PHOTOSYSTEM II: THE OXYGEN EVOLVING SYSTEM OF PHOTOSYNTHESIS

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1. INTRODUCTION

The function of Photosystem II (PS II) is to oxidize water molecules to molecular O_2, and reduce plastoquinone (PQ) molecules: $2H_2O + 2PQ \rightarrow O_2 + 2PQH_2 + 4H^+$. This uphill transfer of electrons requires 4 light quanta ($h\nu$), which is used via the excitation of the reaction center (RC) chlorophyll (Chl) a of PS II, P680; the latter leads to the primary charge separation: $P680 \cdot I + h\nu \rightarrow P680^* \cdot I \rightarrow P680^+ \cdot I^-$, where, I includes a pheophytin molecule. The requirement of two light reactions for the electron transport from H_2O to CO_2 was first suggested by the discovery of the Enhancement effect in algal cells by Emerson and his coworkers (Govindjee, Govindjee, 1975; Govindjee, Whitmarsh, 1982). Later, Emerson enhancement was discovered in the H_2O to $NADP^+$ reaction in isolated thylakoids (R. Govindjee et al., 1964), confirming the existence of two light reactions in chloroplasts. Hill, Bendall (1960) presented the now-famous Z-scheme, and Duysens et al. (1961) provided direct evidence for light reactions I and II. This review is concerned solely with the light reaction and the electron transport in PS II. For earlier reviews, see Govindjee (1980) and Velthuys (1980). Figure 1 shows a current picture of electron flow in PS II, along with the suggested times of reactions (Govindjee, 1982; Inoue et al., 1983). Electron carriers are placed vertically according to their approximate known or estimated redox midpoint potential ($E_{m,7}$). The main path of electron flow is as follows (other names are in the figure): H_2O/O_2 ($E_{m,7}$ + 0.8 V) $\rightarrow$ $M/M^+ \rightarrow Z/Z^+ \rightarrow$ $P680/P680^+$ (+1.2 V) $\rightarrow$ $Pheo/Pheo^-$ (- 0.6 V) $\rightarrow$ Q_A/Q_A^- (0 to -30 mV) $\rightarrow$ Q_B/Q_B^- $\rightarrow$ PQ/PQH_2 (+80 mV), with the release of O_2 and H^+s. Here, M represents the charge accumulating entities necessary for water oxidation; this may include the necessary or stimulatory polypeptides (33, 24 and 18 kD), Mn^{2+}/Mn^{3+}, Cl^-, entities producing absorbance changes at 320 nm (Y-320), etc. M is often referred to as the S-states, where S_0, S_1, S_2, S_3 and S_4 represent the five different states with increasing positive equivalents on them. Z represents the entities that donate electrons directly to P680; the oxidized form of Z, Z^+, is described by its ESR signal labeled II very fast (II_{vf}), and is suggested to be PQH_2^+ molecule. P680 is a monomer or a dimer Chl a attached, in a special environment, to a polypeptide having MW of 47-56 kD. Pheo is a Pheophytin molecule (in monomer form), and Q_A is a bound plastoquinone molecule. Z, P680 and Q_A are suggested to be on the same protein; an iron atom seems to be associated with Q_A. Q_B is another bound plastoquinone molecule, located on a 32 kD polypeptide that may span the membrane 7 times just like bacteriorhodopsin; it is free of lysine and binds, we think, HCO_3^- and herbicides like diuron/atrazine/ioxynil; other phenolic herbicides may bind to the 47-51 kD polypeptide; Q_B needs to be doubly reduced before the reaction will proceed further. This doubly reduced Q_B, Q_B^{2-}*, exchanges with a PQ molecule. HCO_3^- is required for efficient electron flow from Q_A^- to $Q_B^{(-)}$, and for the exchange of Q_B^{2-} with PQ. A working model for the organization of PS II (excluding the light harvesting Chl a/Chl b complex) is shown in Fig. 2. In most, if not all plants, PS II is located mainly in the "appressed" membranes; this is, perhaps, the PS II_α (Anderson, Melis, 1983). 7 polypeptides are recognized here having MW of (I) 32 kD (the Q_B-binding protein); (II) 43 kD (the core antenna, CP43); (III) 47 kD (the RC II-containing polypeptide, CP47, containing

*No distinction is made between Q_B^{2-} and its protonated form.

