1. Introduction

1.1. Photosynthetic reaction center from purple bacteria (bRC)

The photosynthetic reaction center from purple bacteria (bRC) consists of a heterodimer L/M (subunit L and M) and subunit H. In bRC from *Rhodobacter sphaeroides* (*Rb. sphaeroides*), the heterodimer L/M contains four bacteriochlorophyll *a* (BChl*a*), two bacteriopheophytin *a* (BPheo*a*) and two ubiquinones. A pair of BChl*a* P_L and P_M in the periplasmic side form a strongly coupled dimer, which constitutes the main pigment that absorbs at 870 nm (P870) (see **8.1**). $P_{L/M}$ are ligated by His-L173/ His-M202. Adjacent to $P_{L/M}$, the accessory BChl*a* B_L and B_M are located and ligated by His-L153/ His-M182. P_L and B_L participate in an electron transfer (ET) chain with BPheo*a* H_A and ubiquinone Q_A (A-branch), while the C_2-symmetry related P_M and B_M form another possible ET chain with H_B and Q_B (B-branch). Note that in bRC from *Blastochloris viridis* (*Bl. viridis,* formerly *Rhodopseudomonas viridis*) the corresponding cofactors are BChl*b*, BPheo*b*, a menaquinone at the Q_A binding site and a ubiquinone at the Q_B binding site.

Forward ET in bRC occurs predominantly in the A-branch. Electronic excitation on P870 leads to a charge-separation process, in which with the delocalization of a positive charge mainly on $P_{L/M}$ an electron transfers via B_L, H_A, Q_A to Q_B (Figure 1-1). After the first ET process (see **3.1, 3.2**), Q_B^- is protonated and forms Q_BH, which is stabilized by the second ET and proton transfer (PT) (see **4.1, 4.2**), resulting in the formation of the doubly protonated dihydroquinone Q_BH_2 (reviewed in ref. (Okamura et al., 2000)). Q_BH_2 binds at bRC weakly. Thus, it is released from the Q_B binding site to the quinone pool in the cytoplasm side, and the Q_B binding site is occupied by a new non-protonated Q_B from the bulk solution. Q_BH_2 released from bRC to the quinone pool is re-oxidized to Q_B in the cytochrome bc_1 complex.

The non-heme iron complex (Fe-complex) is situated equidistantly from both Q_A and Q_B (Figure 1-1). The involvement of the Fe-complex in ET is still an open question (see **6.1**) (Remy and Gerwert, 2003; Ishikita and Knapp, 2005f).

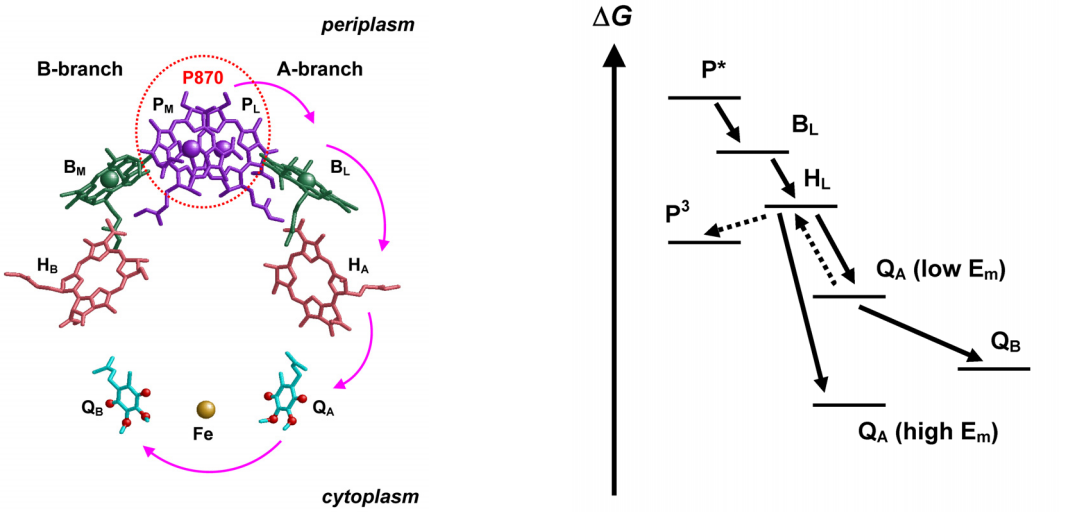

Figure 1-1. Forward ET in bRC. **Left)** Cofactor arrangement (Stowell et al., 1997). **Right)** Free energy level (ΔG) of redox-active cofactors and ET. Forward/backward ET are indicated with solid/dotted arrows. P* and P^3 denote the electronically excited and triplet states, respectively.

1.2. Photosystem II (PSII)

In photosynthetic organisms from cyanobacteria to higher plants, conversion of the solar energy to chemical energy is performed in large protein-pigment complexes located in the thylakoid membrane, Photosystem I (PSI) and Photosystem II (PSII).

