

*Minireview*

## **Apparatus and mechanism of photosynthetic oxygen evolution: a personal perspective\***

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### **Abstract**

This historical minireview describes basic lines of progress in our understanding of the functional pattern of photosynthetic water oxidation and the structure of the Photosystem II core complex. After a short introduction into the state of the art about 35 years ago, results are reviewed that led to identification of the essential cofactors of this process and the kinetics of their reactions. Special emphasis is paid on the flash induced oxygen measurements performed by Pierre Joliot (in Paris, France) and Bessel Kok (Baltimore, MD) and their coworkers that led to the scheme, known as the Kok-cycle. These findings not only unraveled the reaction pattern of oxidation steps leading from water to molecular oxygen but also provided the essential fingerprint as prerequisite for studying individual redox reactions. Starting with the S. Singer and G. Nicolson model of membrane organization, attempts were made to gain information on the structure of the Photosystem II complex that eventually led to the current stage of knowledge based on the recently published X-ray crystal structure of 3.8 Å resolution in Berlin (Germany). With respect to the mechanism of water oxidation, the impact of Gerald T. Babcock's hydrogen abstractor model and all the considerations of electron/proton transfer coupling are outlined. According to my own model considerations, the protein matrix is not only a 'cofactor holder' but actively participates by fine tuning via hydrogen bond networks, playing most likely an essential role in water substrate coordination and in oxygen-oxygen bond formation as the key step of the overall process.

**Abbreviations:** ADRY – acceleration of the deactivation reactions of watersplitting enzyme system Y; CCCP – carbonylcyanide-m-chlorophenylhydrazone; Chl – chlorophyll; CP43 and CP47 – chlorophyll-*a* containing proteins of the PS II core, with molecular masses of 43 kD and 47 kD, respectively; D1 and D2 – polypeptides of the PS II reaction center; ENDOR – electron-nuclear double resonance; EPR – electron paramagnetic resonance; EXAFS – extended X-ray fine structure; FTIR – Fourier transform infrared; P680 – primary electron donor Chls of PS II; PQ-9 – plastoquinone-9; PS II – Photosystem II; Q<sub>A</sub> – primary quinone acceptor of PSII; Q<sub>B</sub> – secondary quinone acceptor of PSII; RC – reaction center; S<sub>i</sub> – redox states of WOC; WOC – water oxidizing complex; Y<sub>Z</sub> and Y<sub>D</sub> – redox active tyrosines of polypeptides D1 and D2, respectively, Y<sub>Z</sub> being the active tyrosine-161 on the D1 protein

### **Prologue**

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\* This historical minireview is dedicated to Jerry Babcock, a pioneer in photosynthesis research. Three photographs of Jerry are shown in Figure 1 (see Yocum et al., 2001, for Jerry's obituary).

To write a historical minireview is an ambitious aim and when reading in this journal several excellent

papers on this topic by the most prominent researchers in our field, I felt somewhat hesitant to start this task. The problem became even more complicated by the extremely sad event of the untimely death of Jerry Babcock who was really a giant in his research activities with the aim of understanding the molecular mechanism of photosynthetic water oxidation. At the 12th International Photosynthesis Congress in Brisbane (Australia), we all were aware of how much we missed not only his brilliant ideas and suggestions but even more important his outstanding personality. Originally, Jerry and I had planned to write this article as a joint paper. After receiving the invitation letter from Govindjee we had a very open and friendly discussion about general organization and content of this article. The final decision was not in our hands and I was left behind to write my own perspective; however, I will try my best to keep his spirit alive with my sincere hope to be able to achieve, at least partly, the original goal. Figure 1 shows in three photographs different activities of Jerry.

