Photochemistry and Photobiology Vol. 42, No. 2, pp. 187 – 210, 1985
Printed in Great Britain.

0031-8655/85 $03.00+0.00

REVIEW ARTICLE

THE ELECTRON DONOR SIDE OF PHOTOSYSTEM II: THE OXYGEN EVOLVING COMPLEX†

GOVINDJEE,* T. KAMBARA and W. COLEMAN

Division of Biophysics, Department of Physiology and Biophysics, and Department of Plant Biology, University of Illinois at Urbana-Champaign, 289 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA

(*Received* 30 *January* 1985; *accepted* 5 *March* 1985)

1. SUMMARY: AN OVERVIEW OF PHOTOSYSTEM II

The O_2-evolving complex (OEC) of photosynthesis resides near the inner side of the thylakoid membrane. Currently, it is believed to be composed of at least four polypeptides: (1) an intrinsic 27–34 kilodalton (kD) polypeptide that may be associated with manganese (Mn); (2) a peripheral 33 kD polypeptide, perhaps also associated with Mn; (3) a peripheral 24 kD polypeptide; and (4) a peripheral 18 kD polypeptide. At least three other polypeptides are associated with this complex to form Photosystem II (PSII): (1) a reaction center (RC) polypeptide, CP-47, of 47–51 kD molecular weight (M_r) containing a chlorophyll *a* (P680), which is the primary electron donor, and a pheophytin (Pheo), which is the primary electron acceptor; also, a bound plastoquinol electron donor (Z), and a bound plastoquinone electron acceptor (Q_A) are suggested to be incorporated in this polypeptide; (2) a 32 kD polypeptide containing a second bound plastoquinone electron acceptor (Q_B); and (3) a 10 kD cytochrome (Cyt) b_{559}, which exists in two forms: a high potential and a low potential.

The primary reaction of PSII is the charge separation: P680·Pheo + $h\nu \rightarrow P680^+Pheo^-$, in which the energy of a photon or an exciton is transformed to achieve the energetically uphill electron transfer. This electron is then transferred to the quinones on the reducing side of PSII. The oxidized reaction center (RC) chlorophyll ($P680^+$) is reduced by removing an electron from Z, an event that can be observed following single-turnover flashes of light. In dark-adapted chloroplasts, electron transfer from Z to $P680^+$ occurs in the first flash in 20–40 ns, but in 400 ns after the second flash. Measurements of the kinetics of this event show oscillations as a function of the flash number. The oxidized species Z^+ accepts electrons from the OEC, which ultimately removes them from water. Electron transfer from the OEC to Z occurs in 50–800 μs, depending upon the redox state of the OEC. The release of O_2 occurs in < 2 ms. The actual chemical steps in O_2 evolution are not yet known, but we do know that this step requires cooperation of four photoacts: O_2 is evolved maximally every fourth flash (with the first maximum being obtained after the third flash, starting from dark-adapted chloroplasts), although protons begin to be released prior to the last step. These reactions require Mn, probably four atoms, and an unknown number of Cl^-. A possible role of Ca^{2+} somewhere on the donor side is now emerging.

Several important observations have recently been made concerning this area of PSII. The dynamic role of Mn has been most clearly shown by the discovery of a low temperature multiline electron spin resonance (ESR) signal, whose amplitude is dependent on the number of flashes given the sample prior to freezing. Chloride may act as an activator of the OEC, perhaps by stabilizing the positive charges on it. However, Cl^--depletion appears to affect mainly the higher oxidation states of the OEC, and interaction of Cl^- with Mn has been suggested by its effect on the multiline Mn signal. A new intermediate, with an ESR signal at $g = 4.1$ ($\Delta H = 360$ Gauss), may act as an electron donor between the S-states (the charge accumulator) and Z.

The intrinsic 34 kD polypeptide, which may sequester or bind Mn, appears to be required for O_2 evolution. The obligatory character of the 24 kD polypeptide is being debated and no apparent role for the 18 kD polypeptide has yet been demonstrated. The observations that the concentration dependence of Cl^- for O_2 evolution is different when 24 kD

Abbreviations: Chl, chlorophyll; Cyt, cytochrome; ESR, electron spin resonance; Kd, kilodalton(s); Mr, molecular weight, OEC, oxygen evolving complex; P680, primary electron donor, a special Chl*a* molecule; Pheo, pheophytin; PQ, plastoquinone; PSII, photosystem II; Q_A, a bound-plastoquinone electron acceptor; Q_B, a second bound-plastoquinone electron acceptor; RC, reaction center; Sn, state of the oxygen evolving complex; Z, a plastoquinol electron donor

†Dedicated to the memory of Warren L. Butler.

*To whom correspondence should be addressed.

polypeptide is absent, that Ca^{2+} may substitute functionally for some of these polypeptides, and that certain treatments are able to release the 33, 24 and 18 kD polypeptides without loss of Mn^{2+} suggest that intricate and important interactions among these components in the OEC still remain to be discovered.

II. INTRODUCTION

A. General

Among others, the early work of Robert Emerson and co-workers on the "Enhancement effect" (see e.g., Emerson, 1958) led to the discovery of two pigment systems and two light reactions in plant photosynthesis (see e.g. Rabinowitch and Govindjee, 1961; Govindjee and Govindjee, 1975; and Govindjee and Whitmarsh, 1982). Duysens *et al.* (1961) and Kok and Hoch (1961) provided the first direct evidence for the two light reaction scheme (Hill and Bendall, 1960). The function of light reaction II is to produce the oxidizing power to oxidize water molecules to molecular O_2, and to produce the reducing power to reduce plastoquinone (PQ) molecules. Protons are released into the interior of the thylakoid vesicles during H_2O-oxidation, and are taken up from the outer suspending medium following the reduction of PQ to plastoquinol (PQH_2). The ΔpH thus produced contributes significantly to the total proton motive force which is used for ATP synthesis. Reviews in the 1980s on various photosystem (PS)II reactions include those by Bouges-Bocquet (1980), Govindjee (1980), Velthuys (1980), Vermaas and Govindjee (1981; electron acceptors); Cramer and Crofts (1982; electron transport); Wydrzynski (1982; O_2 evolution); Amesz (1983; Mn); Inoue *et al.*, eds. (1983; all aspects); Babcock *et al.* (1984; electron donors); Cammarata *et al.* (1984; polypeptides); Govindjee (1984; an overview); Izawa (1985; mechanism of Cl^-); Renger and Govindjee (1985; mechanism of O_2 evolution); Renger and Weiss (1984; mechanism of O_2 evolution); van Gorkom (1985; PSII overview); Ghanotakis and Yocum (1985; polypeptides); and many others, to be cited later, in Sybesma, Ed. (1984; Volume I, Chapter 3).

B. Opening the photosystem II box: the window openers

Photosystem II (PSII) has been a black box for a long time. We do not yet understand the molecular mechanism of O_2 evolution, but several researchers have managed to chisel a few openings, and the crowds are now flocking around the new peep holes or windows into the PSII box (Fig. 1). The following list is incomplete because deciding which work has opened a window, or made only a tiny, but very important hole, is not always easy and clear, and depends upon the perspective of the authors and how they perceive the problems at the time of this review.

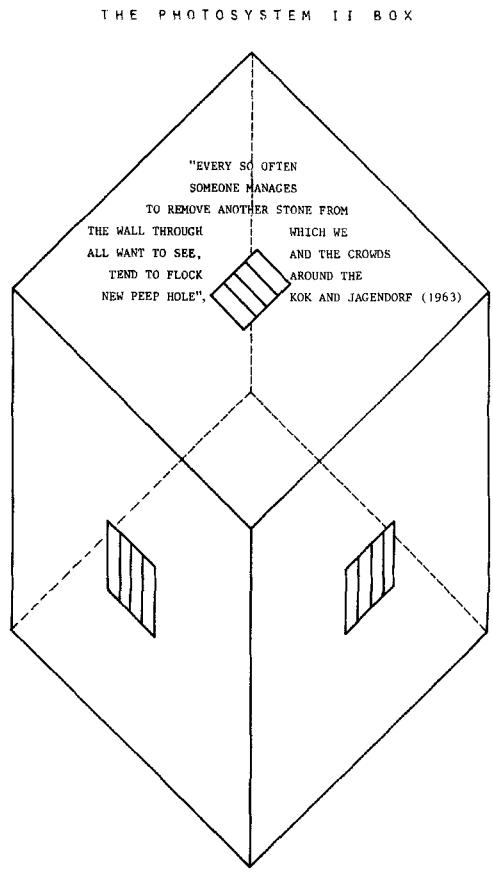


Figure 1. The "Black-Box" of Photosystem II. (Govindjee, presented at the *6th International Congress of Photosynthesis*, Brussels, 1983, unpublished.)

A major window into PSII was opened when Joliot *et al.* (1969) discovered that the O_2 yield/flash as a function of flash number, with a suitable dark-time between flashes, oscillated with a period of 4, with the first maximum on the 3rd flash. Kok *et al.* (1970) provided the insight necessary to understand this phenomenon: they proposed that the OEC exists in two redox states S_0 (25%) and S_1 (75%) in darkness, and that these undergo synchronized sequential one-electron oxidations as follows (see e.g., Wydrzynski, 1982):

$$75\%\ S_1 \xrightarrow{^1h\nu} S_2 \xrightarrow{^2h\nu} S_3 \xrightarrow{^3h\nu} S_4 \xrightarrow{O_2} S_0 \xrightarrow{^4h\nu} S_1 \quad (1a)$$

$$25\%\ S_0 \xrightarrow{^1h\nu} S_1 \xrightarrow{^2h\nu} S_2 \xrightarrow{^3h\nu} S_3 \xrightarrow{^4h\nu} S_4 \xrightarrow{O_2} S_0 \quad (1b)$$

To explain the observed damping of the oscillations, probability factors for "misses" (α) and double advancements (β) were included.

Further insight into the functioning of PSII was provided when Velthuys and Amesz (1974) and Bouges-Bocquet (1973) independently showed that Q_A, the first quinone electron acceptor of PSII, transferred electrons to a two electron acceptor, Q_B. These reactions, also in two synchronous channels, are currently written as (see e.g. Cramer and Crofts, 1982:

$$70\%\ Q_A\ Q_B \xrightarrow{^1h\nu} Q_A^-\ Q_B^- \longrightarrow Q_A\ Q_B^-\ (H+) \xrightarrow{^2h\nu} \quad (\text{stable};\ H+)$$

$$Q_A^-\ Q_B^-\ (H^+) \rightarrow\rightarrow Q_A\ Q_BH_2 \rightarrow Q_A\ Q_B \quad (2a)$$

(H^+; PQ PQH_2)

$$30\%\ Q_A\ Q_B^-\ (H^+) \xrightarrow{^1h\nu} Q_A^-\ Q_B^-\ (H^+) \rightarrow\rightarrow \quad (H+)$$

$$Q_A\ Q_BH_2 \longrightarrow Q_A\ Q_B \xrightarrow{^2h\nu} Q_A\ Q_B \longrightarrow \quad (\text{PQ } PQH_2;\ H+)$$

$$Q_A\ Q_B^-\ (H^+) \quad (\text{stable}) \qquad (2b)$$

The above model is an excellent working hypothesis, although the sites where protonation occurs are not yet known. The reducing power for Q_A^- formation and the oxidizing power for $S_n \rightarrow S_{n+1}$ transitions are provided by light energy at the RC Chl *a* molecule P680, discovered by Döring *et al.* (1967). It took several years before the window of primary photochemical reactions of PSII was opened further; the discovery that $P680^+$ recovered its electron from another donor Z (it has also been called Z_1) within 50 ns, at least after the first flash, was most clearly made by van Best and Mathis (1978). More recently, Brettel *et al.* (1984) have shown that $P680^+$ is reduced in 20 ns during transitions from $S_0 \rightarrow S_1$, and $S_1 \rightarrow S_2$, and in 250–300 ns during transitions from $S_2 \rightarrow S_3$, and from $S_3 \rightarrow (S_4) \rightarrow S_0$. The proton release pattern of 1,0,1,2, originally measured by Fowler (1977) with a sensitive pH electrode, during transitions from $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow (S_4) \rightarrow S_0$, respectively, is consistent with these observations. In this model S_2 and S_3 are positively charged, so that electron flow to $P680^+$ may be slower because of coulombic and other interactions.

On the electron acceptor side, however, the discovery of pheophytin (Pheo) as an intermediate electron acceptor by Klimov *et al.* (1977) (see e.g. Klimov and Krasnovskii, 1981; Klimov, 1984) opened to view the primary photochemical reaction of PSII (see e.g. Parson and Ke, 1982; Govindjee, 1984):

$$P680\ Pheo + h\nu\ (\text{or exciton}) \rightarrow P680^*\ Pheo \quad (3a)$$

$$P680^*Pheo \rightarrow P680^+Pheo^- \quad (3b)$$

$$P680^+Pheo^- + Q_A \rightarrow P680^+Pheo + Q_A^- \quad (3c)$$

$$P680^+Pheo + Z_1 \rightarrow P680\ Pheo + Z_1^+ \quad (3d)$$

In the reaction 3b, the light energy is transformed to redox energy, followed by charge separation across the membrane.

The nature and kinetics of Z_1 (often labeled as Z or D_1) has been most extensively studied by G. Babcock and coworkers (see e.g. Babcock *et al.* 1976, 1984) by ESR spectroscopy; these workers have suggested it to be a plastoquinol molecule.

Several researchers have been busy trying to get a glimpse of the biochemical machinery of O_2 evolution. The work of Spector and Winget (1980) on the 65 kD polypeptide in the O_2-evolving enzyme has not been substantiated (see e.g. Cammarata *et al.*, 1984), nor has the dynamic role of Mn by flash-induced NMR observations of Wydrzynski *et al.* (1976) (*cf.* e.g. Govindjee *et al.*, 1977; Khanna *et al.*, 1981a; and Robinson *et al.*, 1981). Nevertheless these investigations have provided significant new impetus to this field. An insight into the functioning of Mn was provided by Dismukes and Siderer (1980, 1981) who succeeded in showing a relationship of the S-states to the changes in the 18-line Mn ESR signal at low temperature. The first application of Cl-NMR to monitor the binding of Cl^- to thylakoids was by Critchley *et al.* (1982) and Baianu *et al.* (1984).

Historical developments in the area of polypeptides associated with the OEC are as follows. Kuwabara and Murata (1979) were the first to isolate a 33–34 kD polypeptide, later discovered to be related to the OEC. Metz and Bishop (1980) and Metz *et al.* (1980) discovered that a 34 kD polypeptide—now suggested to be different from that of Kuwabara and Murata—was absent in a *Scenedesmus* mutant that lacked O_2 evolution and had a low level of Mn. Two years later Åkerlund *et al.* (1982), using inside-out vesicles, observed that salt-washing releases a 23 kD and an 18 kD polypeptide, and that this is accompanied by a decrease in O_2 evolution activity; readdition of the 23 kD polypeptide reconstituted the system.

The isolation and refinement of a more manageable biological system has proven to be as much of a window opener as the discovery of a new phenomenon or component, or the development of an analytical method. Boardman and Anderson (1964) had succeeded in physically separating O_2-evolving

PSII and NADP-reducing PSI activities by digitonin treatment of thylakoids; and Briantais (1969) obtained, by Triton X-100 treatment, an O_2-evolving PSII preparation. Recent progress in PSII biochemistry has been made possible by the development of such methods to prepare (a) highly active and stable O_2-evolving PSII preparations (Stewart and Bendall, 1979; Berthold *et al.*, 1981; England and Evans, 1981; Yamamoto *et al.*, 1981, 1982; Klimov *et al.*, 1982; Kuwabara and Murata, 1982, 1983; see e.g. Dunahay *et al.*, 1983 for a comparison of some of these methods; Koike and Inoue, 1983); and (b) inside-out vesicles (Åkerlund and Jansson, 1981; Åkerlund *et al.*, 1982). On the other hand, the study of PSII photochemistry has benefited most from the simple non-O_2-evolving RC complex preparations, e.g. those of Satoh and Butler (1978; also see Satoh, 1982), Diner and Wollman (1980), Nakatani (1983a), Yamagishi and Katoh (1983a,b), and Green and Camm (1984).

The above list would remain highly incomplete without the mention of the early pioneering work of Vernon and co-workers and by Wessels and co-workers on PSII preparations (see e.g. Vernon *et al.*, 1969; and Wessels *et al.*, 1973).

In what follows, we shall summarize our current view of the oxygen-evolving complex in relation to the electron donor side of PSII, i.e. the structure and function of the components involved in electron flow from H_2O to $P680^+$ (see Fig. 2). We shall specifically present the current state of knowledge regarding the localization of the components of the OEC (the polypeptides, manganese, chloride, calcium, and other ions) and their function (Section III); a discussion of the mechanism of oxygen evolution (Section IV); and a brief description of the other components on the donor side of PSII (electron donor Z; a component with an ESR signal at $g = 4.1$; cytochrome b_{559}; etc.) (Section V).

