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Species dependent differences in the secondary electron-donation reactions of photosystem II.

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Introduction

The photochemical reactions of photosystem II (PS II) leading to the oxidation of water involve the primary electron donors Mn_4 and Y_Z (see solid arrows in Figure 1). PS II is unique among photosynthetic reaction centers in having secondary electron donors that compete with the primary electron donors for reduction of P_{680}^+ (see dashed arrows in Figure 1). These secondary electron donors include two accessory chlorophylls, two β-carotene molecules, cytochrome b_{559} and tyrosine D (Y_D). The two redox-active accessory chlorophylls in the reaction center are bound to D1-His118 and D2-His117 and they are referred to as chlorophyll Z (Chl_Z) and chlorophyll D (Chl_D), respectively (Stewart et al., 1998). There are two β-carotene molecules bound to the D1/D2 reaction center complex. These are depicted in Figure 1 in symmetric positions on either side of P_{680}, although it is not yet clear where these β-carotene molecules are located or whether only one or both are redox-active (for review, see (Tracewell et al., 2001b)).

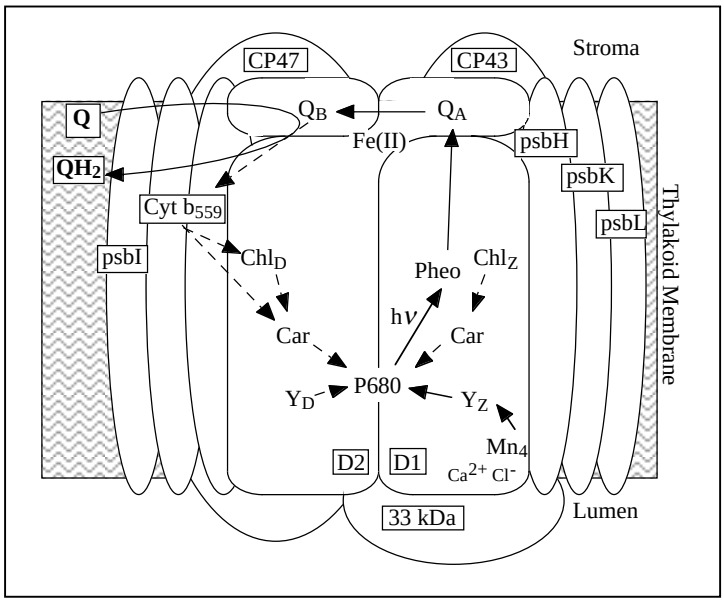

Figure 1. Structural model of photosystem II. The primary electron-transfer pathway involving the photoactive chlorophyll a species P_{680}, tyrosine Z (Y_Z) and the tetramanganese cluster (Mn_4) is shown in solid arrows. The secondary electron-transfer pathways involving β-carotene (Car), the accessory chlorophyll a's (Chl_Z in the D1 subunit and Chl_D in the D2 subunit) and cytochrome b_{559} (Cyt b_{559}) are shown in dashed arrows.

Under physiological conditions, the rates of electron donation to P_{680}^+ by the secondary electron donors are much slower than the rate of electron donation to P_{680}^+ by Y_Z. As a result, the initial photochemical reaction produces a charge separation in which one of the primary electron donors is oxidized. Subsequently, the secondary electron donors can become oxidized via redox equilibration of the hole among the various donors during the lifetime of the charge-separated state (Buser et al., 1990;

Buser et al., 1992). On the other hand, the secondary electron donors can be photooxidized in high yield under conditions when electron transfer from the primary donors is blocked, such as occurs at cryogenic temperatures. It has been proposed that the secondary electron-transfer pathway involving Cyt b_{559} may function in photoprotection of PS II (Stewart and Brudvig, 1998). Recent studies of Cyt b_{559} mutants that lack the heme support this hypothesis (Morais et al., 2001).

