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 O₂, and reduce plastoquinone (PQ) molecules: 2H₂O + 2PQ → O₂ + 2PQH₂ + 4H⁺. This uphill transfer of electrons requires 4 light quanta (hv), 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⁺ - I + hv → P680⁺ - I - P680⁺ - I⁻, where, I includes a pheophytin molecule. The requirement of two light reactions for the electron transport from H₂O to CO₂ was first suggested by the discovery of the Enhancement effect in algae cells by Emerson and his coworkers (Govindjee, Govindjee, 1975; Govindjee, Whitmarsh, 1982). Later, Emerson enhancement was discovered in the H₂O 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 Duyssens 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 (Em, 77). The main path of electron flow is as follows (other names are in the figure): H₂O/O₂ (Em, 77 + 0.8 V) → M/M⁺ → Z/Z⁺ → P680/P680⁺ (+1.2 V) → Pheo/Pheo⁺ (-0.6 V) → QA/Q'B (0 to -30 mV) → QB/Q'B → PQ/PQH₂ (+80 mV), with the release of O₂ and H⁺. Here, M represents the charge accumulating entities necessary for water oxidation; this may include the necessary or stimulatory polypeptides (33, 24 and 18 kD), Mn²⁺/Mn³⁺, Cl⁻, entities producing absorbance changes at 320 nm (Y–320), etc. M is often referred to as the S-states, where S₀, S₁, S₂, S₃ and S₄ 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 (Iᵥ, f), and is suggested to be PQH₂ 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 QA is a bound plastoquinone molecule. Z, P680 and QA are suggested to be with the same protein; an iron atom seems to be associated with QA. QB is another bound plastoquinone molecule, located on a 32 kD polypeptide that may span the membrane 7 times just like bacteriorhodospin; it is free of lysine and binds, we think, HCO₃⁻ and herbicides like diuron/atrazine/ioxynil; other phenolic herbicides may bind to the 47-51 kD polypeptide; QB needs to be doubly reduced before the reaction will proceed further. This doubly reduced QB, QB²⁻, exchanges with a PQ molecule. HCO₃⁻ is required for efficient electron flow from QA to QB⁻, and for the exchange of QB⁻ 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 (Anderson, Melis, 1983). 7 polypeptides are recognized here having MW of (I) 32 kD (the QB-binding protein); (II) 43 kD (the core antenna, CP43); (III) 47 kD (the RC II-containing polypeptide, CP47, containing

*No distinction is made between QB²⁻ and its protonated form.

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P680, I, QA, and Z, the quinol electron donor; (IV) 33 kD (containing Mn, responsible for O₂ evolution, at least, in several plants); (V) 24 kD (stimulatory to O₂ evolution, (VI) 18 kD (involved in stimulating O₂ evolution, according to some authors); and, finally (VII) 10 kD (cytochrome b₅₅₉, 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 O₂ evolution function. The major electrogenic event in PS II is currently suggested to be due to electron flow from Pheo to QA. Meiburg, van Gorkom (1983) have discovered that, in thylakoid blebs, the half-saturation of electrical field dependence for electrically-stimulated reduction of Pheo by QA (reverse of that in photosynthesis) amounts to ~ 330 mV. This could conceivably correspond to Δψ between Pheo/Pheo⁻ and QA/QA⁺. Since this corresponds to Δψ (membrane potential) created by light excitation in the membrane, it implies that electrically QA and Pheo are on the two sides of the electric layer. Trissi et al. (1982) have discovered that a fast component of Δψ 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 QA, stand for herbicide-binding protein and bicarbonate binding protein, respectively. For other symbols, see Govindjee, 1982, Vol. 1, and the text.

