7. The non-heme iron complex near quinones

7.1. Fe-complex in bRC

7.1.1. Fe-complex with Glu

The non-heme iron complex (Fe-complex) is situated equidistantly from both Q_A and Q_B (Figure 3-1-1). Two symmetrical pairs of His residues, His-L190/His-M219 and His-L230/His-M266, and one acidic residue Glu-M234 are ligands of the Fe-complex (Figure 7-1-1). The two His residues of the former pair form an H bond with Q_B and Q_A, respectively.

The depletion of the Fe-complex (Fe-depleted bRC) resulted in a dramatic decrease in the rate of ET from H_A^- to Q_A by a factor of at least 15 (Kirmaier et al., 1986) but the rate of the ET from Q_A^- to Q_B ($k_{AB}^{(1)}$) decreases by only a factor of 2 (Debus et al., 1986). Hence, the conformational gating and PT events of kinetic phase 1 are essentially not affected by Fe depletion, and the underlying ET process is still too fast to be rate limiting for kinetic phase 1. Furthermore, in Fe-depleted bRC reconstitution with Fe^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} recovered the rate of the ET (kinetic phase 1) from Q_A^- to Q_B to the essentially the same level of wild type bRC (WT-bRC), implying the lack of a dominant role of Fe^{2+} or other metals in this ET event (Debus et al., 1986).

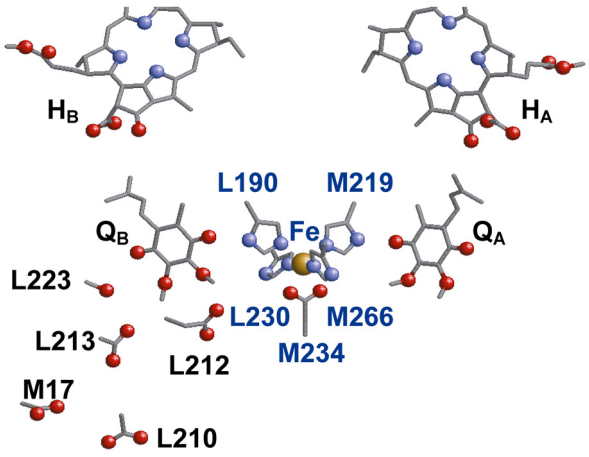

Figure 7-1-1. The Fe-complex in bRC (Stowell et al., 1997).

However, recent FTIR studies of Remy and Gerwert (Remy and Gerwert, 2003) suggested that Q_B is not reduced directly by Q_A^- such that another electron donor (X) should be involved. The electron donor X could be for instance Fe^{2+} or Glu-M234 in the Fe-complex while the partial protonation of $R\text{-}COO^{\delta-}H^{\delta+}$ occurs at Asp-L210 to complete a full protonation of Glu-L212 (Remy and Gerwert, 2003). The direct evidence for the redox activity of the Fe-complex in bRC has not been reported yet. Even if it is redox-active, the oxidized state Fe^{3+} should be only transient as Remy and Gerwert proposed (Remy and Gerwert, 2003). Beijer and Rutherford proposed that the absence of the evidence of the Fe-complex redox-activity in bRC might be explained by its larger inaccessibility of the Fe-complex relative to PSII (Beijer and Rutherford, 1987), being covered with the subunit H.

7.1.2. $E_m(Q_A)$ in Fe-depleted bRC

The calculated $E_m(Q_A)$ in Fe-depleted bRC is significantly lower, by ~210 mV, than that in the WT-bRC (Table 7-1-1). In spectroscopic studies, Fe-depleted bRC showed a

20-fold increase in the life time of the $P^+H_A^-$ state and a corresponding 50% decrease in the yield of the intermediate product state $P^+Q_A^-$ (Kirmaier et al., 1986). For WT-bRC, it was reported that the yield of $P^+Q_A^-$ is essentially 100%. Thus, formation of triplet state was observed specifically in the Fe-depleted bRC (Debus et al., 1986; Kirmaier et al., 1986).

Table 7-1-1. Calculated $E_m(Q_{A/B})$ for WT-bRC and Fe-depleted bRC in mV units.

		$E_m(Q_A)$	$E_m(Q_B)$	ΔE_m
Ser-L223-H•••O=Q_B [a]	Fe-depleted bRC	–386	–205	–181
	WT-bRC	–170	–129	–41
Ser-L223-H•••O-Asp-L213 [b]	Fe-depleted bRC	–378	–302	–76
	WT-bRC	–168	–237	+69

[a] The bRC conformer with an H bond between Ser-L223 and Q_B.
[b] The bRC conformer with an H bond between Ser-L223 and Asp-L213.