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P680, I, Q_A, and Z, the quinol electron donor); (IV) 33 kD (containing Mn, responsible for O_2 evolution, at least, in several plants); (V) 24 kD (stimulatory to O_2 evolution, (VI) 18 kD (involved in stimulating O_2 evolution, according to some authors); and, finally (VII) 10 kD (cytochrome b_{559}, which acts as an electron donor to P680 at 77 K, or when normal electron flow is blocked). One possibility is that IV may span the membrane and a "snake-like" portion of it may serve as what had been called a shielding protein. However, a more reasonable possibility is that there are two such polypeptides, one accessible from the outside, and, the other from the inside -- the latter related to the O_2 evolution function. The major electrogenic event in PS II is currently suggested to be due to electron flow from Pheo to Q_A. Meiburg, van Gorkom (1983) have discovered that, in thylakoid blebs, the half-saturation of electrical field dependence for electrically-stimulated reduction of Pheo by Q_A^- (reverse of that in photosynsthesis) amounts to ~ 330 mV. This could conceivably correspond to ΔE_m between Pheo/Pheo$^-$ and Q_A/Q_A^-. Since this corresponds to $\Delta\psi$ (membrane potential) created by light excitation in the membrane, it implies that electrically Q_A and Pheo are on the two sides of the electric layer. Trissl et al. (1982) have discovered that a fast component of $\Delta\psi$ in PS II is very rapid (< 200 ps).

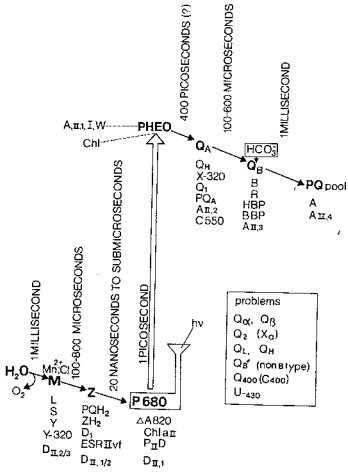

Figure 1. Electron flow in PS II. The symbols used in the text are shown in bold. Other alternate symbols are also given; HBP and BBP, under Q_B, stand for herbicide-binding protein and bicarbonate binding protein, respectively. For other symbols, see Govindjee, 1982, Vol. 1, and the text.

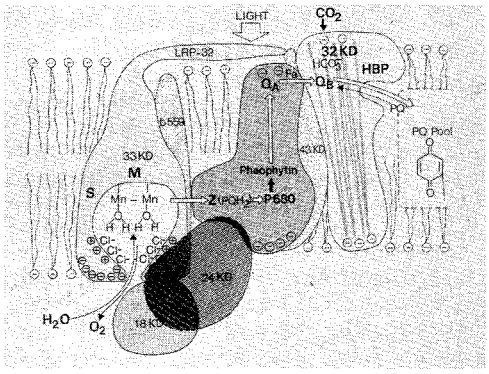

Figure 2. A working model for the organization of PS II components in the membrane. Among the several alternatives for the lysine-rich 33 kD polypeptide(s), two are mentioned in the text. Here, we show a 33 kD "M" complex accessible from the inside, and a LRP-32 from the outside. See Figure 1 and the text.

2. THE DONOR SIDE: THE "M-COMPLEX"

The S-states. (Mar, Govindjee (1971), Joliot, Kok (1975), Diner, Joliot (1977), Radmer, Cheniae (1977), Govindjee (1980) and Wydrzynski (1982)). In dark-adapted algae or chloroplasts, one observes a period of 4 with the 3rd flash showing the first maximum in O_2 evolution/flash. These initial observa-

tions by Joliot et al. (1969) have been a landmark in our understanding of how the O_2 system works. Kok et al. (1970) suggested that dark-adapted systems contain a mixture of 2 S-states S_0 and S_1 in a ratio of 25:75; thus the third flash gives the first maximum: $S_1 \overset{1}{\downarrow} h\nu\, S_2 \overset{2}{\downarrow} h\nu\, S_3 \overset{3}{\downarrow} h\nu\, S_4 \rightarrow S_0 + O_2$. The concept of charge accumulation and independence of O_2 centers from one another was made clear. Additional parameters like α ("misses") and β ("double hits") were introduced to explain the observed damping of the O_2 yield oscillation pattern.

