. This does not appear to be simply a kinetic effect (faster B-side transfer from the initial excited state competing with A-side transfer) because even when P is excited directly (which does not give rise to B-side transfer in this mutant), no A-side transfer is observed. In fact, very little P* is observed, presumably because it is quenched rapidly on the time scale of excitation. It is true that the total oscillator strength of P appears to be diminished somewhat in the mutant, but it is possible to observe substantial A-side transfer at low pH in the detergent LDAO where the reaction center spectrum is essentially the same as it is under conditions of high pH in LDAO where exclusively B-side transfer is observed. The lower oscillator strength in LDAO is not due to partial oxidation of P as determined from EPR measurements. How the mutations introduced give rise simultaneously to both stabilization of $B_B^+H_B^-$ and the loss of the ability to initiate A-side transfer by direct excitation of P remains unclear. Apparently, the dimer is in a different form or conformation at high pH that is either not strongly coupled to the A-side cofactors or is quenched so rapidly by some other mechanism that electron transfer has no chance to occur.

Detergent Effects on the Product of Electron Transfer. A number of previous studies have identified changes in the properties of P as a function of its environment. The influence of detergents in particular on P has been investigated in some detail. Two distinct conformations of P, both in terms of its ground-state spectra[32,38] and the electronic distribution of the radical cation following electron transfer, are strongly influenced by the choice of detergent.[28,39] Although species variation exists in the detailed experimental conditions necessary to arrive at the two conformers, the underlying quality of the two states in different species is essentially the same suggesting that they may play a role in reaction center function.[39]

The stabilization of the B-side charge-separated state in the L168HE/L170ND mutant (as well as the loss of A-side electron transfer) requires a charged detergent. The high pH data described above that display B-side charge separation (Figures 2A and 3B) were all acquired with use of the zwitterionic detergent LDAO. In contrast to the long-lived B-side charge-separated states observed with LDAO, in Triton X-100, no long-lived B-side charge separation is observed, even at a high pH (Figure 2B). Additionally, P photochemistry returns essentially to normal under these conditions. These findings indicate that fairly subtle electronic properties of the environment surrounding the cofactors involved in primary electron transfer have a great influence on the electronic structure and energetics of the system. It is likely that both the ionizable residues that were introduced and the change in detergent are causing local electrostatic field differences.

The Electrostatic Environment Plays a Key Role in Dictating the Products of Electron Transfer. It is clear that electrostatics plays a major role in dictating the products of electron transfer in the reaction center. This is shown by the simple fact that the introduction of two potentially charged amino acids near P and B_B in the L168HE/L170ND mutant alters the long-lived product of electron transfer dramatically. The additional observation that both shifts in pH (presumably altering the charge of the introduced amino acids) and the use of charged vs uncharged detergents result in dramatic changes in the products of electron-transfer further supports this concept. It is possible that alterations in the electrostatic environment in vivo (binding of proteins or other factors, membrane potentials, etc.) could also have significant effects on the electron transfer reactions, resulting in significant changes in photochemistry. In this respect, it is particularly interesting that the ability of the reaction center to perform A-side photochemistry initiating at P and the ability to generate the state $B_B^+H_B^-$ appear to be orthogonal: electrostatic conditions which promote one activity apparently discourage the other.

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