The correlation of the $E_m(Q_A)$ level with triplet yield was established in a number of studies in PSII. The down-shift in $E_m(Q_A)$ decreases the E_m difference between H_A and Q_A, reducing the driving-energy of the corresponding ET process and leading to the charge recombination of the $P^+H_A^-$ state and triplet formation in PSII (Rutherford and Krieger-Liszkay, 2001) (Figure 1-1 and 10-1-1). An up-shift of $E_m(Q_A)$ by ~140 mV in PSII is known to be sufficient to minimize the triplet formation (Krieger and Weis, 1992; Krieger et al., 1995).

Fe depletion may down-shift also $E_m(H_A)$. Based on the Fe depleted bRC model the computed down-shift in $E_m(H_A)$ is less than 40 mV. Even if we take this down-shift in $E_m(H_A)$ into account, the E_m difference between H_A and Q_A in the Fe depleted bRC is by 170 mV smaller than that in WT-bRC. This E_m difference in Fe depleted bRC is sufficiently small to enhance triplet yield. Therefore, we conclude that the existence of the Fe-complex in bRC and PSII is necessary for efficient forward ET from H_A^- to Q_A and suppression of triplet formation by up-shifting the $E_m(Q_A)$ with respect to $E_m(H_A)$ to generate a significant energy barrier for the backward ET from Q_A^- to H_A. Under strong illumination, triplet state suppression is particularly important as photoprotection (see also **10.1**).

7.1.3. $E_m(Q_B)$ in Fe-depleted bRC

The calculated $E_m(Q_B)$ in the Fe-depleted bRC is by ~70 mV lower than that in WT-bRC. Together with the down-shift of ~210 mV in $E_m(Q_A)$, this results in an increased driving-energy for the ET from Q_A^- to Q_B by ~140 meV relative to the WT-bRC (Table 7-1-1). The significantly larger ET driving-energy in the Fe-depleted bRC indicates that Fe^{2+} is not necessary to yield a large E_m difference between Q_A and Q_B. In turn, Fe^{2+} constrains the E_m difference to a smaller E_m range in WT-bRC. Based on ET rates for charge recombination between Q_A^-/Q_B^- and P^+, Debus et al. (Debus et al., 1986) estimated an increase of up to 100 meV in ET driving-energy upon depletion of Fe^{2+}, which is essentially consistent with our results.

From the empirical equation of Page *et al*. (Page et al., 1999), the characteristic time for the ET from Q_A^- to Q_B in Fe depleted bRC is estimated to be 2 μs (with reorganization energy $\lambda = 0.85$ eV (Li et al., 2000)), which is sufficiently small relative to 350 μs for kinetic phase 1 in the Fe depleted bRC (Debus et al., 1986), i.e. the rate-limiting step is not the ET but the conformational gating step as in WT-bRC (Graige et al., 1998). From this estimate it can be concluded that the first ET in Fe

depleted bRC is also independent of the ET driving-energy (i.e. E_m difference between Q_A and Q_B).

The question arises why the calculated down-shift in $E_m(Q_A)$ is by ~140 mV larger than that in $E_m(Q_B)$ in spite of the pseudo-C_2 symmetry in the $Q_{A/B}$ positions with respect to the Fe-complex (see Figure 7-1-1). As expected from the structural symmetry, the direct influences of the Fe^{2+} charge in WT-bRC on $E_m(Q_A)$ and $E_m(Q_B)$ that is computed for a fixed protonation pattern are essentially the same, yielding up-shifts of +186 mV and +169 mV for $E_m(Q_A)$ and $E_m(Q_B)$, respectively (Table 7-1-2). In turn, this indicates that changes in protonation pattern of titratable residues in the Fe-depleted bRC are the main factors that increase the E_m difference between Q_A and Q_B with respect to the WT-bRC. Especially, contributions of protonation pattern changes upon Fe depletion to $E_m(Q_B)$ are significant, resulting in an up-shift of 94 mV for $E_m(Q_B)$ (Table 7-1-2). Hence, if the protonation pattern of titratable residues did not change upon Fe depletion, the calculated $E_m(Q_B)$ of –205 mV in Fe-depleted bRC would be 94 mV lower. Indeed, in the Fe depleted bRC, we observed changes in the protonation pattern of His residues. His-L230, His-M219 and His-M266 become protonated by ~0.4 H^+ upon formation of Q_A^- while His-L190, His-L230 and His-M266 protonate by ~0.3-0.5 H^+ upon formation of Q_B^-. In WT-bRC, all four His are ligands to the Fe-complex and therefore not allowed to change their protonation states.

Table 7-1-2. Contributions to $E_m(Q_{A/B})$ for WT-bRC [a] and Fe depleted bRC [a] in mV units.