H^+ release. It was implied in Kok's model that S is a sequential charge accumulator (see, however, Wydrzynski et al., 1977). This is not strictly correct since all the H^+s are not released in the last step, but in the earlier steps. In dark-adapted thylakoids, the most probable H^+ release pattern is 1, 0, 1, 2 for $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions (Fowler, 1977; Saphon, Crofts, 1977; Bowes, Crofts, 1978; Förster et al., 1981; Wille, Lavergne, 1982; Govindjee et al., 1983a). Two points need to be emphasized. (1) In Tris-washed chloroplasts, Renger, Voelker (1982) have observed H^+ release in the first flash; also see Tiemann et al., 1981, for H^+ release in inside-out vesicles. (2) The H^+ release pattern that is measured by a pH electrode or by an absorbance change of a dye need not reflect precisely the H^+ release during the S-state transitions.

H_2O: The ultimate electron donor. Radmer, Ollinger (1980) and Stemler, Radmer (1975) concluded from ^{18}O experiments that all O_2 evolved comes ultimately from H_2O. Several analogs of H_2O have been used by Radmer, Ollinger (1983) and it has been concluded that H_2O sits in a cleft which is ~ 4 Å wide and ~ 2.5 Å deep. There is also the suggestion that 2 H_2O molecules 1.47 Å apart sit in this cleft ($2\, H_2O \equiv NH_2\, NH_2$). Low $[NH_3]$ is able to replace H_2O and still allow S-state transitions (Velthuys, 1980; Radmer, 1983).

U.V. Absorbance Changes. Pulles et al. (1976) discovered an absorbance change in the UV region that oscillated, in a sequence of light flashes, with a period of 4. Mathis, Havemann (1977) characterized this further. By using hydroxylamine, which blocks changes in the S-state, but allows the operation of the Q_B protein, absorbance changes, due to Q_B, could be subtracted from controls to gain data on UV changes due to the S-state transitions. Velthuys (1981a) suggested that there are two components M and L, in the "M complex" and the oxidation of L is required before M can be oxidized. Thus, $S_1 \rightarrow S_2 \rightarrow S_3$ transition was suggested to be as follows: $LM \rightarrow L^+M \rightarrow L^+M^+$ followed by L^+ reduction to L. Renger, Weiss (1983) studied the absorbance changes involved in the S-state by suppressing UV changes in the Q_B region by using trypsinized thylakoids and FeCy to accept electrons from Q_A^-. A component, oscillating with a period of 4, was dubbed Y-320. I wonder if one should consider a Mn-quinone complex as a component involved in the "M complex' (Lynch et al., 1981).

Manganese. Amesz (1983) has reviewed the role of Mn in the "M complex". Two major approaches have been attempted to look at Mn: one by proton relaxation rates by NMR techniques and the other by ESR (for the observations and problems of NMR studies see Govindjee, Wydrzynski, 1981; Khanna et al., 1983). It has been generally found that ESR does not monitor Mn of the M complex under normal conditions. There are two exceptions: (1) when thylakoids are exposed to different flashes of light, and are heated, the released (or released and converted) Mn^{2+} shows oscillations with a period of 4 suggesting a dynamic role of Mn in photosynthesis (Wydrzynski, Sauer, 1980; Sauer, 1980); and (2) low temperature ESR of thylakoids (Dismukes, Siderer, 1980, 1981; Dismukes et al., 1982, 1983; Hansson, Andreasson, 1982; Ke et al., 1982); this seems to be the most promising approach. A flash pattern is observed with maxima after 1st and 5th flashes in a 16-line ESR signal for Mn. The peak appears when the S_2 state is created. The probing of the S_2 state by this technique was established by