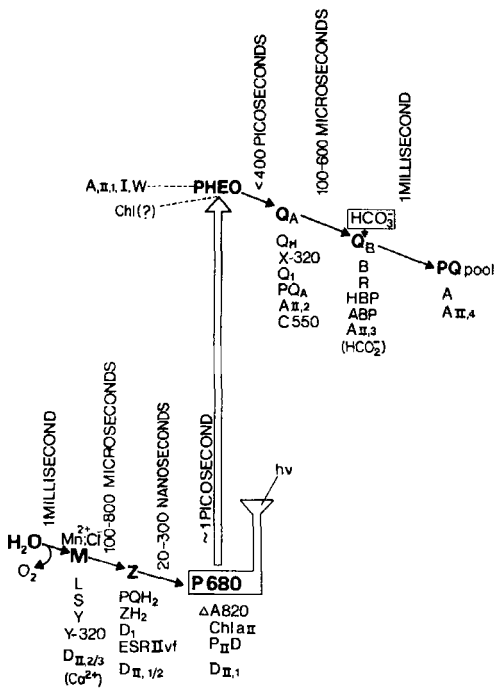


Figure 2. A contemporary scheme of electron flow in Photosystem II showing the major components in bold. Estimated or directly measured times for various reactions are also indicated. The symbols (from left-to-right) are as defined below. (For the benefit of the readers in this field of research, all equivalent symbols, used in the literature, are also defined.) "M" stands for an all-purpose complex, the "M complex" or the oxygen evolving complex (OEC), but it specifically reflects the electron carriers that undergo redox reactions and charge accumulation—it includes the particular component M (Renger), i.e. M of Renger, and the component L (Velthuys); the symbol "S" was coined by Kok to express the redox state of the oxygen evolving system, and to express the charge accumulation process; it is still the favored symbol for the "M complex", but since one often speaks about the deactivation of the "S" states that involve all components including Q_A, we prefer to use a separate symbol for the charge accumulator; Witt and Renger have consistently used the symbol Y for the OEC, whereas Lavorel uses Y for the immediate electron donor to $P680^+$; thus Y (Renger) ≠ Y (Lavorel); Y-320 stands for Y (Renger) having one of its absorption bands at 320 nm; D_{II}, 2/3 stands for the electron donor of Photosystem II (PSII), which is the second or the third electron donor depending upon which donor is counted as the first electron donor; the present authors do not favor the use of this symbol; Mn^{2+}, Cl^- (or other anions) and several polypeptides (see Fig. 3) are parts of the "M complex" or the OEC. The next major symbol Z (used originally by Rabinowitch) stands for the immediate electron donor to $P680^+$; it may be a plastoquinol (PQH_2) molecule and is thus, represented as ZH_2; the symbol D_1 (first donor to $P680^+$) has also been used for Z, although several investigators use "D" for an auxiliary donor on a side path; $ESRII_{vf}$ (Babcock) stands for an "Electron Spin Resonance Signal II very fast" which is a monitor of Z^+; the present authors prefer to use Z_1 (as did Jursinic and Govindjee) for Z because there is the possibility of another intermediate between the "M complex" and Z—which we suggest be called Z_2; the problem of nomenclature is not solved since Bouges-Bocquet used Z_1 and Z_2 for two parallel donors to $P680^+$. The reaction center chlorophyll *a* is labelled as *P680*, where P stands for pigment at the photochemical reaction center and 680 for its red-most

III. THE LOCATION AND COMPOSITION OF THE OXYGEN-EVOLVING COMPLEX

A. Localization of components

The oxygen evolving apparatus that produces O_2 and H^+s from H_2O is thought to be located on the inner side of the thylakoid vesicle for several reasons. Among these are: (a) the H^+s released during these reactions can be monitored from the outside only when a leakage pathway is provided, as, for example, by the addition of gramicidin (Fowler and Kok, 1974); (b) an antibody that specifically inhibits the O_2-evolving system shows a very weak inhibitory effect (~10–15%) with thylakoids, which is doubled (~30%) with sheet-like photosystem II particles

(Braun-Zilinskas and Govindjee, 1972; Zilinskas and Govindjee, 1974); (c) results from ^{17}O-measurements in thylakoids and disrupted thylakoids (Wydrzynski *et al.*, 1978); and (d) experiments with trypsin treatment of chloroplasts suggest that the OEC is toward the inner side of the membrane (Renger, 1976). For other arguments supporting the localization of the O_2-evolving system on the inner side of thylakoid membranes, see Diner and Joliot (1977).

A definite localization of the polypeptides associated with O_2 evolution on the inner side of the membrane came from the work of Åkerlund *et al.* (1982) on inside-out vesicles. A salt-wash of inside-out vesicles, but not of right-side out vesicles, released the 24 kD and 18 kD polypeptides; the polypeptides could be rebound only to the inside-out vesicles. This work has been confirmed by specific heavy agglutination of inside-out vesicles, but not of right-side out vesicles, with specific antibodies against these two polypeptides (Larsson *et al.*, 1984).

The arrangement of the various polypeptides with respect to each other has also been explored. Binding studies of the 18 kD polypeptide with and without the 24 kD polypeptide bound to the membrane have revealed that the amount of the 18 kD polypeptide that rebinds is greater if the 24 kD polypeptide is already bound. The 24 kD polypeptide binds better to the membrane if the 33 kD polypeptide is already present (see e.g. Larsson *et al.*, 1984). This and the earlier studies of Murata *et al.* (1983) on the sequential and independent binding of these polypeptides on PS II particles (which have their O_2-evolving side exposed to the medium, see e.g. Murata *et al.*, 1984a) have led to the wide-angle picture shown in Fig. 3. It has been assumed in this picture, however, that there are two 33–34 kD polypeptides, one of which is hydrophilic (as evidenced by its polarity index and amino acid composition) and extrinsic (because the ease with which some workers have extracted it by washing with high concentration of divalent salts, or by osmotic shock). The location of this hydrophilic 33 kD protein shown in the diagram is purely arbitrary.

In maize PSII preparations, Bricker *et al.* (1982) have shown the existence of an intrinsic 34 kD protein in addition to an extrinsic 33 kD protein. The location of the intrinsic 34 kD polypeptide is also uncertain. It is possible that a Mn-containing 34 kD polypeptide is located within the membrane and that this polypeptide is the one that is missing in a *Scenedesmus* mutant (see e.g. Metz and Bishop, 1980; Metz *et al.*, 1980) which lacks O_2 evolving capacity and Mn. This idea has recently been confirmed (Metz and Seibert, 1984).

The apparent multiplicity of 33–34 D polypeptides and the variability in their extraction are obvious: Washing PSII particles or inside-out vesicles with 250 m*M* NaCl, which releases the 18 and 24 kD polypeptides, does not extract any 33 kD polypeptide. But very high concentrations of divalent salts (see e.g. Ono and Inoue, 1983a) extract these two polypeptides along with a 33 kD polypeptide. On the other hand, urea is able to extract a 33 kD polypeptide, but only a small fraction of the 18 and 24 kD polypeptides (Murata *et al.*, 1983); and Møller *et al.* (1984) have shown that 100 m*M* Tris specifically releases the 33 kD polypeptide.

The location of the Mn binding site or sites is not yet clear. The 33 kD polypeptide isolated by Ono and Inoue (1983a) contains no Mn. But Dismukes *et al.* (1983) have found that osmotic shock can release a 34 kD protein from spinach grana thylakoids or O_2-evolving PS II particles that retains non-dialyzable Mn if isolated in the presence of mild oxidizing agents. Furthermore, Yamamoto and Nishimura (1984) have isolated from PS II preparations a Mn-containing 34 kD protein that co-isolates with a 31 kD protein by a method using butanol. Thus, there appear to be at least two different 33–34 kD polypeptides in the OEC, one with Mn and another without Mn; or alternatively, it may be that only certain conditions extract Mn along with the protein, so that their association may not be significant. Although only future experiments will answer these questions, most of the data, taken together, suggest that there is indeed a Mn-containing protein(s) which participate(s) in O_2 evolution, because the Mn that appears to function in the water-splitting reaction is

---

absorbance band at 680 nm; in the oxidized state ($P680^+$), there is a positive absorbance change (ΔA) around 820 nm—thus, it is also called $\Delta A820$; $Chla_{II}$ (Witt) stands for P680, but, it is not favored by us, because it has also been used by other investigators for one of the antenna chlorophyll *a* complexes of PSII (e.g. core $Chla_{II}$), and because it implies that we know its chemical structure. *Pheo* stands for pheophytin; $A_{II,1}$ stands for the first electron acceptor of PSII; I stands for "Intermediate" electron acceptor because Q_A (=Q) was discovered earlier and was named the primary electron acceptor of PSII; W(Duysens) is a kinetic component suggested to precede Q_A. Q_A is the first bound-plastoquinone electron acceptor of photosystem II; it is usually referred to as "Q" (Duysens); it is suggested that Q_A is the high potential form of Q (Q_H)—this is, however, controversial; X-320 (Stiehl and Witt) represents the semiquinone form of Q (Q^-)—an intermeidate X having one of its absorbance bands at 320 nm; Q_1 (Joliot) is in the main pathway, and thus, $Q_A = Q_1$; we do not favor the symbol PQ_A for Q_A since it may be confused with the chemical species (PQ-A, PQ-B, PQ-C, etc.); C550 (Knaff and Arnon) is the symbol for a component with one of its absorbance changes at 550 nm—it is due to a shift (not a bleaching) in absorbance in a pheophytin molecule when Q_A is reduced to Q_A^- and the pheophytin molecule experiences an electric field (van Gorkom; Klimov). Q_B is the second bound-plastoquinone electron acceptor of photosystem II; it is the two-electron acceptor, and it is bound, although weakly, on a 32 kD polypeptide; it is equivalent to the symbol B (Bouges-Bocquet) and R (Velthuys and Amesz); the protein is referred to as the herbicide-binding protein (HBP), or as the anion-binding protein (ABP), as both HCO_2^- and HCO_3^- seem to bind to it; it can also be called BBP (bicarbonate-binding protein), although direct evidence does not exist. *PQ* pool is the free plastoquinone (PQ) molecules—in the early days, it was referred to as the "A" pool. (Modified after Govindjee, 1984.)

almost certainly bound (see also Section III.C). Further evidence for a Mn bound in a specific site is the fact that the S_2 multiline ESR signal is oriented in the membrane (A. W. Rutherford, personal communication).

If we accept that there are two 33 kD proteins per RC, an interesting question arises as to how many copies of the 24 and 18 kD polypeptides are present. Cammarata *et al.* (1984) argue for 2 copies of each. In their model, 4 Mn atoms lie in a cleft, bound to two-34 kD polypeptides and to the "Z" region of the RC complex (CP 47). This would also be in agreement with the stoichiometry of 2 Mn/34 kD polypeptide suggested by Dismukes *et al.* (1983). The model in Fig. 3 is not specific on this point, although we do show 2 Mn/34 kD polypeptide. The stoichiometry of 33 kD:24 kD:18 kD polypeptide is not settled yet. Murata *et al.* (1983, 1984a,b) have observed a ratio of 1:1:1 per RC, whereas Larsson *et al.* (1984) favor 2 copies of each per RC. What is clear from the work of Ljungsberg *et al.* (1984) is that the large complex to which the 33, 22 and 18 kD polypeptides are attached has some hydrophobic polypeptides of M_r 27 kD and 22 kD, along with the RC and antenna complexes (47 and 43 kD) and Cyt b_{559} (10 kD). An association of the 23 kD polypeptide with Cyt b_{559} has also been suggested because the release and rebinding of the 23 kD protein affects the high potential (HP) Cyt b_{559} (Larsson *et al.*, 1984). As mentioned earlier, Yamamoto and Nishimura (1984) have reported a 31 kD polypeptide that co-isolates with their 34 kD polypeptide—whether Mn is on the 31 or the 34 kD protein is not yet known.

It has been demonstrated in a number of ways that chloride is intimately involved in O_2 evolution (see e.g. Govindjee *et al.*, 1983; Izawa *et al.*, 1983). However, it is not yet known whether Cl^- is associated with the same protein that binds Mn. Brudvig *et al.* (1984) have shown that Cl^--depletion causes gross changes in the multiline ESR signal of Mn (see section III.C). In the model of Fig. 3, Cl^- ions are shown to be in a cleft (or a pocket) where H_2O

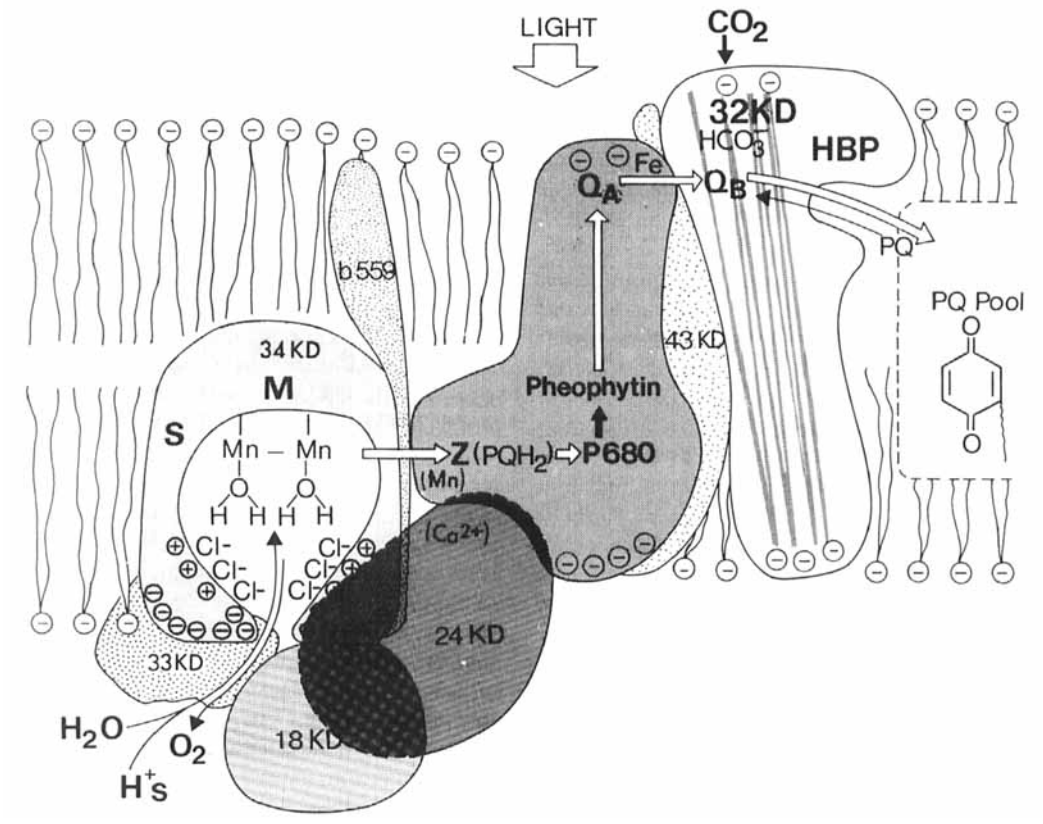


Figure 3. A working model for the organization of Photosystem II components in the thylakoid membrane. The upper side of the diagram is the outer side of the membrane, and the lower side the lumen side. KD stands for kilo Daltons, b-559 for cytochrome *b*-559; other symbols have the same meaning as that described in the legend of Fig. 2. The P680-containing polypeptide has a Mr of 47–51 kD. See text for details. (Modified after Govindjee, 1984.)

Table 1. Summary of 18, 24 and 33 kD polypeptides

	33 kD	24 kD	18 kD
Molecular mass			
SDS PAGE*†	33	23–24	16–18
Gel filtration*	34	23	23
Isoelectric point	5.2*†	6.5*; 7.3†	9.5*; 8.5†
Absorption Maximum (λ_{max})	276 nm*, 277 nm†	277 nm*†	277 nm*†
Extinction Coefficient,	20000*	26000†	13000†
M^{-1} cm^{-1} (at λ_{max})	18000†	24000†	12000†
Polarity index	49%*†	49%*†	52%*; 49%†
Extracted with 250 m*M* NaCl*†	No	Yes	Yes
Extracted with 0.8 *M* Tris, pH 8.0*†	Yes	Yes	Yes
Relation with Mn	Yes	No	No
Extracted with 2.5 *M* Urea*	Yes	No	No
Comments on amino acid composition* (see Table 2)	Lacks histidine; is lysine-rich	Rich in aspartic acid, asparagine, lysine and glycine	Rich in leucine; poor in glycine; lacks methionine; contains lysine
Cysteine residue†	3–4	1	1

*Kuwabara and Murata, 1983; Murata *et al.*, 1983, 1984a,b; †Jansson *et al.*, 1983; Jansson, 1984).

oxidation and H^+ release occurs—this picture is based on a model by Homann *et al.* (1983). The release of H^+s, and the reversible binding of Cl^- may occur in the same location.