	$E_m(Q_A)$	$E_m(Q_B)$	ΔG [b]
E_m in Fe-depleted [a]	**–386**	**–205**	**–181**
(influence of protonation shift from native)	(–30)	(+93)	(–123)
E_m in Fe-depleted [a] without protonation change from native	**–356**	**–298**	**–58**
(direct influence of Fe^{2+} in native)	(+186)	(+169)	(+17)
E_m in native [a]	**–170**	**–129**	**–41**

[a] The bRC conformer with an H bond between Ser-L223 and Q_B.
[b] $\Delta G = E_m(Q_A) - E_m(Q_B)$.

Furthermore, in absence of these protonation pattern changes, the E_m difference between Q_A and Q_B is 58 mV, which is almost the same difference as that for the WT-bRC (Table 2). The much larger E_m modulation of Q_B with protonation pattern changes is obviously due to the existence of the cluster of titratable residues in the Q_B side (Figure 4-2-1, 4-2-2 and 5-1-1c). Thus, it is concluded that the computed increase of the E_m difference between Q_A and Q_B, which was also suggested from kinetic studies (Debus et al., 1986), is due to significant contributions of the accompanied protonation pattern changes near Q_B, up-shifting $E_m(Q_B)$.

7.2. Fe-complex in PSII

7.2.1. Fe-complex with bicarbonate

The non-heme iron complex (Fe-complex) is situated equidistantly from both Q_A and Q_B (Figure 7-2-1). Two symmetrical pairs of His residues, D1-His215/D2-His214 and D1-His272/D2-His268, are ligands of the Fe-complex and the two His of the former pair form an H bond with Q_B and Q_A, respectively. In addition, the Fe-complex has one bicarbonate as non-protein ligand. In spite of a large degree of structural similarity between PSII and bacterial photosynthetic reaction centers (bRC) (Michel and Deisenhofer, 1988), the bicarbonate is absent in the latter. Instead, in bRC Glu is ligated

to the Fe-complex (Figure 7-1-1).

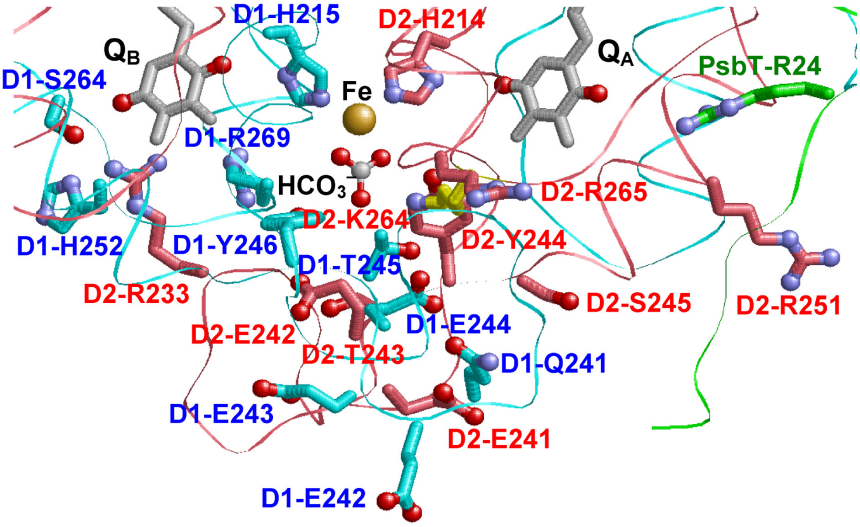

Figure 7-2-1. Residues in the neighborhood of the Fe-complex in PSII. D1, D2 and PsbT are colored in cyan, pink and green, respectively. For clarity, D2-Lys264 is colored in yellow. The two His ligands D1-His272 and D2-His268 above and below the drawing plane are not shown.

7.2.2. D-*de* loop near the Fe-complex in PSII

The specific position of the Fe-complex in the vicinity of the hydrophilic loops that connect the transmembrane helix D with helix *de* (D-*de* loop) parallel to the membrane plane in the D1/D2 proteins is of great interest. In the light-induced degradation process of the D1 protein, the D-*de* loop was proposed to be the first target for cleavage of the D1 protein under strong illumination (Greenberg et al., 1987). The D-*de* loop in PSII is rich in titratable and polar residues. Such a cluster of strongly interacting titratable residues often hinders exact assignment of an apparent pK_a to a specific residue.

The D-*de* loop of the D1/D2 proteins is an insertion specific to PSII that is absent in bRC. Both D1-Glu243 and D1-Glu244 are components of the highly conserved 5 Glu residues (D1-242 to D1-244 and D2-241 to D2-242) in the D-*de* loops (Figure 7-2-1). The QEEET motif between D1-241 and D1-245 has, particularly, physiological importance as being the predominant motive for cleavage of the D1 protein. The exact mechanism invoking D1 degradation is unclear but it becomes necessary under strong illumination with light (Greenberg et al., 1987), which leads to triplet state accumulation at the Chl*a* in D1 resulting ultimately in harmful singlet oxygen.

