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Accelerated Publications

Cytochrome *b*-559 May Function To Protect Photosystem II from Photoinhibition†

Lynmarie K. Thompson and Gary W. Brudvig*
Department of Chemistry, Yale University, New Haven, Connecticut 06511
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ABSTRACT: Although cytochrome *b*-559 is an integral component of the photosystem II complex (PSII), its function is unknown. Because cytochrome *b*-559 has been shown to be both photooxidized and photoreduced in PSII, one of several proposals is that it mediates cyclic electron transfer around PSII, possibly as a protective mechanism. We have used electron paramagnetic resonance spectroscopy to investigate the pathway of photooxidation of cytochrome *b*-559 in PSII and have shown that it proceeds via photooxidation of chlorophyll. We propose that this photooxidation of chlorophyll is the first step in the photoinhibition of PSII. The unique susceptibility of PSII to photoinhibition is probably due to the fact that it is the only reaction center in photosynthesis which generates an oxidant with a reduction potential high enough to oxidize chlorophyll. We propose that the function of cytochrome *b*-559 is to mediate cyclic electron transfer to rereduce photooxidized chlorophyll and protect PSII from photoinhibition. We also suggest that the chlorophyll(s) which are susceptible to photooxidation are analogous to the monomer chlorophylls found in the bacterial photosynthetic reaction center complex.

Naturally occurring intensities of visible light have been shown to cause inhibition of photosynthesis in vivo [reviewed by Powles (1984)]. When a photosynthetic organism is exposed to higher light levels than those to which it is adapted, or to some stress such as lack of water or carbon dioxide, it cannot catalyze electron transport fast enough to utilize all of the excitation energy it absorbs. The excess energy can cause reactions that damage the pigments (called photooxidation) or the photosystems (called photoinhibition). The most susceptible component of the photosynthetic apparatus to damage by light is photosystem II (PSII).[1] Photosynthetic organisms have several mechanisms for coping with excess excitation energy: changes in antenna size are used to limit or redistribute energy to prevent photoinhibition damage, and carotenoid pigments quench triplet chlorophyll, which sensitizes the formation of singlet oxygen, to prevent photooxidation damage (Powles, 1984). In this paper, we present an explanation for the unique susceptibility of PSII to photoinhibition and propose a mechanism that may be used in PSII for protection from this process.

The main electron transport chain in PSII is shown schematically in the box in Figure 1. Photoinhibition of PSII is thought to damage some component other than the oxygen-evolving complex, because artificial electron donors (which provide a source of electrons when the oxygen-evolving complex has been inactivated) do not restore electron-transfer activity (Powles, 1984). There is some controversy over the site that is damaged during photoinhibition. It has been suggested that the primary damage occurs at the Q_B site via reactions of oxygen radicals (Kyle et al., 1984; Kyle, 1987). This is based on evidence that photoinhibition decreases the binding of inhibitors to the Q_B site and decreases Q_B-dependent electron transfer. However, some laboratories have obtained conflicting results (Tytler et al., 1984; Callahan et al., 1986; Arntz & Trebst, 1986). One problem is that the mechanism of photoinhibition may vary with conditions such as anaerobicity (Krause et al., 1985), light intensity, and the intactness of the preparation. An alternate view is that photoinhibition damages some component involved in the primary charge separation; photoinhibition has been shown to inhibit photoreduction of Q_A (Cleland et al., 1986) and pheophytin (Demeter et al., 1987). Susceptibility to photoinhibition has also been correlated with conditions that prolong the lifetimes of $P680^+$ and

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[1] Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; Chl, chlorophyll; cyt b_{559}, cytochrome *b*-559; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; MES, 2-(*N*-morpholino)ethanesulfonic acid; Pheo, pheophytin; PQ, plastoquinone; PSII, photosystem II; P680, primary electron donor in PSII; P870, primary electron donor in reaction centers from *Rhodobacter sphaeroides*; Q_A and Q_B, primary and secondary quinone electron acceptors in PSII and the reaction center of purple non-sulfur bacteria; Z, secondary electron donor in PSII.

