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MINI-REVIEW

Electron Transfer in the Water-Oxidizing Complex of Photosystem II

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Abstract

An overview is presented of secondary electron transfer at the electron donor side of Photosystem II, at which ultimately two water molecules are oxidized to molecular oxygen, and the central role of manganese in catalyzing this process is discussed. A powerful technique for the analysis of manganese redox changes in the water-oxidizing mechanism is the measurement of ultraviolet absorbance changes, induced by single-turnover light flashes on dark-adapted PS II preparations. Various interpretations of these ultraviolet absorbance changes have been proposed. Here it is shown that these changes are due to a single spectral component, which presumably is caused by the oxidation of Mn(III) to Mn(IV), and which oscillates with a sequence $+1$, $+1$, $+1$, -3 during the so-called $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ redox transitions of the oxygen-evolving complex. This interpretation seems to be consistent with the results obtained with other techniques, such as those on the multiline EPR signal, the intervalence Mn(III)–Mn(IV) transition in the infrared, and EXAFS studies. The dark distribution of the S states and its modification by high pH and by the addition of low concentrations of certain water analogues are discussed. Finally, the patterns of proton release and of electrochromic absorbance changes, possibly reflecting the change of charge in the oxygen-evolving system, are discussed. It is concluded that nonstoichiometric patterns must be considered, and that the net electrical charge of the system probably is the highest in state S_2 and the lowest in state S_1.

Key words: Photosystem II; water oxidation; electron transfer; manganese; oxygen evolution.

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Introduction

The electron transfer chain at the donor side of PS II[4] has been a subject of continuous interest in photosynthesis research. While the acceptor side of PS II shows remarkable similarities with that of the photosystem of purple bacteria, the high potential donor side is unique, being able to oxidize water to molecular oxygen.

In PS II, electron transfer starts with the oxidation of a special chlorophyll *a* molecule having its red absorbance maximum at 680 nm (Döring *et al.*, 1969), the primary electron donor P-680. The electron is first transferred to a pheophytin *a* molecule called I, thereby generating its radical anion (Klimov *et al.*, 1977), and then to the traditional "primary" electron acceptor (Duysens and Sweers, 1963), a tightly bound plastoquinone molecule (Stiehl and Witt, 1968) called Q_A that is reduced to the (unprotonated) semiquinone anion (Van Gorkom, 1974). The formation of the state P^+-680-Q_A^- takes a few hundred picoseconds only (Nuijs *et al.*, 1986).

Subsequently, the electron on Q_A^- is transferred to a second plastoquinone molecule, which is assumed to be a member of the mobile pool of plastoquinone molecules present in thylakoid membranes (Velthuys, 1982). The semiquinone anion Q_B^- is formed (Van Gorkom *et al.*, 1982), which stays firmly bound to PS II until a second photoreaction takes place. Unlike Q_A^-, Q_B^- is able to accept another electron and, after the uptake of two protons, leaves PS II as plastoquinol. Both Q_A^- reoxidations usually take place between a few hundred microseconds and a few milliseconds (Bowes and Crofts, 1980). A nonheme iron with unknown function is associated with both semiquinones, and largely determines the EPR properties of these (Nugent *et al.*, 1981). Normally, this iron has a valence of +2, but certain oxidants like ferricyanide may induce its oxidation to Fe^{3+}. The latter has been identified recently as the electron acceptor Q-400 (Petrouleas and Diner, 1986).

The picture of the route of electron transfer at the acceptor side is relatively consistent now, and the highly resolved structure of the *Rps. viridis* reaction center (Deisenhofer *et al.*, 1984, 1985) may be regarded as a model system for it. No such model is available for the donor side yet, and many gaps and inconsistencies remain to be resolved.

The oxidized primary electron donor P^+-680 is re-reduced by a tightly bound electron donor called Z. The oxidized form of this species is characterized by a specific EPR signal, called signal II (Babcock and Sauer, 1975), and the absorbance difference spectrum has been reported (Diner and

[4] Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone; DCMU 3(3′,4′-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone; PS, photosystem.

De Vitry, 1984; Dekker *et al.*, 1984c; Weiss and Renger, 1986). Both the lineshape of the EPR signal (O'Malley and Babcock, 1984; O'Malley *et al.*, 1984; Brok *et al.*, 1985) and the absorbance difference spectrum suggested that Z is a bound plastoquinol molecule that is oxidized to the semiquinone cation. Apparently, the reaction center proteins are built in such a way that the protons are retained on the cationic semiquinone, a strong acid with $E_{m,7} = 1.0\,V$ (Wood and Bendall, 1976).

In PS II devoid of oxygen-evolving capacity, experimentally brought about, for example, by washing with concentrated Tris at alkaline pH, Z is the terminal electron donor. Under these conditions, the decay half-time of P^+-680 coincides with that of the oxidation of Z (Reinman *et al.*, 1981; Boska *et al.*, 1983). This half-time varies, depending on the pH, between 2 and 20 μs. In oxygen-evolving PS II, no consistent picture of electron transfer in this part of PS II has emerged yet. Many properties seem to indicate the existence of a second electron donor, located either between Z and P-680, or at a second route parallel to Z (Bouges-Bocquet, 1980; Brettel *et al.*, 1984; Lavergne, 1984). P^+-reduction occurs in the nanosecond-time range (Sonneveld *et al.*, 1979), the rate being dependent on the redox state of the oxygen-evolving complex (Brettel *et al.*, 1984). Z oxidation also is more rapid in oxygen-evolving PS II (Boska and Sauer, 1984), but the kinetics remain to be resolved.

Z^+-reduction in Tris-washed PS II takes place between tens of milliseconds and a few seconds, presumably as a result of back-reaction with the PS II electron acceptors, and/or electron donation by artificial donors. Z^+ may also be able to oxidize a second tightly bound plastoquinol called D. This reaction takes a millisecond (Boussac and Etienne, 1982a), and once formed, D^+ is stable for hours. This component, responsible for the EPR signal II_{slow} (Babcock and Sauer, 1973), seems always to be present in oxygen-evolving PS II, and its function is unknown.

Normally, Z^+ is involved in the oxidation of water, generating molecular oxygen. Z has to be oxidized four times in order to catalyze the formation of oxygen out of water. The reaction centers do not cooperate in this respect, as indicated by the "classical" period 4 oscillation of oxygen release upon illumination with short, saturating light flashes (Joliot *et al.*, 1969). Thus, every reaction center must be able to store four positive equivalents before oxygen can be formed (Kok *et al.*, 1970):

$$S_0 \xrightarrow{h\nu} S_1 \xrightarrow{h\nu} S_2 \xrightarrow{h\nu} S_3 \xrightarrow{h\nu} S_4 \longrightarrow S_0 \qquad (2H_2O \rightarrow O_2 + 4H^+)$$

Of these so-called S states, S_0 and S_1 were believed to be stable in the dark, while S_2 and S_3 revert to S_1 in minutes. S_4 is not stable, and reverts to S_0 in

about a millisecond, releasing oxygen (Joliot and Kok, 1975). Dark adaptation of PS II having a mixed S-state population thus leads to S_1 in 75% and S_0 in 25% of the centers, thereby explaining Joliot's original observation that oxygen is released primarily after the third flash. The period 4 oscillation is damped, due to misses and double hits. The former are caused by the (small) probability that the charge separation is unsuccesful, and the latter are dependent on the length of the light flashes.