7.2.3. pH-dependence of E_m(Fe) observed experimentally

With redox titration by ferricyanide, the E_m of the Fe-complex in PSII for one-electron oxidation (E_m(Fe)) was determined to be +400 mV *versus* NHE at pH 7.0 (Bowes et al., 1979; Wraight, 1985). Despite its redox-activity, the Fe-complex is unlikely relevant in the functional ET process in PSII, since E_m(Fe) is too high with respect to $E_m(Q_{A/B})$ (reviewed in Ref. (Nugent, 1996)).

It was also demonstrated in EPR studies (Zimmermann and Rutherford, 1986; Petrouleas and Diner, 1987) that, only after replacement of native Q_B with a high-potential quinone, the Fe-complex can be oxidized by Q_B. Nevertheless, the sensitivity of the Fe-complex on EPR signals enables this complex to serve as a probe for the $Q_{A/B}$ redox state, which elucidated many details of related reactions.

The pH dependence of E_m(Fe) with –60 mV/pH is a strong indication that titratable groups in the neighborhood of the Fe-complex deprotonate upon oxidation of the

Fe-complex (Bowes et al., 1979; Wraight, 1985; Petrouleas and Diner, 1986). The bicarbonate (Figure 7-2-1) might be a factor responsible for this pH-dependence, but FTIR studies suggested that the bicarbonate ligand does not deprotonate upon oxidation of the Fe-complex (Hinerwadel and Berthomieu, 1995). Alternatively, the bicarbonate may influence the pK_a of nearby titratable residues and these induced changes in the protonation pattern of PSII may affect the redox properties of the Fe-complex (Nugent et al., 1988).

7.2.4. Deprotonation of D1-Glu244 upon oxidation of Fe-complex

Upon oxidation of the Fe-complex at pH 7 in the $Q_A^0Q_B^0$ state, we observed deprotonation of 0.7 H^+ for D1-Glu244 and a small amount of protonation of 0.1 H^+ for D1-Glu243 (Ishikita and Knapp, 2005d). Other residues are not significantly affected. Therefore, D1-Glu243 and especially D1-Glu244 are the most important residues for the redox reaction of the Fe-complex in PSII.

Wraight (Wraight, 1985) observed a pH-dependence of E_m(Fe) with a slope of –60 mV/pH and an apparent pK_a value of 8 or 5.3 depending on the Fe-complex redox state Fe^{2+} or Fe^{3+}. The calculated pK_a value for D1-Glu244 is dramatically shifted by varying the Fe-complex redox state, yielding 7.5 in the Fe^{2+} state and 5.5 in the Fe^{3+} state (Ishikita and Knapp, 2005d).

On the other hand, with replacement of the bicarbonate by other small carboxylate anions, Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994) observed changes in E_m(Fe) and its pH-dependence, and interpreted these altered E_m(Fe) properties as a consequence of pK_a shifts of titratable groups by bicarbonate replacement. They also suggested that the same groups were likely responsible for the pH-dependence of E_m(Fe).

It is unlikely that the pK_a of the bicarbonate or reconstituted carboxylate anions determine properties of E_m(Fe) directly, since upon replacement of the bicarbonate with carboxylate anions the measured E_m(Fe) and its pH-dependence exhibited only small correlations with the pK_a of the reconstituted carboxylate anions (Deligiannakis et al., 1994). Even if the reconstituted carboxylate anions have a similar level of pK_a values, their individual molecular structures may require structural rearrangements of nearby titratable side-chains to allow ligation at the Fe-complex. Presumably, the pK_a of these residues are shifted due to these structural rearrangements caused by binding of carboxylate anions. In this context, our computation suggests that D1-Glu244 is likely to be one of those whose pK_a will be significantly affected by bicarbonate replacement with other carboxylate anions, and thus it influences E_m(Fe) (Ishikita and Knapp, 2005d).

7.2.5. Residues responsible for pH dependence of E_m(Fe)

Our computation shows a pH-dependence of –60 mV/pH in the $Q_A^0Q_B^0$ redox state (Ishikita and Knapp, 2005d). To elucidate the origin of this pH-dependence in more detail, in the present study we consider a more simplified system, in which only the D1/D2 proteins are titratable and the other PSII subunits are kept invariant in standard protonation state.

