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Proton release upon oxidation of tyrosine in reaction centers from *Rhodobacter sphaeroides*

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Abstract Markedly different light-induced protonational changes were measured in two reaction center mutants of *Rhodobacter sphaeroides*. A quadruple mutant containing alterations, at residues L131, M160, M197, and M210, that elevate the midpoint potential of the bacteriochlorophyll dimer was compared to the Y_M mutant, which contains these alterations plus a tyrosine at M164 serving as a secondary electron donor [Kálmán et al., Nature 402 (1999) 696]. In the quadruple mutant, a proton uptake of 0.1–0.3 H^+/reaction center between pH 6 and 10 resulted from formation of the oxidized bacteriochlorophyll donor and reduced primary quinone. In the Y_M mutant, a maximal proton release of −0.5 H^+/reaction center at pH 8 was attributed to formation of the tyrosyl radical and modeled using electrostatic and direct proton-releasing mechanisms.

Key words: Photosynthesis; Electron transfer; Proton transfer; Amino acid radical; Photosystem II

1. Introduction

In purple photosynthetic bacteria the conversion of light energy into chemical free energy occurs in a membrane-spanning pigment–protein complex called the reaction center (for reviews see [1,2]). Upon light absorption by the bacteriochlorophyll dimer (P), electron transfer takes place from P through intermediates to the primary quinone (Q_A) and then the secondary quinone (Q_B). In the absence of Q_B the charge-separated state $P^+Q_A^-$ undergoes charge recombination within 100 ms in the wild type. However, in the presence of a secondary electron donor, fast reduction of P^+ prevents charge recombination, resulting in the state PQ_A^-, which is stable for minutes (reviewed in [3]). Proton uptake is observed in reaction centers upon reduction of Q_A, although the semiquinone is not protonated directly [4,5]. The uptake of protons contributes to the solvation of the charge on the quinone within the protein, stabilizing Q_A^- (reviewed in [6]). Similarly, on the periplasmic side, P^+ is stabilized by the release of protons to the bulk. Both protonational events are based on electrostatic Bohr effects, which result in changes of the pK_a values of protonatable residues upon interaction with the light-induced charged states [4,5]. Other factors, such as protein relaxation and conformational changes, might also contribute to changes in pK_a values of residues [7–9].

The bacterial reaction center has a structural similarity to the core subunits of photosystem II [10–12], but critical functional differences exist. Different secondary donors reduce the oxidized primary donors in the two photosystems, namely cytochrome c_2 in the bacterial system compared to a tyrosine residue in photosystem II. The generation of a tyrosyl radical requires a high midpoint potential, such as the ~1.0 V value for the chlorophyll donor of photosystem II, compared to the relatively low midpoint potential of 0.5 V for the bacteriochlorophyll donor P in wild-type bacterial reaction centers. However, mutants of *Rhodobacter sphaeroides* have been shown to have a significantly increased P/P^+ midpoint potential of over 0.8 V [13]. In one of these mutants, termed the quadruple mutant, four amino acid residues near P are altered. The mutations in the quadruple mutant are Leu L131 to His, Leu M160 to His, Phe M197 to His, and Tyr M210 to Trp. In the Y_M mutant, a fifth mutation, Arg M164 to Tyr, places a tyrosine residue in a position analogous to the redox-active tyrosine residues found in photosystem II (Fig. 1). The highly oxidizing dimer was shown to be capable of oxidizing the introduced tyrosine, forming the tyrosyl radical $Y^{\bullet}_{M164}$ [13]. Under the conditions of these experiments, upon light excitation either the state $Y_{M164}P^+Q_A^-$ or $Y^{\bullet}_{M164}PQ_A^-$ is formed, with the relative amount being highly pH-dependent. A pH dependence for tyrosine oxidation in photosystem II arises because formation of a stable tyrosyl radical requires the transport of the phenolic proton from the tyrosine to a proton acceptor, generally involving nearby protonatable amino acid side chains (reviewed in [14–16]). Upon oxidation, the phenolic proton of the tyrosyl radical can either be distributed over several acceptors within the protein, resulting in proton release to the bulk based on the electrostatic Bohr effect, or released into the bulk solution by deprotonation through a network of proton acceptors.