The forward, light-induced S-state transitions proceed in the (sub)-millisecond time range. EPR experiments on signal II have indicated that Z^+ oxidizes S_3 in a millisecond (the same half-time in which oxygen is released), and that Z^+ oxidizes S_2 in about 400 μs (Babcock *et al.*, 1976). The other S-state transitions were thought to proceed in 100 μs, or less.

There is increasing evidence that the redox state of manganese at least partially determines the S state (Amesz, 1983). The S-state dependence of the amount of released Mn(II) after a mild heat-shock treatment (Wydrzynski and Sauer, 1980) was the first indication of Mn valence changes during the S-state cycle. A multiline EPR signal, observable at very low temperature, was attributed to the S_2 state only, and ascribed to a mixed-valence Mn dimer or tetramer (Dismukes and Siderer, 1981; Andréasson *et al.*, 1983; Zimmermann and Rutherford, 1984). Illumination of PS II at 200 K induces, in addition to the multiline signal an EPR signal at $g = 4.1$ (Casey and Sauer, 1984); also this signal may arise from the Mn cluster (De Paula *et al.*, 1985; Zimmermann and Rutherford, 1986). Manganese *K*-edge X-ray absorption spectra indicated an oxidation of manganese on the $S_1 \rightarrow S_2$ transition (Goodin *et al.*, 1984). Srinivasan and Sharp (1986) reported a change in the NMR proton relaxation rate upon the $S_1 \rightarrow S_2$ transition, which they interpreted as a result of a Mn(III) $\rightarrow$ Mn(IV) valence change.

The same transition was reported to be accompanied by an absorbance change in the ultraviolet (Pulles *et al.*, 1976). Dekker *et al.* (1984c) determined the spectrum and extinction coefficient of this change and found it consistent with the oxidation of one Mn(III) to Mn(IV). Dismukes and Mathis (1984) reported a concomitant absorbance increase in the near-infrared, which they attributed to the intervalence absorption band of a Mn(III)–Mn(IV) dimer.

Of these methods, the measurement of ultraviolet absorbance changes is perhaps most likely to yield unambiguous information on changes on manganese redox state, relatively independent of interactions with other Mn atoms or ligands. Several authors have now reported on the sequence of ultraviolet absorbance changes during the four S-state transitions, and various interpretations have been proposed.

In this review, we discuss in more detail the results obtained with UV (and visible) absorbance difference measurements, and the redox state of the oxygen-evolving complex in the dark. More detailed reviews were,

among others, published by Joliot and Kok (1975), Bouges-Bocquet (1980), Wydrzynski (1982), Amesz (1983), Van Gorkom (1985), Govindjee *et al.*, (1985), and Dismukes (1986).

The S-State Distribution in the Dark

In the original model of the "oxygen clock" (Kok *et al.*, 1970), S_0 and S_1 were assumed to be stable in the dark, while S_2 and S_3 react back to S_1. Later, it was shown that the decay route $S_3 \rightarrow S_2 \rightarrow S_1$ is at least the main one (Joliot and Kok, 1975; Hanssum *et al.*, 1985), and a detailed analysis of the oxygen release patterns tended to confirm the long-term stability of both S_0 and S_1 (Forbush *et al.*, 1971), although a long-term equilibration of S_1 and S_0 leading to, peculiarly, 75% S_1 and 25% S_0 also seemed to be possible (Joliot and Kok, 1975).

Velthuys and Visser (1975) suggested that the presence of S_0 in the dark may be apparent only: certain reductants induce a slow reduction of D^+, the compound responsible for EPR signal II_{slow}. D is oxidized again after a charge separation in PS II with a half-time of about 2 s (Babcock and Sauer, 1973), so its oxidation may induce extra misses on the first flashes in a flash series, thus mimicking S_0 in the dark in part of the centers. Vermaas *et al.* (1984) reported that indeed a small fraction of S_2 and S_3 decay in a few seconds, although part of this decay also may be explained by back-reaction with Q_A^-. This back-reaction takes also about 2 s (Bennoun, 1970), and the $Q_A^- Q_B \rightleftarrows Q_A Q_B^-$ equilibrium constant is rather moderate (see, e.g., Robinson and Crofts, 1984; Schatz and Van Gorkom, 1985). Nevertheless, a flash series with frequency high enough to overcome the 2-s decay of S_2 and S_3 yielded an oxygen release pattern that could be fitted with almost 100% S_1 in the dark (Vermaas *et al.*, 1984).

It is not known which compound causes the proposed oxidation of S_0 to S_1. A possible candidate is D^+ itself. It may also be possible that the equilibrium $S_0 + O_2 + 4H^+ \rightleftarrows S_4 + 2H_2O$, in combination with the decay of S_4, S_3, and S_2 to S_1, plays a role in setting the S_0/S_1 equilibrium (Beck *et al.*, 1985). It should be noted that the dark S_0 oxidation may be apparent also. Very weak background light, giving rise to, for example, one turnover in 5 min, must be able to accumulate S_1 in the dark. The question of the S_1/S_0 ratio in the dark is still open, and, in fact, every amount of S_0 between 0 and 25% can be expected.

Additional processes become important at higher pH values. In chloroplasts, D^+ is stable at pH 6.0, but is reduced slowly at pH 8.3 (Boussac and Etienne, 1982b). The reductant involved is not known. In the so-called BBY preparations (Berthold *et al.*, 1981), D^+ is stable at pH 8.3, but S_1 is reduced

to S_0 instead (De Groot *et al.*, 1986; Plijter *et al.*, 1986). The dark reduction of S_1 reversed (in the dark) when the system was brought back to pH 6.0 again (Plijter *et al.*, 1986), suggesting that S_1 and S_0 equilibrate rather quickly in this case (within 15 min). Also in this case, the redox compound responsible for setting the S_1/S_0 equilibrium remained unknown. It was suggested that these processes at higher pH values may be related to the reported conformational change of PS II above pH 7.6 (Völker *et al.*, 1985).