The main photosynthetic reaction center of PSII consists of the D1/D2 proteins, which have a large structural similarity with subunits L/M in bRC. The amino acid sequences of the corresponding proteins D1 and D2 in PSII (Zurawski et al., 1982) resemble subunits L and M in bRC, respectively, implying that both PSII and bRC are derived from a common ancestor (Michel and Deisenhofer, 1988; Baymann et al., 2001; Rutherford and Faller, 2003). Therefore, structural details of PSII, especially for the D1 and D2 proteins, had been mainly provided by a series of structural modeling studies (Trebst, 1987; Bowyer et al., 1990; Svensson et al., 1996; Xiong et al., 1996, 1998) based on the crystal structures of the L and M subunits of bRC until PSII crystal structures at higher resolutions (Ferreira et al., 2004; Loll et al., 2005) became available.

However, except for protein subunits D1/D2 and L/M, PSII and bRC differ considerably. For instance, PSII has no protein corresponding to subunit H of bRC that binds at the cytoplasmic side of L/M heterodimer (near $Q_{A/B}$). As a consequence, the arrangement of titratable residues near Q_B in PSII differs drastically from that in bRC. A cluster of titratable residues in the neighborhood of Q_B in bRC, which is suggested to function as proton transfer pathway to Q_B (Okamura et al., 2000), is apparently absent in PSII. Nevertheless, the corresponding proton uptake by Q_B occurs also in PSII.

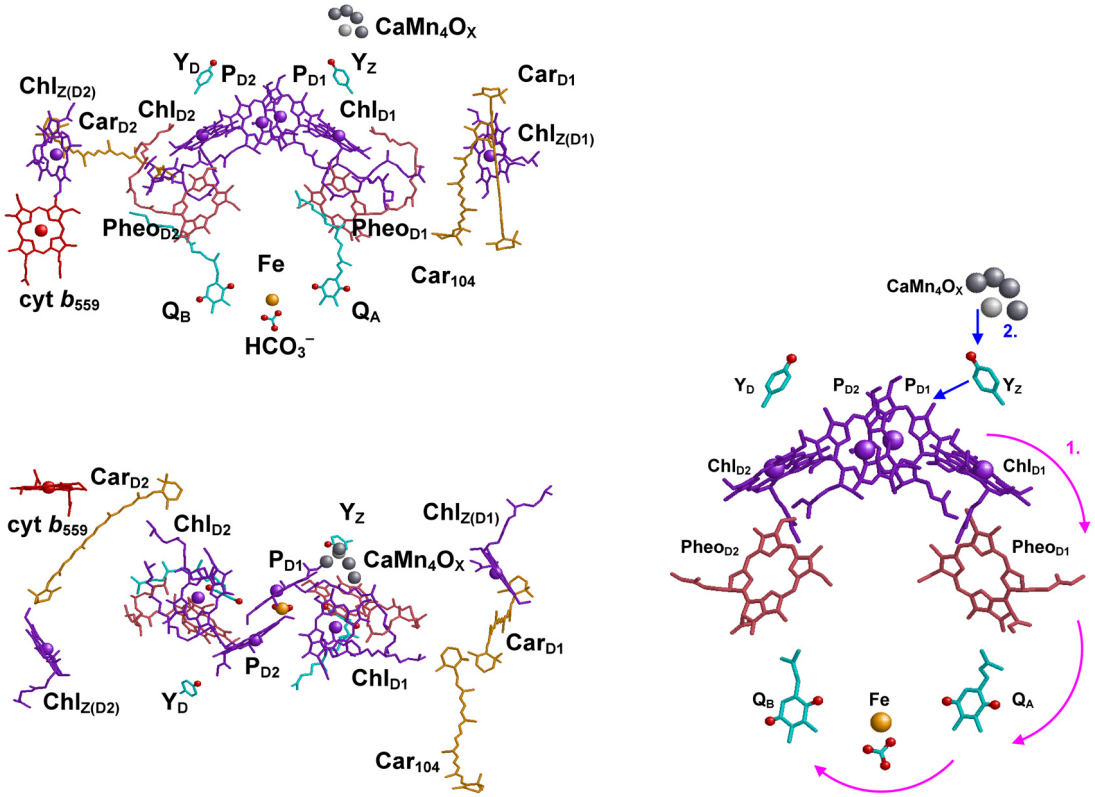

Figure 1-2. Arrangement of cofactors along D1/D2 proteins of PSII (Loll et al., 2005). **Left, upper)** Side view. **Left, bottom)** Top view. **Right)** Forward ET in PSII. **1** and **2** denotes the ET for charge-separated electron and positive charge, respectively.