Research in this area of the OEC is still at the stage of isolating and characterizing the polypeptide components. Soon, however, it should be possible to obtain their complete physical and chemical characteristics, to assign a definite function to each of these polypeptides and to determine their spatial relationships. Some of the attempts at determining function are described in the next section.

B. The polypeptides and their involvement in O_2-evolution

The three polypeptides that are best known thus far have M_r of 32–34 kD, 23–24 kD and 15–18 kD; for convenience they (have been and) will be referred to as 33, 24 and 18 kD polypeptides. Table 1 summarizes some of the information available on them. The major points are: (a) all three are hydrophilic (polarity index is 48–49%)—although this does not mean that they may not have "hydrophobic patches"; (b) determination of their isoelectric points show that the 33 kD polypeptide is acidic (it may be H-bonded to the membrane); the 24 kD polypeptide is neutral or slightly acidic; and the 18 kD polypeptide is basic; (c) none of them contains any metal ions and none has any absorption in the visible region; the absorption maxima are at 275–276 nm; (d) they are monomeric; and (e) they are peripherally located on the lumen side of thylakoids, as discussed in section III.A. Table 2 shows their amino acid compositions; they all contain lysine, but the 33 kD polypeptide lacks histidine, whereas the 18 kD polypeptide lacks methionine. The 33 kD polypeptide has 29 lysine residues as opposed to none in the 33 kD Q_B apoprotein.

The details in Tables 1 and 2 are in general agreement with those obtained by Yamamoto *et al.* (1983). The most striking discrepancy is in the considerably higher values for tyrosine obtained by the latter authors in all the polypeptides, and the difference in the contents of arginine and phenylalanine in their 24 kD polypeptide.

There had been a general agreement that the 33 kD polypeptide is necessary for O_2 evolution because (a) removal of all 33 kD protein leads to a total loss of O_2 evolution (see e.g. Murata *et al.*, 1983); and (b) when the 18 kD and 24 kD polypeptides are removed, membranes containing the 33 kD polypeptide still evolve substantial amounts of oxygen when Ca^{2+} and Cl^- ions are present (Nakatani, 1984). Experiments with removal and reinsertion of the 33 kD polypeptide have met with only partial success, although a correlation between the rebinding of the 33 kD polypeptide and a partial restoration of O_2 evolution has been observed by Ono and Inoue (1984a). The 33 kD polypeptide has been reported to be associated with 2 Mn atoms (see sections III.A, III.C and IV.C, and Abramowicz and Dismukes (1984)) although direct proof is still lacking, especially since Ono and Inoue (1984b) have succeeded in washing out a 33 kD polypeptide using high concentrations of divalent cations without the release of any Mn. Franzen and Andreasson (1984) have also reported that the 33 kD polypeptide is not responsible for Mn binding in the OEC. Recently, Ono and Inoue (1984c) have even removed this 33 kD polypeptide and demonstrated O_2 evolution with an

optimal concentration of $CaCl_2$ present. It appears to us that Mn may be associated with this protein, but not bound inside it. The release of both Mn and the protein may lead to loss of O_2 evolution.

The role of the 24 kD polypeptide is certainly controversial. There are two opposing views. In one view, this polypeptide plays an obligatory role in O_2 evolution because its removal leads to an almost complete inhibition of O_2 evolving capacity (see e.g. Larsson *et al.*, 1984). Rebinding of the 24 kD polypeptide restores this capacity. Åkerlund *et al.* (1984a) have further shown, based on experiments with single turnover flashes, that the 24 kD protein supports the functional connection of the OEC with RC II. Studies of the kinetics of electron donation to $P680^+$ in salt-washed inside-out thylakoids also indicate that this protein functions on the oxidizing side of PS II (Åkerlund *et al.*, 1984b). Åkerlund (1984) has proposed, based on the O_2 flash-yield pattern in salt-washed membranes, that the release of 24 kD protein from inside-out thylakoids inhibits the conversion of S_2 to S_3, but does not inhibit the S_0 to S_1 and S_1 to S_2 transitions. Wensink *et al.* (1984) have also found that removal of the 24 and 18 kD polypeptides reduces the efficiency of the higher S-state transitions. These ideas need to be tested further because of their possible connection with the Cl^- effect (see e.g. Wensink *et al.*, 1984; and section III.A, III.D and IV.D). Andersson *et al.* (1984) have shown that addition of the 24 kD polypeptide to membranes depleted of this protein lowers their Cl^- concentration requirement for optimal O_2 evolution. It has also been suggested recently (see Itoh *et al.*, 1984; Theg *et al.*, 1984) that Cl^- depletion specifically blocks the higher S-state transitions. Thus, it is possible that the 24 kD protein is needed to keep Cl^- in its proper place, and that this is the reason for its requirement. Furthermore, the effect of the 24 kD protein on the S-states may simply be due to the effect of Cl^- on the S-states.

In the other view, the 24 kD polypeptide has only a stimulatory role because release of *all* of the 24 kD protein was found to diminish O_2-evolution by only 50% (Murata *et al.*, 1983; Murata *et al.*, 1984a). As noted above, O_2 evolution can proceed without this component when enough Ca^{2+} and Cl^- are present.

There is much evidence that the 18 kD protein does not play a crucial role in O_2 evolution (at least in PSII particles or inside-out vesicles), because its complete absence has no effect on O_2 evolution (Murata *et al.*, 1983; Larsson *et al.*, 1984). In cholate-treated particles, however, Toyoshima *et al.* (1983) have observed a stimulation of O_2 evolution by the 18 kD protein. Furthermore, Izawa *et al.* (1984) have shown the importance of *both* 18 kD and 24 kD polypeptides, since only in the presence of a certain combination of the two is maximum O_2 evolution obtained.

C. *Manganese*

There is substantial evidence (Cheniae, 1980; Sauer, 1980) that Mn plays an essential role in photosynthetic O_2 evolution in green plants. There are several pools of bound Mn in chloroplasts (Cheniae and Martin, 1970; Sharp and Yocum, 1980; Khanna *et al.*, 1981b), but only one of them is functional in the O_2 evolving process. These pools are often referred to as (1) a "very-loosely" (or weakly)-bound pool, which is non-functional in O_2

Table 2. Amino acid composition of 33, 24 and 18 kD polypeptides

	33 kD		24 kD		18 kD	
Lys	9.2*	(8.3)†	10.9	(10.6)	9.2	(9.3)
His	0.0	(0.1)	0.8	(0.6)	0.9	(0.9)
Arg	2.7	(2.4)	1.7	(1.7)	5.6	(5.4)
Asx (Asp/Asn.)	8.8	(8.9)	11.7	(11.1)	11.2	(10.6)
Glx (Glu/Gln.)	13.2	(13.5)	9.1	(9.2)	11.1	(11.7)
Thr	7.7	(7.8)	5.9	(6.0)	5.3	(5.3)
Ser	7.4	(7.2)	8.6	(9.0)	8.6	(7.8)
Pro	6.1	(5.9)	4.6	(4.5)	8.8	(8.5)
Gly	12.3	(12.0)	10.1	(9.7)	5.1	(5.2)
Ala	6.0	(6.6)	8.3	(8.0)	9.0	(8.9)
Val	7.2	(6.7)	7.9	(8.5)	3.7	(4.4)
Met	0.4	(0.5)	0.7	(0.6)	0.0	(0.1)
Ile	3.0	(3.2)	2.0	(2.1)	3.8	(4.4)
Leu	7.1	(7.1)	6.0	(5.8)	11.4	(11.6)
Tyr	3.2	(2.8)	4.2	(4.3)	3.6	(2.6)
Phe	5.3	(5.3)	6.6	(6.5)	2.1	(2.2)
Trp	0.4	(0.2)	1.0	(0.8)	0.6	(0.2)
(Cys)		(1.3)		(0.7)		(0.7)
[Total residues/protein (mole/mole)]	268		213		126	

*Data from Kuwabara and Murata (1984); †Data from Jansson (1984); confirmed by Møller *et al.* (1984).

evolution and is removed by EDTA or high-salt washing (Radmer and Cheniae, 1977; Yocum *et al.*, 1981; Khanna *et al.*, 1981b); (2) a "loosely" (or strongly)-bound pool (⅔ of the rest) which is related to O_2 evolution and is released by NH_2OH, alkaline Tris, and heat treatment (Radmer and Cheniae, 1977; Yocum *et al.*, 1981; Khanna *et al.*, 1983); and (3) a "tightly" (or very strongly)-bound pool, some of which may be removed by harsher treatments under (2), but whose function is not clear; it may be related to a Mn-requirement for electron donation reactions involving Z, and/or it may play a key role in maintaining the compactness and integrity of chloroplasts (Khanna *et al.*, 1981b). The OEC is assumed to consist of a Mn-containing protein whose metal centers are associated with a specific coordination shell and which act as functional redox groups (see literature cited in Govindjee, 1984; Renger and Govindjee, 1985).

The properties of Mn which enable it to oxidize H_2O are its higher oxidation states (Mn^{3+} and Mn^{4+}) and variable coordination numbers (Cheniae, 1970; Lawrence and Sawyer, 1978). Thus, it is quite possible that Mn complexes act as charge-accumulating intermediates during H_2O oxidation (Radmer and Cheniae, 1977), and that a specific coordination shell is constructed around the Mn ions in order to stabilize the highly reactive intermediates (Renger, 1978). A unique property of Mn in its reaction with H_2O is that the thermal equilibrium constant in the reaction: $M^{3+}(H_2O) + H_2O \rightarrow M^{3+}(OH)^- + H_3O^+$ is much larger (by two orders of magnitude), compared to that for other transition metal ions (Wells, 1965). $Mn^{3+}OH^-$ is, therefore, quite stable in aqueous solution. Another point to remember is that the spin state of molecular O_2 is triplet, while that of H_2O is singlet. Thus, although the rate limiting process in each $S_i \rightarrow S_{i+1}$ transition appears to be the electron transfer from Mn to Z (van Gorkom, 1985), in order for H_2O oxidation to proceed smoothly, electron spin conservation has to be satisfied by coupling of the transition metal ion with any unpaired electrons (Malmström, 1982). Furthermore, bimetallic centers in many transition metal complexes may bring two oxygen atoms into close enough proximity to form an O–O bond, since such centers reversibly bind molecular oxygen (Lawrence and Sawyer, 1978; Jones *et al.*, 1979). This is convenient for H_2O oxidation, since a four electron oxidation of two water molecules, which can bypass the thermodynamically unfavorable intermediates, can occur through the bimetallic complex formation (see e.g. Radmer and Cheniae, 1977). Although it has not yet been resolved how the Mn ions function in the H_2O oxidation process in chloroplasts, it is evident from many experiments that the above-mentioned properties of Mn probably are involved.

ESR measurements of Mn ions that are released by treatment of chloroplasts with NH_2OH or Tris-washing suggest that four Mn atoms per PSII RC are necessary for O_2 evolution (Yocum *et al.*, 1981). It has been reported that the addition of only 2 Mn atoms per PSII is enough to restore completely the capacity for PSII donor reactions in Mn-deficient samples (Klimov *et al.*, 1982). First, these experiments do not necessarily provide information on the number of Mn required for O_2 evolution. Secondly, Klimov *et al.* showed that normally 4 Mn atoms per PSII RC is necessary out of which 2 can be replaced, although with less efficiency, by other metal cations. A heterogeneity of the 4 Mn pool has also been recently shown by Ono and Inoue (1984c); it is suggested that there are 2 subpools of 2 Mn each, and both are required for maximal O_2 evolution. The problem of the minimum number of Mn atoms necessary for one unit of the OEC has long been actively debated. Renger and Weiss (1984), Murata *et al.* (1984a), Miller and Cox (1984), Abramowicz *et al.* (1984), and Kusunoki (1984) appear to favor two Mn, while Govindjee (1984), Yocum (1984), and Cammarata *et al.* (1984) favor four Mn. A part of this controversy may be apparent only because some of the data deal with the number of Mn atoms per 33 kD polypeptide (Murata *et al.*, 1984; Abramowicz *et al.*, 1984) and this would translate into 4 Mn per OEC if there are two copies of this protein (see section III.A).

Although the requirement for Mn in the four-electron oxidation of two water molecules to form molecular O_2 is well established (see the review by Renger and Govindjee, 1985), we do not know the chemical form of Mn in each oxidation step. One of the reasons for this ignorance is that the functional Mn *in situ* is ESR silent, at least at physiological temperatures. There are several methods by which the electronic state corresponding to each S-state had been surmised (see review by Amesz, 1983). For example, the functional Mn has been extracted from the H_2O oxidizing enzyme by extensive heat-treatment. The spectrum of the extracted Mn is quite similar to that of $[Mn(H_2O)_6]^{2+}$ (Lozier *et al.*, 1971; Blankenship *et al.*, 1975). The amount of released Mn oscillates with a period of four when the samples are given a series of light flashes prior to heating, and the observed pattern is similar, although not identical, to the O_2 yield flash pattern (Wydrzynski and Sauer, 1980). This result suggests that a cycling of Mn oxidation states is involved in the O_2 evolving process. A direct demonstration of Mn involvement has been through low temperature ESR spectroscopy. An eighteen-line, light-induced ESR signal observed at low temperatures (4–8 K) in spinach chloroplasts has been assigned to Mn on the basis of its hyperfine structure (Dismukes and Siderer, 1981; Hansson and Andreasson, 1982). A flash pattern for the formation of this signal has been observed (Fig. 4), with maxima after the 1st and 5th flashes (Dismukes and Siderer, 1981; Zimmerman and Rutherford, 1984). The peak corresponds to the creation of the S_2 state (Dismukes and Siderer, 1981; Brudvig *et*

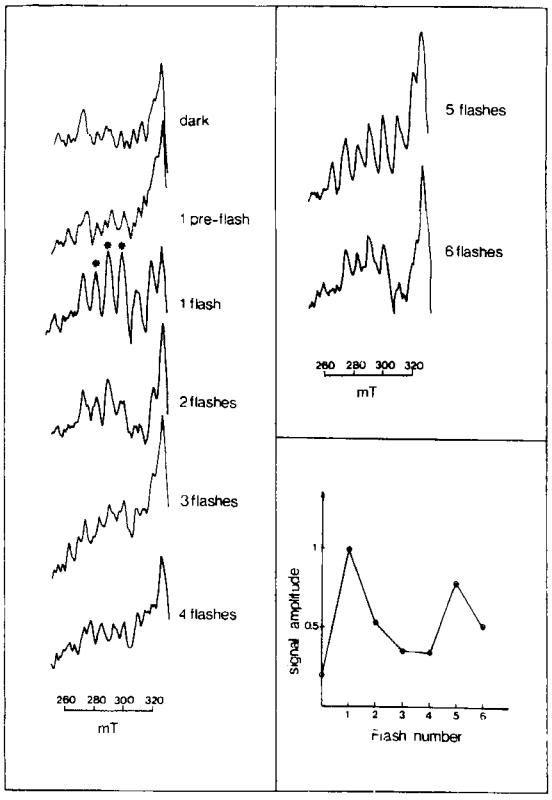


Figure 4. Multiline ESR signals produced by a series of light flashes. Samples of Photosystem II membranes were given one preflash at 20°C, dark-adapted for 15 min, and then illuminated by 1–6 flashes at 0°C. Temperature of measurement, 8 K; frequency, 9.44 GHz; microwave power, 31.5 mW; modulation amplitude, 25 G. The amplitudes of the signal, measured as the heights of the three downfield peaks marked by *, as a function of flash number are shown in the lower right-hand panel. (After J. L. Zimmerman and A. W. Rutherford, 1984, personal communication.)

al., 1983). Brudvig (1984) has shown that this S_2 state in the active OEC is created maximally at 170 K, whereas it is created maximally at 200 K in the resting OEC; and there are distinct differences in the lineshapes of their ESR signals. The oxidation state of Mn corresponding to the S_2 state has been interpreted in terms of a weakly exchange-coupled Mn(III)-Mn(IV) binuclear or $Mn(III)_3Mn(IV)$ tetranuclear manganese center (Dismukes *et al.*, 1982).

Based on a comparison with model compounds, X-ray-absorption-edge measurements and EXAFS (Extended X-ray Absorption Fine Structure) studies (Kirby *et al.*, 1981), it was suggested that in dark-adapted thylakoids Mn is in a mixture of Mn^{2+} and Mn^{3+} states having a Mn-Mn separation, with O-atoms in between, of 2.7 Å. Earlier, Wydrzynski *et al.* (1975) had also obtained, from measurements of H_2O proton relaxation, the conclusion that dark-adapted thylakoids had a mixture of Mn^{2+} and Mn^{3+}.