In the dark-adapted BBY preparations at pH 8.3, the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition is observed after the fourth flash, as a result of the reduction of S_1 to S_0. In PS II in the presence of low concentrations of certain water analogues, like NH_2OH (Bouges, 1971), NH_2NH_2, and H_2O_2 (Velthuys and Kok, 1978), this transition is shifted even to the fifth flash. The delay can probably not be attributed to an oxidation of these compounds after the first two flashes. With H_2O_2, O_2 is not evolved after the first or second flash (Velthuys and Kok, 1978), and with NH_2OH, the detected N_2 on the first flash (Radmer and Ollinger, 1982) may be due to inactive centers only (Saygin and Witt, 1985b). The binding of NH_2NH_2 and NH_2OH is rather slow (Hanssum and Renger, 1985) and involves several molecules per reaction center (Förster and Junge, 1985c; Hanssum and Renger, 1985).

At low concentrations of NH_2OH, the second flash appears to induce the $S_0 \rightarrow S_1$ transition (Förster and Junge, 1985b; Saygin and Witt, 1985b). After the first flash, two protons are released with a half-time of a few milliseconds (Förster and Junge, 1985b), and an electrochromic shift is observed that is just opposite to that of the $S_1 \rightarrow S_2$ transition (Saygin and Witt, 1985b) (see below). Presumably, an "over-reduced" state S_{-1} is formed (Velthuys and Kok, 1978), but its chemical characterization has not yet been achieved (Witt *et al.*, 1986).

The Period 4 Oscillation of Ultraviolet Absorbance

A period 4 oscillation can be detected in ultraviolet absorbance (Pulles *et al.*, 1976), but a period 2 oscillation is detectable also in this spectral region. The latter oscillation has been ascribed to the two-electron gate at the acceptor side of PS II (Pulles *et al.*, 1976; Mathis and Haveman, 1977). Several methods have been used to eliminate this acceptor side contribution in order to analyze the donor side oscillations in more detail. Velthuys (1981) subtracted changes in the presence of 1 mM hydroxylamine from those without. This component acts as an efficient electron donor, and does not cause absorbance changes upon oxidation (Van Gorkom, 1976). Therefore, the subtraction supposedly yields the period 4 oscillation of the donor side alone, but it should be kept in mind that a small part of the acceptor changes

are possibly not subtracted due to the ability of hydroxylamine to inhibit the electron transport from Z to P^+-680 (Den Haan *et al.*, 1974).

By using this method, Velthuys (1981) reported that the donor side absorbance increased with a half-time of 70 μs on the first flash (in dark-adapted chloroplasts), stayed relatively unchanged after the second, and decayed in about a millisecond after the third, suggesting an oxidation on the $S_1 \rightarrow S_2$ transition and a reduction during oxygen release. The UV spectrum of the $S_1 \rightarrow S_2$ transition was reported to consist of a broad absorbance increase near 300 nm with $\Delta\varepsilon_{max} = 6000\,M^{-1}\,cm^{-1}$ (Dekker *et al.*, 1984c), possibly a Mn(III) $\rightarrow$ Mn(IV) transition.

A quantitative analysis has been presented of the oscillating changes in purified PS II preparations, supplied with the electron acceptor DCBQ (Dekker *et al.*, 1984d). Due to the use of this acceptor, the contamination of acceptor side oscillations is strongly diminished. The S-state parameters (the dark S-state ratio and the number of misses and double hits) were determined from the oscillation pattern of the millisecond transient accompanying oxygen release. Knowing how much of each S-state transition takes place after each flash, one needs four flashes to calculate the change due to each S-state transition, or only three if one assumes that the four transitions should add up to zero. All sets of oscillating changes due to the subsequent four or three flashes beginning with flash numbers 2, 3, 4, and 5 gave nearly the same results (see also Fig. 1), and were averaged. The results of this averaging are shown in Fig. 2A (the spectrum of the $S_3 \rightarrow S_0$ transition is multiplied by $-1/3$). They were interpreted to consist of an acceptor side contribution with a spectrum resembling that due to the reaction $Q_B^- + \frac{1}{2}DCBQ + H^+ \rightarrow Q_B + \frac{1}{2}DCBQH_2$, the sequence of which was roughly $-1, +1, -1, +1$ for the S-state transitions $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$, and of a donor side contribution with a spectrum similar to that published before (Dekker *et al.*, 1984c), and with sequence $+1, +1, +1, -3$. It was concluded that each of the first three S-state transitions involve the oxidation of a Mn(III) ion to Mn(IV). The spectrum of the millisecond transient accompanying oxygen release could be fitted with that of the reduction of the three Mn(IV) ions, together with that of the semiquinone cation Z^+ (Dekker *et al.*, 1984d). The kinetics of the four successive transitions were found to occur with half-times of about 30, 110, 350, and 1300 μs in spinach PS II preparations (Dekker *et al.*, 1984b), i.e., roughly consistent with the reduction times of Z^+ in oxygen-evolving PS II (Babcock *et al.*, 1976; Boska and Sauer, 1984).

Recently, similar experiments were carried out by Renger and Weiss (1985, 1986) using trypsinized PS II particles supplied with the electron acceptor ferricyanide. The trypsinization procedure presumably blocks electron transfer from Q_A^- to the secondary quinone to a great extent, but leaves the donor side relatively unchanged. The results of Renger and Weiss