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

The S-states. (Mar, Govindjee (1971), Joliot, Kok (1975), Diner, Joliot (1977), Rädmer, 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 O₂ evolution/flash. These initial observa-
tions by Joliot et al. (1969) have been a landmark in our understanding of how
the O₂ system works. Kok et al. (1970) suggested that dark-adapted systems
contain a mixture of 2 S-states S₀ and S₁ in a ratio of 25:75;
thus the third flash gives the first maximum: $S₁ \uparrow S₂ \uparrow S₃ \uparrow S₄ \rightarrow S₀ \uparrow O₂$.
The concept of charge accumulation and independence of O₂ centers from one
another was made clear. Additional parameters like $a$ ("misses") and $b$ ("double
hits") were introduced to explain the observed damping of the O₂ yield oscilla-
tion 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 S₀ → S₁, S₁ → S₂, S₂ → S₃ and S₃ → S₀ 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⁺ re-
lease 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.

H₂O₂: The ultimate electron donor. Radmer, Ollinger (1980) and Stebler, Radmer
(1975) concluded from ¹⁸O experiments that all O₂ evolved comes ultimately from
H₂O. Several analogs of H₂O have been used by Radmer, Ollinger (1983) and it
has been concluded that H₂O sits in a cleft which is ~ 4 Å wide and ~ 2.5 Å
deep. There is also the suggestion that 2 H₂O molecules 1.47 Å apart sit in this
cleft (2 H₂O ≡ NH₂ NH₂). Low [NH₃] is able to replace H₂O and still allow
S-state transitions (Velthuys, 1980; Radmer, 1983).

U.V. Absorbance Changes. Pulles et al. (1976) discovered an absorbance change
in the U.V. region that oscillated, in a sequence of light flashes, with a period of
4. Mathis, Havemann (1977) characterized this further. By using hydroxy-
amine, which blocks changes in the S-state, but allows the operation of the Qb
protein, absorbance changes, due to Q₈, could be subtracted from controls to
gain data on U.V. changes due to the S-state transitions. Velthuys (1981a) sug-
gested 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, S₁ → S₂ → S₃
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 U.V. changes in the Q₈ region by using trypsinized
thylakoids and FeCy to accept electrons from Q₈. 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) Mn²⁺ 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 S₂ state
is created. The probing of the S₂ 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 S₂ 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 Mn²⁺ and Mn³⁺ states. The question whether 4 Mn atoms are necessary for O₂ evolution or 2 Mn atoms and 2 other atoms (e.g., Ca⁺⁺, 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 O₂ 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 O₂ 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⁻ > NO₃⁻ > 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 ³⁵Cl-NMR as a tool to study Cl⁻-binding in thylakoids. Our major conclusions (Critchley et al., 1982; Balany et al., 1983) are: (1) Cl⁻ binds reversibly (exchange rate > 1,000 sec⁻¹) to thylakoids of halophytes with a K_b of ~ 1 M⁻¹ and a ΔE of binding of ~ 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 (PO₄³⁻, SO₄²⁻) may not work because they cannot enter the "Cl⁻ pocket" (Hommann et al., 1983). We have calculated that, at least in halophytes, there are 20-40 Cl⁻ bound per O₂ evolving center. Cl⁻ active in S-state activation may be very few (e.g., 4). Heat treatment, in general, is assumed to inactivate O₂ 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 O₂ 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. Zilinskis, 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 O₂ evolving enzyme. Although this work could not be reproduced 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 (Ackelund 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 O₂ evolving enzyme (Ackelund, 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 O₂ evolution and (3) a 18 kD polypeptide that may (Toyoshima et al., 1983) or may not stimulate O₂ 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 O₂ evolution, and therefore, it may only have a stimulatory function. Ackerlund and C. Yokum and coworkers, however, find a total inactivation. The universality of 33 kD polypeptide as the Mn-containing O₂ 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 H₂O 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 O₂ evolution. D. Blaubaugh (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 O₂ 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 O₂ evolution.

Cytochrome b₅₅₉. Widger et al. (1983) have isolated and chemically characterized cyt b₅₅₉. 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 O₂ 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 (Fe²⁺) to HP H⁺ cyt b (Fe⁺).