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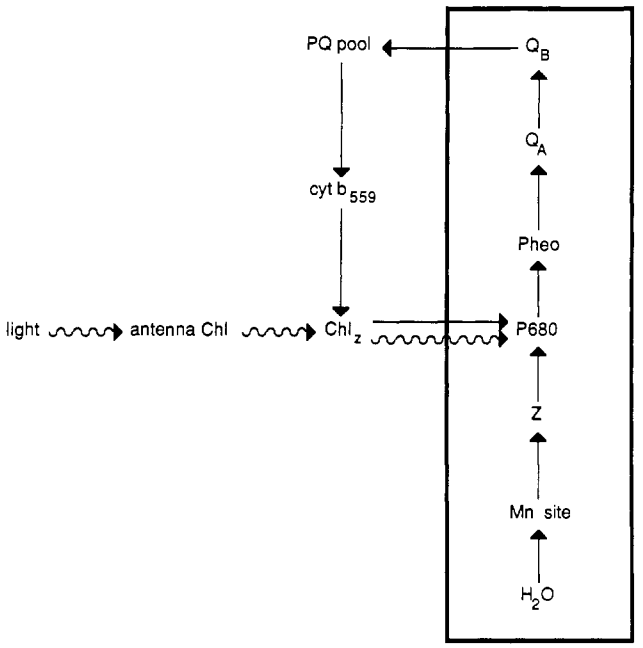


FIGURE 1: Pathways of energy and electron transfer in PSII and proposed model for the mechanism of photoinhibition and the function of cyt b_{559}. The main electron transport chain is shown in the box (solid arrows indicate electron transfer; wavy arrows indicate energy transfer); upon absorption of a photon of light, P680 (probably a Chl dimer) transfers an electron to a pheophytin, which then transfers the electron to Q_A (a bound plastoquinone) and finally to a second plastoquinone, which binds at the Q_B site. Meanwhile, $P680^+$ oxidizes Z (tyrosine), which in turn oxidizes a Mn cluster (the catalytic site of the oxygen-evolving complex), which ultimately oxidizes water. We propose that Chl_z facilitates energy transfer from the antenna to P680 but is then also susceptible to oxidation by $P680^+$; cyt b_{559} mediates cyclic electron transfer around PSII to maintain Chl_z reduced and protect PSII against photoinhibition.

Z^+ (Theg et al., 1986; Callahan et al., 1986). For example, the rate of photoinhibition is increased by inhibition of the oxygen-evolving complex (Theg et al., 1986; Callahan et al., 1986), which slows rereduction of $P680^+$. In contrast, photoinhibition is decreased by conditions that maintain P680 reduced, such as the presence of the inhibitor DCMU (Kyle et al., 1984), which binds at the Q_B site and causes a rapid charge recombination reaction between $P680^+$ and Q_A^- or artificial electron donors (Callahan et al., 1986) which rereduce $P680^+$. Therefore, photoinhibition is thought to result from damage to the oxidizing side of the reaction center, such as a second oxidation of $P680^+$ (Theg et al., 1986; Cleland, et al., 1987) or Z^+ (Callahan et al., 1986).

The sensitivity of PSII to photoinhibition is probably due to its unique role in oxidizing water, which requires that it generate the most powerful oxidant in photosynthesis, $P680^+$. This is illustrated in Figure 2, which shows that the reduction potential of $P680/P680^+$, estimated at 1.17 V (Klimov et al., 1980), is much greater than the reduction potentials of the primary electron donors of photosystem I and photosynthetic bacteria (0.25–0.5 V). The reduction potential of chlorophyll (Chl/Chl^+) is greater than that generated in all the reaction centers except for PSII. Therefore, PSII is the only reaction center capable of oxidizing its antenna Chl. Photosynthetic complexes must contain some antenna Chl(s) that are close to the reaction center to promote efficient energy transfer from the antenna to the reaction center. But if any of these antenna Chl(s) are too close, they will be susceptible to oxidation. Because it must generate such a powerful oxidant, PSII may have a unique difficulty achieving efficient energy transfer without photooxidizing Chl.