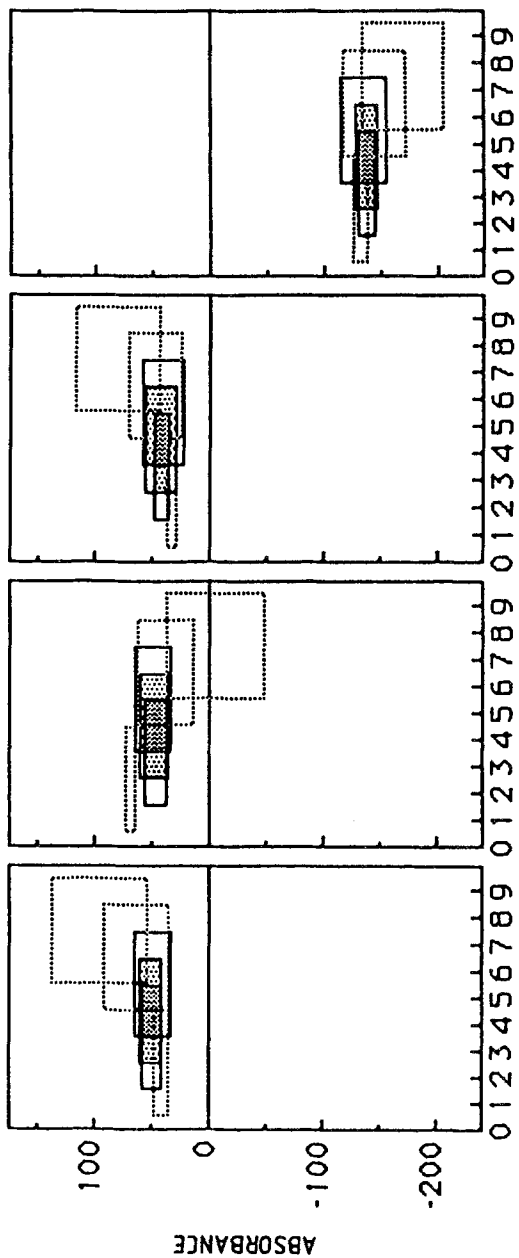

Fig. 1. Example of calculated differential extinction coefficients of the four S-state transitions. The rectangles show the results obtained with the original data of Dekker *et al.* (1984d) at 295 nm for all groups of four successive flashes. The height of each rectangle shows the upper and lower limits allowed by the noise in the four data points used. Note that inclusion of the first flash led to significantly different results, whereas subsequent flash groups yielded consistent results as far as the increasing uncertainty due to the damping of the oscillation allowed. The groups of the four successive flashes beginning with flash numbers 1 and 6 were not used for the mathematical analysis reported by Dekker *et al.* (1984d). The units on the vertical scale are relative.

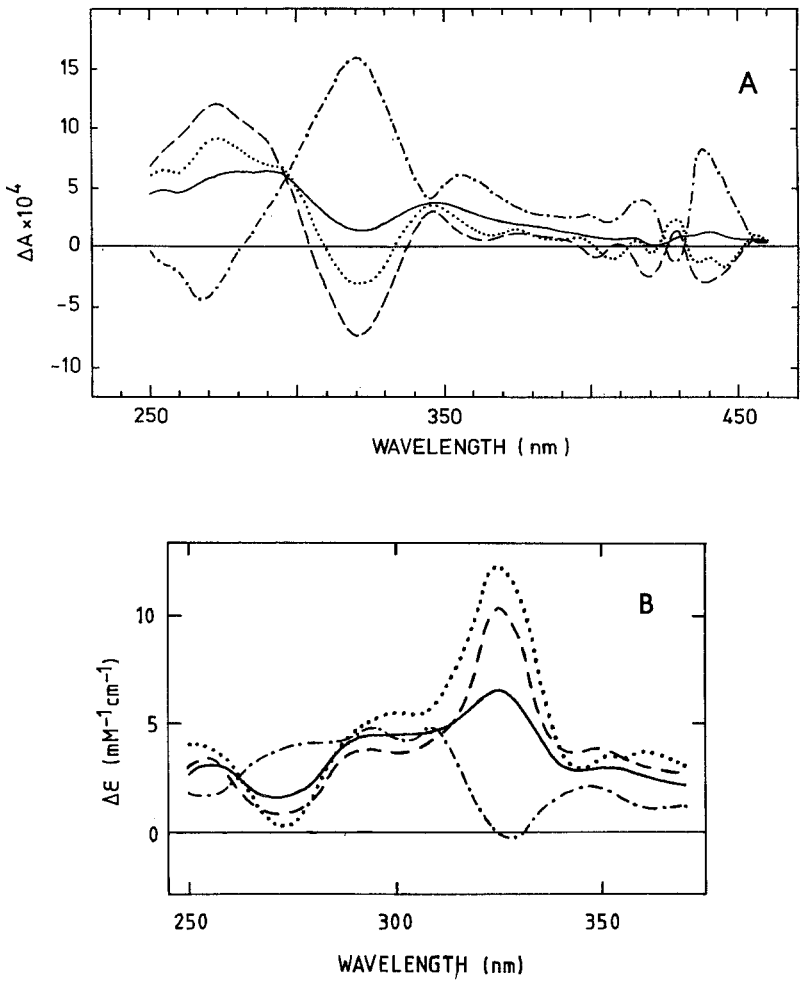

Fig. 2. Absorbance difference spectra associated with the $S_0 \rightarrow S_1$ (dotted line), $S_1 \rightarrow S_2$ (dash-dot line), $S_2 \rightarrow S_3$ (dashed line), and $S_3 \rightarrow S_0$ (after multiplying by $-1/3$; full line) transitions. The data points were adapted from the original data of (A) Dekker *et al.*, (1984d), who used dark-adapted PS II preparations supplied with the electron acceptor DCBQ and of (B) Renger and Weiss (1986), who used dark-adapted, trypsinized PS II preparations supplied with the electron accepor ferricyanide.

are shown in Fig. 2B (again, the spectrum of the $S_3 \rightarrow S_0$ transition is multiplied by $-1/3$). Also these spectra may consist of a species having a broad absorbance increase near 300 nm with sequence $+1, +1, +1, -3$, and of a species showing a period 2 oscillation, peaking near 325 nm. Peculiarly, this period 2 oscillation is just opposite to that shown in Fig. 2A, but it can

still be ascribed to the acceptor side: in the trypsinized particles, ferricyanide must be able to oxidize the reduced form of Q-400, the quinone-bound Fe^{2+} ion (Petrouleas and Diner, 1986), efficiently. In a flash series, the Fe^{3+} ion is reduced first, thereby shifting the period 2 oscillation, if present, with one unit. The kinetics of the S-state transitions reported by Renger and Weiss (1985) largely coincided with those reported by Dekker *et al.* (1984b).

Renger and Weiss (1986) suggested an alternative explanation for the spectra shown in Fig. 2B, based on the author's view (Renger, 1977) that only two Mn ions are needed for the oxidation of water to oxygen, and that the 1, 1, 1, −3 model badly explains the stability of the S_1 state. They suggested a period 2 oscillation of an, as yet, unidentified Mn ligand with sequence +1, −1, +1, −1, and a period 4 oscillation of Mn with sequence 0, +2, 0, −2. This suggestion does not explain the phase shift due to the addition of ferricyanide, and fits poorly the reported "period 2-free" oscillations (Dekker *et al.*, 1984a; Saygin and Witt, 1985b) (see below).

The discrimination between both alternatives depends on the interpretation of the period 2 oscillation as an acceptor side contamination or an intrinsic donor side contribution. In the latter case, the spectrum of the millisecond transient accompanying oxygen release should contain the spectrum of the species showing the period 2 oscillation. The spectrum reported by Renger and Weiss (1986) indeed contains such a contribution, in line with their hypothesis, but this contribution may also be caused by a millisecond decay phase of Q_A^- (Dekker *et al.*, 1984d). No evidence is presented that such a Q_A^- decay phase can be excluded (for example by taking the difference between the third and the fifth flash), and, in fact, it is likely that such a transient is present in millisecond transient reported by Renger and Weiss (1986), in view of measurements of Weiss (1985) at 325 and 360 nm. Therefore, and because of the phase shift of the period 2 oscillation mentioned above, the interpretation of Renger and Weiss seems unlikely.