Brudvig et al. (1983a,b). Both the temperature dependence of its reaction and its deactivation match closely the character of the S_2 state. Another technique to monitor Mn is X-ray-absorption-edge measurement. Kirby et al. (1981) have implied, by comparison with data on model Mn compounds, that in thylakoids Mn may be in a mixture of Mn^{2+} and Mn^{3+} states. The question whether 4 Mn atoms are necessary for O_2 evolution or 2 Mn atoms and 2 other atoms (e.g., Ca^{2+}, etc.) may be enough for the efficient operation of the M complex needs to be settled. Klimov et al. (1982) showed that 2 Mn/RC is enough to completely restore the functioning of PS II (DCPIP reduction). Data on 33 kD polypeptide and Mn release suggested that 2 Mn are associated with this release (N. Murata, personal communication). Thus, 2 Mn/RC could be considered sufficient for O_2 evolution. But the highly active PS II particles and chloroplasts contain a minimum of 4 Mn/RC (Yocum et al., 1981; G.M. Cheniae, personal commun.). Thus, I believe 4 Mn/RC should be considered as the minimum requirement for O_2 evolution until proven otherwise. (See a model in Govindjee et al., 1977.)

Chloride. Izawa et al. (1983) and Govindjee et al. (1983b) have summarized their findings on the role of Cl^- in the "M" complex. It is clear that the order of effectiveness of anion ($Cl^- > Br^- > NO_3^- > I^- > F^-$, etc.) on the M complex follows the order for activation of several *in vitro* enzymatic systems. Thus, it is easy to imagine that they may play a similar role *in vivo*. The major function of Cl^- may be to stabilize the M complex (Mn) when a positive charge arrives there from P680; when a H^+ leaves the M complex, a Cl^- may also leave. Furthermore, it is suggested that Cl^- activates the S-states (Izawa et al., 1983; Coleman et al., 1983). We have, for the first time, introduced the use of ^{35}Cl-NMR as a tool to study Cl-binding in thylakoids. Our major conclusions (Critchley et al., 1982; Baianu et al., 1983) are: (1) Cl^- binds reversibly (exchange rate, > 1,000 sec^{-1}) to thylakoids of halophytes with a K_b of $\sim 1\ M^{-1}$ and a ΔE of binding of $\sim$ 9 Kcal/mole. The weakly ionic binding is a necessary condition for its action. Ions which bind too tightly (F^-) may inhibit the reaction as they are not easily released when H^+ is released; and large ions (PO_4^{2-}, SO_4^{2-}) may not work because they cannot enter the "Cl^- pocket" (Homann et al., 1983). We have calculated that, at least in halophytes, there are 20-40 Cl^- bound per O_2 evolving center. Cl^- active in S-state activation may be very few (e.g., 4). Heat treatment, in general, is assumed to inactivate O_2 evolution by the release of Mn. However, Hind et al. (1969) observed that 30°C treatment allowed a better Cl^- depletion. Coleman et al. (1983) have systematically measured Hill activity of thylakoids after treatment at various temperatures with and without Cl^- or other anions present. The new point is that the order of effectiveness of these anions in stabilizing thylakoids against thermal inactivation follows that of their effectiveness in stimulating electron flow in Cl^- depleted samples; Cl^- may activate the M-complex.

Polypeptides. It has long been surmised that a protein(s) is (are) involved on the O_2 evolution side (Wydrzynski, 1982). Attempts to isolate the Mn-containing oxygen evolving enzyme have not yet succeeded, but many exciting observations have been made and pieces are being put together. Zilinskas, Govindjee (1974) had succeeded in obtaining an antibody that was specific against the "M" complex: it gave a 15% inhibition with thylakoids and a 30% inhibition with PS II membranes. Spector, Winget (1980) claimed to have isolated a 65 kD-Mn-containing protein suggested to be the O_2 evolving enzyme. Although this work could not be reprodcued in any of the major laboratories in the field, yet it provided an incentive to many to look for the enzyme again. A major impetus has come from the use of "inside-out" thylakoid vesicles (Åkerlund et al., 1982). These have permitted the removal and reinsertion of 24 and 18 kD polypeptides. The current status of the polypeptides associated with the O_2 evolving enzyme (Åkerlund, 1983; Yamamoto, Nishimura, 1983; Kuwabara, Murata, 1983;