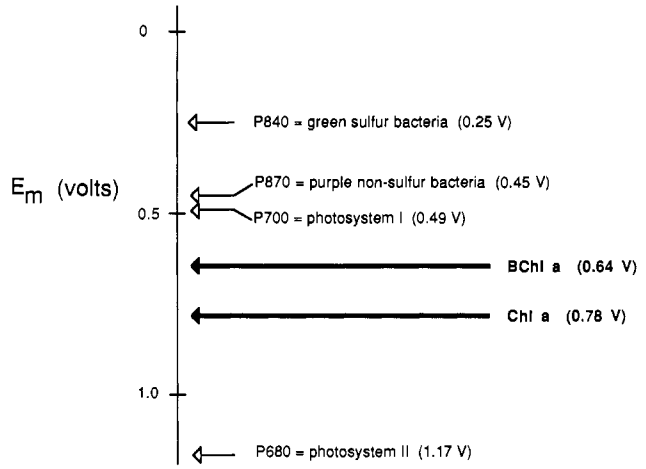


FIGURE 2: Comparison of the reduction potentials of the primary electron donors (P/P^+) and chlorophylls (Chl/Chl^+) in photosynthesis [reduction potentials from Blankenship and Prince (1985) and references cited therein and Klimov et al. (1980)].

Photooxidation of Chl by PSII has been reported by several groups (Visser & Rijgersberg, 1975; de Paula et al., 1985). A 10 G wide EPR signal at $g = 2.0024$, which is characteristic of a Chl cation radical, is produced upon illumination of samples in which photooxidation of both the oxygen-evolving complex and cytochrome *b*-559 (cyt b_{559}) are blocked (de Paula et al., 1985). The visible difference spectrum produced under the same conditions confirms that the 10 G wide EPR signal arises from a Chl cation radical (Visser et al., 1977). We propose that this oxidation of a nearby Chl by $P680^+$ is the first event in photoinhibition.

We also propose that PSII has a mechanism for protecting against damage due to Chl oxidation; this is the function of cyt b_{559} in PSII. The function of cyt b_{559} has been a long-standing puzzle [reviewed by Cramer and Whitmarsh (1977) and Cramer et al. (1986)]. Cyt b_{559} is known to be closely associated with the reaction center of PSII, since it copurifies with even the simplest preparation, consisting of only the D1, D2, and cyt b_{559} proteins (Nanba & Satoh, 1987). Yet it is probably not a component of the main electron transport chain of PSII, since the extent and kinetics of its photooxidation under physiological conditions are very small and slow (Cramer & Whitmarsh, 1977). Because it is photooxidized by P680 (Knaff & Arnon, 1969) and reduced by plastohydroquinone from the electron acceptor side of PSII (Whitmarsh & Cramer, 1978), it has been proposed that the function of cyt b_{559} is to mediate cyclic electron transfer around PSII. It has been suggested that this cycle could protect PSII from damage due to high light intensities (Heber et al., 1979).

In order to test whether such a cycle could protect PSII from photoinhibition, we have investigated the pathway of electron transfer between cyt b_{559} and P680. We have used EPR experiments to show that the photooxidation of cyt b_{559} occurs via photooxidation of Chl. This suggests that cyt b_{559} could serve to reduce the Chl that is oxidized during photoinhibition. Finally, we present a model for the mechanism of photoinhibition and for the function of cyt b_{559}.