The data described above practically exclude the 0, +1, 0, −1 sequence, proposed originally by Velthuys (1981). The spectra of the $S_0 \rightarrow S_1$ and of the $S_2 \rightarrow S_3$ transitions in Fig. 2A do not resemble any familiar acceptor side spectrum, and have clearly different zero transitions. Nevertheless, Lavergne (1985) argued that the 0, +1, 0, −1 sequence must still be considered as well. Lavergne (1986) measured absorbance changes at 295 nm and analyzed the response of the pattern of the period 4 oscillation upon changing the S-state parameters. The data were in favor of the 0, +1, 0, −1 sequence, but the interpretation, however, strongly depends on the properties of the changes due to the first flash. In the analyses described above, these were excluded, simply because if and when they were included in the mathematical analysis, different results were obtained. Probably, a small part of the centers are blocked after the first flash, the amount being dependent on the preparation

used and on the acceptor system used. When neglecting the changes due to the first flash, Lavergne's data do not discriminate between both models.

In spinach PS II preparations washed with 2 M NaCl, the period 2 oscillation of the acceptor side was not detectable anymore, but the period 4 oscillation still appeared in the majority of the reaction centers (Dekker *et al.*, 1984a). The oscillating changes of these particles with DCBQ at 310 nm (where the period 2 oscillation should interfere strongly—see Fig. 2A) favor the +1, +1, +1, −3 sequence (Fig. 3). In a PS II preparation from a thermophilic cyanobacterium, the electron acceptor silicomolybdate could reoxidize Q_A^- in all centers in the presence of DCMU without destroying the photosystem (Saygin and Witt, 1985b). The changes at 384 nm could be interpreted with the 1, 1, 1, −3 sequence if 75% S_1 and 25% S_0 in the dark were assumed, and with a 0, 1, 1, −2 sequence if 100% S_1 in the dark was assumed. After the addition of low concentrations of hydroxylamine, also in these particles the 1, 1, 1, −3 sequence of manganese absorbance changes was favored (Saygin and Witt, 1985b).

Recently, Saygin and Witt (1987) presented the absorbance difference

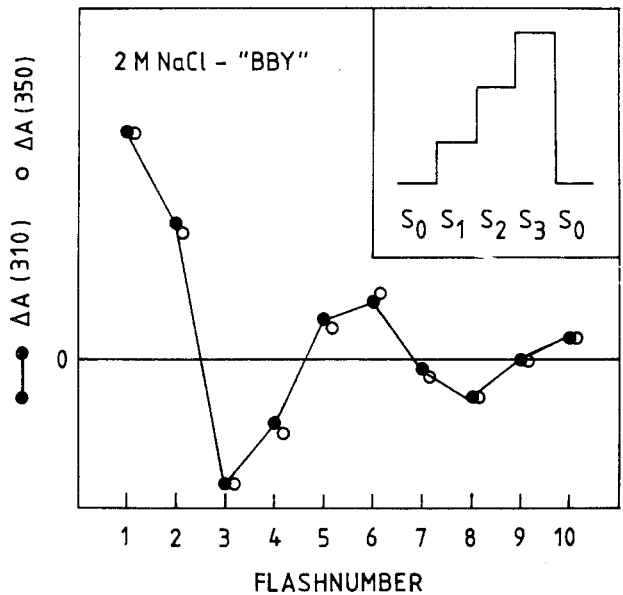

Fig. 3. Sequence of oscillating absorption changes 30 ms after the flashes in dark-adapted 2 M NaCl-washed PS II preparations at 310 nm (closed circles) and at 350 nm (open circles) (Dekker *et al.*, 1984a). The patterns were normalized at the third flash and show that the relative absorption change due to each individual S-state transition is approximately the same at both wavelengths. Inset: absorption changes at 310 nm due to the successive S-state transitions, calculated from flashes 2–8 as described in the text.

spectra of the electron donors oxidized after each of the first four flashes, both in the absence and presence of 24 μM hydroxylamine. Their data suggest that the spectra of $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ peak near 340 nm with $\Delta\varepsilon$ about $3000\,M^{-1}cm^{-1}$ and that $S_0 \rightarrow S_1$ (in the presence of hydroxylamine) peaks near 310 nm with $\Delta\varepsilon = 2000\,M^{-1}cm^{-1}$.

The amplitude and shape of these spectra differ from those measured in spinach PS II preparations, although the general characteristic, a broad increase in the UV, is retained. The differences may be due to the PS II preparation used (i.e., cyanobacterial vs. spinach PS II). Saygin and Witt (1987) proposed that $S_0 \rightarrow S_1$ represents a Mn(II) $\rightarrow$ Mn(III) valence change, while the others represent Mn(III) $\rightarrow$ Mn(IV) valence changes. The changes in the spectra are relatively small, so on the basis of the spectra alone, there may be no need to postulate different valence changes.

The difference spectrum has been reported of the ultimate electron donor oxidized after the first flash in BBY preparations at pH 8.3 (Plijter *et al.*, 1986), i.e., largely the $S_0 \rightarrow S_1$ transition (see above). The shape of this spectrum suggests that the spectra of $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are very similar, even at the very different pH values. Also at pH 6.0, these spectra are very similar (Figs. 2A and 3) in spinach PS II preparations.

In summary, the available data show that all four S-state transitions cause absorbance changes in the ultraviolet, and strongly suggest that these changes are largely due to a single spectral component which oscillates with a sequence $+1, +1, +1, -3$ during the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ cycle. Most likely, this component represents the oxidation of Mn(III) to Mn(IV).

Water Oxidation

The data described above imply that three Mn ions undergo redox changes during the S-state cycle, but a change of the fourth Mn ion was not detected. It was proposed (Dekker *et al.*, 1984d) that this fourth Mn ion is oxidized by Z^+ in state S_3, yielding the transient state S_4 with four Mn(IV) ions. This state leads within a millisecond to the complete oxidation of water to oxygen in a concerted reaction, the details of which remain beyond detection, and which leaves behind a state S_0 characterized by four Mn(III) ions.

This interpretation seems to be consistent with the results obtained with other spectroscopic techniques, as pointed out recently by Dismukes (1986). Presumably, two Mn ions are relatively strongly coupled, and one of these is oxidized in the $S_1 \rightarrow S_2$ transition, giving rise to a mixed-valence Mn(III)–Mn(IV) dimer, which is responsible for the multiline EPR signal, and for the intervalence transition in the infrared (Dismukes and Mathis,

1984). EXAFS studies (Goodin *et al.*, 1984) reveal two Mn ions at 2.7 Å distance which are oxidized on this transition. The $S_2 \rightarrow S_3$ transition retains the intervalence absorption, but wipes out the multiline EPR signal, possibly as a result of magnetic interaction of the Mn(IV) ion formed on this transition with the strongly coupled dimer (Dismukes, 1986).