It is also useful to compare the unique properties of well-characterized model complexes to the biological system. A Mn-semiquinone complex, in which the intramolecular charge separation may easily occur, has been studied in this regard (Lynch *et al.*, 1984). It has been pointed out (Renger and Weiss, 1983; Govindjee, 1984) that a Mn-quinone complex might be included as a component in the OEC. For other Mn models of O_2 evolution, see section IV.C.

D. Chloride

There are at least two locations at which anions affect electron flow through Photosystem II: in the region of Q_B, where HCO_3^- depletion or HCO_2^- addition inhibits electron flow (see e.g. Govindjee *et al.*, 1983), and in the OEC, where Cl^- is necessary for steady-state oxygen evolution (see e.g. Hind *et al.*, 1969; Kelley and Izawa, 1978; Govindjee *et al.*, 1983; Izawa *et al.*, 1983; Critchley, 1985). In the latter case, the requirement is not completely specific for Cl^-; anions of other strong acids may partially substitute for it. The effectiveness of the anions follows the order $Cl^- > Br^- \gg NO_3^- > I^-$; anions such as PO_4^{3-} and SO_4^{2-} have no effect (Hind *et al.*, 1969; Kelley and Izawa, 1978; Critchley *et al.*, 1982), whereas F^- has inhibitory effects (Kelley and Izawa, 1978; Critchley *et al.*, 1982).

Depletion of Cl^- has been shown to have several effects on O_2 evolution. It makes the OEC more sensitive to inhibition by hydroxylamine (Kelley and Izawa, 1978), Tris (Izawa *et al.*, 1983), and heat treatment (Coleman *et al.*, 1983). In the latter case, it was shown that the degree of protection provided by Cl^-, Br^-, and NO_3^- is roughly in accord with their ability to stimulate the Hill reaction in unheated, Cl^--depleted thylakoids. As before, SO_4^{2-} and PO_4^{3-} are without effects. Furthermore, Krishnan and Mohanty (1984) have shown that Hill activity of heat-inactivated (40°C, 3 min) and Tris-washed (0.8 *M*, pH 8.3) thylakoids of *Beta vulgaris* is partially restored if they are incubated with 150 m*M* $MgCl_2$ [not with $MgSO_4$ or $Mg(NO_3)_2$] prior to the assay. Cl^--depletion has also been shown to inhibit the transition from $S_2 \rightarrow S_3$ (Theg *et al.*, 1984; Itoh *et al.*, 1984). The addition of 0.6 m*M* Cl^- is sufficient to restore half-maximal Hill activity in depleted thylakoids (Kelley and Izawa, 1978; Baianu *et al.*, 1984).

Chloride appears to exert its effect by binding to the membrane in a pH-dependent manner (see e.g. Critchley *et al.*, 1982; Theg and Homann, 1982; Govindjee *et al.*, 1983). In thylakoids that are isolated from salt-tolerant plants (halophytes), the binding has been characterized by ^{35}Cl-NMR (Critchley *et al.*, 1982; Govindjee *et al.*, 1983; Baianu *et al.*, 1984) as being weakly ionic in character, with a binding energy of about 3 kcal mol^{-1} and a binding constant $K_b \cong 7\ M^{-1}$ at 25°C (Baianu *et al.*, 1984). The relative specificity for Cl^- is attributed to ionic field effects and steric factors, which affect the binding (Critchley *et al.*, 1982). The stimulation of the Hill reaction by Cl^- is also strongly pH-dependent in halophytic thylakoids (Critchley, 1983). The intricate interactions between H^+s and

Cl^- are now being thoroughly investigated (Homann and Johnson, 1984; Izawa *et al.*, 1984).

Little is known about the polypeptides involved in Cl^- binding. It has been shown that adding the 24-kD polypeptide from spinach to peptide-depleted inside-out thylakoid vesicles from halophytes dramatically increases the apparent affinity of the OEC for Cl^- (Andersson *et al.*, 1984; also *c.f.* Ghanotakis *et al.*, 1984a). Izawa *et al.* (1984) have, in contrast to Anderson *et al.*, shown that both the 18 kD and 24 kD polypeptides are needed to sequester Cl^- in PSII. It has also been reported that the removal of the 24 kD polypeptide from spinach PSII particles does not eliminate the Cl^- stimulation of the Hill reaction (Nakatani, 1984).

An understanding of the role of Cl^- in PSII in spinach will require more intensive study of the Cl^- binding properties of the OEC and identification of the functional groups involved in the Cl^- stimulation of O_2 evolution. A working hypothesis by Coleman and Govindjee (1984) is briefly discussed in Section IV.D.

E. Copper, calcium, and other cations

A possible role of copper in O_2 evolution has not yet been explored. Copper is present in plants at 6 p.p.m., and it is a constituent of plastocyanin and superoxide dismutase (see Table 1 in Gerwick, 1982). Ono *et al.* (1984) showed that Tris-treatment of PSII O_2-evolving particles led to a loss not only of Mn, but also Cu; the Cu level dropped from 2.1 Cu/400 Chl to 1 Cu/400 Chl. Just as for Mn, a good correlation between Cu content and O_2 evolution was observed. Thus, the role of Cu in O_2 evolution should be examined. In this connection, it is worth mentioning that Holdsworth and Arshad (1977) have observed the presence of Cu in PSII protein complexes of brown algae, and Ramaswamy and Nair (1978) have suggested a possible involvement of Cu in PSII reactions of greening potato tubers.

During the last several years, the role of calcium on the electron donor side has been investigated. Piccioni and Mauzerall (1976, 1978 a,b) showed that the depletion of Ca^{2+} from preparations of the cyanobacterium (blue-green alga) *Phormidium luridum* blocked O_2 evolution. Yu and Brand (1980) showed the stimulatory effect of Ca^{2+} on PSII reactions. England and Evans (1983) have shown a requirement for Ca^{2+} in the extraction of O_2-evolving PSII preparations from the cyanobacterium *Anacystis nidulans*. However, Pakrasi and Sherman (1984) found no such requirement, perhaps, because their preparations did not easily lose bound Ca^{2+}. Critchley *et al.* (1982) showed that in Cl^--depleted halophytic thylakoids, addition of $CaCl_2$ gave a much greater reactivation of O_2 evolution than $MgCl_2$, suggesting a specific role of Ca^{2+} in PSII.

Barr *et al.* (1982) have also shown an inhibition of electron transport by calmodulin antagonists such as chlorpromazine, phenothiazine, or trifluoroperazine. Pakrasi and Sherman (1984) also showed an inhibition of PSII activity by chlorpromazine in fractions from *Anacystis nidulans R2*. Barr *et al.* (1983) showed that in acid-treated chloroplasts, electron flow from H_2O to 2,5 dimethylbenzoquinone DMBQ (in the presence of dibromothymoquinone, DBMIB) could be restored 20–25% by 20–50 m*M* $CaCl_2$. Barr and Crane (1984) have now shown that a protein extracted by the acid treatment of chloroplasts restores electron flow activity if added along with $CaCl_2$. Although the data in their Table 1 does not show any enhancement of the $H_2O_2 \rightarrow$ DMBQ reaction by the addition of 20 m*M* $CaCl_2$, they still conclude that Calmodulin/calcium effects are on the reaction center II, not on water oxidation. It is also difficult to imagine how they support this conclusion by their observation that the $H_2O \rightarrow$ siolicomolybdate reaction at pH 6.0 in acid-treated thylakoids responded better to Mg^{2+} than Ca^{2+}. Brand *et al.* (1983) have also reached the conclusion that Ca^{2+} effects are close to the reaction center II. Incisive experiments are needed to prove the conclusion that Ca^{2+} or calmodulin effects in thylakoids are on the PSII reaction center.

More recently, it has been shown that salt-washing of PSII particles (which inhibits oxygen evolution) not only releases the 18 and 24 kD proteins, but also reduces the Ca^{2+} content of the particles by about 40% (Ghanotakis *et al.*, 1984a). The oxygen-evolving activity can be restored to 80–90% of the original level merely by adding 10–15 m*M* $CaCl_2$ (Ghanotakis *et al.*, 1984a; Miyao and Murata, 1984). Several other divalent cations are not as effective, and Mn^{2+} is slightly inhibitory. Moreover, the apparent affinity of the oxygen evolving enzyme for Ca^{2+} is substantially increased in the presence of the 18 and 24 kD proteins (Ghanotakis *et al.*, 1984b), suggesting that Ca^{2+} might be most active when a polypeptide binding site is available.

Another interesting role of Ca^{2+} has been shown during the photoactivation of the latent O_2-evolving enzyme in intact chloroplasts (see e.g. Ono and Inoue, 1983b,c). Experiments with EGTA [ethylene glycol bis (β-aminoethyl ether)-N,N^1-tetra acetic acid], a chelating agent with a high affinity for Ca^{2+}, showed that Ca^{2+} was required for this photochemical reaction. Ono and Inoue proposed that both Mn and Ca^{2+} must be bound to the latent OEC before it can be photoactivated by light. The question remains whether the OEC in the already-activated system may retain this Ca^{2+}.

Thus, it remains to be proven whether Ca^{2+} is required for O_2 evolution or for the reaction center activity, or for both, and/or whether a calmodulin-type protein indeed functions on the donor side of PSII.

IV. THE MECHANISM OF OXYGEN EVOLUTION

We do not know how water molecules are oxidized

in the O_2 evolving system. Further studies of this system are required to understand the mechanism completely (see Renger and Govindjee, 1985 for a review). In this section, however, we will provide a discussion of possible mechanisms of water oxidation, based on current knowledge of the chemistry of water in manganese complexes, the kinetics of the turnover of the S-states and proton release in the OEC, and the observations of the involvement of Mn and Cl^- in O_2 evolution. In view of the incomplete information currently available, one of the best ways to obtain a better understanding of the mechanism is to construct a model that incorporates all of the information from the various observations, which allows for a coherent understanding of all of the available data, and which makes predictions that can be experimentally and theoretically tested.

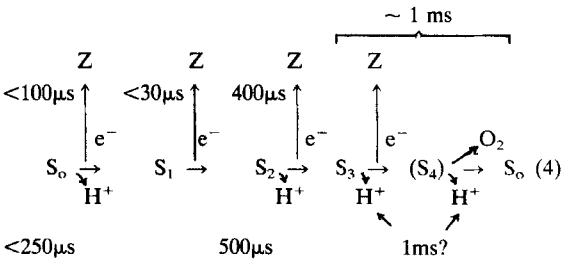
A. *Water oxidation*

The free energy change (ΔG) in the combustion of two hydrogen molecules, $2H_2 + O_2 \rightarrow 2H_2O$ (aqueous) is 4.92 eV (see e.g., Metzner, 1978). Since four electron exchanges occur between hydrogen and oxygen, the average energy requirement for one charge transfer step in the water-splitting process becomes 1.23 eV in a chemical system. Water oxidation in chloroplasts takes place at an average of 0.81 eV (at pH = 7.0) for each step through the coupling of H_2O with the OEC. However, the energy requirement of the four steps are quite different. In the first step of water oxidation, $H_2O \rightarrow OH^{\cdot} + H^+ + e^-$, the one electron release, with the formation of $OH^{\cdot}$, requires 2.3 eV in chemical systems (see e.g., Radmer and Cheniae, 1977). The energy for the second step, $H_2O + OH^{\cdot} \rightarrow H_2O_2 + H^+ + e^-$, is only 0.37 eV. The first step thus involves the highest energy barrier along the path (Renger, 1978). This seems to be the reason why Mn is used in water oxidation in chloroplasts: The equilibrium constant for the reaction $M^{3+}(H_2O) \Leftrightarrow M^{3+}OH^- + H^+$ in aqueous solution is the largest for the case in which M = Mn (Wells, 1965). It is not yet known whether H_2O molecules in chloroplasts are bound to the Mn ions directly or indirectly.

Recently, Radmer and Ollinger (1983; see Radmer, 1983) have observed the effect of various H_2O analogs such as NH_2OH and NH_2NH_2 on the flash yield of O_2 in order to investigate the geometry of the O_2 evolving site. Radmer has observed that the ability of a molecule to interact with the O_2 evolving site correlates with the shape of the molecule rather than with its chemical activity. By using several different molecules with various shapes, Radmer has concluded that the H_2O binding site lies in a cleft 4 Å wide and 2.5 Å deep. Furthermore, two H_2O molecules at a distance of 1.47 Å may be in this cleft. The existence of a cleft around the binding sites may be important in preventing the decay of S-states through reactions of the intermediates in H_2O oxidation with other molecules.

B. *The S-states and proton release*

The S-state transitions, the H^+ release pattern, and the suggested times for electron transfer and H^+ release may be represented as (see e.g. Junge and Jackson, 1982):



Here, the times for Z reduction are mostly taken from Babcock *et al.* (1976) and that for H^+ release from Förster *et al.* (1981).

The general picture of the S-states has remained essentially the same since Kok *et al.* (1970) provided the initial model. The only major change relates to the H_2O oxidation: In the absence of any knowledge to the contrary, H_2O oxidation was earlier considered to occur in the last step as: $S_4 + 2H_2O = S_0 + 4H^+ + O_2$. Fowler (1977), Saphon and Crofts (1977), Bowes and Crofts (1978), Förster *et al.* (1981), and Wille and Lavergne (1982) have shown that in dark-adapted thylakoids, the H^+ release pattern follows 1, 0, 1, 2 pattern for $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow (S_4) \rightarrow S_0$ transitions, respectively (see Eq. 4). Proton release from S_2 to S_3, but not from S_1 to S_2, was confirmed by measuring thermoluminescence peak positions as a function of pH (Rutherford *et al.*, 1984a). These results suggest that the water-splitting process may begin earlier than the last step, an idea also presented, independent of the H^+ release data, by Wydrzynski *et al.* (1977). Whether the measured 1, 0, 1, 2 H^+ release pattern is the true pattern during S-state transitions remains to be proven (see Renger and Govindjee, 1985). Brudvig *et al.* (1984) and Brudvig (1984) have suggested that the OEC exists in both an "active" and a "resting" state: long-darkness produces the resting state. Therefore, it is necessary to remeasure the H^+ release pattern under these two conditions.

In dark-adapted chloroplasts, the only S-states are S_0 and S_1 because both S_2 and S_3 deactivate to S_1 (see e.g. Joliot and Kok, 1975)—this deactivation occurs within 5s in intact systems and within 60s in isolated thylakoids. The S_2 state has been found to be very sensitive to alkaline pH (Briantais *et al.*, 1977) and to Tris (Frasch and Cheniae, 1980). This is interesting because Tris extracts the 33, 24 and 18 kD polypeptides (section III.B) and alkaline pH aids in the release of Cl^- (section III.D). Another interesting observation, using lauryl choline chloride, has raised the possibility that the S-state cycling can be

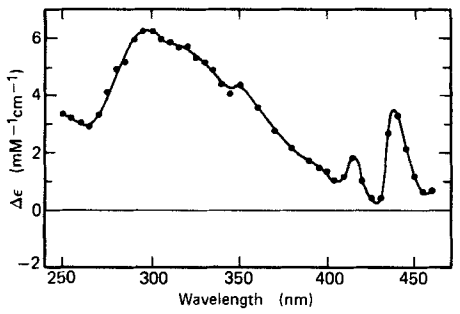


Figure 5. Absorption spectrum attributed to each of the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions in spinach PSII particles. (Redrawn and modified from Dekker *et al.*, 1984a.)

uncoupled from the O_2 evolution step (Wydrzynski *et al.*, 1984).

There is one problem with the operation of the S-states—it has to do with the "misses". Many workers (see Wydrzynski, 1982) have accepted the idea that the probability of "misses" is the same after each transition. However, others (see e.g., Delrieu, 1983, 1984) consider the misses to be different in different S-states. In the opinion of the present authors, crucial experiments are still needed to settle this question. Lavorel (1976) has pointed out another inconsistency: In algae where the quantum yield of O_2 evolution is very high, there are more, rather than fewer, "misses" than in chloroplasts. An interesting observation is that at non-saturating flash intensities, S_2 is most abundant and $[S_1]$ and $[S_3]$ are constant at steady state (Lavorel and Maison-Peteri, 1983). The existence of a charge carrier **C** was proposed to explain the high quantum yield of O_2 evolution at low intensities as well as to explain the high $[S_2]$. As S_3 deactivates to S_1, the charges are not lost, but are stored on **C**; these can be recovered during the transition of S_o to S_2. The nature of this **C** is not specified.

There are several recent papers that deal with the question of the S-state distribution in dark adapted chloroplasts or algae. In chloroplasts, dark-adapted for a long time, it has been shown that the system starts from 100% S_1 (Vermaas *et al.*, 1984).