In this model computation, we obtained a pH-dependence of E_m(Fe) with a slope of –50 mV/pH in the pH of 5-9, suggesting that most of the pH-dependence originate from titratable residues in the D1/D2 complex. When the protonation state of D1-Glu244 is constrained (either to ionized or protonated) in the same model system, the pH-dependence of E_m(Fe) becomes considerably smaller exhibiting a slope of only –26 mV/pH. This implies that D1-Glu244 is significantly responsible for the pH-dependence

of E_m(Fe) but is not alone responsible. To suppress the pH-dependence of E_m(Fe) in PSII completely, we had to increase the number of titratable residues whose protonation states are constrained, and finally reached a conclusion that a cluster of titratable residues, not a single residue, is responsible for the pH-dependence of E_m(Fe). These important residues are delocalized around the Fe-complex in the D1/D2 unit. We obtain a relatively small pH-dependence of E_m(Fe) with a slope of –17 mV/pH only when we constrain the protonation states of the following residues whose importance in the PSII protein complex were suggested formerly: five Glu residues (D1-242 to 244 and D2-241 to 242) in the D-*de* loop (Greenberg et al., 1987; Shipton et al., 1989) and six basic residues (D2-Arg233, D2-Arg251 (Cao et al., 1991), D1-His252 (Sigfridsson et al., 2004), D2-Lys264, D2-Arg265 (Diner et al., 1991; Deligiannakis et al., 1994) and D1-Arg269 (Hutchison et al., 1996; Xiong et al., 1997)) (Figure 7-2-1).

7.2.6. Proton network for the Fe-complex

The necessity of several titratable residues in accounting for the pH-dependence of E_m(Fe) are in a line with the conclusion of Berthomieu and Hienerwadel (Berthomieu and Hienerwadel, 2001). They suggested that modeling the changes of E_m(Fe) upon bicarbonate replacement by other carboxylate anions requires a delocalized network of titratable residues (Berthomieu and Hienerwadel, 2001). Indeed, forcing D1-Glu244 to be deprotonated invoked D1-Glu243 to be protonated as a consequence of charge compensation (Ishikita and Knapp, 2005d). Furthermore, simultaneous enforced deprotonation of both D1-Glu243 and D1-Glu244 result in protonation of D2-Glu242 (Ishikita and Knapp, 2005d). These strong interactions among a cluster of Glu residues in the D-*de* loop imply that they serve as proton network and internal proton reservoir for the Fe-complex. This delocalized network of titratable residues could explain the mutant studies of Mäenpää et al. (Mäenpää et al., 1995) where a single mutation of D1-Glu243 to Lys did not alter the phenotype of PSII but modulation of the ET process from Q_A^- to Q_B resulted from the deletion of three Glu (D1-242, D1-243 and D1-244).

7.2.7. Influence of the Q_A/Q_B redox state on E_m(Fe)

We obtained E_m(Fe) = +302 mV for the $Q_A^-Q_B^0$ state and +268 mV for the $Q_A^0Q_B^-$ state at pH 7, while E_m(Fe) = +400 mV for the $Q_A^0Q_B^0$ state. These results suggest that Q_B^- state stabilizes the oxidized state of the Fe-complex more effectively than Q_A^- state. Apparently, the location of Q_A and Q_B is symmetric with respect to the Fe-complex and bicarbonate (Ferreira et al., 2004; Loll et al., 2005). Therefore, the different influence of the two quinones on E_m(Fe) should be attributed to different arrangements of titratable residues at the Q_A/Q_B side or differences in the protonation pattern upon formation of Q_A^-/Q_B^-.

For each redox state $Q_A^0Q_B^0$, $Q_A^-Q_B^0$ and $Q_A^0Q_B^-$, we calculated protonation pattern of titratable residues in the whole PSII complex. The formation of negatively charged Q_A^- result in increased protonation by 0.4 H^+ at D1-Glu244 and by 0.3 H^+ at PsbT-Arg24 (Ishikita and Knapp, 2005d). The latter is a residue outside of the D1/D2 proteins but relatively close to Q_A (O_{QA}-N_{Arg} distance of 6.6 Å) (Figure 7-2-1). Protonation of these two residues remains unaffected by formation of Q_B^- i.e., D1-Glu244 remains deprotonated and PsbT-Arg24 partially deprotonated. On the other hand, D1-His252 becomes more protonated by 0.5 H^+ upon formation of Q_B^- (Ishikita and Knapp, 2005d). This resembles a proton uptake ability of Glu-L212 in bRC from *Rb. sphaeroides* (Rabenstein et al., 2000; Ishikita et al., 2003; Ishikita and Knapp, 2004).