Experimental Procedures

PSII membranes were isolated from market spinach leaves by the method of Berthold et al. (1981) as modified by Beck et al. (1985). The final storage buffer consisted of 20 mM MES–NaOH, pH 6.0, 20 mM NaCl, and 30% (v/v) ethylene glycol. The PSII membranes were treated with 2 M NaCl to remove the 17- and 23-kDa extrinsic polypeptides as previously described (Thompson et al., 1986) except that 15 mM

$CaCl_2$ was included in all buffers to prevent loss of calcium. Oxygen evolution activities were determined in a buffer consisting of 25 mM MES–NaOH, pH 6.0, and 10 mM NaCl. The activities of the untreated PSII preparations (under a light intensity of 1200 W/m^2) were 400–500 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, with less than 10% increase upon addition of 5 mM $CaCl_2$. The polypeptide-depleted PSII activities were 40% of the untreated PSII activity, which increased to 80% upon addition of 5 mM $CaCl_2$.

Polypeptide-depleted PSII membranes were treated with ascorbate to partially reduce cyt b_{559}. Two different ascorbate treatments were used. In one case 7 mM ascorbate was added to the samples, which were then incubated about 10 min before being frozen in EPR tubes. This treatment reduced about 60% of the cyt b_{559}. In the other case ascorbate treatment consisted of centrifugation at 35000g for 10 min, followed by resuspension to a Chl concentration of about 0.5 mg/mL in a buffer consisting of 15 mM ascorbate, 20 mM MES–NaOH, pH 6.0, 15 mM $CaCl_2$, and 30% (v/v) ethylene glycol. The centrifugation and resuspension were repeated once, followed by a final centrifugation and resuspension to 4–5 mg of Chl/mL. This treatment reduced over 90% of the cyt b_{559}. In both cases 100 μM DCMU was added before the samples were loaded into EPR tubes. The PSII samples were kept at 4 °C under dim green light throughout all manipulations.

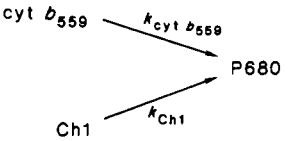
The following were used as standards for the quantitations of the EPR signals. A sample of PSII treated with 2 M NaCl was further treated with 2 mM K_2IrCl_6 to ensure that all of the cyt b_{559} was oxidized. The cyt b_{559} EPR signal present in the dark was then assumed to correspond to two spins per PSII (de Paula et al., 1985). Integration of the g_z turning point was used to quantitate cyt b_{559}. The EPR intensity standard for Chl was an untreated PSII sample illuminated for 2 min at 4 °C and rapidly frozen at 77 K. The double-integrated area of the EPR signal II present in the dark was then assumed to correspond to one spin per PSII (Babcock et al., 1983). The spectra of Chl and signal II_s were recorded within 1 h of illumination, since these signals slowly decrease during dark storage at 77 K (Miller et al., 1987). The Chl difference spectra (Figure 3a) were corrected for a small contribution due to signal II_s (less than 12% of one spin per PSII and probably a subtraction artifact). An untreated sample containing 100 μM DCMU and illuminated 2 min at 200 K was used as the standard corresponding to formation of the S_2 state multiline EPR signal in all PSII complexes. The S_2 state multiline EPR signal intensity was measured as the sum of the peak heights of six peaks as indicated in Figure 3b.

Illuminations of EPR samples (at an intensity of 700 W/m^2) were performed in a liquid nitrogen cooled nitrogen gas jet (100–200 K), in a liquid nitrogen bath (77 K), or in a dry ice–acetone bath (200 K). The sample was kept in darkness for 2 min for temperature equilibration prior to the illumination. Samples were illuminated until a single charge separation was complete, at least 10 min (77–120 K), 8–10 min (140–155 K), 4 min (160–180 K), or 2 min (220 K), and then immediately frozen at 77 K.