In this paper, we review the results of recent studies of the chlorophyll and β-carotene radical cations that are formed photochemically in PS II at cryogenic temperatures. A comparison of these reactions in spinach and cyanobacterial PS II reveals that the type and quantity of chlorophyll radical cations formed at cryogenic temperatures vary among photosynthetic species (Tracewell et al., 2001a). These differences are interesting in light of the proposed role of the secondary electron donation pathway in photoprotection of PS II because plant and cyanobacterial PS II have different photoprotective mechanisms. We consider the emerging structural data on plant and cyanobacterial PS II and how differences in the organization of the antennae proteins may relate to differences in the secondary electron-donation reactions.

Photooxidation of chlorophylls and carotenoids at cryogenic temperatures

In *Synechocystis* PS II core complexes, one type of chlorophyll radical cation is formed which absorbs near-IR light at 820 nm (Stewart et al., 1998) (Figure 2B). However, two types of chlorophyll radical cation are formed in spinach PS II (Figure 2A). The same 820 nm feature seen in *Synechocystis* PS II core complexes is also observed in spinach PS II membranes. In addition, a second chlorophyll cation radical is formed in spinach PS II which has an absorbance maximum at 850 nm (Hanley et al., 1999; Tracewell et al., 2001a). Hanley *et al.* (1999) assigned both absorbance features observed in spinach PS II to a single Chl^+ species, Chl_Z^+. We observed that the 850 nm peak increases and the 820 nm peak decreases when the temperature of the sample is increased (Tracewell et al., 2001a). This experiment

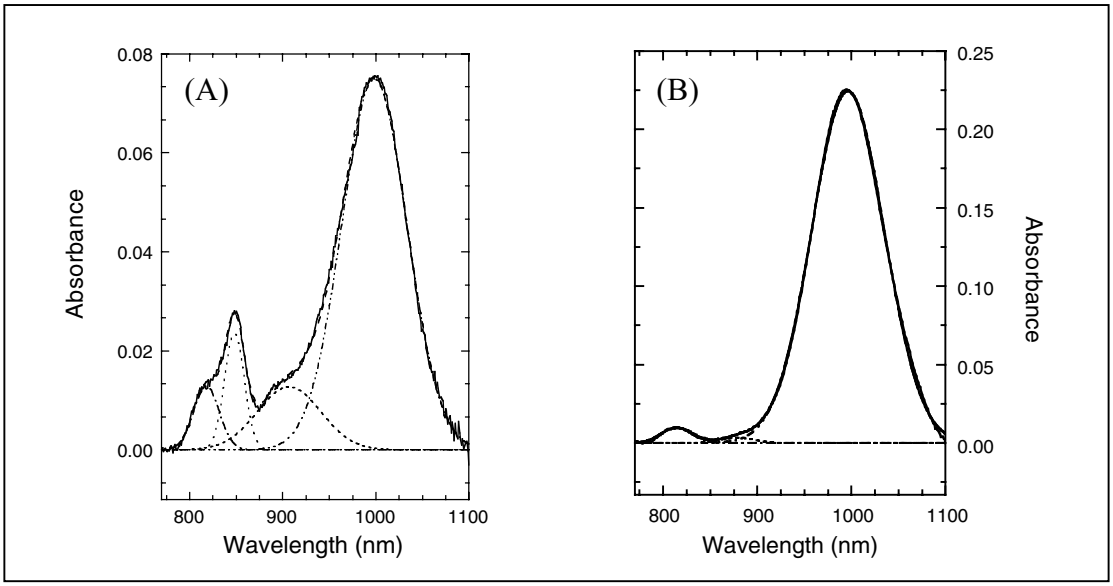

Figure 2. Near IR spectra of spinach PS II membranes (A), and *Synechocystis* PS II core complexes (B) following illumination with a 200 W quartz halogen lamp for 15 minutes at 120 K (Tracewell et al., 2001). The spectra were deconvoluted using four and three gaussian curves, respectively.

demonstrated that the 850 nm and 820 nm features arise from independent species. Based on the locations of these Chl's in the reaction center core complex and mutagenesis data from *Synechocystis* PS II (Stewart et al., 1998), we assigned the 820 and 850 nm absorbing chlorophyll cation radicals to Chl_Z^+ and Chl_D^+, respectively (Tracewell et al., 2001a).