Since D1-Glu244 is the acidic residue that is closest to the bicarbonate (~5 Å) and the Fe-complex (~9 Å from Fe) (Ferreira et al., 2004; Loll et al., 2005), obviously, the

protonation state of D1-Glu244 has a direct impact on the redox behavior of the Fe-complex, and vice versa. According to the crystal structure (Ferreira et al., 2004; Loll et al., 2005), Q_B is via D1-Ser264 involved in an H-bond network with D1-His252 (Figure 7-2-1). This Q_B H-bond network facilitates protonation of the remote, solvent-exposed D1-His252 upon formation of Q_B^- as a consequence of charge compensation. In this case, D1-Glu244 does not have to protonate. The solvent-exposure of D1-His252 and its relatively large distance from the Fe-complex (~14 Å from Fe) diminish its direct electrostatic influence on E_m(Fe).

On the other hand, such an H-bond network extending over ~14 Å is absent in the Q_A side of PSII. Therefore, to compensate the negative charge on Q_A^-, PSII has only the possibility to react with protonation of D1-Glu244. Due to the proximity of D1-Glu244 to the Fe-complex (~9 Å from Fe), protonation at D1-Glu244 up-shifts E_m(Fe) considerably as compared to a protonation of the more distant D1-His252.

7.2.8. Light-induced oxidation of the Fe-complex by exogenous Q_B

Zimmermann and Rutherford (Zimmermann and Rutherford, 1986) found that, after reconstituting high-potential exogenous quinone at the Q_B site (for instance by phenyl-*p*-benzoquinone) light-induced oxidation of the Fe-complex could be observed without support of ferricyanide. Later, Petrouleas and Diner also observed reversible oxidation of the Fe-complex by similar exogenous Q_B (Diner and Petrouleas, 1987; Petrouleas and Diner, 1987). It also has been established that inhibition of ET from Q_A^- to Q_B by DCMU (Figure 7-2-2), a herbicide, results in inhibition of Fe-complex oxidation by ferricyanide (Petrouleas and Diner, 1986). Based on these facts, Petrouleas and Diner proposed the following sequential reactions (Petrouleas and Diner, 1987);

(i) in the first flash, ET from Q_A^- to Q_B occurs in the Fe^{2+} state and the $Q_A{}^0Fe^{2+}Q_B^-$ state forms

$$[Q_A^-Fe^{2+}Q_B] \rightarrow [Q_AFe^{2+}Q_B^-] \qquad \text{(Eq. 7-1)}$$

(ii) ET from Fe^{2+} to Q_B^- coupled to net protonation of Q_B by two H^+ results in $Q_A{}^0Fe^{3+}Q_BH_2$

$$[Q_AFe^{2+}Q_B^-] + 2H^+ \rightarrow [Q_AFe^{3+}Q_BH_2] \qquad \text{(Eq. 7-2)}$$

(iii) in the second flash, ET from Q_A^- to Fe^{3+} completes the $Q_A{}^0Fe^{2+}Q_BH_2$ state.

$$[Q_A^-Fe^{3+}Q_BH_2] \rightarrow [Q_AFe^{2+}Q_BH_2] \qquad \text{(Eq. 7-3)}$$

Indeed, the $E_m(Q^-/QH_2)$ of +573 mV for phenyl-*p*-benzoquinone (Zimmermann and Rutherford, 1986) would be high enough to reduce the oxidized Fe-complex (in step (ii)). However, the value of $E_m(Q^-/QH_2)$ cannot fully explain the efficiency to form $Fe^{3+}Q_BH_2$ with respect to the $E_m(Q^-/QH_2)$ for other exogenous quinones, implying involvement of other factors (Zimmermann and Rutherford, 1986).

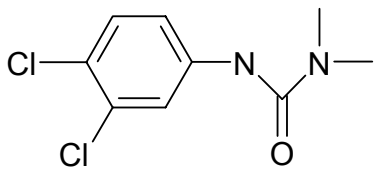

Figure 7-2-2. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea).

7.2.9. Proton release from D1-His252

We consider that, to enable this ET from Fe^{2+} to Q_B (Eqs. 7-1, 7-2 and 7-3), besides a

high-potential quinone, protonation of D1-His252 is probably important to up-shift the $E_m(Q_B)$, making Q_B energetically more suitable as electron acceptor. The lack of such an H-bond network in the Q_A side makes it more difficult for PSII to recruit other distant titratable residues besides D1-Glu244 to stabilize the negatively charged Q_A^-. Here we propose that proton release from D1-His252 is related to the efficiency of the Fe-complex oxidation.