Murata et al., 1983; Sayre, Cheniae, 1982; Bishop, 1983) is as follows. There are 3 polypeptides: (1) a 33 kD-lysine containing polypeptide, associated indirectly with Mn binding; (2) a 24 kD polypeptide that has been shown to stimulate O_2 evolution and (3) a 18 kD polypeptide that may (Toyoshima et al., 1983) or may not stimulate O_2 evolution. Murata's and of Cheniae et al.'s results show that removal of 24 kD polypeptide does not lead to a total absence of O_2 evolution, and therefore, it may only have a stimulatory function. Ackerlund and C. Yocum and coworkers, however, find a total inactivation. The universality of 33 kD polypeptide as the Mn-containing O_2 evolving enzyme is also not yet clear. Okada and Asada (1983) have isolated a 13 kD Mn-polypeptide from a blue-green alga; it has catalase activity and seems to function on the H_2O side. A possible inhibition by KCN on the water side has been shown, among others, by H. Nakatani (personal commun.). Thus, a KCN-sensitive component may be involved in O_2 evolution. D. Blubaugh (in my laboratory) has recently observed inhibitions by certain inhibitors of the alternate cytochrome oxidase pathway. These aspects need to be pursued to understand the biochemistry of the O_2 evolving system. In addition, the effects of and interactions of heavy metals (like Zn and Ni; Tripathy and Mohanty, 1980; Tripathy et al., 1983) on the water side of PS II needs to be explored to further probe the biochemistry of O_2 evolution.

Cytochrome b_{559}. Widger et al. (1983) have isolated and chemically characterized cyt b_{559}. It has a MW of 10 kD on SDS-urea gradient gel; there are 2 polypeptide chains/heme and its amino acid sequence is known, at least up to 33 residues from the N-terminus. Its association with PS II activity has been known for some time; it donates electron to P680 at 77 K (Butler et al., 1973), and mutants lacking PS II activity also lack cyt b559 (Maroc and Garnier, 1981). Butler, Matsuda (1983) have speculated that it aids in O_2 evolution although it may not be absolutely required for it. Butler suggests that its function may lie in accepting a H^+ from the S-states converting the LP cyt b (Fe^{2+}) to HP H^+ cyt b (Fe^{2+}).

3. REACTION CENTER COMPLEX: Z, P680, Pheo and Q_A

The general impression is that P680, Z, Pheophytin and Q_A are all located on the reaction center complex (47-51 kD polypeptide). The CP47 has a fluorescence band at 695 nm (F695) at 77K, whereas CP43 has F685 (Nakatani, 1983; Yamagishi, Katoh, 1983) (for earlier literature on these bands, see Govindjee, Yang, 1966).

Z. Whether there are two Zs (Jursinic and Govindjee, 1977a; Bouges-Bocquet, 1980) or one (Conjeaud et al., 1979) is not certain. Boussac and Etienne (1982) have provided evidence for two Zs and explained why one sees only one under other experimental conditions. The nature of Z is being actively investigated. The oxidation of $P680^+$ is accompanied by an ESR signal II vf in normal samples and II f in Tris-washed samples (Babcock et al., 1976). Z^+ accepts electrons from the M complex. The g-value and ΔH of the ESR signal suggests its quinone character. Ghanotakis et al. (1983) have shown, by comparison with model quinone compounds, that Z^+ may be PQH_2^+. Its suggested redox potential favors this possibility. Dekker et al. (1983), Diner et al. (1983a), and Renger, Weiss (1983b) have obtained absorbance spectra of Z^+, similar to that of PQH_2^+. Boska et al. (1983) have shown that, at least in Tris-washed materials, the kinetics of Z^+ formation and the reoxidation of $P680^+$ to P680 at different pHs are the same establishing the identity of Z as electron donor to $P680^+$. (Babcock et al. (1983) obtained the same conclusions in PS II RC preparations.) This awaits confirmation by the measurements of ΔA due to $Z \rightarrow Z^+$ and that due to $P680^+ \rightarrow P680$ reactions under fast (ns to sub μs) measuring

conditions. Data of K. Sauer and coworkers (these proceedings) favor this conclusion.