Apparently, the water-oxidizing complex functions in such a way that first four oxidizing equivalents are "stored" on Mn ions before water is oxidized to the relatively harmless product oxygen. Recently, experiments have been presented suggesting that water is not oxidized before the S_4 state is reached (Radmer and Ollinger, 1986; Bader *et al.*, 1987). The concerted reaction of water oxidation probably needs the presence of calcium ions. In the absence of Ca^{2+} ions, only the state S_3Z^+ can be reached (Boussac *et al.*, 1985). In the absence of chloride ions, the results of Ono *et al.* (1986a, b) indicate that only a somewhat modified and more stabilized S_2 state can be reached (Homann *et al.*, 1986) and that the $S_2 \rightarrow S_3$ transition is inhibited.

Charge Accumulation

In the original model of Kok *et al.* (1970), it was proposed that the four protons of the two water molecules are released together with oxygen in the $S_4 \rightarrow S_0$ transition. Proton release measurements soon indicated that this proposition needs refinement, and it is now generally accepted that the sequence of proton release is 1, 0, 1, 2 for the successive S-state transitions (see, e.g., Förster and Junge, 1985a). This pattern implies that in the $S_0 \rightarrow S_1$ and $S_2 \rightarrow S_3$ transitions the addition of a positive charge to the system is compensated by the release of a proton. The kinetics of proton release were reported to occur with half-times of 250 μs ($S_0 \rightarrow S_1$), 200 μs ($S_2 \rightarrow S_3$), and 1.2 ms ($S_3 \rightarrow S_0$) (Förster and Junge, 1985a), i.e., the first being slower than the electron transport, and the others occurring in the same time range. Possibly, the charge accumulation on the $S_1 \rightarrow S_2$ transition is compensated by the binding of a chloride ion (Preston and Pace, 1985), in which case the total charge of the system would be equal in all S states.

The protons observed on the $S_0 \rightarrow S_1$ and $S_2 \rightarrow S_3$ transitions cannot be released as a result of water oxidation, in view of the sequence of manganese absorbance changes and the recent experiments of Radmer and Ollinger (1986). Presumably, they are released as a result of changes in their dissociation constants.

More information on the charge distribution within the oxygen-evolving complex may be obtained by analysis of the properties of electrochromic band shifts. In PS II, just as in other photosystems, several of such shifts have been reported. The most well known is "C-550" (Knaff and Arnon, 1969), a

blue shift of a pheophytin *a* molecule, probably the intermediary electron acceptor I, upon reduction of Q_A (Van Gorkom, 1974; Schatz and Van Gorkom, 1985). Photoaccumulation of I^- was reported to be accompanied by a bandshift of Chl *a* (Ganago *et al.*, 1982), and several authors have reported that the Soret band of Chl *a* is red-shifted upon oxidation of Z (Dekker *et al.*, 1984c; Diner and De Vitry, 1984; Lavergne, 1984; Schatz and Van Gorkom, 1985).

Also the S-state transitions are accompanied by electrochromic band shifts. The $S_1 \rightarrow S_2$ transition induces a red shift of the Soret band of Chl *a* (Dekker *et al.*, 1984c), which is probably reversed on the $S_3 \rightarrow S_0$ transition (Lavergne, 1984). A blue shift with a similar oscillation pattern was observed for the red absorption band of Chl *a* in a cyanobacterial PS II preparation (Saygin and Witt, 1985a). From the oscillation pattern of this shift, and from that of the absorbance changes at 514 nm (Saygin and Witt, 1984), it was concluded that the states S_2 and S_3 both have an extra charge compared to S_0 and S_1, in line with what one would expect from the 1, 0, 1, 2 sequence of proton release. Apparently, the binding of a chloride ion upon the $S_1 \rightarrow S_2$ transition (Preston and Pace, 1985) has no significant influence on the shifting pigment.

Figure 4 shows the result of a calculation as described above for the UV changes, based on the data of Saygin and Witt (1985a) concerning the oscillation pattern of the electrochromic shift in the red. Irrespective of the dark S-state distribution, the sequence is not exactly 0, +1, 0, −1 (Figs. 4A and 4B). The same can be observed for the pattern of the shift around 435 nm in Fig. 2A: the only positive change (i.e., 440–430 nm) is seen in the $S_1 \rightarrow S_2$ transition, the others being smaller, and negative (Fig. 5). The contaminating period 2 oscillation of the acceptor side is not expected to give a significant contribution in this region of the spectrum, since Q_B^- does not

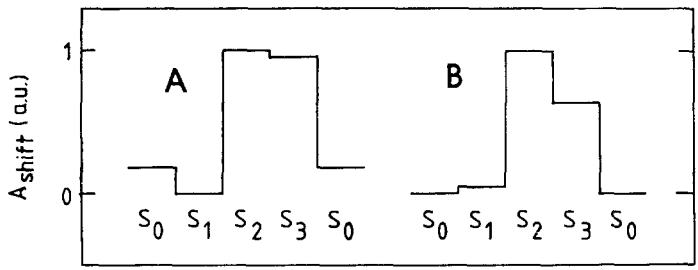

Fig. 4. (A) Sequence of the electrochromic bandshift (average of 672 minus 676 nm and of 706 minus 694 nm) in PS II particles from a *Synechococcus sp.*, calculated as described in the text on the basis of the data reported by Saygin and Witt (1985a), assuming 100% S_1 in the dark and 7% misses and 4% double hits upon each flash. (B) Same as (A), except that 75% S_1 and 25% S_0 in the dark are assumed.

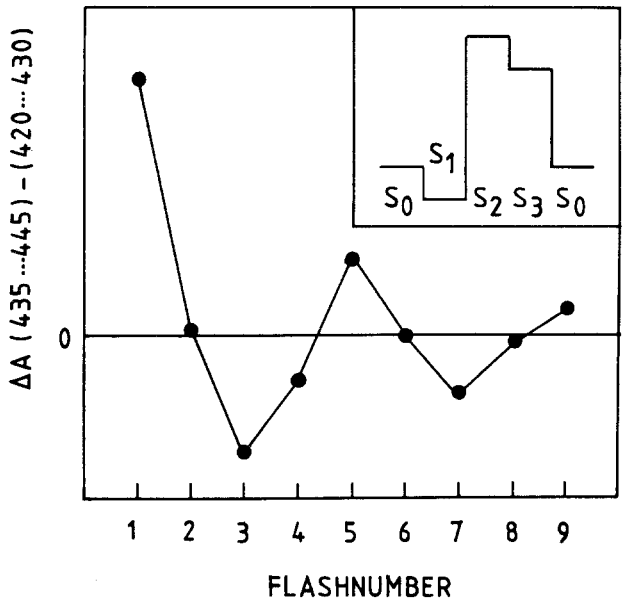

Fig. 5. Sequence of the Chl *a* bandshift 30 ms after the flashes in dark-adapted PS II preparations, measured at 435, 440, and 445 nm minus 420, 425, and 430 nm (Dekker *et al.*, 1984d). Inset: as for Fig. 3, but for the wavelengths mentioned here.

cause electrochromism in the Soret region (Lavergne, 1984; Schatz and Van Gorkom, 1985).