In this work we measured the pH-dependent stoichiometry of the light-induced proton uptake and release in the quadruple and Y_M mutants. The light-induced pH changes were measured both optically using pH-sensitive dyes and potentiometrically with a pH electrode. The pK_a values and stoichiometry of the protonational changes associated with the tyrosine oxidation are discussed in terms of different possible mechanisms of proton release.

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Abbreviations: P, bacteriochlorophyll dimer; Q_A and Q_B, primary and secondary quinones, respectively

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2. Materials and methods

2.1. Protein isolation and preparation of samples

The construction of the quadruple and Y_M mutant strains has been previously described [13]. Wild-type reaction centers were isolated using wild-type genes expressed in the *R. sphaeroides* ΔLM1 deletion strain [17]. Reaction centers from cultures grown semi-aerobically were prepared as described previously [17], except that the detergent Triton X-100 was used for the ion exchange chromatography instead of lauryldimethylamine oxide. The buffer (15 mM Tris–HCl) and the EDTA were removed by either ion exchange chromatography or a long (24–48 h) dialysis against 0.05% Triton X-100, 100 mM NaCl, pH 8.0. The salt present in the assay solution was always kept at a concentration of 100 mM in order to obtain stable readings of the pH with a glass electrode and also to suppress possible electrochromic effects of the charge-separated states on some dye molecules. Terbutryn was added at a concentration of 100 μM to block interquinone electron transfer. For some measurements, 200 μM ferrocene was added as an external electron donor to P^+ to generate the PQ_A^- state. Absorption of carbon dioxide was prevented by the use of degassed solutions and saturating the gas phase over the samples with nitrogen gas. The concentration of the photochemically active reaction centers was determined by monitoring the absorbance changes at 865 and 450 nm and using the extinction coefficients $\Delta\varepsilon_{865} = 112\ mM^{-1}\ cm^{-1}$ for the P/P^+ transition and $\Delta\varepsilon_{450} = 4.9\ mM^{-1}\ cm^{-1}$ for the Q_A/Q_A^- transition [18,19].

2.2. Proton uptake/release measurements

For optical detection, the absorption changes of pH-sensitive dyes (bromocresol purple, pH 5.6–7.0; *o*-cresol red, pH 7.0–8.5 and *o*-cresol phthalein, pH 8.6–9.6) were measured at approximately 586 nm, where the $PQ_A \rightarrow P^+Q_A^-$ transition has an isobestic point [20]. A Cary 5 spectrophotometer (Varian) was utilized to measure the optical absorbance changes. A magnetic stirrer was mounted under the cell holder for rapid distribution of added buffer or acid.

For the potentiometric detection, pH changes were directly measured with a semi-micro combination glass pH electrode (Corning Ross, 8103) connected to a pH meter (Orion SA 720) [5,6,20]. A differential amplifier of local design was used to amplify (100 times) and electronically filter the signals. Since the steady-state values of the absorption changes were detected, the relatively slow response time (1 s) of the electrode did not limit the measurements.

For both optical and potentiometric measurements, excitation of the samples was achieved with short (24 s) illumination from a tungsten lamp (Oriel) with an interference filter (860 ± 15 nm). The yield of the $Y^{\bullet}_{M164}PQ_A^-$ state is very low, but it is stable once formed, so a measurable amount of this state can be generated with continuous illumination [13]. The light intensity was set such that it resulted in only 30% of charge separation in wild-type reaction centers. The mutants degrade under high light intensity, whereas this relatively low-intensity illumination resulted in fully reversible changes of the optical spectra of the mutants. The net H^+ binding/release was determined at each pH value as the difference of the dye/electrode responses between unbuffered and buffered (10 mM) samples. The following buffers were used: Mes (2-(*N*-morpholino) ethane sulfonic acid) for pH 5.6–6.7, HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′(2-ethane)sulfonic acid) for pH 7.0–8.0, Tris for pH 7.6–8.8, and Ches (2-(*N*-cyclohexylamino) ethane sulfonic acid) for pH 8.6–9.6. To determine the buffering capacity of the entire system, a known amount of strong acid (HCl) was added during extensive stirring of the sample solution.