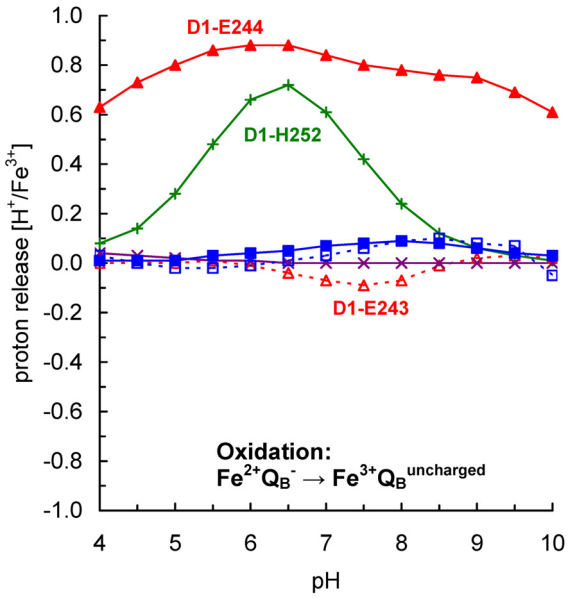

Figure 7-2-3. pH-dependence of PSII proton release upon oxidation of the Fe-complex ($Fe^{2+} \rightarrow Fe^{3+}$). The individual influence from dominant titratable residues is shown. (e) for the transition $Fe^{2+}Q_B^- \rightarrow Fe^{3+}Q_B^{uncharged}$. $Q_B^{uncharged}$ should represent Q_B^0, Q_BH^0 and $Q_BH_2^0$, although the computations were done for the Q_B^0 state only. Curves: D1-Glu243 (dotted line and open △ in red), D1-Glu244 (solid line and closed ▲ in red), D1-His252 (solid line and + in green), D2-Lys264 (solid line and × in purple), D2-Arg265 (dotted line and open □ in blue) and PsbT-Arg265 (solid line and closed ■ in blue).

Upon the $Fe^{2+}Q_B^- \rightarrow Fe^{3+}Q_B^{uncharged}$ reaction, we observe significant proton release from D1-His252 at pH 5-8 (Figure 7-2-3). Note that $Q_B^{uncharged}$ stands for the states Q_B^0, Q_BH^0 and $Q_BH_2^0$. Zimmermann and Rutherford observed that at pH 5.5 light-induced oxidation of the Fe-complex by exogenous Q_B^- was drastically decreased to a yield of 20 % (Zimmermann and Rutherford, 1986). In this connection, Wraight reported the occurrence of an apparent $pK_{Fe(oxidized)}$ at 5.3 and the absence of proton release below pH 5 (Wraight, 1985). Consistent with these experimental findings, we observed in our computations a significant decrease of proton release from D1-His252 below pH 5 (Figure 7-2-3) where D1-His252 is strongly protonated for the reduced and oxidized Fe-complex (Ishikita and Knapp, 2005d). Furthermore, we obtain maximum proton release from D1-His252 at pH 6-7 (Figure 7-2-3). Indeed, Petrouleas and Diner obtained maximum efficiency of the formation of $Fe^{3+}Q_BH_2$ in the same pH range of 6-7 (Petrouleas and Diner, 1987), which is considered as the typical physiological pK_a for His.

Hence, loss of proton release from D1-His252 at low pH may result in inefficient Q_B^- protonation. Neutralization of the negatively charged Q_B^- by proton release from D1-His252 should be coupled to or even be a prerequisite for efficient Fe-complex oxidation. Otherwise, Fe^{2+} should be oxidized to Fe^{3+} simultaneously with electron donation to the negatively charged Q_B^-, which is energetically very unfavorable (i.e. $Fe^{2+}Q_B^- \xrightarrow{unfavorable} Fe^{3+}Q_B^{2-}$).

7.2.10. EPR signals at g = 1.82 and g = 1.9 relate to ability of D1-His252 to deprotonate or not

EPR studies showed that PSII samples in the $Fe^{2+}Q_A^-$ state consist of conformers with g = 1.82 (≈1.84 in ref. (Petrouleas and Diner, 1987)) or g = 1.9 (Rutherford and Zimmermann, 1984). Rutherford and Zimmermann proposed that the two forms of the

$Fe^{2+}Q_A^-$ state (g = 1.82 and g = 1.9 conformers) reflect different protonation states of a titratable residue near the Fe-complex or $Q_{A/B}$, since (i) lowering the pH resulted in population increase of the PSII conformer with g = 1.82 at the expense of that with g = 1.9 (ii) an apparent pK_a of 7-8 obtained from the ratio of the two conformers excluded a direct protonation event of Q_B (Rutherford and Zimmermann, 1984).

Petrouleas and Diner demonstrated that it was more difficult to oxidize the Fe-complex of PSII in the g = 1.82 conformer than in the g = 1.9 conformer (Petrouleas and Diner, 1987). As discussed in **7.2.9**, proton release from D1-His252 could be necessary for efficient $Fe^{3+}Q_BH_2$ formation (Eq. 7-3). At low pH, D1-His252 is not able to release a proton (Figure 7-2-3) due to its persistent protonation for the reduced and oxidized Fe-complex.