Carotenoid radical cations are formed in both spinach and *Synechocystis* PS II and the properties of the carotenoid radicals are quite similar in the two systems. The Car^+ exhibits an absorption maximum at 994 nm in PS II at 120 K. An additional peak near 890 nm, with an intensity that tracks that of the 994 nm peak, is attributed to a vibronic band of Car^+. This feature is observed in both spinach PS II and *Synechocystis* PS II, although it is much less pronounced for *Synechocystis* samples at an illumination temperature of 120 K, as shown in Figure 2.

Comparison of transmembrane α-helices in spinach and cyanobacterial PS II

The differences between the types of chlorophyll radical cations formed in spinach and cyanobacterial PS II may be related to differences in the organization of transmembrane α-helices in the PS II reaction centers. The structure of PS II has been investigated in both plant and cyanobacterial complexes: spinach (Rhee et al., 1998; Nield et al., 2000b) and *Synechococcus* (Zouni et al., 2001). Plant PS II contains the LHC II subunits and several additional light-harvesting proteins, CP29 and CP26, which are absent from cyanobacterial PS II. The S subunit, which is unique to plants, appears to play a significant role in non-photochemical quenching of chlorophyll fluorescence, but it is not found in the LHC II - PS II supercomplex (Nield et al., 2000a). The positions of the transmembrane α-helices have been identified within samples of the LHC II - PS II supercomplex from spinach by using electron microscopy (see Figure 3). Some of the helices have been assigned to specific transmembrane polypeptides by using a combination of HPLC and mass spectrometry of membrane fractions (Zheleva et al., 1998) and by cross-linking experiments (Harrer et al., 1998).

The black-shaded cylinders in Figure 3 represent transmembrane α-helices that may contact the accessory chlorophylls, Chl_Z and Chl_D, in the PS II reaction center. Based on mutagenesis studies, it was concluded that Chl_Z and Chl_D are bound to D1-His118 and D2-His117, respectively (Stewart et al., 1998). These residues are located in the B helices of the reaction center polypeptides. The crystal structures of spinach and *Synechococcus* PS II confirmed that Chl_Z and Chl_D are located next to each of the B helices in the D1 and D2 subunits (Rhee et al., 1998; Zouni et al., 2001). It is interesting that the small subunits that bind to the PS II reaction center next to the B helices of the D1 and D2 subunits appear to be somewhat different in the plant PS II complex compared to the cyanobacterial PS II complex. In the *Synechococcus* PS II structure, Chl_Z is sandwiched between helix B of D1 and an unidentified transmembrane α-helix, while Chl_D is sandwiched between helix B of D2 and two other transmembrane α-helices, one of which is assigned to be the PsbI subunit (see model in Figure 3B). However, in the structure of the spinach LHC II - PS II supercomplex, there are two α-helices near the B helix of the D1 subunit and three α-helices located near the B helix of D2 (see model in Figure 3A). Neither of the two α-helices near the B helix of D1 in the spinach structure have the same tilt in the