The molecular composition of the OEC (the "M" complex) is unknown, but it may include bound H_2O, Mn (section III.C), Cl^- (section III.D), and several polypeptides (section III.B). In addition, however, it contains a chromophore having an absorbance in the UV region (see Pulles *et al.*, 1976; Mathis and Havemann, 1977; Velthuys, 1981). In trypsin-treated chloroplasts (Renger, 1976)—where the binary oscillations on the electron acceptor side do not occur—light-induced UV absorbance changes, which are characterized by 1 ms relaxation kinetics, oscillate synchronously with the oxygen yield (Renger and Weiss, 1983). This is suggested to reflect the $Z^+ \cdot S_3 + 2H_2O \rightarrow Z \cdot S_o + O_2$ reaction, and was dubbed Y-320. Weiss and Regner (1984a) have obtained a difference spectrum for S_3 minus S_o by subtracting the Z^+ minus Z spectrum discussed later in section V.A. This spectrum has a rather broad peak around 320 nm; it might involve a Mn(IV) $\longleftrightarrow$ Mn(III) transition, as suggested by the authors or it may involve Mn-semiquinone complexes (see section IV.C; Lynch *et al.*, 1984). It has been known for some time that plastoquinones* may be involved on the electron donor side of PSII as well (see e.g. Okayama, 1974; Sadewasser and Dilley, 1978). Dekker *et al.* (1984a) have recently obtained a somewhat different spectrum for the S-state transitions, after making extensive corrections for various overlapping signals (Figure 5).

Thermoluminescence (TL) has become a powerful tool to monitor the changes in the S-states because of the discovery of the period 4 dependence of TL as a function of flash number by Y. Inoue and K. Shibata (see e.g. a review by Inoue and Shibata, 1982; and Inoue, 1983). Rutherford *et al.* (1982) have successfully used TL to probe the ratio of $Q_B^- : Q_B$ and the deactivation of S-states in thylakoids. The deactivation of S-states can now be monitored in leaves by this technique (Rutherford *et al.*, 1984b). In this way it was shown that the deactivation of S_2 centers associated with Q_B^- had a halftime of 20–30 s, but those associated with Q_B had a halftime of ~ 150 s. It is thus obvious from the above that the deactivation of S-states is dependent upon the electron acceptor side of PSII. In addition, these times are much longer than the 5 s quoted for the green alga *Chlorella*. In a thermophilic alga *Synechococcus vulcanus*, the deactivations of S_3 and S_2 states occur with still longer half-times of 200 and 75 seconds, respectively (Govindjee *et al.*, 1984). Recent advances on thermoluminescence will be reviewed by Sane and Rutherford (1986) in a forthcoming book (Govindjee *et al.*, Eds., 1986).

C. The participation of manganese

Many models for the participation of Mn have been proposed based on the available data and on thermodynamic considerations of the properties of Mn and water (see e.g. Govindjee *et al.*, 1977; Renger, 1977; Lawrence and Sawyer, 1978; Wydrzynski and Sauer, 1980; Goodin, 1983; Renger *et al.*, 1983; Hansson *et al.*, 1984; Kusonoki, 1984). These models show how the state of the Mn complex that binds the H_2O molecules is changed in the process of the four electron steps, according to Kok's four-photon scheme of the redox cycle. The chemical forms of the complex which correspond to the intermediates of H_2O oxidation, and the redox states of Mn are shown for each model in Fig. 6; the steps

*It is worth noting that Plastoquinone-A (9) (0.018/Chl) is the major quinone present in PSII particles (Okayama, 1983; Murata *et al.*, 1984b); and Phylloquinone is present only at a ten-fold lower concentration.

that involve the binding and release of protons, H_2O, and O_2 during the H_2O oxidation process are also included.

In the S_o state, H_2O molecules are shown (Fig. 6) to be bound to the Mn cluster in all of the models except those by Goodin (Gd) and Hansson and Andreasson (H-A), in which reaction, $H_2O \rightarrow HO^- + H^+$, may have occurred in the hydrophilic phase. If the Mn cluster of the OEC is only in the hydrophobic phase, then the models other than those by Gd and H-A are favored. In the Lawrence and Sawyer (L-S), Gd, Kusunoki (K), and H-A models, H_2O molecules are bound directly to the Mn ions, whereas the binding site of H_2O is not explicitly shown in the other models. The state of Mn ions in the S_o state is Mn^{2+} in the L-S, Wydrzynski and Sauer (W-S) and K Models, whereas it is a mixture of Mn^{2+} and Mn^{3+} in the Govindjee *et al.* (Gv), Gd, and H-A models. Renger (R) does not denote the valance state of Mn explicitly. A proton release ($H_2O \rightarrow OH^- + H^+$) occurs during the transition from S_o to S_1 states (Gv, L-S, W-S, and K models). The S_1 state in these models is, in our opinion, stable in the dark as Kok *et al.* had proposed since the equilibrium constant for $MnOH_2 + H_2O \rightleftharpoons MnOH^- + H_3O^+$ is quite large, as mentioned in section III.C. However, the S_1 state in the Gd and H-A models may be unstable, compared to the S_o state. Thus, these two models may not be in agreement with the usual stability of the S_1 state. The valence state of Mn in the S_2 state is Mn^{2+} $3Mn^{3+}$ (Gv), $2Mn^{3+}$ (L-S and W-S), or $Mn^{3+}Mn^{4+}$ (Gd, K and H-A). The valence state $Mn^{3+}Mn^{4+}$ is favored by Dismukes and Siderer (1981), who based their conclusion on their analysis of the multiline Mn ESR signals. It is apparent that the highest valency of Mn ions in the H_2O oxidation process is Mn^{3+} in the Gv, L-S, and W-S models, and Mn^{4+} in the other models.

In the R, L-S, Gd and H-A models, the two oxygen

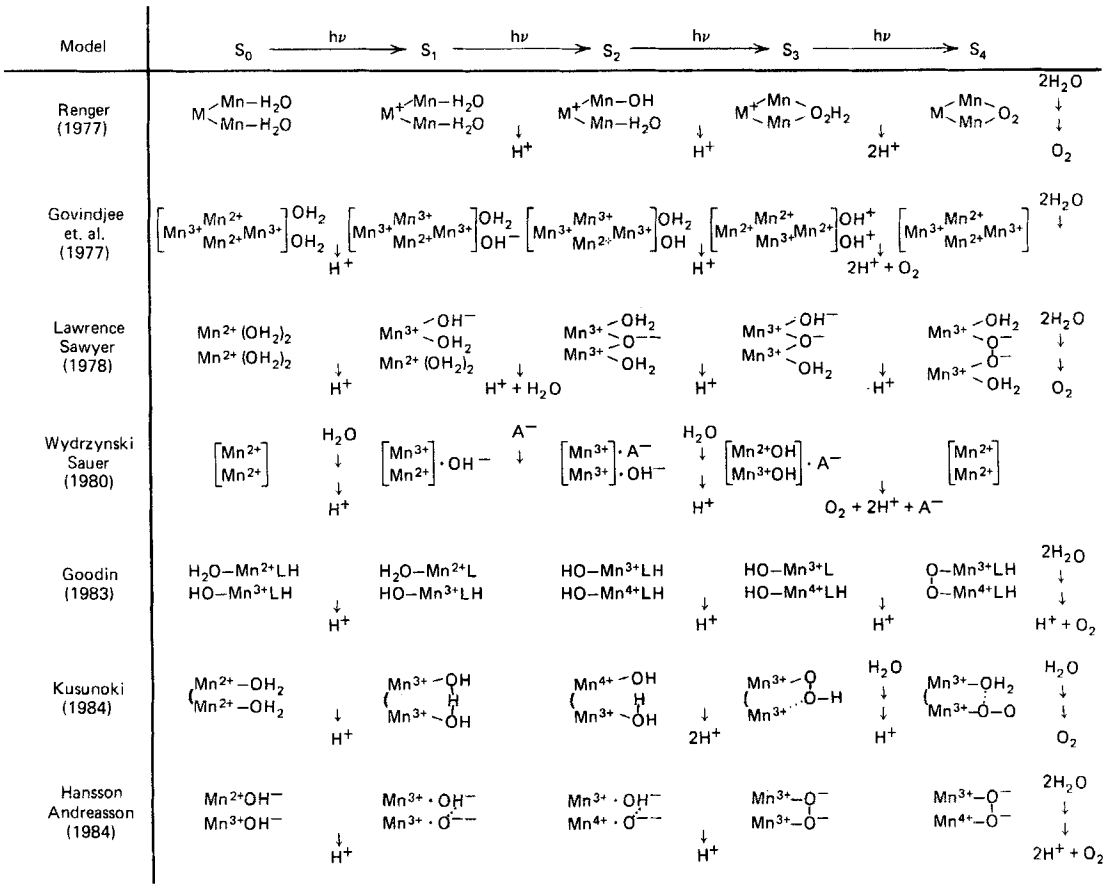


Figure 6. Models for the molecular mechanism of photosynthetic water oxidation. The models, from top to bottom, are those by Renger (1977), Govindjee *et al.* (1977), Lawrence and Sawyer (1978), Wydrzynski and Sauer (1980), Goodin (1983), Kusunoki (1984) and Hansson and Andreasson (1984). The number of Mn (2 or 4) involved, the valence state of Mn cluster, the oxidized (intermediate) state of water molecules, and the proton release pattern are shown for each of Kok's S-states (see top of the figure). M (in Renger's model) stands for a redox-active bridging ligand, L (in Goodin's model) for a ligand, and A^- (in Wydrzynski and Sauer's model) for a counter ion.

atoms, coupled with each other, are bound to two Mn ions, prior to O_2 evolution from the S_4 state; this type of binding has been found in the mixed-valence Mn^{3+}, Mn^{4+} dimer containing bipyridine ligands (Cooper and Calvin, 1977; Cooper *et al.*, 1978; for references to other artificial models, see Wohlgemuth *et al.*, 1984). The Mn–Mn separation (2.7 Å) in this artificial complex is identical with that estimated from the EXAFS studies on chloroplasts (Kirby *et al.*, 1981). Finally, the H^+ release pattern is 0,1,1,2 in the R model; 1,0,1,2 in the Gv, W-S, Gd, and H-A models; 1,1,1,1 in the L-S model; and 1,0,2,1 in the K model, during the $S_o \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow (S_4) \rightarrow S_o$ transitions, respectively. If the proton concentration around the OEC is in equilibrium with that on the outside of the membrane, then the observed (see section IV.B) pattern of 1,0,1,2 in dark-adapted chloroplasts would favor only the Gv, W-S, Gd and H-A models, provided it is the pattern for active, not resting OEC.

Dekker *et al.* (1984b) have obtained evidence that all S-state transitions have the same difference absorption spectrum in the ultraviolet, and Dekker *et al.* (1984c) have tentatively ascribed these changes to the oxidation of Mn (III) to Mn (IV). If this is proven to be correct, then all the models presented in Fig. 6 would have to be questioned. Furthermore, we must await an analysis of the information being obtained from studies on the interaction of Mn ions with $Pheo^-$ (Kulikov *et al.*, 1983): this interaction is minimal with two or four Mn atoms bound, but it is maximal with one or three Mn atoms bound.

Kambara and Govindjee (1985) have recently proposed a model for water oxidation in the OEC that utilizes redox-active ligand chemistry and the chemistry of Mn in two different environments to explain the known aspects of electron transfer from H_2O to Z. The major features of this model are: (1) The four functional Mn atoms are divided into two groups of two Mn each: (a) [Mn] complexes in a hydrophobic cavity in the intrinsic 34 kD protein, and (b) (Mn) complexes on the hydrophilic surface of the extrinsic 33 kD protein. The oxidation of H_2O is carried out by the two [Mn], and the protons are released from [Mn] to (Mn) along the hydrogen bond between their respective ligand H_2O molecules. (2) Each of the two [Mn] in the cavity binds one redox-active ligand (RAL), such as a semiquinone (e.g. catechol; more plausibly, an aromatic amino acid, such as histidine, may carry out this function). Electron transfer occurs from the reduced RAL in [Mn] to Z^+. (3) The two (Mn) are similar to Mn^{3+} complexes in aqueous media; Cl^- stabilizes the system by being indirectly coordinated to a (Mn) as an outer sphere ligand. (Mn) stabilizes the water oxidation reactions by pulling H_2O protons away and releasing them into the lumen.

Before any model for O_2 evolution can be finalized, it will have to include the possibility that it can run in reverse at times, i.e. it may be capable of converting O_2 into H_2O although with extremely low efficiency. Brudvig (1984) showed that thylakoids with active OEC take-up O_2 1–2 μmol O_2/mg Chl h. Vidaver *et al.* (1984) have recently concluded that "the *in vivo* functioning of the water-splitting system is dependent on the presence of O_2 partial pressures of 0.01 atm. or less." Obviously, we have a long way to go before the "window" of the "O_2 clock" will be opened for us.

D. The participation of chloride

The role of Cl^- in chloroplast electron transport was discovered by Warburg and Lüttgens (1944, 1946). Although a large amount of data has recently been made available concerning the Cl^- effect in thylakoids (see section III.D), there have been relatively few detailed suggestions about how Cl^- functions in the water-splitting reactions. Certainly, any mechanism proposed to explain the function of Cl^- must also explain the following set of results: (1) Inactivation by Cl^- depletion is reversible (Hind *et al.*, 1969; Kelley and Izawa, 1978); (2) Cl^--depletion is accelerated by incubating the thylakoids at high pH (Izawa *et al.*, 1983) and by adding uncouplers (Theg and Homann, 1982); (3) Cl^--binding is pH-dependent and reversible (Critchley *et al.*, 1982); (4) activation of the Hill reaction by added Cl^- shows hyperbolic kinetics, indicating saturation (Kelley and Izawa, 1978); (5) activation of the Hill reaction by anions is relatively specific for Cl^-, but not exclusive (Hind *et al.*, 1969; Kelley and Izawa, 1978; see also section III.D); (6) The pH optimum of the Hill reaction is shifted to more alkaline pH by Cl^--binding (Hind *et al.*, 1969; Critchley, 1983); and (7) the number of Cl^- per PSII RC may be as large as 40 (Izawa *et al.*, 1984).

There are several possible roles that could be envisioned for Cl^- in the mechanism of the water-splitting reactions, some of which have already appeared in the literature (see e.g. Govindjee *et al.*, 1983; Izawa *et al.*, 1983; Itoh *et al.*, 1984; Theg *et al.*, 1984). The lack of information about Cl^- binding at the molecular level makes it difficult to decide among the possibilities, and therefore most of these suggestions must be considered as merely working hypotheses.