### Early stages

#### *Entry into Max-Volmer-Institute and measurements of flash induced oxygen evolution*

My personal activity in photosynthesis research started at the end of 1965 with entry as a student into Horst Witt's research group at the Max-Volmer-Institute of the Technical University in Berlin (see Witt 1991). At that time, a very active group of PhD students in the Institute were working hard and strongly discussing different facets of the primary processes of photosynthesis. Among these people the names of Günter Döring, Wolfgang Junge, Hans-Henrich Stiehl, Ulrich Siggel, Jochen Vater and Christoph Wolff are well known to experts in the field. Our attempts were highly stimulated by Bernd Rumberg who was working as post doc at that time. Figure 2 compiles photos from the late 1960s/early 1970s of the former PhD students and of Bernd Rumberg, at Berlin.

When I started my work, very little was known about the functional *pattern* of water cleavage and the *structure* of Photosystem II (PS II); its water oxidizing complex (WOC) was virtually a black box and measurements of light induced oxygen evolution was a major tool to analyze the reaction properties.

During my diploma thesis and subsequent PhD work, I performed, predominately, measurements of

average oxygen yield under repetitive flash excitation using a membrane covered Clark-type platinum electrode (Clark et al. 1953). Several interesting conclusions were gathered from these experiments: i) the functional integrity of the WOC is only weakly dependent on pH in the range from 5 to 8 with maximum activity at 6.5–7.0 and steeply declines in alkaline (between pH 8–9) and in acidic (below pH 5) environment (Renger 1969a); ii) a nonlinear relationship exists between electron transport rate under saturating continuous (CW) illumination and the number of functionally fully competent WOCs owing to coupling of different PS II complexes with PS I via a common plastoquinone (PQ) pool (Siggel et al. 1972a); iii) two PS II complexes cooperate with respect to diuron type inhibitor action (Siggel et al. 1972b); and iv) the rate limiting step of PS II electron transport from H<sub>2</sub>O to plastoquinone (PQ) exhibits a biphasic kinetics with a dominating (normalized amplitude, 70–75%) 600  $\mu$ s component (Vater et al. 1969) that is characterized by an activation energy of about 20 kJ/mol and an almost pH independent half-life time (Renger 1969a).

The most exciting result in this type of experiments, however, was obtained when Bernd Rumberg asked me to perform comparative control measurements of oxygen evolution in the presence of the protonophoric uncoupler carbonylcyanide-*m*-chlorophenylhydrazine (CCCP). In this case, the rate under CW light of saturating intensity drastically increased (by a factor of up to 10) as predicted owing to powerful uncoupling effect by CCCP, but I simultaneously observed a rather unexpected phenomenon: a drastic decrease of the average oxygen yield per flash when the dark time between the flashes was increased (Renger 1969a, b). This effect turned out to be fully reversible, i.e., oxygen evolution could be 'switched off' and 'on' simply by varying the frequency of the repetitive excitation flashes. The only reasonable explanation of this phenomenon was to assume that water oxidation under repetitive excitation conditions includes the formation of metastable intermediate(s) with lifetime(s) that is (are) drastically shortened by CCCP. This idea was entirely new and a significant extension of the concept of a 'dark silent' WOC (Allen and Franck 1955; Whittingham and Brown 1958) that needs a 'priming' reaction (Joliot 1965) by a flash to be functionally competent in oxygen evolution.