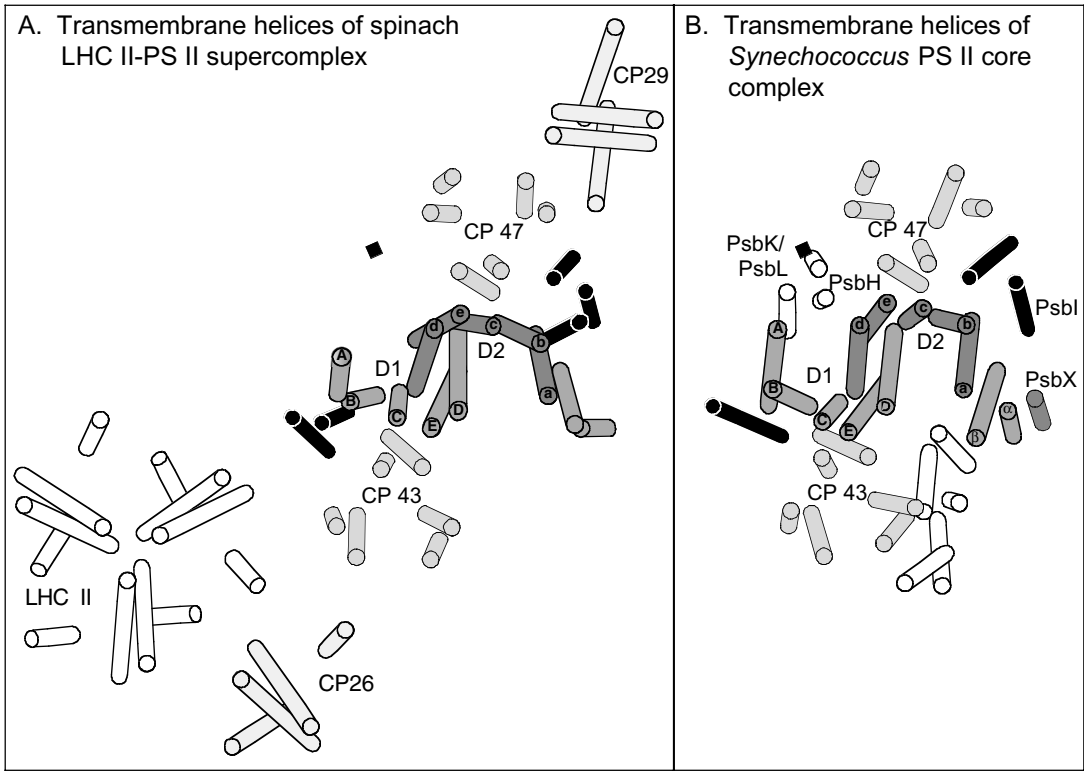

Figure 3. Arrangement of transmembrane polypeptides in photosystem II. The spinach model of the LHC II - PS II supercomplex is based on the helical organization of subunits observed by electron crystallography (Nield et al., 2000a and 2000b). The model of the PS II core complex helical organization is based on the X-ray crystal structure of *Synechococcus* PS II at 3.8 Å resolution (Zouni et al., 2001).

membrane as the unidentified helix in the cyanobacteria structure. Similarly, the α-helix closest to the B helix of D2 does not have the same orientation in the two PS II structures. Electron crystallography of spinach PS II at 6 Å resolution has been presented recently (Rhee, 2001) and the X-ray derived structure from *Synechococcus* is at 3.8 Å resolution. The variations in α-helix positions may indicate differences in the Chl_Z-protein and Chl_D-protein contacts between the two species. Differences in the amino acids of these polypeptides that are adjacent to the accessory chlorophylls are expected to affect the spectroscopic properties of the chlorophyll cation radicals. In addition, variations in the positions of charged or polar residues or in the hydrogen bonding between the chlorophylls and their surrounding amino acids will modulate the redox potentials of the chlorophyll cations. Such variations in the chlorophyll-amino acid contacts could account for the species dependent differences in the secondary electron-donation reactions of PS II.