P680-P690. Döring et al. (1967) were the first to observe changes due to P680. The Z to P680 reaction in control thylakoids has been measured by two methods: (1) Chl a fluorescence rise; and (2) absorbance change due to $P680^+$ to P680 reaction either at 690 nm ($-\Delta A$) or at 820 nm ($+\Delta A$). Mauzerall (1972) was the first to observe a Chl a fluorescence rise time of 20-30 ns in dark-adapted algae. Butler (1972) explained this data by suggesting that $P680^+$ is a quencher of fluorescence and the rise may be due to the conversion of $P680^+$ to P680. Sonneveld et al. (1979), in an elegant analysis of Chl a fluorescence rise during a flash excitation at different intensities, established that in dark-adapted thylakoids, this rise is best explained by a 20-40 ns reduction time of $P680^+$ to P680. However, in preilluminated samples, this rise is 400 ns (0.4 μs). Van Best, Mathis (1978) observed a 30 ns component for the reduction of $P680^+$ to P680 by direct absorption ($+\Delta A$) measurements at 820 nm in dark-adapted thylakoids. Recently, Eckert, Renger and H.T. Witt (see Renger et al., 1983) have been able to "eliminate" the fluorescence "artifact" and measure the ns component at 690 nm. An ESR signal, due to $P680^+$, was demonstrated by van Grokom et al. (1974); it has a g value of 2.003, and $\Delta H = \sim 8G$. No fast kinetic work has yet been possible. The redox potential of $P680/P680^+$ was estimated to be + 1.1 Volt by Jursinic, Govindjee (1977b) from measurements of activation energy for the delayed light emission, and from the E_m of known components. Klimov et al. (1979) have come to a similar value from the E_m of $Pheo/Pheo^-$ and from ΔE between Pheo and P680. A triplet state from P680 was discovered by Rutherford et al. (1981a) by ESR measurements. It is suggested to be formed from the radical pair $P680^+ \cdot I^-$.

Pheophytin. At present Pheo is considered as the primary electron acceptor of PS II (Klimov, Krasnovsky, 1981; Parson, Ke, 1982; Ke, 1983) although it is possible that a Chl a molecule may precede Pheo (Rutherford, 1981). Klimov et al. (1977) succeeded in showing that $Pheo^-$ can be accumulated in PS II if $P680^+$ is reduced by an external donor and Q_A is chemically reduced prior to illumination. Accumulation of $Pheo^-$ leads to a quenching of Chl a fluorescence. This is because $Pheo^-$ can trap excitons. In this picture, variable fluorescence is delayed fluorescence by charge recombination of $P680^+Pheo^- \rightarrow P680 \cdot Pheo + h\nu$. The $Pheo^-$ molecule is a monomer as evidenced by its ESR characteristics ($g = 2.0333$, $\Delta H = 13$ G) (Klimov et al., 1980). Although electron flow from P680 to Pheo has not been measured, it has to be in the picosecond time scale (certainly several orders of magnitude faster than the back reaction of $P680^+Pheo^- \rightarrow P680 \cdot Pheo$, which is of the order of 2-4 ns). Direct detection of Pheo changes in the ns range was made by Shuvalov et al. (1980). The reduction time of $Q_A:Pheo^-Q_A$ to $Pheo \cdot Q_A^-$ is suggested to be less than 400 ps as the lifetime of fluorescence for the constant fluorescence (F_0) is < 400 ps (Haehnel et al., 1982) or ~ 200 ps (Fenton et al., 1982). A split $Pheo^-$ ESR signal was discovered by Klimov et al. (1980) and was suggested to be due to an interaction with Fe in Q_AFe complex. From the redox potential dependence of the triplet state EPR signal, Rutherford et al. (1981b) have confirmed the $E_{m,7}$ of $Pheo^-/Pheo$ to be -0.6 Volt, as earlier found by Klimov et al. (1979).