3. Results and discussion

Both spectrophotometric and potentiometric methods were applied to determine the extent of light-induced proton uptake and release between pH 6 and 10 in highly oxidizing reaction centers. Although the spectrophotometric and potentiometric methods have different sensitivities and kinetic responses over the investigated pH range, the results using these two different techniques agreed well. The use of both methods ensured that the high P/P^+ midpoint potential did not cause irreversible changes in the measuring system, such as oxidation of pH indicator dyes. Distinct differences in the direction and rate of the signal formation and disappearance indicate that the protonational changes in the Y_M mutant are altered compared to the quadruple mutant (Fig. 2A). The observed pH-dependent protonational changes of each mutant are discussed below in terms of the relative contributions of the individual P^+, $Y^{\bullet}_{M164}$, and Q_A^- components.

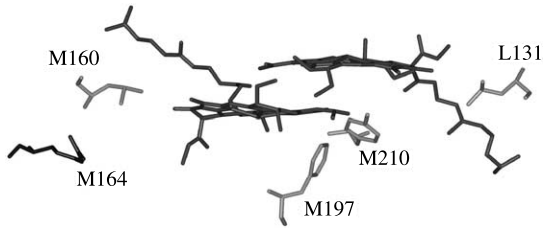

Fig. 1. Three-dimensional structure of the reaction center from *R. sphaeroides* showing P and the amino acid residues altered in the mutants, with the view looking down at the approximately two-fold symmetry axis of the protein. The quadruple mutant has four mutations, Leu L131 to His, Leu M160 to His, Phe M197 to His and Tyr M210 to Trp, that lead to the high oxidation/reduction midpoint potential of the dimer. The imidazole nitrogen atoms of His L131, His M160, and His M197 in the mutants are all within hydrogen bonding distance of P (within 3.5 Å). The phenolic oxygen of Tyr M210 is within 3.5 Å of the conjugated system of P in the wild-type reaction center, but is not hydrogen-bonded to P. The Y_M mutant has the additional fifth mutation of a tyrosine residue substituted at Arg M164. A structural model of the Y_M mutant places the phenolic oxygen of Tyr M164 approximately 10 Å from the nearest conjugated carbon of P [25].

3.1. Proton uptake by the quadruple mutant

The pH dependence of the light-induced proton uptake was measured in both the PQ_A^- and $P^+Q_A^-$ states of the quadruple mutant and was similar to that observed in wild-type reaction centers (Fig. 2B). The proton uptake associated exclusively with formation of Q_A^-, measured by forming the PQ_A^- state in the presence of an external donor to P^+, showed a binding of 0.2–0.4 H^+/Q_A^-. Without an external donor, in the $P^+Q_A^-$ state, the net proton uptake at each pH was lower, 0.1–0.3 H^+/reaction center, than that measured for the PQ_A^- state. The difference between these two measurements, representing the proton release due to the formation of P^+ alone, had a maximum value of $-0.2\ H^+/P^+$ at approximately pH 7 and was almost independent of pH above pH 8 (Fig. 3A). The proton release by P^+ in the quadruple mutant was nearly the same as that from wild type. The results from the wild-type reaction centers agree well with earlier studies performed using reaction centers isolated from the Ga and carotenoidless R-26 strains of *R. sphaeroides* and reaction centers isolated from *Rhodobacter capsulatus* [4,5,26].

The pH-dependent stoichiometry of the observed proton uptake in the quadruple mutant (Fig. 2B) was fitted with the assumption of three hypothetical independent residues following established procedures [4,5,20,21,26]. Three residues represent the minimum number of residues required to explain the pK_a shifts, although the data could be equally well fitted using more residues that each have smaller individual p*K* shifts [5]. This simple modeling describes the data but does not provide identification of the residues participating in the uptake or release of protons. The actual residues involved in the electrostatic interactions most probably interact with each other, as demonstrated by more detailed calculations of elec-

trostatic interactions between residues in proteins [22–24]. In these models, the pK_a of any given residue would depend on the protonation states of all the other residues, and not just on the charge on the dimer and quinone. In principle the data could be fitted with models that require the calculation of every pK_a, but the simple model is sufficient to show that the data can be explained by electrostatic interactions.