Results

Low-temperature illumination of PSII can be used to "trap" a charge-separated state. This occurs when the rate of the reverse charge recombination reaction is decreased sufficiently that the forward charge separation reaction is essentially irreversible. These studies can provide kinetic information by examining the relative yields of different charge-separated states, which are determined by the relative rates of the competing irreversible reactions producing those states. It has been shown previously that under low-temperature illumination conditions (77–220 K) the following charge separations are produced: $S_2Q_A^-$ (produced by oxidation of the Mn site of the oxygen-evolving complex), (ferricytochrome b-559)Q_A^-, and $Chl^+Q_A^-$ (de Paula et al., 1985). Thus cyt b_{559}, Chl, and the Mn site are competing electron donors to $P680^+$. Photooxidation is limited to a single electron transfer in these experiments by the presence of DCMU, which prevents electron transfer from Q_A to Q_B (Joliot & Kok, 1975). A comparison of the yields of the different photooxidized electron donors as a function of the temperature of illumination leads to the following kinetic model: below 100 K, $k_{Mn} \ll k_{cyt\ b_{559}}$ or k_{Chl}; above 200 K, $k_{Mn} \gg k_{cyt\ b_{559}}$ or k_{Chl}, where k_x is the rate of photooxidation of x (de Paula et al., 1985).

One issue that remains unresolved by these studies is the pathway of photooxidation of the alternate electron donors cyt b_{559} and Chl—are these donors in series or in parallel? It is known that Chl is not photooxidized unless cyt b_{559} is already oxidized (de Paula et al., 1985, 1986). It has been shown that in untreated PSII when cyt b_{559} is fully reduced, *only* cyt b_{559} is photooxidized in a single charge separation at 77 K. After chemical oxidation of cyt b_{559} in these samples, illumination at 77 K oxidizes Chl (de Paula et al., 1985). NaCl treatment, which removes the 17- and 23-kDa extrinsic polypeptides of PSII, is known to lower the reduction potential of cyt b_{559} such that it is oxidized in the dark by the ambient potential (Larsson et al., 1984). In these polypeptide-depleted samples containing oxidized cyt b_{559}, illumination at 77 K also oxidizes Chl (de Paula et al., 1986). This preferential photooxidation of cyt b_{559} suggests that, if the two alternate electron donors are in parallel pathways, the rate of oxidation of cyt b_{559} is much greater than the rate of oxidation of Chl:



(1)

where $k_{cyt\ b559} \gg k_{Chl}$. The other possibility is that the two alternate electron donors are in a single sequential pathway of electron transfer:

$$\text{cyt } b_{559} \rightarrow \text{Chl} \rightarrow \text{P680} \qquad (2)$$

Because cyt b_{559} rereduces Chl^+, oxidation of Chl will never be observed in a single charge separation unless cyt b_{559} is already oxidized.

We have used low-temperature illumination studies to investigate the relative rates of photooxidation of Chl and cyt b_{559} and resolve the question of whether Chl and cyt b_{559} are in sequential or parallel pathways. The yield of photooxidation of each alternate electron donor is determined by its competition with photooxidation of the Mn site. Thus we can compare the rates of photooxidation of the two alternate electron donors by using the Mn site as a reference. In order to examine the competition between Mn and Chl photooxidation, cyt b_{559} must be oxidized. However, chemical oxidation of cyt b_{559} in an untreated sample will also oxidize the Fe on the electron acceptor side of PSII, since the reduction potentials are similar, 380 mV (Cramer & Whitmarsh, 1977) and 360 mV (Petrouleas & Diner, 1986), respectively. In a sample with the Fe on the electron acceptor side oxidized, two electron-transfer reactions can occur, even in the presence of DCMU. To avoid this problem, we have chosen to use NaCl-treated PSII samples, in which cyt b_{559} is fully oxidized by the ambient potential but the acceptor-side Fe is fully reduced. In order to photooxidize cyt b_{559} and Chl in the same