An indirect role for Cl^- in O_2 evolution might be as an allosteric effector, stimulating the water-splitting reactions by binding to a site (or sites) other than the active site. This is the mechanism suggested for the effect of Cl^- binding on the O_2-binding affinity of hemoglobin (Chiancone *et al.*, 1972). Binding of an allosteric ligand (or other ligands) to an enzyme also protects the enzyme against inactivation in many cases (see e.g. Ginsburg and Carroll, 1965; Zyk *et al.*, 1969). This behavior has been observed in the OEC, where Cl^--binding protects against the inactivation by hydroxylamine, Tris and heat treatment (see section III.D). Allosteric mechanisms, however, are

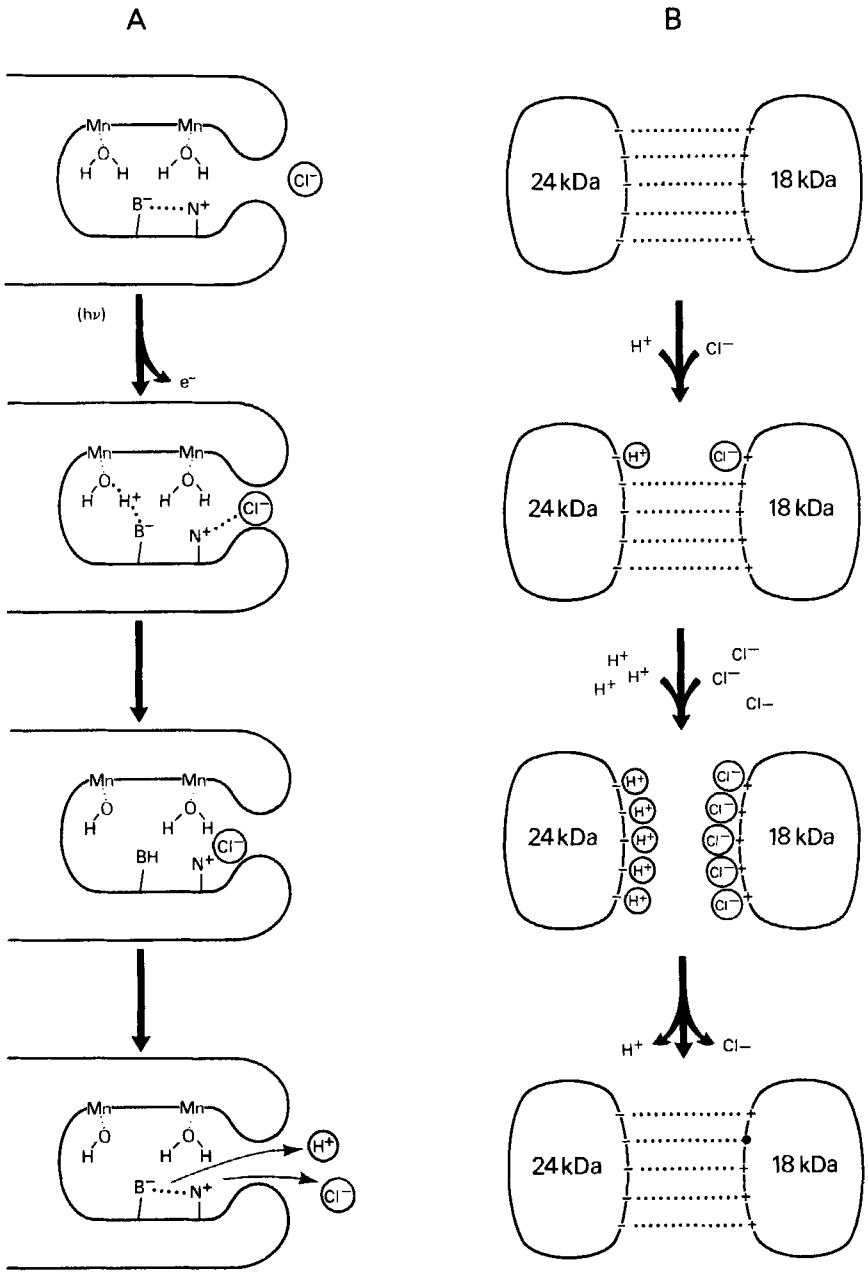


Figure 7. A model for chloride-ion activation of O_2 evolution in which binding of the anion induces shifts in the apparent pk_a's of reactive groups on the polypeptide of the oxygen evolving complex. (A) In the active site, the binding of Cl^- to the protonated amino group N^+ promotes an "attack" by B^- (representing a basic group of the protein) on a water proton. The proton is made more labile as a result of the removal of an electron from water by Mn at the active site. Unbinding of the Cl^- causes the simultaneous release of H^+ (*cf.* Govindjee *et al.*, 1983) and enables the salt-bridge between B^- and N^+ to re-form. (B) Proton release into the lumen of the thylakoid in the presence of Cl^- disrupts salt-bridges between opposite charges on the 18 and 24 kD polypeptides. Binding of Cl^- to the excess positive charges on the 18 kD polypeptide (isoelectric pont, 8.5–9.5) promotes the binding of H^+s to the excess negative charges on the 24 kD polypeptide (isoelectric point, 6.5–7.3) (and *vice versa*). The subsequent removal of H^+s causes the Cl^- ions to be released and enables the salt-bridges to re-form (After Coleman and Govindjee, 1984).

usually associated with regulatory (Monod *et al.*, 1963) or transport proteins (Kyte, 1981). Nevertheless, this possibility should be further explored (see also Fig. 7).

A more direct role for Cl^- in the active site is possible. For example, halides have been shown to participate as substrates in the peroxidative formation of molecular iodine, bromine and chlorine,

catalyzed by the heme-containing enzyme chloroperoxidase (Hager, 1970): $2X^- + H_2O_2 \rightarrow X_2 + 2\,OH^-$. Although peroxo-intermediates have been suggested to participate in the water-splitting mechanism (see Fig. 6, section IV.C) there is no firm evidence yet for their involvement. However, Nakatani (1983b) has reported that KCN can specifically inhibit the water-splitting site and has suggested that a heme is involved in the reactions. Also, a 13 kD Mn-protein with catalase activity has been isolated from a cyanobacterium; an antibody to it inhibits H_2O to dichlorophenol-indophenol (DCPIP), but not diphenyl carbazide to DCPIP reaction that bypasses water oxidation (Okada and Asada, 1983).

Sandusky and Yocum (1983) have suggested, based on competition experiments between NH_3 and Cl^-, that Cl^- participates in the reactions at the active site as a bridging ligand between Mn atoms. They propose that Cl^- stimulates O_2-evolution by facilitating charge transfer between Mn atoms in the cluster.

Another possible model (Coleman and Govindjee, 1984) is presented in Fig. 7; it involves a dual role for Cl^-: (1) indirect activation of base catalysis (enhancement of H^+ release from water) at the active site of the OEC (33 kD-Mn-containing polypeptides) (Fig. 7A); and (2) activation of proton binding at other sites in the complex (e.g. at 24 and 18 kD polypeptides) (Fig. 7B). This kind of role is consistent with the known action of Cl^- (and other anions) in soluble enzymes. The key to this model is the postulated ability of anions to shift the pk_a's of essential reactive groups in enzymes by suppressing adjacent positive charges on other groups. This behavior has been suggested to explain the effect of anions on the velocity-pH curves of salivary amylase and fumarate hydratase (Dixon and Webb, 1979).

A thorough evaluation of the validity of this dual model for Cl^- action in PSII *in vivo* (and for other models) will perhaps require a knowledge of the three-dimensional structure of the polypeptide components, and more sophisticated studies of their ion-binding properties and protein-protein interactions. At present, this particular hypothesis serves simply as a working model for further investigation. For detailed reviews on the role of Cl^- and O_2 evolution, see Critchley (1985) and Izawa (1985).

V. OTHER COMPONENTS OF THE DONOR SIDE OF PSII

A. The component "Z"

The electron donor(s) to $P680^+$ include a component Z, responsible for the ESR signal II very fast (vf) (Babcock *et al.*, 1976); this donor appears to be equivalent to a donor labeled D_1, based on optical studies of the recovery of $P680^+$ to P680 (see e.g. Mathis and Paillotin, 1981). This conclusion comes from the exact equivalence of $P680^+$ decay and Z^+ rise kinetics in Tris-washed chloroplasts (Babcock *et al.*, 1983; Boska *et al.*, 1983) and a rough equivalence in normal thylakoids (Boska and Sauer, 1984; Sauer *et al.*, 1984). The <3 μs (instrument limited) kinetic component observed for Z^+ formation may or may not be slower than the 0.02 to 0.4 μs components for $P680^+$ reduction (see Brettel *et al.*, 1984). Based on the similarity in the ESR spectrum of signal II slow (at 150°C), which resembles IIvf, with that of a 2,5-dimethyl semiquinone cation radical, and on consideration of detailed differences between the two, O'Malley and Babcock (1983) have suggested that Z^+ is a semiquinone cation PQH_2^+ anchored to a protein (see e.g. Babcock *et al.*, 1984). Since such a species should have characteristic optical absorption changes when Z is converted to Z^+, three research groups have independently measured such spectra. Dekker *et al.* (1984b), using Tris-washed PSII particles, diphenylcarbazide (DPC) as a donor, and ferricyanide as an acceptor, have calculated the spectra for Z^+ minus Z after subtracting the changes due to Q_A^- minus Q_A, and due to the oxidation of ferrocyanide to ferricyanide, from the 50 ms phase of UV absorption changes (Fig. 8). This spectrum, when compared with that of the oxidation of duroquinol (DQH_2) in 9 *M* H_2SO_4 and 1 *M* methanol, obtained by radiolysis (E. J. Land, unpublished), shows remarkable similarity. Much less similarity was observed by a comparison with the production of $PQH^\cdot$ from PQH_2 in ethanol (Bensasson and Land, 1973). Although not conclusive, these results suggest that Z^+ may indeed be PQH_2^+. Diner and de Vitry (1984) using a simpler PSII particle that lacks the OEC, with ferricyanide as an electron acceptor and benzidine as an electron donor, obtained a difference spectrum for Z^+ minus Z by comparing the absorbance measured at 0.5 ms after the flash with that at 10 ms when all of the Z^+ is reduced by benzidine. After subtracting a small contaminating contribution by Q_A^- minus Q, the spectrum obtained was similar to that of Dekker *et al.*, although peaks around 280 nm and around 350 nm were clearly observed only in Diner and de Vitry's data. These details were absent

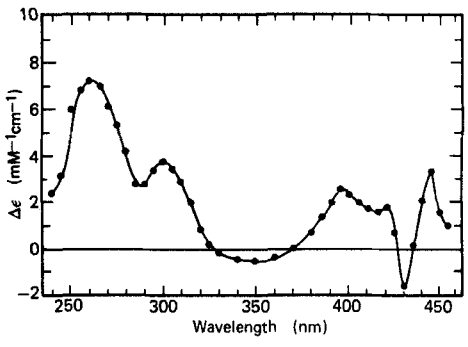
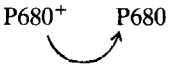


Figure 8. Absorbance difference spectrum of $Z \rightarrow Z^+$ in Tris-treated PSII particles. (Redrawn and modified from Dekker *et al.*, 1984b.)

in the difference spectrum of PQH_2^+ (trimethyl-*p*-benzoquinol cation) minus PQH_2, but were present in the spectrum for 2,3-dimethyl-1,4 napthoquinol cation minus its quinol. Thus, Diner and de Vitry favor Z^+ to be related to the cation quinol of vitamin K type. Weiss and Renger (1984a,b) have also measured the spectrum of Z^+ minus Z; in this work, the peak at 350 nm was present, but the peak at 280 nm was less pronounced, and the absorbance ratio of 260 nm to 300 nm was much lower than that obtained by Diner and de Vitry and Dekker *et al.* Thus, it seems that a unique difference spectrum of Z^+ minus Z has not yet been obtained, perhaps, because of problems of corrections and overlapping bands due to electrochromic effects.

Renger and Govindjee (1985) have discussed the difficulties of exact assignments, although it appears that the "window" for the "Z" has been opened. The problem with the identification of Z^+ with PQH_2^+ lies with the observation of proton release in Tris-washed chloroplasts (Renger and Völker, 1982), which would suggest the following reaction:



$PQH_2 \rightarrow PQH_2^+ \rightarrow PQH^{\cdot} + H^+$. This problem can be solved, however, if we assume that the H^+ observed by Renger and Völker came from the apo-protein of Z (see discussions by Babcock *et al.*, 1984). Furthermore, it is possible that Z^+ may turn out to be a mixture of PQH_2^+ and $PQH^{\cdot}$. If the time-dependent spectra for Z to Z^+ and of Z^+ to Z could be optically measured, we would know more about its chemical identity.

An additional argument supports the idea that $Z^+ = PQH_2^+$, since the redox potential of cation quinone radicals has been shown to be + 950 mV (Wood and Bendall, 1976); this is consistent with its position in the redox scheme between that of H_2O/O_2 (+ 800 mV) and that of $P680/P680^+$ (estimated to be around + 1100 mV; Jursinic and Govindjee, 1977a; Klimov *et al.*, 1979).

The literature abounds with suggestions that there is more than one electron donor between the OEC ("M complex") and P680. We shall discuss in Section V.B. a suggestion that Cyt *b*-559 may be such a donor (Bishop, 1984). Among others, Jursinic and Govindjee (1977b) had suggested the following scheme:

$$\begin{matrix} H_2O \\ O_2 \end{matrix} \rightarrow \text{"M"} \rightarrow Z_2 \rightarrow Z_1\ (Z) \rightarrow P680 \qquad (5)$$
complex (OEC)

The idea of two Zs was discussed fully by Bouges-Bocquet (1980) who supported the concept of a parallel pathway:

$$\begin{matrix} H_2O \\ O_2 \end{matrix} \rightarrow \text{"M complex"} \left\{ \begin{matrix} S_0,S_1 \text{ fast} \rightarrow Z_1 \rightarrow P680 \\ S_2,S_3 \text{ slow} \rightarrow Z_2 \end{matrix} \right. \qquad (6)$$
(OEC)

Boussac and Etienne (1982) have provided evidence that two donors D and Z operate at pH 8.5, but that only one operates at pH 6.0. The relationship of Z and Z_2 to the other donor D (which is not to be confused with D_1, and which is responsible for ESR signal II slow) is not at all clear. The additional donor D is simply an auxiliary donor, which may not be connected to the S-states.

Recently, a new ESR Signal has been discovered at $g = 4.1$ with a linewidth of 360 Gauss (Casey and Sauer, 1984; Zimmermann and Rutherford, 1984). Zimmermann and Rutherford (1984) have shown that this signal is formed by illumination at 200 K, not by flash excitation at room temperature; it does not occur in the presence of diuron (which blocks electron flow from Q_A^- to Q_B); and it is absent in Tris-washed membranes (which releases the 33, 24 and 18 kDa polypeptides, abolishing O_2 evolution). Casey and Sauer have attributed it to a precursor of the S_2 state of the OEC, whereas Zimmermann and Rutherford have suggested that it arises from an intermediate donor between the S-states and the Z.

As mentioned earlier, Brettel *et al.* (1984) have now established that electron flow from Z to $P680^+$ occurs in 20 ns when the S_0, S_1 states are present, and in 400 ns when the S_2, S_3 states are present. Such observations are also consistent with the parallel pathway model dicussed by Bouges-Bocquet (1980), although a linear electron flow chain could easily explain these data.

B. Cytochrome b-559

Cytochrome *b*-559 is one component whose chemistry is well-known, but not its function (see e.g. Cramer *et al.*, 1979; Bendall, 1982). Widger *et al.* (1983) have isolated it in a pure form and have established its amino acid sequence, heme content, etc. There are several observations that suggest a definite importance of Cyt b_{559} for PSII reactions. Mutants that lack Cyt b_{559} also lack PSII activity (Maroc and Garnier, 1981). Bishop (1984) has studied a mutant LF-9 of *Scenedesmus* that lacks *only* Cyt b_{559}; the Mn content, Cyt b_{563}, Cyt f_{553} and the 34 kD polypeptide are all normal. This mutant is fully functional in the DPC (diphenyl carbazide) to DCPIP (dichlorophenol indophenol) reaction (i.e. RC II is normal), and it is normal in the PSI reaction, but electron flow involving water oxidation is totally inhibited. Thus, Bishop (1984) has suggested that Cyt b_{559} may function as an intermediate between the OEC and "Z"; this of course should be tested. Another interesting observation, as noted earlier, is that the release and rebinding of the 23 kD protein reversibly affects the HP (high-potential) Cyt b_{559} (Larsson *et al.*, 1984). Butler and Matsuda (1983) have argued that statements dealing with the negative correlation between HP Cyt b_{559} and O_2 evolution should be examined more carefully because there may be ambiguity in the definition of high potential in the protonated and the unprotonated

forms; these may have very different midpoint potentials.

The electron-donating group of Cyt b_{559} may be "close" to the RC P680 (at least at 77 K). It can efficiently donate electrons to $P680^+$ under these conditions (see e.g. Butler *et al.*, 1973); furthermore, Butler and Matsuda (1983) have discussed a hypothesis in which it may act as a temporary reservoir of H^+s from the S-states. This hypothesis, if proven true, would locate Cyt b_{559} on the inner side of the membrane, and question whether intrinsic H^+ release is the same as measured by pH electrode and other methods. However, Cyt b_{559} also functions on the electron acceptor side in this model; we must therefore allow it to communicate with the outer side (the Q_A side) of the membrane. There are two Cyt b_{559} molecules per RC (see e.g. Whitmarsh and Ort, 1983)—the second one was left out in Fig. 3 so as not to crowd the picture. However, there are a number of reports which suggest that there is only one cyt b_{559} per RC. For example, A. W. Rutherford (personal communication), using ESR, finds only 1 cyt b_{559}/RC in PSII particles prepared according to the method of Berthold *et al.* (1981). Selak *et al.* (1984) have observed a heterogeneity in HP Cyt b_{559} on the basis of a differential reactivity towards the externally-added oxidant ferricyanide; thus, the second Cyt b_{559} may be in a slightly different local environment. Whitford *et al.* (1984) have observed that only half of the available HP Cyt b_{559} undergoes photooxidation in the presence of FCCP (Flurocarbonyl cyanide phenylhydrazone) and have suggested that this fraction must be exposed to the outer surface. Thus, Cyt b_{559} must have access to both the outer and the inner side of the membrane, based on the current experiments and ideas. It may span most of the membrane. The "window" of cytochrome b_{559} is still hazy, and maybe some sort of serendipidity will be needed to improve the view.