The fits from the simple model are also useful for comparison with results from measurements on reaction centers with wild-type amino acid sequences. The pK_a values of the residues obtained from the fits for both the $P^+Q_A^-$ and PQ_A^- states deviated only slightly from those determined in other studies [4,5,20]. The small differences between the current and previous measurements are mainly due to differences in the extent of the investigated pH range, resulting in a fit for three protonatable residues for the current measurements rather than four residues for measurements with a more extensive pH range. The mutants had a lower stability than wild type at extreme pH values, limiting the extension of the pH range. The similarity in the proton uptake by the quadruple mutant and the wild type indicates that the four mutations that increase the midpoint potential do not alter the proton uptake and release, which is thought to be due mainly to surface residues.

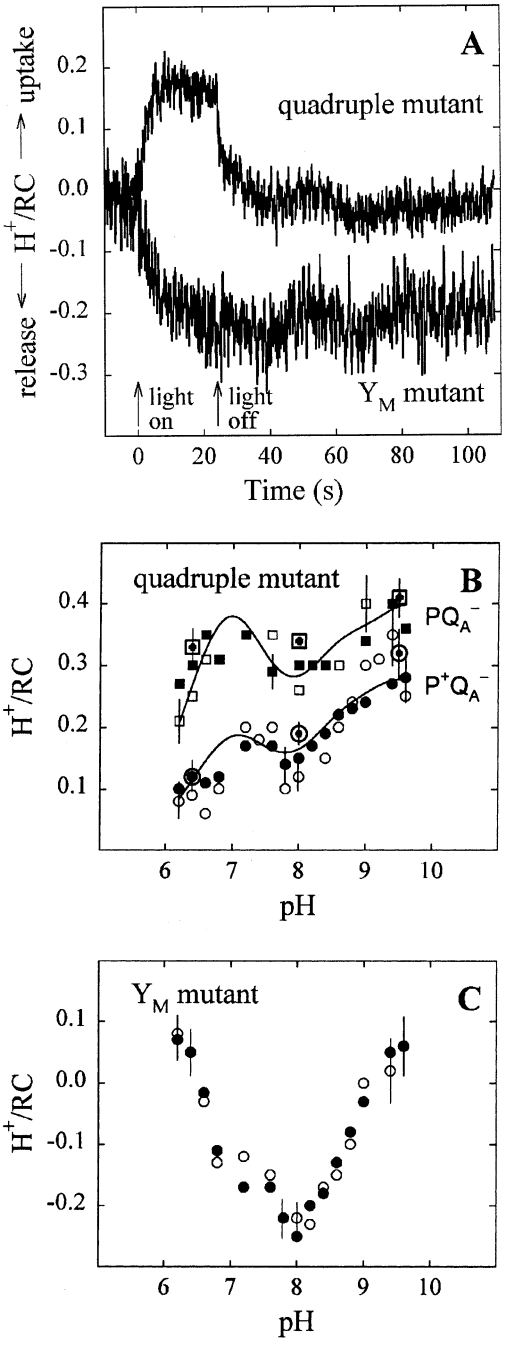

Fig. 2. Representative kinetic traces and pH dependence of the light-induced proton uptake and release by the quadruple and Y_M mutants. A: The kinetic traces represent the optical responses of the pH-sensitive dye (*o*-cresol red) at pH 8.0 in the unbuffered samples and were recorded at 585 and 592 nm for the quadruple and the Y_M mutants, respectively. The buffered signals at the selected wavelengths were zero and therefore are not shown. The pH-dependent stoichiometries were determined by spectrophotometric (open symbols) and potentiometric (closed symbols) assays. B: The proton uptake in the quadruple mutant in P^+QA^- and PQ_A^- states was determined in the absence (circles) and in the presence (squares), respectively, of ferrocene as external electron donor to P^+. Representative data points determined for wild-type reaction centers are shown (large crossed squares and circles). Solid lines represent the fit of the observed proton binding measured with the potentiometric method to the sum of three Henderson–Hasselbalch curves, representing the contribution of the hypothetical protonatable residues. Each residue is assumed to have a pK_a in the ground state that shifts due to the presence of the charge-separated state generated by the light. The $P^+Q_A^-$ state was fitted with three protonatable residues having pK_a values shifted from 6.85 to 7.15, from 8.5 to 8.75, and from 9.5 to 9.9, in the ground state compared to the light-induced charge-separated state. For the PQ_A^- state, the fitted pK_a values in the ground and charge-separated states, respectively, for three protonatable residues were 6.6 and 7.25, 8.4 and 8.8, and 9.5 and 10.1. Errors were calculated at each pH based upon four individual measurements with error bars shown at representative pH values. C: In the Y_M mutant the measurements were done without the addition of an external donor. The protonational changes arise from the reduced quinone and either the oxidized dimer or tyrosyl radical, depending on the pH. Conditions: 0.05% Triton X-100, 100 mM NaCl, 100 μM terbutryn, 40 μM pH indicator dye (in the spectrophotometric method), 200 μM ferrocene (only for panel B, squares), 10 mM buffers. The reaction center concentration for the wild type was 2 μM, and the concentrations for the mutants were 7 and 10 μM in the spectrophotometric and potentiometric methods, respectively.