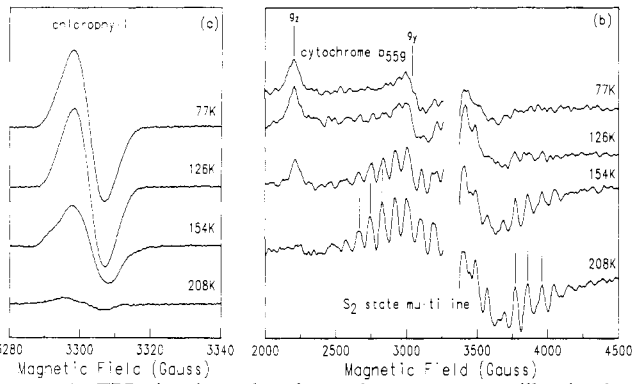


FIGURE 3: EPR signals produced upon low-temperature illumination over a range of temperatures in polypeptide-depleted, ascorbate-treated PSII: illuminated minus dark spectra of (a) the Chl cation radical and (b) oxidized cyt b_{559} (g_z and g_y turning points indicated) and Mn site S_2 state multiline (six lines indicate peaks measured). The cyt b_{559} EPR signal is most clear in the 77 K spectrum, which contains no S_2 state multiline signal; the S_2 state multiline signal is most clear in the 208 K spectrum, which contains no cyt b_{559} signal. Instrument conditions: microwave frequency, 9.1 GHz; microwave power, (a) 0.4 mW and (b) 1.3 mW; modulation frequency, 100 kHz; modulation amplitude, (a) 4 G and (b) 20 G; sample temperature, (a) 70 K and (b) 10 K.

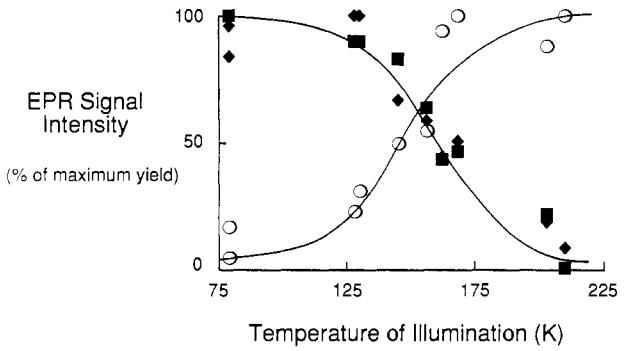


FIGURE 4: Effect of illumination temperature on the photooxidation of competing electron donors in PSII as monitored by EPR signal intensities: (◆) Chl; (■) cyt b_{559}; (○) Mn S_2 state multiline. Each signal was normalized to its own maximum yield in a given series of identical samples.

sample, we have treated these samples with ascorbate to partially reduce cyt b_{559}. Figure 3 shows the EPR signals produced in these samples upon illumination throughout the range of temperatures. At low temperatures predominantly cyt b_{559} and Chl are photooxidized. As the temperature of illumination is increased, oxidation of the alternate electron donors decreases and oxidation of the Mn site increases. This is consistent with the kinetic model discussed above (de Paula et al., 1985). Standards were used to determine that the sum of the different photooxidized electron donors at each temperature corresponded to 101% ± 11% of one charge separation. Therefore, only one electron-transfer reaction occurred, which indicates that the Fe on the electron acceptor side remained reduced in these samples. The yield of each signal, as a percent of its maximum yield in a series of identical samples, is plotted in Figure 4. It is apparent that photooxidation of cyt b_{559} decreases in parallel with photooxidation of Chl. The same result was observed in samples with 60% and over 90% of cyt b_{559} reduced; data from both are shown in Figure 4. The two alternate electron donors are exhibiting the same relative yield of photooxidation and therefore the same ability to compete with the Mn throughout the temperature range.

The parallel pathway (1) is ruled out by our data, because it cannot account for both a preferential photooxidation of cyt b_{559} and equivalent yields of photooxidation of both alternate electron donors. The sequential pathway (2) is consistent with both observations. The rereduction of Chl by cyt b_{559} explains the preferential photooxidation of cyt b_{559}. The electron transfer from Chl to P680 is the irreversible step that competes with the Mn oxidation pathway, which will determine the yield of photooxidation of both alternate electron donors. We conclude that the two alternate electron donors are in a sequential pathway (2), in which electron transfer proceeds from cyt b_{559} to Chl to P680.