Q_A. Since Q is made up of several components, we define Q_A as the major electron carrier between Pheo and Q_B, the second quinone electron acceptor. Q_A is generally monitored by fluorescence. When Q_A is Q_A^-, Chl a emission is high and when it is Q_A, it is low. The fluorescence rise kinetics measures, in principle, both the $P680^+$ to P680 reaction, and the Q_A to Q_A^- reaction. Since $P680^+$ to P680 reaction is in the 20-30 ns range, reduction time of Q_A to Q_A^- could not be measured by fluorescence. The reoxidation of Q_A^- to Q_A can be measured by the decay in Chl a fluorescence. Zankel (1973) and Mauzerall

(1972) have made such measurements, and the time is in the range of 200-600 μs. Bowes, Crofts (1980) have, however, measured this time as a function of flash number and have obtained evidence that $Q_A^- Q_B \rightarrow Q_A Q_B^-$ is 200-400 μs and $Q_A^- Q_B^- \rightarrow Q_A Q_B^{2-}$ is 600-1000 μs. The Q_A^- formation can also be directly measured by absorbance change due to semiquinone anion formation (Stiehl, Witt, 1968; van Gorkom, 1974; Farineau, Mathis, 1983). It is often called X-320, 320 nm being an absorbance maximum. Both Q_A^- and Q_B^- should give this signal (Siggel et al., 1977). Thus, in first flash, when absorbance changes due to Y-320 is abolished, $Q_A Q_B \xrightarrow{h\nu} Q_A^- Q_B \rightarrow Q_A Q_B^-$ should give an absorbance change which should last a long time (lifetime of Q_B^-), but after the 2nd flash, when $Q_A^- Q_B^-$ is converted to $Q_A Q_B^{2-}$, the absorbance change should decay rapidly and there should be a binary oscillation of this phenomenon (Mathis, Haveman, 1977). An ESR signal due to Q_A^- was discovered by Klimov et al. (1981) when Fe was removed from the sample. It's g value and ΔH are 2.0044 and 9 G, respectively. It is normally not observed due to an interaction with Fe^{2+}. An ESR signal due to $Q_A^- Fe^{2+}$ was discovered by Nugent et al. (1981) (also see Rutherford, Mathis, 1983); it can be observed when illumination is done at > 5 K, most of the signal being formed at > 200 K. The existence of Fe in PS II has now been shown by Mössbauer spectroscopy (Petrouleas, Diner, 1982). C550, an absorbance change due to a bandshift of Pheo (van Gorkom 1974; Klimov et al., 1977) reflects the reduction of Q_A^-. It can be titrated, as done recently by Diner and Delosme (1983a,b). Its redox potential is ~ 0 to -30 mV, equivalent to the so-called Q_H (the high potential Q). No C550 change is associated with Q_L (the low potential Q), which may be equivalent to Q_2 (Joliot, Joliot, 1983).

4. THE Q_B-PROTEIN

Q_B is generally assumed to be bound to a 32 kD lysine-free protein; the latter is also a herbicide-binding protein. Its amino acid composition is now known by DNA-sequencing (Zurawski et al., 1982), and it has been suggested that it spans the membrane 7 times just as bacteriorhodopsin does (Rao et al., 1983). That Q_B is a 2 electron "gate" was shown independently by Bouges-Bocquet (1973) and Velthuys and Amesz (1974). A binary oscillation in Chl a fluorescence, measured after diuron addition, following a number of preilluminating flashes, is the easiest measure of this phenomenon. Fig. 3 shows a working model for the operation of this cycle.
An important concept of how electrons are transferred from Q_B to PQ was suggested by Velthuys (1981b). In this concept, after Q_B^- is formed, it exchanges with a PQ molecule on the Q_B-protein. The herbicide and other inhibitors (I) are assumed to act by replacing Q_B. Q_B^- is more tightly bound than Q_B or Q_B^{2-}. The picture of Q_B function, as stated above, is confirmed by experiments of Lavergne (1982a) who showed that diuron acts faster with Q_B than with Q_B^- present. An interaction of herbicides with various quinones at the binding site (Vermaas et al., 1983; Oettmeier, Soll, 1983) also supports the picture that quinones and herbicides interact with each other at the Q_B-protein(s). Different herbicides can replace each other on this protein (Oettmeier, Trebst, 1983) suggesting that they have common binding environments. However, it is possible that they bind in such a way that binding to one site changes the conformational state of the other. The ratio of Q_B^-/Q_B in thylakoids is normally 3/7 (Wollman, 1978). However, in intact algae, this could be more, as the PQ pool seems to be reduced even in darkness. Thermoluminscence (TL) peak "B" monitors $S_2 Q_B^-$ recombination reactions (Rutherford et al., 1982). We have used the flash dependence of this peak to monitor this ratio in leaves; in addition, deactivation of the S_2 states by recombination with Q_B^- was also measured in leaves. Rutherford et al. (1983) have indeed observed that Q_B^-/Q_B ratio is

extremely high in dark-adapted leaves. The same is true for intact chloroplasts (Govindjee et al., 1983c).