Due to a number of remarkable features consistent with experimental results, the protonation behavior of D1-His252 observed in the present study is an indication that the EPR signals at g = 1.82 and g = 1.9 can be attributed to two different D1-His252 populations, the former being capable of releasing a proton and the latter not. Indeed, addition of the phenolic herbicide DINOSEB (Figure 7-2-4) at pH 8.5 resulted in an increased population of the g = 1.82 conformer, indicating that the titratable residue responsible for the g = 1.82 and g = 1.9 conformers should be a key residue associated with ET between Q_A and Q_B (Rutherford and Zimmermann, 1984). Therefore, Rutherford and Zimmermann proposed that this titratable residue is the same residue that is responsible for proton uptake upon formation of the Q_B^- state (Rutherford and Zimmermann, 1984), which is in agreement with our assignment of D1-His252 to the origin of the g = 1.82 and g = 1.9 conformers.

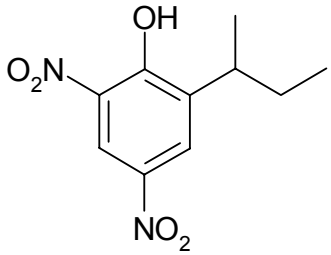

Figure 7-2-4. DINOSEB (2-(1-methylpropyl)-4,6-dinitrophenol).

The involvement of a His residue near Q_B in oxidation of the Fe-complex was also suggested by FTIR studies of Berthomieu and Hienerwadel (Hinerwadel and Berthomieu, 1995; Berthomieu and Hienerwadel, 2001). They observed a stoichiometric one-proton release from a His residue upon oxidation of the Fe-complex by ferricyanide. Although their measurements did not refer to PSII samples reconstituted with high-potential exogenous quinones, we assume that D1-His252 could also contribute to proton release under these conditions as observed in the FTIR studies.

In **7.2.4**, we referred to D1-Glu244 as one of the titratable residues responsible for variation of the pK_a with the Fe-complex redox state, discussed by Wraight (Wraight, 1985) or Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994). However, an uncertainty remains in the interpretation of experimental redox titrations, because the redox change of the Fe-complex is accompanied with that of $Q_{A/B}$ (Zimmermann and Rutherford, 1986; Hallahan et al., 1991; Deligiannakis et al., 1994) (see discussion in previous part). This may include the case where the oxidation of the Fe-complex is coupled to the Q_B redox state discussed here. Due to a number of remarkable features consistent with experimental results, D1-His252 can be the titratable residue.

On the other hand, D1-His252 is obviously more distant from the bicarbonate binding site than D1-Glu244 (Figure 7-2-1). As discussed in the present study, both D1-Glu244 and D1-His252 are in the same network of residues serving as internal proton reservoir. Therefore, we assume that both of the two residues are, more or less, related to the titratable residue discussed by Wraight (Wraight, 1985) or Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994). It might well be that the residue of Wraight (Wraight, 1985) and that of Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994) are identical. Nevertheless, it should again be noted that the titratable residue discussed by Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994) referred to the one whose pK_a was affected by the bicarbonate replacement. This background of their original suggestion can be indicative of a residue, not necessarily but preferentially, is located close to the bicarbonate binding site. At this point, we consider that D1-Glu244 is more likely the titratable residue proposed by Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994), possibly together with other titratable residues in the D-*de* loop.

Conclusion:

The protonation state of D1-Glu244 is significantly coupled to the oxidation state of the Fe-complex. However, **to account for the pH-dependence of the calculated E_m(Fe)**, other residues, **as for instance D1-Glu243, D1-His252, D2-Lys264, D2-Arg265 and PsbT-Arg24, need to be considered**, implying the existence of **a network of residues in the D-*de* loop serving as internal proton reservoir**. With the $Fe^{2+}Q_B^- \rightarrow Fe^{3+}Q_B^{uncharged}$ ET reaction, which was suggested to occur photochemically in PSII after reconstitution of native Q_B with high-potential exogenous quinone, we observed significant proton release from D1-His252. It is likely that **EPR signals for the $Fe^{2+}Q_A^-$ state at g = 1.82 and g = 1.9 can be attributed to two different D1-His252**, the former being capable of releasing a proton and the latter not. Therefore, **the titratable residue discussed by Wraight** (Wraight, 1985) **can be D1-His252**, especially when the oxidation of the Fe-complex is associated with a redox change of Q_B. On the other hand, Deligiannakis et al. (Deligiannakis et al., 1994; Petrouleas et al., 1994) suggested that, instead of the bicarbonate or an other carboxylate anion, **a residue whose pK_a is affected by bicarbonate replacement** should directly influence the redox behavior of the Fe-complex. We propose that **D1-Glu244 could play this role**, possibly together with other titratable residues in the D-*de* loop.