VII. CONCLUDING REMARKS

Photosynthetic oxygen evolution is the source of O_2 on Earth and sustains all oxygenic life. The ability of researchers to isolate and characterize several of the polypeptides, to measure Mn by low temperature ESR Spectroscopy and by EXAFS, to measure Cl^- by NMR, to measure several intermediates by fast kinetic ESR and sensitive optical spectroscopy, and to use simpler reaction center complexes and highly active O_2-evolving PSII preparations has opened many windows in the PSII box (see section I and II.B). However, many more windows remain to be opened before we can understand the water oxidation process. For example, very little is known about the molecular intermediates in water oxidation, the molecular role of manganese, chloride and plastoquinone, and cytochrome b_{559}. The oxygen evolving complex has not been isolated, and its arrangement in the membrane has not yet been delineated.

Acknowledgements—Govindjee acknowledges financial support from the National Science Foundation (NSF), U.S.A. (PCM 83-06061) and from the Research Board of The University of Illinois at Urbana-Champaign; Coleman is thankful, in addition, to Professor H. S. Gutowsky, and his NSF grant (PCM79-11148), a College work-study grant, and a NIH Biomedical Research grant (RR7030; 1984–1985). Kambara is grateful to the University of Electrocommunications, Tokyo, and to the Ministry of Education, Science and Culture, Japan, for financial support. We thank John Whitmarsh, H. S. Gutowsky, Danny Blubaugh, G. Renger and A. W. Rutherford for suggestions during the preparation of this manuscrpt. The authors are highly indebted to Debbie Fisher and Kim Pribble, of the UIUC Biophysics Office, for typing this manuscript.

REFERENCES

Abramowicz, D. A. and G. C. Dismukes (1984) *Biochim. Biophys. Acta* **765**, 318–328.

Abramowicz, D. A., T. K. Raab and G. Dismukes (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 349–351. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Åkerlund, H.-E. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 391–394. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Åkerlund, H. E. and C. Jansson (1981) *FEBS Lett.* **124**, 229–232.

Åkerlund, H.-E., C. Jansson and B. Anderson (1982) *Biochim. Biophys. Acta* **681**, 1–10.

Åkerlund, H.-E., G. Renger, W. Weiss and R. Hagemann (1984a) *Biochim. Biophys. Acta* **765**, 1–6.

Åkerlund, H.-E., K. Brettel and H. T. Witt (1984b) *Biochim. Biophys. Acta* **765**, 7–11.

Amesz, J. (1983) *Biochim. Biophys. Acta* **726**, 1–12.

Andersson, B., C. Critchley, I. J. Ryrie, C. Jansson, C. Larsson and J. M. Anderson (1984) *FEBS Lett.* **168**, 113–117.

Babcock, G. T., R. E. Blankenship and K. Sauer (1976) *FEBS Lett.* **61**, 286–289.

Babcock, G. T., D. F. Ghanotakis, B. Ke and B. A. Diner (1983) *Biochim. Biophys. Acta* **723**, 276–283.

Babcock, G. T., W. J. Buttner, D. F. Ghanotakis, P. J. O'Malley, C. T. Yerkes and C. F. Yocum (1984) In *Advances in Photosynthesis Research* (C. Sybesma), Vol. I, pp. 243–252. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Baianu, I. C., C. Critchley, H. S. Gutowsky and Govindjee (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3713–3717.

Barr, R. and F. L. Crane (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 441–448, Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Barr, R., K. S. Troxel and F. L. Crane (1982) *Biochim. Chiphys. Res. Commun.* **104**, 1182–1188.

Barr, R., K. S. Troxel and F. L. Crane (1983) *Plant Physiol.* **72**, 309–315.

Bendall, D. (1982) *Biochim. Biophys. Acta* **683**, 119–151.

Bensasson, R. and E. J. Land (1973) *Biochim. Biophys. Acta* **325**, 175–181.

Berthold, D. A., G. T. Babcock and C. F. Yocum (1981) *FEBS Lett.* **134**, 231–234.

Bishop, N. I. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 321–328. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Blankenship, R. E., G. T. Babcock and K. Sauer (1975) *Biochim. Biophys. Acta* **387**, 165–175.

Boardman, N. K. and J. M. Anderson (1964) *Nature (Lond.)* **203**, 166–170.

Boska, M. and K. Sauer (1984) *Biochim. Biophys. Acta* **765**, 84–87.
Boska, M., K. Sauer, W. Buttner and G. T. Babcock (1983) *Biochim. Biophys. Acta* **722**, 327–330.
Bouges-Bocquet, B. (1973) *Biochim. Biophys. Acta* **314**, 250–256.
Bouges-Bocquet, B. (1980) *Biochim. Biophys. Acta* **594**, 85–103.
Boussac, A. and A.-L. Etienne (1982) *FEBS Lett.* **148**, 113–116.
Bowes, J. M. and A. R. Crofts (1978) *Z. Naturforschg., C. Biosci.* **33C**, 271–275.
Brand, J. J., P. Mohanty and D. C. Fork (1983) *FEBS Lett.* **155**, 120–124.
Braun-Zilinskas, B. and Govindjee (1972) *FEBS Lett.* **25**, 143–146.
Brettel, K., E. Schlodder and H. T. Witt (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 295–298. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Briantais, J.-M. (1969) *Physiol. Veg.* **1**, 135–180.
Briantais, J.-M., C. Vernotte, J. Lavergne and C. J. Arntzen (1977) *Biochim. Biophys. Acta* **461**, 61–74.
Bricker, T. M., J. G. Metz, D. Miles and L. A. Sherman (1982) *Biochim. Biophys. Acta* **724**, 447–455.
Brudvig, G. (1984) Paper presented at the *9th International Congress on Photobiology*, 1–6 July, 1984, Philadelphia.
Brudvig, G. M., J. L. Casey and K. Sauer (1983) *Biochim. Biophys. Acta* **723**, 366–371.
Brudvig, G. M., W. F. Beck and J. dePaula (1984) *Biophysic. J.* **45**, 258a.
Butler, W. L. and H. Matsuda (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and Ki. Satoh), pp. 113–122. Academic Press, Tokyo.
Butler, W., J. W. M. Visser and H. L. Simmons (1973) *Biochim. Biophys. Acta* **292**, 140–151.
Cammarata, K., N. Tamura, R. Sayre and G. Cheniae (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 311–320, Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Casey, J. L. and K. Sauer (1984) *Biophys. J.* **45**, 271a.
Cheniae, G. N. (1970) *Ann. Rev. Plant. Physiol.* **21**, 467–498.
Cheniae, G. N. (1980) *Meth. Enzymol.* **69**, 349–362.
Cheniae, G. N. and I. F. Martin (1970) *Biochim. Biophys. Acta* **197**, 219–239.
Chiancone, E., J. E. Norne,, S. Forsen, E. Antonini and J. Wyman (1972) *J. Mol. Biol.* **70**, 675–688.
Coleman, W. J., I. C. Baianu, H. S. Gutowsky and Govindjee (1983) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 283–286. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Coleman, W. and Govindjee (1984) In *Proceedings of the 16th FEBS Congress (Moscow, USSR).* VNU Science Press BV, Utrecht, The Netherlands. In press.
Cooper, S. R. and M. Calvin (1977) *J. Am. Chem. Soc.* **99**, 6623–6630.
Cooper, S. R., G. C. Dismukes, M. P. Klein and M. C. Calvin (1978) *J. Am. Chem. Soc.* **100**, 7248–7252.
Cramer, W. and A. R. Crofts (1982) In *Photosynthesis Vol. I—Energy Conversion by Plants and Bacteria* (Edited by Govindjee), pp. 387–467. Academic Press, New York.
Cramer, W., J. Whitmarsh and P. Horton (1979) In *The Porphyrins* (Edited by D. Dolphin), Vol. 7, pp. 71–106. Academic Press, New York.
Critchley, C. (1983) *Biochim. Biophys. Acta* **724**, 1–5.
Critchley, C. (1984) *Biochim. Biophys. Acta Reviews* **811**, 33–46.
Critchley, C., I. C. Baianu, Govindjee and H. S. Gutowsky (1982) *Biochim. Biophys. Acta* **682**, 436–445.
Dekker, J. P., H. J. van Gorkom, J. Wensink and L. Ouwehand (1984a) *Biochim. Biophys. Acta* **764**, 301–309.
Dekker, J. P., M. Brok and H. J. van Gorkom (1984b) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 171–174. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Dekker, J. P., H. J. van Gorkom, M. Brok and L. Ouwehand (1984c) *Biochim. Biophys. Acta.* In press.
Delrieu, M.-J. (1983) *Z. Naturforsch. C. Bioscience* **38c**, 247–258.
Delrieu, M.-J. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 291–294. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Diner, B. and C. de Vitry (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 407–411. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Diner, B. and P. Joliot (1977) In *Photosynthesis I. Photosynthetic Electron Transport and Photophosphorylation* (Edited by A. Trebst and M. Avron), Encyclopedia of Plant Physiology, New Series, Vol. 5, pp. 187–205. Springer-Verlag, Berlin.
Diner, B. and F. A. Wollman (1980) *Eur. J. Biochem.* **110**, 521–526.
Dismukes, G. C. and Y. Siderer (1980) *FEBS Lett.* **121**, 78–80.
Dismukes, G. C. and Y. Siderer (1981) *Proc. Natl. Acad. Sci. U.S.A* **78**, 274–278.
Dismukes, G. C., K. Ferris and P. Watnick (1982) *Photobiochem. Photobiophys.* **3**, 243–256.
Dismukes, G. C., D. A. Abramowicz, K. F. Ferris, P. Mathur, Y. Siderer, B. Upadrashta and P. Watnick (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 145–164. Academic Press, Tokyo.
Dixon, M. and E. C. Webb (1979) *The Enzymes* Third Edn, pp. 395–467. Academic Press, New York.
Döring, G., H. H. Stiehl and H. T. Witt (1967) *Z. für Naturforschung* **22b**, 639–644.
Dunahay, T. G., L. A. Staehlin, M. Seibert, P. D. Ogilvie and S. P. Berg (1983) *Biochim. Biophys. Acta* **764**, 179–193.
Duysens, L. N. M., J. Amesz and B. M. Kamp (1961) *Nature (Lond.)* **190**, 510–511.
Emerson, R. (1958) *Annl. Rev. Plant Physiol.* **9**, 1–24.
England, R. R. and E. H. Evans (1981) *FEBS Lett.* **134**, 175–177.
England, R. R. and E. H. Evans (1983) *Biochem. J.* **210**, 473–476.
Förster, V., Y.-Q. Hong and W. Junge (1981) *Biochim. Biophys. Acta* **638**, 141–152.
Fowler, C. F. (1977) *Biochim. Biophys. Acta* **462**, 414–421.
Fowler, C. F. and B. Kok (1974) *Biochim. Biophys. Acta* **357**, 299–307.
Franzen, L.-G. and L.-E. Andreasson (1984) *Biochim. Biophys. Acta* **765**, 166–170.
Frasch, W. D. and G. M. Cheniae (1980) *Plant Physiol.* **65**, 735–745.
Gerwick, B. C. (1982) In *Handbook of Biosolar Resources* (O. R. Zaborsky, Chief Editor), Vol. I, Part 2, pp. 213–222. CRC Pres, Inc., Boca Raton, Florida.
Ghanotakis, D. F. and C. F. Yocum (1985) *Photosynth. Res.* In press.
Ghanotakis, D. F., G. T. Babcock and C. F. Yocum (1984a) *FEBS Lett.* **167**, 127–130.
Ghanotakis, D. F., J. N. Topper, G. T. Babcock and C. F. Yocum (1984b) *FEBS Lett.* **170**, 169–173.

Ginsburg, A. and W. R. Carroll (1965) *Biochemistry* **4**, 2159–2174.

Goodin, D. B. (1983) Ph.D. Thesis, University of California, Lawrence Berkeley Laboratory.

Govindjee (1980) *Plant Biochemical Journal (India) Sircar Memorial Volume*, pp. 7–30.

Govindjee (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 227–238. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Govindjee and R. Govindjee (1975) In *Bioenergetics of Photosynthesis* (Edited by Govindjee), pp. 1–50. Academic Press, New York.

Govindjee and J. Whitmarsh (1982) In *Photosynthesis*, Vol. I, pp. 1–16, Energy Conversion by Plants and Bacteria, (Edited by Govindjee). Academic Press, New York.

Govindjee, T. Wydrzynski and S. B. Marks (1977) In *Bioenergetics of Membranes* (Edited by L. Packer, G. Papageorgiou and A. Trebst), pp. 305–316. Elsevier, North Holland, Amsterdam.

Govindjee, I. C. Baianu, C. Critchley and H. S. Gutowsky (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 303–315. Academic Press, Tokyo.

Govindjee, H. Koike and Y. Inoue (1984) *Photochem. Photobiol.* **39** (Supplement), 32S.

Govindjee, J. Amesz and D. C. Fork Eds. (1986) *Light Emission by Plants and Bacteria*. Academic Press, Orlando, in press.

Green, B. and E. Camm (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. II, pp. 95–98. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Hager, L. P. (1970) *Meth. Enzymol.* **17A**, 648–652.

Hansson, O. and L. E. Andreasson (1982) *Biochim. Biophys. Acta* **679**, 261–268.

Hansson, O., L. E. Andreasson and T. Vänngard (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 307–310. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Hill, R. and F. Bendall (1960) *Nature (Lond.)* **186**, 136–137.

Hind, G., H. Y. Nakatani and S. Izawa (1969) *Biochim. Biophys. Acta* **172**, 277–289.

Holdsworth, E. S. and J. H. Arshad (1977) *Arch. Biochem. Biophys.* **183**, 361–373.

Homann, P. H., J. D. Johnson and V. R. Pfister (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 275–284. Academic Press, Tokyo.

Homann, P. H. and J. D. Johnson (1984) *Photochem. Photobiol.* **39** (supplement), 33S.

Inoue, Y. (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 439–450. Academic Press, Tokyo.

Inoue, Y. and K. Shibata (1982) In *Photosynthesis Vol. I.—Energy Conversion by Plants and Bacteria* (Edited by Govindjee), pp. 507–533. Academic Press, New York.

Inoue, Y., A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh, Editors (1983) *The Oxygen Evolving System of Photosynthesis*. Academic Press, Tokyo.

Itoh, S., C. T. Yerkes, H. Koike, H. H. Robinson and A. R. Crofts (1984) *Biochim. Biophys. Acta.* **766**, 612–622.

Izawa, S. (1985) *Photosynthesis Research*. In Preparation.

Izawa, S., A. Muallem and N. K. Ramaswamy (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 293–302. Academic Press, Tokyo.

Izawa, S., N. K. Ramaswamy and P. M. Nair (1984) *Paper presented at the 9th International Congress on Photobiology*, 1–6 July, 1984, Philadelphia.

Jansson, C. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 375–378. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Jansson, C., H.-E. Åkerlund and B. Andersson (1983) *Photosyn. Res.* **4**, 271–279.

Joliot, P. and B. Kok (1975) In *Bioenergetics of Photosynthesis* (Edited by Govindjee), pp. 387–412. Academic Press, New York.

Joliot, P., G. Barbieri and R. Chabaud (1969) *Photochem. Photobiol.* **10**, 309–329.

Jones, R. D., D. A. Summerville and F. Busalo (1979) *Chem. Rev.* **79**, 139–179.

Junge, W. and J. B. Jackson (1982) In *Photosynthesis. Vol. I.—Energy Conversion by Plants and Bacteria* (Edited by Govindjee), pp. 589–646. Academic Press, New York.

Jursinic, P. and Govindjee (1977a) *Photochem. Photobiol.* **26**, 617–628.

Jursinic, P. and Govindjee (1977b) *Biochim. Biophys. Acta* **461**, 253–267.

Kambara, T. and Govindjee (1985) *Biophys. J.*, **47**, 419a. (*Proc. Natl. Acad. Sci.*, USA, in press).

Kelley, P. M. and S. Izawa (1978) *Biochim. Biophys. Acta* **502**, 198–210.

Khanna, R., S. Rajan, Govindjee and H. S. Gutowsky (1981a) In *Photosynthesis II. Photosynthetic Electron Transport and Photophosphorylation* (Edited by G. Akoyunoglou), pp. 307–316. Balaban International Science Services, Philadelphia.

Khanna, R., S. Rajan, K. E. Steinback, S. Bose, Govindjee and H. S. Gutowsky (1981b) *Israel J. Chem* **21**, 291–295.

Khanna, R., S. Rajan, Govindjee and H. S. Gutowsky (1983) *Biochim. Biophys. Acta* **725**, 10–18.

Kirby, J. A., D. B. Goodin, T. Wydrzynski, A. S. Robertson and M. P. Klein (1981) *J. Am. Chem. Soc.* **103**, 5537–5542.

Klimov, V. V. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 131–138. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Klimov, V. V. and A. A. Krasnovskii (1981) *Photosynthetica* **15**, 592–602.

Klimov, V. V., A. V. Klevanik, V. A. Shuvalov and A. A. Krasnovskii (1977) *FEBS Lett.* **82**, 183–186.

Klimov, V. V., S. I. Allakhverdiev, S. Demeter and A. A. Krasnovskii (1979) *Doklady Akad. Nauk SSR* **249**, 227–230.