Discussion

The EPR experiment presented above demonstrates that the pathway of photooxidation of cyt b_{559} in PSII is via photooxidation of Chl. This evidence, coupled with evidence that cyt b_{559} can be photoreduced via plastoquinone (Whitmarsh & Cramer, 1978), suggests a model for the function of cyt b_{559} in PSII (Figure 1). We propose that photoinhibition of PSII is due to the fact that $P680^+$, the strongest oxidant in photosynthesis, is capable of oxidizing its own antenna Chl. The last antenna Chl, Chl_z, which serves to link the antenna to the reaction center, is susceptible to photooxidation, but is kept reduced by cyclic electron transfer through cyt b_{559}. This cyt b_{559} cycle may fulfill a critical role in protecting oxygen-evolving photosynthetic organisms from photoinhibition.

Our proposal that Chl oxidation is the first step in photoinhibition is consistent with various characteristics of photoinhibition that have been reported. Inhibition of oxygen evolution would favor the Chl oxidation pathway, leading to the observed increase in photoinhibition. An artificial electron donor would compete with the Chl pathway and decrease photoinhibition. DCMU treatment would decrease the lifetime of $P680^+$, decreasing Chl oxidation and photoinhibition.

The model for the mechanism of protection against photoinhibition is also supported by a recent report that cyclic electron transfer around PSII occurs in vivo under high light intensities. Falkowski et al. (1986) have compared fluorescence and oxygen yields from intact alga cells over a range of light intensities. They conclude that at saturating light intensities about 15% of electron flow cycles around PSII. They suggest that this cycling is mediated by cyt b_{559} and may serve to prevent photodamage.

If Chl oxidation is the initial event of photoinhibition, the Chl cation radical must subsequently undergo some damaging reaction that inhibits primary charge separation and/or damages the Q_B (D1) protein. Photoinhibition has been shown to first decrease light-limited rates of electron transport and subsequently decrease light-saturated rates (Kok et al., 1956; Horton et al., 1987). This would occur if the primary event of photoinhibition were a decrease in the coupling of energy transfer from the antenna to the reaction center and the secondary event(s) damaged the reaction center itself. This is consistent with our model in which the primary event of photoinhibition is the oxidation of Chl_z, which provides the link between the antenna and the reaction center. Chl^+ is known to be unstable in solution in the presence of oxygen; an irreversible spectral change occurs within seconds (Goedheer, 1966). If Chl_z^+ undergoes such a reaction that modifies the macrocycle, there would no longer be an efficient flow of energy to the reaction center. The secondary event(s) of photoinhibition may be the oxidation of other molecules or amino acids in the vicinity by $P680^+$, which would damage the reaction center itself.

The damage to the reaction center can be more easily explained upon consideration of the proposed similarity of the structure of the PSII reaction center to that of the purple

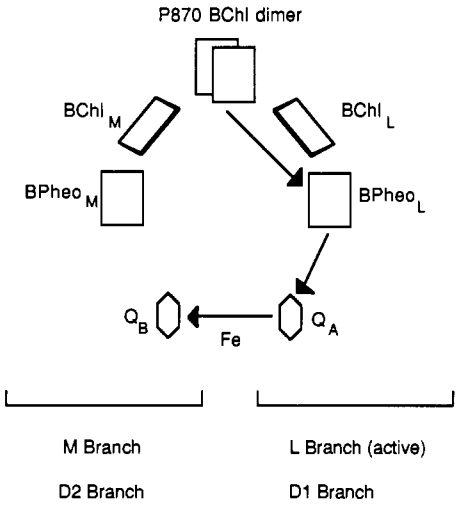


FIGURE 5: Schematic representation of the arrangement of the cofactors in the reaction center of the purple non-sulfur bacterium *Rb. sphaeroides* [based on Chang et al. (1986) and Allen et al. (1987)]. The arrows indicate the pathway of electron transfer.