In the heterodimer D1/D2 proteins, four chlorophyll *a* (Chl*a*), two pheophytin *a* (Pheo*a*) and two plastoquinones form two ET chains (D1-branch and D2-branch). These are a weakly coupled dimer of Chl*a* $P_{D1/D2}$, the accessory Chl*a* $Chl_{D1/D2}$, $Pheo_{D1/D2}$, and

plastoquinones $Q_{A/B}$ in D1/D2-branches. The presence of the O_2-evolving center $CaMn_4O_X$ (Mn-cluster) in the lumenal side of the D1 protein is characteristic of PSII (Figure 1-2).

Electronic excitation in the photosynthetic reaction center of PSII initiates at the pigment P680, which possesses an absorption peak at 680 nm (see **8.3**). Although the identification of P680 is an open question, according to a number of recent studies P680 consist of four Chl*a*, the pseudo-dimer Chl*a* $P_{D1/D2}$ and the accessory Chl*a* $Chl_{D1/D2}$ (Dekker and van Grondelle, 2000; Prokhorenko and Holzwarth, 2000; Barber and Archer, 2001; Barber, 2002; Frese et al., 2003; Barber, 2004). At room temperature the cationic state of $P680^+$ is delocalized mainly on P_{D1}, while Chl_{D1} is likely to function as the initial electron donor to $Pheo_{D1}$. Thus, forward ET in PSII proceeds from P680 via $Pheo_{D1}$, Q_A and Q_B (Figure 1-2, right). As a consequence, Q_B becomes doubly protonated (see **5.2**), forms Q_BH_2 and releases to quinone pool in the stromal side. The re-oxidation of Q_BH_2 to Q_B is performed by the neighboring protein cytochrome b_6f complex in the thylakoid membrane.

As in bRC, the Fe-complex is situated equidistantly from both Q_A and Q_B (Figure 1-2). In contrast to bRC, the redox activity of the Fe-complex in PSII has been observed (e.g. its redox potential is measured to be +400 mV versus normal hydrogen electrode (NHE) (Bowes et al., 1979; Wraight, 1985)), although the mechanism of Fe oxidation mediated by protein residues is still unknown. Nevertheless, the existence of a specific region near the Fe-complex, which is rich in titratable residues, is of interest (see **7.2**).

As a consequence of charge separation, P680 becomes cationic, and is reduced by a proton-coupled ET from the Mn cluster via the redox-active tyrosine D1-Tyr161 (Y_Z) (Figure 1-2, right). The Mn-cluster is initially in the lowest oxidation state S0, and each electron donation from the Mn cluster to $P680^+$ increases the S state by 1 until the S4 state (via S1, S2 and S3 state). When the Mn-cluster reaches to the S4, the highest oxidized state, it decays to the S0 state generating O_2 by oxidation of water. The redox potential (E_m) required for water oxidation is ~+820 mV versus NHE. Therefore, E_m(P680) should be sufficiently high (+1100-1300 mV (Rappaport et al., 2002; Ishikita et al., 2005b; Ishikita et al., 2005c)), and the oxidation of the water on the Mn cluster is ultimately achieved by the high E_m of P680 (see **8.4**).

The Mn-cluster is in the lumenal side of the D1/D2 proteins. In PSII from cyanobacteria, this region is surrounded by other extrinsic protein subunits of PSII, PsbO, PsbU and PsbV. These extrinsic proteins have been suggested to optimize the availability of Ca^{2+} and Cl^- for the Mn-cluster. PsbO is also thought to contribute the structural stability of the Mn-cluster (reviewed in refs. (Seidler, 1996; De Las Rivas et al., 2004)) (see **9.1**).

Oxidation of water to O_2 is accompanied by the production of protons. Although it is an open question how these protons are finally released to the lumenal bulk solution (see **9.1**), the photocycle in PSII evidently results in accumulation of proton in the lumenal bulk solution, lowering the pH. Especially, excessive illumination overactivates the photocycle of PSII. This results in overacidification of the lumen and generation of long-lived $P680^+$, leading to the formation of triplet state. The triplet state of Chl*a* ultimately generates harmful singlet oxygen. To avoid this potential danger, in PSII from higher plants (but not from cyanobacteria) the acidification of the lumen activates the xanthophyll cycle in the *peripheral* antenna complexes of PSII, in which the light-harvesting carotenoide violaxanthin is de-epoxidized to the energy-dissipating carotenoids antheraxanthin or zeaxanthin (reviewed in ref. (Hieber et al., 2000)).