Klimov, V. V., S. I. Allakhverdiev, V. A. Shuvalov and A. A. Krasnovskii (1982) *FEBS Lett.* **148**, 307–312.

Koike, H. and Y. Inoue (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 257–263. Academic Press, Tokyo.

Kok, B. and G. Hoch (1961) In *Light and Life* (Edited by W. D. McElroy and B. Glass), pp. 397–423. Johns Hopkins Press, Baltimore.

Kok, B. and A. Jagendorf (Chairman and Organizer, respectively) (1963) *Photosynthetic Mechanisms of Green Plants*, Papers presented at the Airlie House, Warrenton, Virginia, National Academy of Science—National Research Council, Washington, D.C., Publication No. 1145.

Kok, B., B. Forbush and M. McGloin (1970) *Photochem. Photobiol.* **11**, 457–475.

Krishnan, M. and P. Mohanty (1984) *Photosyn. Res.* **5**, 185–198.

Kulikov, A. V., V. R. Bogatyrenko, G. I. Likhtenshtein, S. I. Allakhverdiev, V. V. Klimov, V. A. Shuvalov and

A. A. Krasnovskii (1983) *Biophysics* **28**, 381–388.
Kusonoki, M. (1984) In *Advances in Photosynthesis Research*, Vol. I. (Edited by C. Sybesma), pp. 275–278. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Kuwabara, T. and N. Murata (1979) *Biochim. Biophys. Acta* **581**, 228–236.
Kuwabara, T. and N. Murata (1982) *Plant and Cell Physiol.* **23**, 533–539.
Kuwabara, T. and N. Murata (1983) *Plant Cell Physiol.* **24**, 741–747.
Kuwabara, T. and N. Murata (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 371–374. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Kyte, J. (1981) *Nature* **292**, 201–204.
Larsson, C., C. Jansson, U. Ljungberg, H.-E. Åkerlund and B. Andersson (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 363–366. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Lavorel, J. (1976) *FEBS Lett.* **66**, 164–167.
Lavorel, J. and B. Maison-Peteri (1983) *Physiol. Veg.* **21**, 509–517.
Lawrence, G. D. and D. T. Sawyer (1978) *Coord. Chem. Rev.* **27**, 173–193.
Ljungberg, U., C. Jansson, C. Larrson, H.-E. Åkerlund and B. Andersson (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 367–370. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Lozier, R., M. Baginsky and W. L. Butler (1971) *Photochem. Photobiol.* **14**, 323–328.
Lynch, M. W., D. N. Hendrickson, B. J. Fitzgerald and C. G. Pierpont (1984) *J. Am. Chem. Soc.* **106**, 2041–2049.
Malmström, B. G. (1982) *Ann. Rev. Biochem.* **51**, 21–59.
Maroc, J. and J. Garnier (1981) *Biochim. Biophys. Acta* **637**, 473–480.
Mathis, P. and J. Havemann (1977) *Biochim. Biophys. Acta* **461**, 167–181.
Mathis, P. and G. Paillotin (1981) In *Biochemistry of Plants* (Edited by M. D. Hatch and N. K. Boardman), Vol. 8, pp. 97–161. Academic Press, New York.
Metz, J. and N. I. Bishop (1980) *Biochim. Biophys. Res. Commun.* **94**, 560–566.
Metz, J. and M. Seibert (1984) *Plant. Physiol.* **76**, 829–832.
Metz, J., J. Wong and N. I. Bishop (1980) *FEBS Lett.* **114**, 61–66.
Metzner, H. (1978) In *Photosynthetic Oxygen Evolution* (Edited by H. Metzner), pp. 59–67. Academic Press, London.
Miller, M. and R. P. Cox (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 355–358. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Miyao, M. and N. Murata (1984) *FEBS Lett.* **168**, 118–120.
Møller, B. L., L. E. A. Henry, P. B. Hoj, F. Koenig and U. Hinz (1984) Paper presented at the *9th International Photobiology Congress*, 1–6 July, 1984, Philadelphia.
Monod, J., J.-P. Changeux and F. Jacob (1963) *J. Mol. Biol.* **6**, 306–329.
Murata, N., M. Miyao and T. Kuwabara (1983) In *Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 213–222. Academic Press, Tokyo.
Murata, N., M. Miyao, T. Omata and T. Kuwabara (1984a) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 329–332. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Murata, N., M. Miyao, T. Omata, H. Matsunami and T. Kuwabara (1984b) *Biochim. Biophys. Acta* **765**, 363–369.
Nakatani, H. Y. (1983a) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata and K. Satoh), pp. 49–54. Academic Press, Tokyo.
Nakatani, H. Y. (1983b) *Plant Cell Physiol.* **24**, 467–472.
Nakatani, H. Y. (1984) *Biochem. Biophys. Res. Commun.* **120**, 299–304.
Okada, S. and K. Asada (1983) *Plant Cell Physiol.* **24**, 163–172.
Okayama, S. (1974) *Plant Cell Physiol.* **15**, 95–101.
Okayama, S. (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 383–390. Academic Press, Tokyo.
O'Malley, P. J. and G. T. Babcock (1983) *Biophys. J.* **41**, 315a.
Ono, T. and Y. Inoue (1983a) *FEBS Lett.* **164**, 255–260.
Ono, T. A. and Y. Inoue (1983b) *Biochim. Biophys. Acta* **723**, 191–201.
Ono, T. A. and Y. Inoue (1983c) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 337–344. Academic Press, Tokyo.
Ono, T. and Y. Inoue (1984a) *FEBS Lett.* **168**, 281–286.
Ono, T. and Y. Inoue (1984b) *FEBS Lett.* **166**, 381–384.
Ono, T. and Y. Inoue (1984c) Paper presented at the *9th International Photobiology Congress*, 1–6 July, 1984, Philadelphia.
Ono, T., H. Nakatani, E. Johnson, C. Arntzen and Y. Inoue (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 381–386. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Pakrasi, H. P. and L. A. Sherman (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 395–398. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Parson, W. W. and B. Ke (1982) In *Photosynthesis Volume I—Energy Conversion by Plants and Bacteria* (Edited by Govindjee), pp. 331–386. Academic Press, New York.
Piccionni, R. G. and D. C. Mauzerall (1976) *Biochim. Biophys. Acta* **423**, 605–609.
Piccionni, R. G. and D. C. Mauzerall (1978a) *Biochim. Biophys. Acta* **504**, 384–397.
Piccionni, R. G. and D. C. Mauzerall (1978b) *Biochim. Biophys. Acta* **504**, 398–405.
Pulles, M. J. P., J. H. van Gorkom and J. G. Willemsen (1976) *Biochim. Biophys. Acta* **449**, 536–540.
Rabinowitch, E. and Govindjee (1961) In *Light and Life* (Edited by W. D. McElroy and B. Glass), pp. 378–391. The John Hopkins Press, Baltimore.
Radmer, R. (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 135–144. Academic Press, Tokyo.
Radmer, R. and G. Cheniae (1977) In *Primary Processes of Photosynthesis* (Edited by J. Barber), Topics in Photosynthesis, Vol. 2, pp. 303–348. Elsevier, Amsterdam.
Radmer, R. and O. Ollinger (1983) *FEBS Lett.* **152**, 39–43.
Ramaswamy, N. K. and P. M. Nair (1978) *Plant. Sci. Lett.* **13**, 383–388.
Renger, G. (1976) *Biochim. Biophys. Acta* **440**, 287–300.
Renger, G. (1977) *FEBS Lett.* **81**, 223–228.

Renger, G. (1978) In *Photosynthetic Oxygen Evolution* (Edited by H. Metzner), pp. 239–248. Academic Press, London.

Renger, G. and Govindjee (1985) *Photosynthesis Research.* **6,** 33–55.

Renger, G. and M. Völker (1982) *FEBS Lett.* **149,** 203–207.

Renger, G. and W. Weiss (1983) *Biochim. Biophys. Acta* **722,** 1–11.

Renger, G. and W. Weiss (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 253–260. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Renger, G., H.-J. Eckert and W. Weiss (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 73–82. Academic Press, Tokyo.

Robinson, H. H., R. R. Sharp and C. F. Yocum (1981) In *Photosynthesis II. Electron Transport and Photophosphorylation* (Edited by G. Akoyunoglou), pp. 317–326. Balaban International Science Services, Philadelphia.

Rutherford, A. W., A. R. Crofts and Y. Inoue (1982) *Biochim. Biophys. Acta* **682,** 457–465.

Rutherford, A. W., G. Renger, H. Koike and Y. Inoue (1984a) *Biochim. Biophys. Acta.* **767,** 548–556.

Rutherford, A. W., Govindjee and Y. Inoue (1984b) *Proc. Natl. Acad. Sci. USA* **81,** 1107–1111.

Sadewasser, D. A. and R. A. Dilley (1978) *Biochim. Biophys. Acta* **501,** 208–216.

Sandusky, P. O. and C. F. Yokum (1983) *FEBS Lett.* **162,** 339–343.

Sane, P. V. and A. W. Rutherford (1986) In *Light Emission by Plants and Bacteria* (Edited by Govindjee, J. Amesz and D. C. Fork), in preparation. Academic Press, Orlando.

Saphon, S. and A. R. Crofts (1977) *Z. Naturforschg, C. Biosciences* **32C,** 617–626.

Satoh, K. (1982) In *Methods in Chloroplast Molecular Biology* (Edited by M. Edelman, R. B. Hallick and N.-H. Chua), pp. 845–856. Elsevier Biomedical Press, Amsterdam.

Satoh, K. and W. Butler (1978) *Plant Physiol.* **61,** 373–379.

Sauer, K. (1980) *Acc. Chem. Res.* **13,** 249–256.

Sauer, K., M. Boska, J. L. Casey and K. K. Karukstis (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 121–126. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Selak, M. A., B. E. Koch-Whitmarsh and J. Whitmarsh (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 493–496. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Sharp, R. R. and C. F. Yocum (1980) *Biochim. Biophys. Acta* **592,** 185–195.

Spector, M. and G. Winget (1980) *Proc. Natl. Acad. Sci. USA* **77,** 957–959.

Stewart, A. C. and D. S. Bendall (1979) *FEBS Lett.* **107,** 308–312.

Sybesma, C. (Ed.) (1984) *Advances in Photosynthesis Research*, Proceedings of the VIth International Congress on Photosynthesis, Aug. 1–6, 1983, Brussels, Belgium, 3480 pages in 4 volumes. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Theg, S. M. and P. Homann (1982) *Biochim. Biophys. Acta* **674,** 221–234.

Theg, S. M., P. Jursinic and P. H. Homann (1984) *Biochim. Biophys. Acta.* **766,** 636–646.

Toyoshima, Y., K. Akabori, E. Fukutaka and A. Imaoka (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 239–244. Academic Press, Tokyo.

van Best, J. A. and P. Mathis (1978) *Biochim. Biophys. Acta* **503,** 178–188.

van Gorkom, H. J. (1985) *Photosynthesis Research.* **6,** 97–112.

Velthuys, B. R. (1980) *Ann. Rev. Plant Physiol.* **31,** 545–567.

Velthuys, B. R. (1981) In *Photosynthesis II* (Edited by G. Akoyunoglou), pp. 75–85. Balaban International Serv., Philadelphia.

Velthuys, B. R. and J. Amesz (1974) *Biochim. Biophys. Acta* **333,** 85–94.

Vermaas, W. F. J. and Govindjee (1981) *Photochem. Photobiol.* **34,** 775–793.

Vermaas, W. F. J., G. Renger and G. Dohnt (1984) *Biochim. Biophys. Acta* **704,** 194–202.

Vernon, L., B. Ke, H. H. Mollenhauer and E. R. Shaw (1969) In *Progress in Photosynthesis Research* (Edited by H. Metzner), pp. 137–148. International Union of Biological Sciences, Tübingen.

Vidaver, W., D. Bruce, K. Colbow and R. Popovic (1984) *Photochem. Photobiol.* **39** (Supplement), 32S.

Warburg, O. and W. Lüttgens (1944) *Naturwissenschaften* **32,** 161, 301.

Warburg, O. and W. Lüttgens (1946) *Biokhimiya (Russian)* **11,** 303; reprinted in *Schwermetalle als Wirkungsgruppen von Fermenten*, Berlin, 1946, and *Heavy Metal Prosthetic Groups and Enzyme Action*, Clarendon Press, Oxford, 1949.

Weiss, W. and G. Renger (1984a) *FEBS Lett.* **169,** 219–223.

Weiss, W. and G. Renger (1984b) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 167–170. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Wells, C. F. (1965) *Nature* **205,** 693–694.

Wensink, J., J. P. Dekker and H. J. van Gorkom (1984) *Biochim. Biophys. Acta* **765,** 147–155.

Wessels, J. S. C., O. von Alphen-van Waveren and D. Voorn (1973) *Biochim. Biophys. Acta* **292,** 741–752.

Whitford, D., K. Gounaris and J. Barber (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 497–500. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Whitmarsh, J. and D. Ort (1983) *Arch. Biochem. Biophys.* **231,** 378–389.

Widger, W. R., W. A. Cramer, M. Hermodson, M. Gullifor, D. Meyer, J. Farchaus and B. Liedtke (1983) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 123–133. Academic Press, Tokyo.

Wille, B. and J. Lavergne (1982) *Photobiochem. Photobiophys.* **4,** 131–144.

Wohlgemuth, R., J. W. Otvos and M. Calvin (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 745–748. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.

Wood, P. M. and D. S. Bendall (1976) *Eur. J. Biochem.* **61,** 337–344.

Wydrzynski, T. (1982) In *Photosynthesis. Vol. I—Energy Conversion by Plants and Bacteria* (Edited by Govindjee), pp. 469–506. Academic Press, New York.

Wydrzynski, T. and K. Sauer (1980) *Biochim. Biophys. Acta* **589,** 56–70.

Wydrzynski, T., N. Zumbulyadis, P. G. Schmidt and Govindjee (1975) *Biochim. Biophys. Acta* **408,** 349–354.

Wydrzynski, T., N. Zumbulyadis, P. G. Schmidt, H. S. Gutowsky and Govindjee (1976) *Proc. Natl. Acad. Sci., USA* **73,** 1196–1198.

Wydrzynski, T., S. B. Marks and Govindjee (1977) *Abst. 4th Int. Congr. on Photosynth.*

4–9 Sept. 1977, Reading, U.K., p. 417.
Wydrzynski, T., S. B. Marks, P. G. Schmidt, Govindjee and H. S. Gutowsky (1978) *Biochemistry* **17**, 2155–2162.
Wydrzynski, T., B. J. Huggins, P. Jursinic, V. Förster and W. Junge (1984) *Photochem. Photobiol.* **39** (Supplement), 32S.
Yamagishi, A. and S. Katoh (1983a) *Arch. Biochem. Biophys.* **225**, 836–846.
Yamagishi, A. and S. Katoh (1983b) In *The Oxygen Evolving System of Photosynthesis* (Edited by Y. Inoue, A. R. Crofts, Govindjee, N. Murata, G. Renger and K. Satoh), pp. 39–48. Academic Press, Tokyo.
Yamamoto, Y. and M. Nishimura (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 333–336. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Yamamoto, Y., M. Doi, N. Tamura and M. Nishimura (1981) *FEBS Lett.* **133**, 265–268.
Yamamoto, Y., T. Ueda, H. Shinkai and M. Nishimura (1982) *Biochim. Biophys. Acta* **679**, 347–350.
Yamamoto, Y., S. Shimada and M. Nishimura (1983) *FEBS Lett.* **151**, 49–53.
Yocum, C. F. (1984) In *Advances in Photosynthesis Research* (Edited by C. Sybesma), Vol. I, pp. 239–242. Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag.
Yocum, C. F., C. T. Yerkes, R. E. Blankenship, R. R. Sharp and G. T. Babcock (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7507–7511.
Yu, CM.-C. and J. J. Brand (1980) *Biochim. Biophys. Acta* **591**, 483–487.
Zilinskas, B. and Govindjee (1974) *Plant Sci. Lett.* **3**, 219–227.
Zimmermann, J. L. and A. W. Rutherford (1984) *Biochim. Biophys. Acta.* **767**, 160–167.
Zyk, N., N. Citri and H. S. Moyed (1969) *Biochemistry* **8**, 2787–2794.

Note added in Proof:
This review was first presented at a symposium of the *16th FEBS Congress*, held in June, 1984 in Moscow (U.S.S.R.), and later at a 5-day photosynthesis symposium of the *Chinese Society of Plant Physiologists*, held in September, 1984 in Yangzhou (People's Republic of China).

The authors thank A. B. Haasdijk and P. van Snippenberg of Landbouwhogeschool (Wageningen, The Netherlands) for drawing the original Figures 1, 2, and 3.