3.2. Proton release by the Y_M mutant

Formation of the state $Y^{\bullet}_{M164}PQ_A^-$ rather than $P^+Q_A^-$ is expected to lead to a distinctive proton release in the Y_M mutant. Significantly different light-induced protonational changes were evident in the absence of an external donor in the Y_M mutant compared to the quadruple mutant at pH 8.0 (Fig. 2A). Furthermore, the pH dependence of the proton release from the Y_M mutant (Fig. 2C) was dramatically different from that of the quadruple mutant (Fig. 2B). A small proton uptake was detected only below pH 7 and above pH 9. As the pH was increased from 6 to 8, the proton release became larger and reached a maximum value of approximately −0.25 H^+/reaction center at pH 8. Above pH 8, the proton release declined rapidly, and above pH 9 a small proton uptake was measured.

The protonational pattern of this mutant arises from the states $Y_{M164}P^+Q_A^-$ and $Y^{\bullet}_{M164}PQ_A^-$ depending on pH. Under the conditions used in this study, Q_A serves as a one-electron acceptor and Q_B is not present, precluding formation of the

doubly oxidized state $Y^{\bullet}_{M164}P^{+}Q_A^{-}Q_B^{-}$. Both optical and electron paramagnetic resonance spectra show that the signals associated with P^{+} decrease with increasing pH while those associated with the tyrosyl radical $Y^{\bullet}_{M164}$ increase with pH, indicating that the ability of the oxidized donor to transfer an electron from Y_{M164} is pH-dependent, probably because of the protonational state of a nearby residue that can act as a proton acceptor for the phenolic proton of the tyrosine [13,25]. Thus at every pH, Q_A^{-} is present in the samples but the relative amounts of P^{+} and $Y^{\bullet}_{M164}$ are determined by the pK_a associated with tyrosyl formation.

The changes in the proton release are assigned to the presence of the tyrosyl radical in the Y_M mutant, as it is likely that both the proton uptake by Q_A^{-} and the proton release due to P^{+} are the same in the quadruple and Y_M mutants. Electrochemical, ENDOR and FTIR studies demonstrate that the replacement of Arg M164 with other amino acids causes only minor changes in the properties of the dimer [27,28]. The small changes in midpoint potential, spin distribution and vibration energies upon replacement of Arg M164 suggest that this residue has a negligible effect on the local electrostatics of P and therefore most probably does not influence the proton release by P^{+} significantly. The proton uptake by Q_A^{-} in the Y_M mutant was measured at pH 8 in the presence of an external electron donor (ferrocene) and found to be 0.27 H^{+}/Q_A^{-}, identical within the experimental error to the proton uptake of the quadruple mutant and wild type in the PQ_A^{-} state. At low pH values in the absence of an external donor, where the state $Y_{M164}P^{+}Q_A^{-}$ is predominantly formed in the Y_M mutant, the measured protonational change is similar in the Y_M and the quadruple mutants. For example, at pH 6.2, 0.1 H^{+}/reaction center was measured in both mutants (Fig. 2B,C), indicating that the pK_a shifts due to P^{+} are not perturbed by the mutation introducing tyrosine at residue M164.