D1/D2 proteins are surrounded by the light-harvesting *core* antenna complexes CP43/CP47. These subunits of PSII transfer energy to P680 in the D1/D2 proteins, and

facilitate charge separation in the photosynthetic reaction center. In these antenna complexes there exist several β-carotene (Car) molecules. These Car are likely to play a photoprotective role in PSII. The existence of two specific Car Car_{D1} (see **10.3**) and Car_{D2} (see **10.2**) in the interface with D1/D2 protein were suggested from a number of spectroscopic studies. Car_{D2}, already present in former crystal structures (Zouni et al., 2001; Kamiya and Shen, 2003; Biesiadka et al., 2004; Ferreira et al., 2004), has been suggested to participate in electron hole transfer pathway (secondary ET pathway) from $P680^+$ to the stromal subunit cytochrome b_{559} (Hanley et al., 1999; Faller et al., 2001; Tracewell and Brudvig, 2003; Ishikita and Knapp, 2005a). The importance of this pathway is pronounced in case the ET from the Mn cluster to $P680^+$ is inactive (see **10.2**). The position of Car_{D1} near D1/CP43 proteins was recently confirmed by the crystal structure of PSII at 3.0 Å resolution (Loll et al., 2005) (see **10.3**).

1.3. Photosystem I (PSI)

PSI belongs to the same superfamily of photosynthetic reaction centers as PSII, i.e. PSI seems to share a common ancestor with PSII. However, PSI has a structural similarity with photosynthetic reaction center from green bacteria rather than PSII or purple bacterial bRC such as. *Rb. sphaeroides* and *Bl. viridis* (Nugent, 1996; Rutherford and Faller, 2003). In this regard, PSII and purple bacterial bRC belong to the *Type II* reaction center, while PSI and green bacterial bRC belong to the *Type I* reaction center.

PSI has six Chl*a* ($P_{A/B}$, $A_{-1A/B}$ and $A_{0A/B}$), two phylloquinones ($A_{1A/B}$) and one Fe_4S_4 center F_X in the heterodimer PsaA/PsaB proteins (A-branch and B-branch), and another two Fe_4S_4 centers ($F_{A/B}$) in the PsaC protein (Figure 1-3). $P_{A/B}$, a hetero dimer of a Chl*a* and an epimer Chl*a*' (Watanabe et al., 1985; Jordan et al., 2001) is the main component of pigment P700, which absorbs at 700 nm. The accessory Chl*a* A_{-1} is also likely to be involved in ET as well as the A_0 Chl*a* (Brettel, 1997).

The phylloquinone A_1 accepts an electron from A_0 (Figure 1-3). Its E_m is significantly low relative to $Q_{A/B}$ in bRC and PSII (see **6.3**). From kinetic studies of the ET from A_1 to F_X, it was suggested that the ET is biphasic, implying that ET from A_1 to F_X occurs in both A and B-branches (Figure 1-3, see **6.1**). Clear evidence for the presence of ET via A_{-1B} and A_{0B} to A_{1B} is not yet available. Unlike $Q_{A/B}$ in bRC and PSII, $A_{1A/B}$ is never protonated and remains in the binding site. ET preceeds from F_X via F_A and F_B to the feredoxin that binds at the stromal subunit PsaD (Brettel, 1997). The photooxidized $P700^+$ is re-reduced by ET from cytochrome c_6 or plastocyanin (Molina-Heredia et al., 2003).

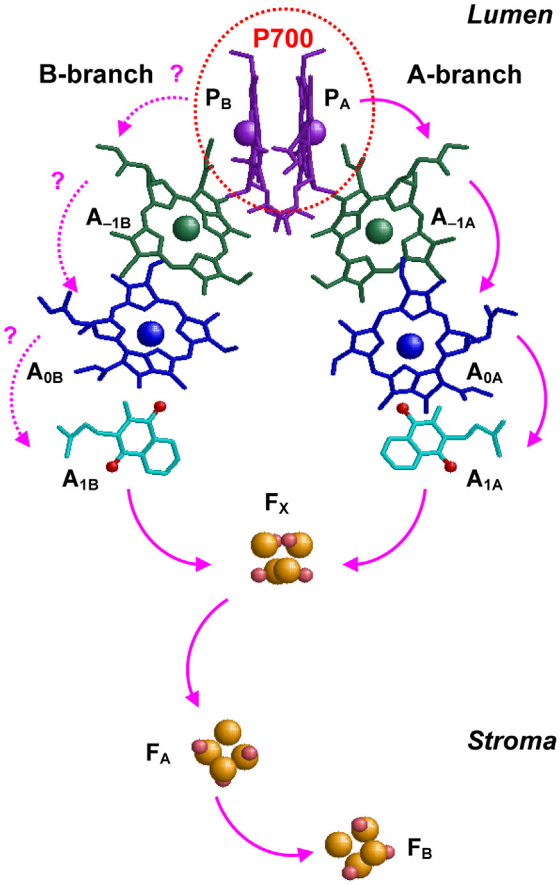

Figure 1-3. Forward ET in PSI (Jordan et al., 2001). The presence of ET to A_{1B} in the B-branch is not yet clear. These processes are indicated with dotted arrow.