If the electrochromic shift due to the S-state changes represents the charge in the manganese complex, then the charge distribution is as follows:

$$S_2 > S_3 \gg S_0 \geqslant S_1$$

This situation may help to explain the relative instability of the S_2 state and stability of the S_1 state.

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References

Amesz, J. (1983). *Biochim. Biophys. Acta* **726**, 1–12.
Andréasson, L. -E., Hansson, Ö., and Vänngård, T. (1983). *Chem. Scr.* **21**, 71–74.

Babcock, G. T., and Sauer, K. (1973). *Biochim. Biophys. Acta* **325**, 504–519.
Babcock, G. T., and Sauer, K. (1975). *Biochim. Biophys. Acta* **376**, 329–344.
Babcock, G. T., Blankenship, R. E., and Sauer, K. (1976). *FEBS Lett.* **61**, 286–289.
Bader, K. P., Thibault, P., and Schmid, G. H. (1987). In *Progress in Photosynthesis Research*, Vol. I, (Biggins. J., ed.). Martinus Nijhoff Publishers. Dordrecht, The Netherlands, pp. 5.549–552.
Beck, W. F., De Paula, J. C., and Brudvig, G. W. (1985). *Biochemistry* **24**, 3035–3043.
Bennoun, P. (1970). *Biochim. Biophys. Acta* **216**, 357–363.
Berthold, D. A., Babcock, G. T., and Yocum, C. F. (1981). *FEBS Lett.* **134**, 231–234.
Boska, M., and Sauer, K. (1984). *Biochim. Biophys. Acta* **765**, 84–87.
Boska, M., Sauer, K., Buttner, W., and Babcock, G. T. (1983). *Biochim. Biophys. Acta* **722**, 327–330.
Bouges, B. (1971). *Biochim. Biophys. Acta* **234**, 103–112.
Bouges-Bocquet, B. (1980). *Biochim. Biophys. Acta* **594**, 85–103.
Boussac, A., and Etienne, A. -L. (1982a). *Biochem. Biophys. Res. Commun.* **109**, 1200–1205.
Boussac, A., and Etienne, A. -L. (1982b). *FEBS Lett.* **148**, 113–116.
Boussac, A., Maison-Peteri, B., Vernotte, C., and Etienne, A. -L., (1985). *Biochim. Biophys. Acta* **808**, 225–230.
Bowes, J. M., and Crofts, A. R. (1980). *Biochim. Biophys. Acta* **590**, 373–384.
Brettel, K., Schlodder, E., and Witt, H. T. (1984). *Biochim. Biophys. Acta* **766**, 403–415.
Brok, M., Ebskamp, F. C. R., and Hoff, A. J. (1985). *Biochim. Biophys. Acta* **809**, 421–428.
Casey, J., and Sauer, K. (1984). *Biochim. Biophys. Acta* **767**, 21–28.
De Groot, A., Plijter, J. J., Evelo, R., Babcock, G. T., and Hoff, A. J. (1986). *Biochim. Biophys. Acta* **848**, 8–15.
Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1984). *J. Mol. Biol.* **180**, 385–398.
Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985). *Nature* (*London*) **318**, 618–624.
Dekker, J. P., Ghanotakis, D. F., Plijter, J. J., Van Gorkom, H. J., and Babcock, G. T. (1984a). *Biochim. Biophys. Acta* **767**, 515–523.
Dekker, J. P., Plijter, J. J., Ouwehand, L., and Van Gorkom, H. J. (1984b). *Biochim. Biophys. Acta* **767**, 176–179.
Dekker, J. P., Van Gorkom, H. J., Brok, M., and Ouwehand, L. (1984c). *Biochim. Biophys. Acta* **764**, 301–309.
Dekker, J. P., Van Gorkom, H. J., Wensink, J., and Ouwehand, L. (1984d). *Biochim. Biophys. Acta* **767**, 1–9.
Den Haan, G. A., Duysens, L. N. M., and Egberts, D. J. M. (1974). *Biochim. Biophys. Acta.* **368**, 409–421.
De Paula, J. C., Innes, J. B., and Brudvig, G. W. (1985). *Biochemistry* **24**, 8114–8120.
Diner, B. A., and De Vitry, C. (1984). In *Advances in Photosynthesis Research* (Sybesma, C., ed.), Vol. I, Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag, The Netherlands, pp. 407–411.
Dismukes, G. C. (1986). In *Manganese in Metabolism and Enzyme Function*, (Wedler, F. C., and Schram, V. L., eds.), Academic Press, New York, in press.
Dismukes, G. C., and Siderer, Y. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 274–278.
Dismukes, G. C., and Mathis, P. (1984). *FEBS Lett.* **178**, 51–54.
Döring, G., Renger, G., Vater, J., and Witt, H. T. (1969). *Z. Naturforsch.* **24**, 1139–1143.
Duysens, L. N. M., and Sweers, H. E. (1963). In *Studies on Microalgae and Photosynthetic Bacteria*, Special Issue of *Plant Cell Physiol.*, University of Tokyo Press, Tokyo, pp. 353–372.
Forbush, B., Kok, B., and McGloin, M. P. (1971). *Photochem. Photobiol.* **14**, 307–321.
Förster, V., and Junge, W. (1985a). *Photochem. Photobiol.* **41**, 183–190.
Förster, V., and Junge, W. (1985b). *Photochem. Photobiol.* **41**, 191–194.
Förster, V., and Junge, W. (1985c). *FEBS Lett.* **186**, 153–157.
Ganago, I. B., Klimov, C. C., Ganago, A. O., Shuvalov, V. A., and Erokhin, Y. E. (1982). *FEBS Lett.* **140**, 127–130.