non-sulfur bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*. There is considerable spectroscopic evidence that the pathway of electron transfer from the primary electron donor to the Q_B site is quite similar in PSII and bacteria (Okamura et al., 1982; Rutherford, 1983). It has also been found that the PSII proteins D1 and D2 contain sequence homologies to the bacterial reaction center proteins L and M (Michel & Deisenhofer, 1988, and references cited therein). On the basis of these similarities, it has been proposed that D1 and D2 form the PSII reaction center and that it has a similar structure to that of *Rps. viridis* (Michel & Deisenhofer, 1986; Hearst, 1986). The recent isolation of a D1/D2/cyt b_{559} preparation of PSII that photoreduces pheophytin (Nanba & Satoh, 1987) provides experimental support for this proposal. The arrangement of the cofactors in the crystal structure of the reaction center of *Rb. sphaeroides* (Chang et al., 1986; Allen et al., 1987) is shown schematically in Figure 5. This arrangement is essentially equivalent in *Rps. viridis* (Deisenhofer et al., 1984). The pathway of electrons is down one branch only, the active L branch. The function of inactive M branch is unknown.

The analogy between the reaction centers of PSII and bacteria suggests that there are two monomer Chls in PSII adjacent to the P680 Chl dimer. This is supported by the presence of four to five Chl in the D1/D2/cyt b_{559} preparation of PSII (Nanba & Satoh, 1987; Barber et al., 1987; Akabori et al., 1988). However, the histidine ligands that bind the monomer BChls in the bacterial reaction center are not conserved in PSII (unlike most of the other amino acids involved in binding the cofactors). Thus the monomer Chls are bound differently in the PSII reaction center (Michel & Deisenhofer, 1988), perhaps to a pair of His residues 10 amino acids away. If the PSII reaction center complex contains these monomer Chls, they would be quite susceptible to oxidation by $P680^+$. This is not a problem in the bacterial reaction center complex, because the reduction potential of $P870/P870^+$ is not high enough to oxidize BChl (see Figure 2). The change in the ligands of the monomer Chls may have occurred in order to decrease the likelihood of oxidizing these Chls in PSII. Perhaps it is the oxidation of a monomer Chl in the PSII reaction center which we observe and which leads to photoinhibition. Photooxidation of the Chl on the inactive D2 branch in PSII would form the charge separation $Chl_{D2}^+Q_A^-$, which might be stable at low temperatures and observable in our EPR experiments. In contrast, $Chl_{D1}^+Q_A^-$ should recombine quite rapidly and is probably not observable in our experiments.

An examination of the roles of the monomer BChls in the bacterial reaction center indicates the damage that might occur upon oxidation of the analogous monomer Chls in PSII. The monomer $BChl_L$ on the active branch is thought to participate in the primary charge separation (Creighton et al., 1988). $BChl_M$ has been shown to be unnecessary for normal electron transfer rates (Holton et al., 1987). It could play a role in energy transfer: because the ring planes of the monomeric BChls are positioned at an angle 70° from the plane of the dimer (Deisenhofer et al., 1984), the two different orientations would together facilitate energy transfer from antenna BChl with a full range of orientations. The function of the inactive M branch may be to facilitate energy transfer from the antenna to the reaction center, via $BChl_M$. In PSII, however, the analogous Chl_{D2} can probably be photooxidized by P680. Oxidation and subsequent damage of Chl_{D2} would then decrease light-limited electron-transfer rates, the first stage of photoinhibition. After Chl_{D2} is damaged, Chl_{D1} would be susceptible to oxidation by $P680^+$. Although rapid recombination would compete with damaging reactions of Chl_{D1}^+, eventually Chl_{D1} would be damaged and primary charge separation would be inhibited. Thus photoinhibition would damage components of both D1 and D2, consistent with recent studies which have shown that both D1 and D2 are synthesized during the recovery from photoinhibition (Callahan et al., 1986). Finally, the presence of two copies of cyt b_{559} in PSII would be explained by the need to rereduce the two photooxidizable monomer Chls, to protect PSII from photoinhibition.

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