The largest proton release in the Y_M mutant was at pH 8, where the $Y^{\bullet}_{M164}PQ_A^{-}$ state predominates, according to previous results [13]. The protonational change attributable to Y_{M164} in the Y_M mutant at pH 8 can be ascertained by subtracting the proton uptake that was measured for the $Y_{M164}PQ_A^{-}$ state (0.27 H^{+}/Q_A^{-}) from the proton release measured for the predominantly $Y^{\bullet}_{M164}PQ_A^{-}$ state (−0.25 H^{+}/reaction center), yielding −0.52 $H^{+}/Y^{\bullet}_{M164}$. Thus, formation of the tyrosyl radical results in a significantly larger release of protons than that measured for P^{+}.

To relate the measured proton release from $Y^{\bullet}_{M164}$ to a molecular mechanism, the pH-dependent stoichiometry of the proton release exclusively due to the tyrosyl radical in the Y_M mutant was calculated. The observed protonational changes for the quadruple mutant measured in the $P^{+}Q_A^{-}$ state were subtracted from the data for the Y_M mutant at each pH where both mutants were measured (Fig. 3B). Assuming that the proton uptake by Q_A^{-} is the same in the quadruple and Y_M mutants, this subtraction removes the contribution of proton uptake due to Q_A^{-} in the Y_M mutant at any pH. As discussed above, the relative contributions of the $Y_{M164}P^{+}Q_A^{-}$ and $Y^{\bullet}_{M164}PQ_A^{-}$ states in the Y_M mutant change with pH, so that at low pH the contribution due to P^{+} is removed by this subtraction, but at higher pH values, where a contribution from P^{+} is present in the quadruple mutant but is absent in the Y_M mutant, this subtraction overestimates the contribution of P^{+}. Above pH 7.5, the proton release due to P^{+} in the quadruple mutant was approximately −0.1 H^{+}/P^{+} and was relatively independent of pH (Fig. 3A), so the overestimation in the Y_M mutant probably has only a small effect. Since the protonational changes in the $Y_{M164}P^{+}Q_A^{-}$ and $Y^{\bullet}_{M164}PQ_A^{-}$ states cannot be measured independently, the difference between the Y_M mutant and the $P^{+}Q_A^{-}$ state of the quadruple mutant is a close approximation of the contribution of $Y^{\bullet}_{M164}$ to the observed protonational changes in the Y_M mutant. A more elaborate calculation, in which the relative contributions of the two states determined from previous data [13] are applied to the measured protonational changes as a function of pH, does not change the results significantly (data not shown). The proton release due to the tyrosyl radical was thus identified as increasing with pH to a maximum value at pH 8, and then decreasing at higher pH.

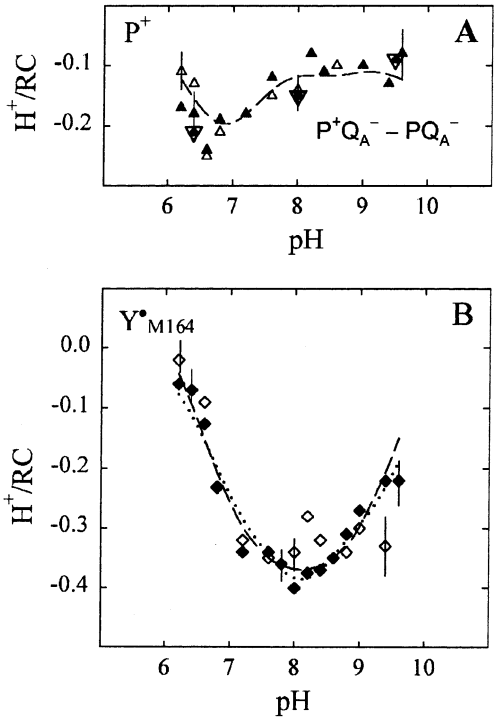

Fig. 3. Calculated proton release by P^{+} in the quadruple mutant (panel A) and by the tyrosyl radical ($Y^{\bullet}_{M164}$) in the Y_M mutant (panel B). The pH-dependent proton release by P^{+} in panel A is represented by the calculated difference (triangles) between the proton uptake measured in the two different states of the quadruple mutant. The dashed line is the difference between the two fits shown in Fig. 2A. For comparison the corresponding data points for wild type are included (large crossed upside-down triangles). Data points in panel B represent the difference between the measured protonational changes in the Y_M mutant and in the quadruple mutant in the absence of an external electron donor using the spectrophotometric (open symbols) and potentiometric (closed symbols) methods. The points were fitted in panel B assuming either an electrostatic (dotted line) or direct (dashed line) proton-releasing mechanism. The electrostatic mechanism used three residues whose pK_a values change upon formation of the tyrosyl radical, with pK_a shifts from 7.3 to 7.0, from 8.4 to 7.9, and from 9.4 to 9.1, in the dark and light, respectively. In the fit assuming direct deprotonation of the phenolic proton via a network of several residues, proton release is controlled by the pK_a values of the proton acceptor and the tyrosine itself. The pK_a values obtained from the fit are 6.6 and 9.5.