Goodin, D. B., Yaccandra, V. K., Britt, R. D., Sauer, K., and Klein, M. K. (1984). *Biochim. Biophys. Acta* **765**, 524–531.
Govindjee, Kambara, T., and Coleman, W. (1985). *Photochem. Photobiol.* **42**, 187–210.
Hanssum, B., and Renger, G. (1985). *Biochim. Biophys. Acta* **810**, 225–234.
Hanssum, B., Dohnt, G., and Renger, G. (1985). *Biochim. Biophys. Acta* **806**, 210–220.
Homann, P. H., Gleiter, H., Ono, T. -A., and Inoue, Y. (1986). *Biochim. Biophys. Acta* **850**, 10–20.
Joliot, P., and Kok, B. (1975). In *Bioenergetics of Photosynthesis* (Govindjee, ed.), Academic Press, New York, pp. 387–412.
Joliot, P., Barbieri, G., and Chabaud, R. (1969). *Photochem. Photobiol.* **10**, 309–329.
Klimov, V. V., Klevanik, A. V., Shuvalov, V. A., and Krasnovsky, A. A. (1977). *FEBS Lett.* **82**, 182–186.
Knaff, D., and Arnon, D. I. (1969). *Proc. Natl. Acad. Sci. USA* **63**, 963–969.
Kok, B., Forbush, B., and McGloin, M. (1970). *Photochem. Photobiol.* **11**, 457–475.
Lavergne, J. (1984). *FEBS Lett.* **173**, 9–14.
Lavergne, J. (1985). *Physiol. Veg.* **23**, 411–423.
Lavergne, J. (1986). *Photochem. Photobiol.* **43**, 311–318.
Mathis, P., and Haveman, J. (1977). *Biochim. Biophys. Acta* **461**, 167–181.
Nugent, J. H. A., Diner, B. A., and Evans, M. C. W. (1981). *FEBS Lett.* **124**, 241–244.
Nuijs, A. M., Van Gorkom, H. J., Plijter, J. J., and Duysens, L. N. M. (1986). *Biochim. Biophys. Acta* **848**, 167–175.
O'Malley, P. J., and Babcock, G. T. (1984). *Biochim. Biophys. Acta* **765**, 370–379.
O'Malley, P. J., Babcock, G. T., and Prince, R. C. (1984). *Biochim. Biophys. Acta* **766**, 283–288.
Ono, T. -A., Zimmermann, J. L., Inoue, Y., and Rutherford, A. W. (1986a). *Biochim. Biophys. Acta* **851**, 193–201.
Ono, T. -A., Conjeaud, H., Gleiter, H., Inoue, Y., and Mathis, P. (1986b). *FEBS Lett.* **203**, 215–219.
Petrouleas, V., and Diner, B. A. (1986). *Biochim. Biophys. Acta* **849**, 264–275.
Plijter, J. J., De Groot, A., Van Dijk, M. A., and Van Gorkom, H. J. (1986). *FEBS Lett.* **195**, 313–318.
Preston, C., and Pace, R. J. (1985). *Biochim. Biophys. Acta* **810**, 388–391.
Pulles, M. P. J., Van Gorkom, H. J., and Willemsen, J. G. (1976). *Biochim. Biophys. Acta* **449**, 536–540.
Radmer, R., and Ollinger, O. (1982). *FEBS Lett.* **144**, 162–166.
Radmer, R., and Ollinger, O. (1986). *FEBS Lett.* **195**, 285–289.
Reinman, S., Mathis, P., Conjeaud, H., and Stewart, A. (1981). *Biochim. Biophys. Acta* **635**, 429–433.
Renger, G. (1977). *FEBS Lett.* **81**, 223–228.
Renger, G., and Weiss, W. (1985). *Biochem. Soc. Trans.* **14**, 17–20.
Renger, G., and Weiss, W. (1986). *Biochim. Biophys. Acta* **850**, 184–196.
Robinson, H. H., and Crofts, A. R. (1984). In *Advances in Photosynthetic Research* (Sybesma, C., ed.), Vol. I, Martinus Nijhoff/Dr. W. Junk Publishers, Den Haag, The Netherlands, pp. 477–480.
Saygin, Ö., and Witt, H. T. (1984). *FEBS Lett.* **176**, 83–87.
Saygin, Ö., and Witt, H. T. (1985a). *FEBS Lett.* **187**, 224–226.
Saygin, Ö., and Witt, H. T. (1985b). *Photobiochem. Photobiophys.* **10**, 71–82.
Saygin, Ö., and Witt, H. T. (1987). In *Progress in Photosynthesis Research*, Vol. I, (Biggins, J., ed.). Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp. 5.537–540.
Schatz, G. H., and Van Gorkom, H. J. (1985). *Biochim. Biophys. Acta* **810**, 283–294.
Sonneveld, A., Rademaker, H., and Duysens, L. N. M. (1979). *Biochim. Biophys. Acta* **548**, 536–551.
Srinivasan, A. N., and Sharp, R. R. (1986). *Biochim. Biophys. Acta* **850**, 211–217.
Stiehl, H. H., and Witt, H. T. (1968). *Z. Naturforsch.* **23**, 220–224.
Van Gorkom, H. J. (1974). *Biochim. Biophys. Acta* **347**, 439–442.
Van Gorkom, H. J., (1976). Thesis, State University of Leiden, The Netherlands.

Van Gorkom, H. J. (1985). *Photosynth. Res.* **6**, 97–112.
Van Gorkom, H. J., Thielen, A. P. G. M., and Gorren, A. C. F. (1982). In *Function of Quinones in Energy-Conserving Systems* (Trumpower, B. L., ed.), Academic Press, New York, pp. 213–225.
Velthuys, B. R. (1981). In *Proceedings of the 5th International Congress on Photosynthesis* (Akoyunoglou, G., ed.), Vol. 2, Balaban International Sciences, Philadelphia, Pennsylvania, pp. 75–85.
Velthuys, B. R. (1982). In *Function of Quinones in Energy-Conserving Systems* (Trumpower, B. L., ed.), Academic Press, New York, pp. 401–408.
Velthuys, B. R., and Visser, J. W. M. (1975). *FEBS Lett.* **55**, 109–112.
Velthuys, B. R., and Kok, B. (1978). *Biochim. Biophys. Acta* **502**, 211–221.
Vermaas, W. F. J., Renger, G., and Dohnt, G. (1984). *Biochim. Biophys. Acta* **764**, 194–202.
Völker, M., Ono, T., Inoue, Y., and Renger, G. (1985). *Biochim. Biophys. Acta* **806**, 25–35.
Weiss, W. (1985). Thesis, Technical University of Berlin.
Weiss, W., and Renger, G. (1986). *Biochim. Biophys. Acta* **850**, 173–183.
Witt, H. T., Schlodder, E., Brettel, K., and Saygin, Ö. (1986). *Photosynth. Res.* **10**, 453–471.
Wood, P. M., and Bendall, D. S. (1976). *Eur. J. Biochem.* **61**, 337–344.
Wydrzynski, T. J. (1982). In *Photosynthesis. I. Energy Conversion by Plants and Bacteria* (Govindjee, ed.), Academic Press, New York, pp. 469–506.
Wydrzynski, T., and Sauer, K. (1980). *Biochim. Biophys. Acta* **589**, 56–70.
Zimmermann, J. L., and Rutherford, A. W. (1984). *Biochim. Biophys. Acta* **767**, 160–167.
Zimmermann, J. L., and Rutherford, A. W. (1986). *Biochemistry* **25**, 4609–4615.