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

The general impression is that P₆₈₀, Z, Pheophytin and QA 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. Bousjac 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 v of 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 PQ₂⁺. 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 PQ₂⁺. 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 → Z⁺ and that due to P₆₈₀⁺ → P₆₈₀ reactions under fast (ns to sub μs) measuring
conditions. Data of K. Sauer and coworkers (these proceedings) favor this conclusion.
P680-P690. Dröge 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 (ΔA) or at 820 nm (ΔA). 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 (ΔA) 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 ΔH = ~ 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 to known components. Klimov et al. (1979) have come to a similar value from the E to 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⁺⁺.
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 QA 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⁻→ P680⁺Pheo + hν. The Pheo⁻ molecule is a monomer as evidenced by its ESR characteristics (g = 2.0333, ΔH = 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⁻→ P680⁺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 QA-Pheo⁻QA to Pheo⁴QA is suggested to be less than 400 ps as the lifetime of fluorescence for the constant fluorescence (F₀) 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 QAFe complex. From the redox potential dependence of the triplet state EPR signal, Rutherford et al. (1981b) have confirmed the E m7 of Pheo⁻/Pheo to be ~0.6 Volt, as earlier found by Klimov et al. (1979).
QA. Since Q is made up of several components, we define QA as the major electron carrier between Pheo and QA, the second quinone electron acceptor. QA is generally monitored by fluorescence. When QA is QA⁻, Chl a emission is high and when it is QA, it is low. The fluorescence rise kinetics measures, in principle, both the P680⁺ to P680 reaction, and the QA to QA⁺ reaction. Since P680⁺ to P680 reaction is in the 20-30 ns range, reduction time of QA to QA⁻ could not be measured by fluorescence. The reoxidation of QA⁻ to QA 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 $Q_A^{aq} \rightarrow Q_A^{aq}$ is 200-400 μs and $Q_B^{aq} \rightarrow Q_B^{aq}$ is 600-1000 μs. The $Q_A^{aq}$ 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 $Q_A^{aq}$ and $Q_B^{aq}$ should give this signal (Sigel et al., 1977). Thus, in first flash, when absorbance changes due to X-320 is abolished, $Q_A^{aq} \rightarrow Q_A^{aq}$ should give an absorbance change which should last a long time (lifetime of $Q_A^{aq}$), but after the 2nd flash, when $Q_A^{aq}$ is converted to $Q_B^{aq}$, the absorbance change should decay rapidly and there should be a binary oscillation of this phenomenon (Mathis, Haveman, 1977). An ESR signal due to $Q_A^{aq}$ 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 $Q_A^{aq}Fe^{2+}$ 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 (Petroules, Diner, 1982). C550, an absorbance change due to a bandshift of Pheo (van Gorkom 1974; Klimov et al., 1977) reflects the reduction of $Q_A^{aq}$. 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 $Q_l$ (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 (Zurawska 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 preillumination 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 (1) are assumed to act by replacing $Q_B$. $Q_l$ is more tightly bound than $Q_B$ or $Q_B$. 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. Thermoluminescence (TL) peak "B" monitors $S_0Q_B$ 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.

5. THE HCO₃⁻ 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 HCO₃⁻ appears to be required for the efficient electron flow from QA to QB⁻ and from QB⁻ to PQ. In my current picture, HCO₃⁻ binds to the QB-protein to provide the proper conformation (allosteric effector) to this protein so that it can efficiently accept electrons from QA and efficiently exchange QB⁻ with PQ(QB). This hypothesis predicts that the absence of HCO₃⁻ should reversibly slow down (a) QA to QA reaction, as already observed (Jursinic et al., 1976; Siggel et al., 1977; Farineau, Mathis, 1983); and (b) the exchange of QB with PQ, as interpreted from the existing data (Govindjee et al., 1976; Farineau, Mathis, 1983). Govindjee et al. (1976) suggested that HCO₃⁻ depletion slows down the latter reaction to 150 ms from a normal time of 1 ms. With better CO₂-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 S₂Q₂) 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 ¹⁴C-herbicide was
first shown by Khanna et al. (1981). We observed that CO₂-depletion of thylakoids leads to a decreased binding of ¹³C-atrazine; this is restored to normal binding upon reconstitution with HCO₃⁻. This was confirmed by Vermaas et al. (1982) with ¹³C 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 O₂ 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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