3.3. Electrostatic or direct proton release mechanism?

Our results show that protons are released to the bulk solution upon formation of a tyrosyl radical in modified bacte-

rial reaction centers. The extent and pH-dependent pattern of this proton release are significantly different from those associated with the formation of the oxidized dimer (Fig. 3). Protons are also released into the bulk solution upon oxidation of the tyrosine Y_Z that serves as the intermediate electron carrier between the chlorophyll donor and the manganese cluster in photosystem II. The two possible mechanisms that have been suggested to explain the proton release in photosystem II are discussed below in terms of the observed pH dependence of the tyrosyl radical of the Y_M mutant.

First, the observed proton release associated with Y_Z has been proposed to be electrostatic in nature [29–31]. In this mechanism, upon oxidation, the phenolic proton of Y_Z is trapped inside the protein, although it might be transferred away from the primary proton acceptor to further acceptors [31]. This locally trapped charge shifts the pK_a values of remote surface residues, and they release protons to the bulk. Depending on the number of residues and the extent of the pK_a shifts, the proton release can be unity or substoichiometric. This mechanism is similar to that used to describe the release of protons due to P^+ and can be fitted in the same way, with three interacting residues necessary to describe the result (Fig. 3B). As with the P^+ data, a more detailed electrostatic model could be applied, but the fit shown clearly demonstrates that electrostatic interactions can explain the observed protonational changes. The larger proton release in the Y_M mutant compared to the quadruple mutant can be attributed in this model to the displacement of the positive charge from the bacteriochlorophylls of P to one or more proton acceptors near Y_{M164}.

Second, the proton release may be due to a direct deprotonation of the tyrosine with release to the surface via a hydrogen-bonded network [32,33]. By this mechanism, one proton should be released to the solution for each protonated tyrosine that is oxidized. In the Y_M reaction center, the extent of formation of the tyrosyl radical is observed to be pH-dependent [13], so the proton release was fitted to an increasing component to account for the increasing extent of tyrosine oxidation from low to high pH. Additionally, at high pH the tyrosine will become deprotonated in its ground state, precluding the release of the proton, so a decreasing component was incorporated to represent the pK_a of the tyrosine. In this model, our data can be characterized with the sum of two Henderson–Hasselbalch titration curves with pK_a values of 6.6 and 9.5 (Fig. 3B). The lower pK_a is similar to the pK_a of 6.9 associated with the tyrosyl radical formation determined in our earlier studies [13,25]. This pK_a was attributed to the formation of the tyrosyl radical being limited by the ability of the nearby residue His M193 to serve as a proton acceptor. The higher pK_a arising from the decline of the proton release agrees with pK_a values of approximately 10 for tyrosine in solution. An additional overall loss of proton release may be due to loss of some fraction of the tyrosyl radical during illumination [13]. There are indications in artificial systems that tyrosyl radicals are photosensitive and unstable after several hundred flashes [34]. For photosystem II, a wide range of pK_a values has been estimated for the proton acceptor D1 His 190 and Y_Z [15]; however, the pK_a values of 6.6 and 9.5 determined in this study are consistent with estimates from site-directed mutation and chemical rescue studies [35–37].

The pH dependence of our data can be explained equally well with both the electrostatic and direct release models. Based on the lack of electrochromic absorption changes around the dimer band at 865 nm in the Y_M mutant [13,25], it is unlikely that the proton is localized in the vicinity of the dimer. This is consistent with the direct proton release mechanism or with an electrostatic mechanism in which the proton is distributed over several distant, protonatable residues within the protein. Regardless of the mechanism, the comparable proton release behavior of the tyrosyl radical in the Y_M mutant of the reaction center and in photosystem II is consistent with both being largely controlled by proton interactions.

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