Model for variations in Car^+/ Chl_Z^+ / Chl_D^+ reduction potentials and locations of hole due to protein-protein interactions

As shown in Figure 2, illumination of PS II complexes at cryogenic temperatures yields a charge separation in which both chlorophyll and carotenoid cation radicals are formed. Because PS II is limited to a single stable charge separation at low temperature, this result indicates that there are some PS II centers in which a

chlorophyll serves as the ultimate electron donor and others in which a carotenoid does. Moreover, the different yields of chlorophyll versus carotenoid cation radicals in spinach and cyanobacterial PS II indicates that the distribution of these PS II centers is different in the two types of PS II complexes. Based on the observation of non-exponential recombination kinetics, we concluded that an ensemble of PS II conformers is trapped as the protein complex is frozen (Tracewell et al., 2001a). In addition, experiments in which the sample was re-illuminated after the $Car^+ Q_A^-$ charge separation was allowed to recombine at low temperature showed that PS II centers in which Car^+ was formed initially only re-form Car^+. These results are consistent with an ensemble of PS II conformers in which the reduction potentials for Car^+ and Chl^+ varies among the reaction centers within the sample. We represent these variations in reduction potentials as a range of values in Figure 4 relative to the reduction potential of P_{680}^+ (~1.2V).

The range of reduction potentials for each chromophore in the reaction center is not known and is represented here by bars. The bars are placed relative to each other according to the steady-state yields of each cation in spinach and *Synechocystis* PS II. A lower reduction potential translates to a higher yield of cation formed by steady-state illumination. Thus, the higher yield of chlorophyll cation radicals in spinach PS II relative to *Synechocystis* PS II is explained by a distribution of the reduction potentials for Chl^+ that is shifted in the spinach PS II complex towards lower values relative to those for Car^+. As discussed above, we attribute this shift in reduction potentials for the chlorophyll cations between the plant and cyanobacterial PS II complexes to be due to differences in the chlorophyll-protein contacts in the two types of systems (Figure 3).

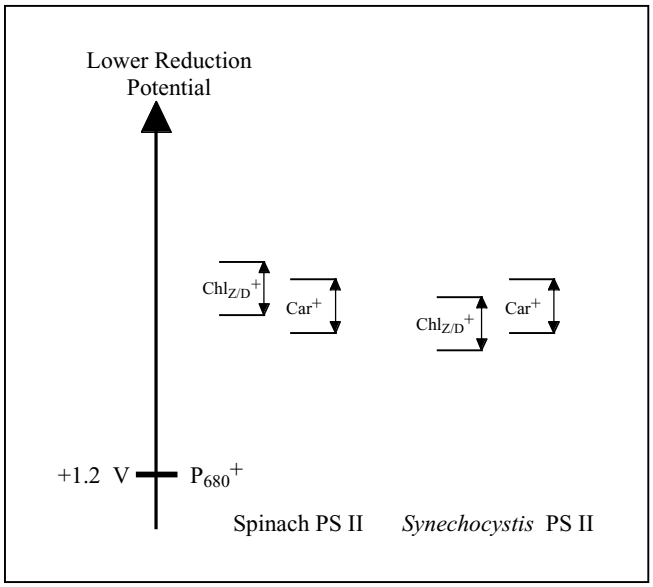

Figure 4. Model for the relative reduction potentials for Car^+ and Chl^+ in photosystem II.

Photoprotection of the photosystem II reaction center

It is known that Chl_Z^+ is a potent fluorescence quencher (Schweitzer et al., 1998). Based on this observation, it has been proposed that Chl_Z^+ formation could protect PS II from light damage by switching off energy flow into the reaction center via quenching of chlorophyll excited states (Stewart and Brudvig, 1998). In this regard, it is interesting that the plant and cyanobacterial systems have different photoprotection mechanisms. In higher plants, it is known that the xanthophyll cycle plays an important role in photoprotection (Niyogi, 1999), but this cycle is absent in cyanobacteria. The observation of differences in the secondary electron-donation reactions involving Car, Chl_Z and Chl_D between plant and cyanobacterial PS II (Tracewell et al., 2001a) may correlate with differences in the photoprotection mechanisms in these two systems. It is notable that the structural differences between

the plant and cyanobacterial PS II complexes that we have highlighted in black in Figure 3 and have discussed above are located at the points where the LHC II and LHC II-related antennae proteins connect to the PS II reaction center.

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