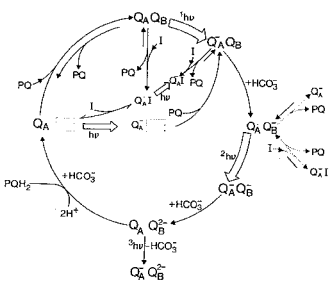

Figure 3. A working model for the electron acceptor quinone complex of PS II. Here I stands for inhibitor. See text and cited references.

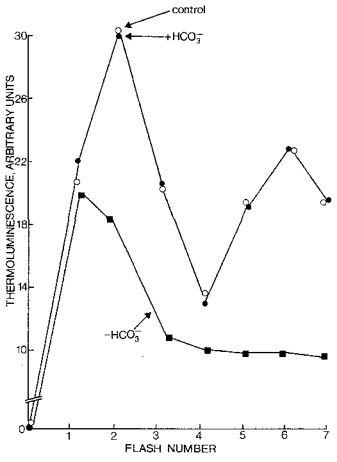

Figure 4. Thermoluminesence of "B" band as a function of flash number in control (○), CO_2-depleted (■) and reconstituted (●) spinach thylakoids (Govindjee et al., 1983d).

5. THE HCO_3^- EFFECT

A very significant phenomenon, that we have studied for several years (Govindjee, van Rensen, 1978; Vermaas, Govindjee, 1981a,b, 1982a; Stemler, 1982) is that HCO_3^- appears to be required for the efficient electron flow from Q_A^- to $Q_B^{(-)}$ and from Q_B^{2-} to PQ. In my current picture, HCO_3^- binds to the Q_B-protein to provide the proper conformation (allosteric effector) to this protein so that it can efficiently accept electrons from Q_A^- and efficiently exchange Q_B^{2-} with PQ(Q_B). This hypothesis predicts that the absence of HCO_3^- should reversibly slow down (a) Q_A^- to Q_A reaction, as already observed (Jursinic et al., 1976; Siggel et al., 1977; Farineau, Mathis, 1983); and (b) the exchange of Q_B with PQ, as interpreted from the existing data (Govindjee et al., 1976; Farineau, Mathis, 1983). Govindjee et al. (1976) suggested that HCO_3^- depletion slows down the latter reaction to 150 ms from a normal time of 1 ms. With better CO_2-depletion and reconstitution methods (J. Snel, J.J.S. van Rensen, and also W. Vermaas, Govindjee, Eaton-Rye), this reaction is suggested to slow down into several seconds region. We are now able to obtain a total block in the PS II reactions after 3 flashes given every 1 or 2 seconds (also see Vermaas, Govindjee, 1982b): Thus, the 4th and subsequent flashes are unable to produce additional change. This has been shown by the absence of cycling in thermoluminescence (due to $S_2Q_B^-$) as a function of flash number (Govindjee, et al., 1983d, Fig. 4), and in the water H^+ release as a function of flash number (Govindjee et al., 1983a). The decrease in the amplitude of X-320 observed by Farineau, Mathis (1983), beginning at the 4th flash, may also be explained by the same phenomenon. The interaction with ^{14}C-herbicide was

first shown by Khanna et al. (1981). We observed that CO_2-depletion of thylakoids leads to a decreased binding of ^{14}C-atrazine; this is restored to normal binding upon reconstitution with HCO_3^-. This was confirmed by Vermaas et al. (1982) with ^{14}C ioxynil.

6. CONCLUDING REMARK

Several unpleasant statements about PS II may now be eliminated, and the gloom over the solution of PS II reactions may now be lifted. The new PS II membranes, active in O_2 evolution, should replace the use of thylakoids for investigations on PS II biochemistry (see e.g., Berthold et al., 1981; Dunahay et al., 1983).

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