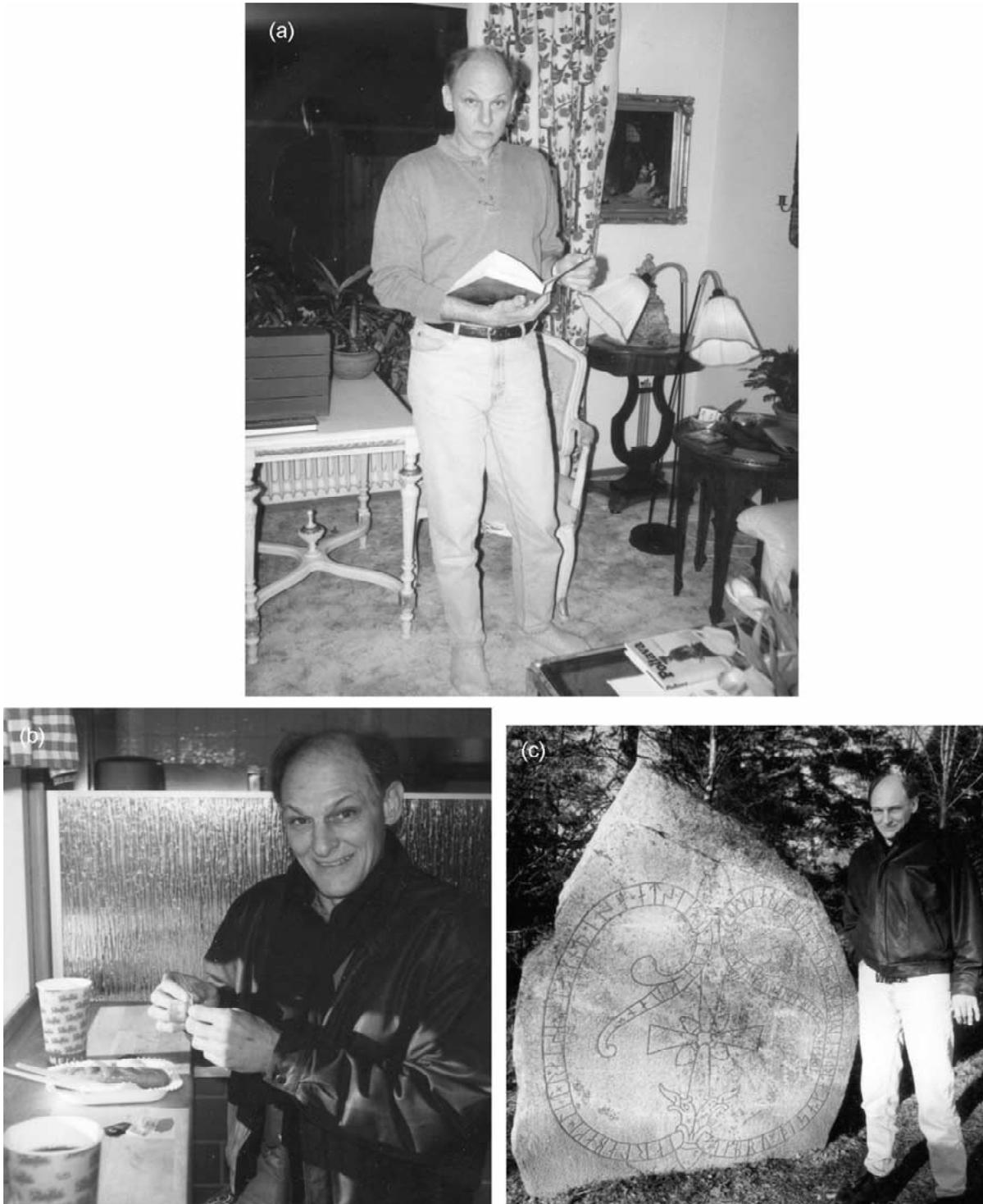


Figure 1. Jerry Babcock in full activity. Figure (a) shows him at home; in (b), he is eating his breakfast; and in (c), Jerry is eager to understand and explain the mysteries of our world to us.

### *The period four oscillation of oxygen evolution*

Almost coincidentally, *the* essential breakthrough was achieved in understanding the general scheme of functional organization of photosynthetic water cleavage when Pierre Joliot et al. (1969) discovered the period four oscillation in oxygen evolution induced by a train of single turnover flashes in dark adapted algae and chloroplasts (see P. Joliot, this issue). Detailed studies of this phenomenon and analyses of the whole period four oscillation pattern led to the conclusion that water oxidation takes place via a sequence of one electron transfer steps until four oxidizing redox equivalents accumulate and molecular oxygen is released (Kok et al. 1970). The redox states of this sequence referred to as Kok-cycle are symbolized by  $S_i$  where  $i = 0, \dots, 4$  indicates the number of redox equivalents accumulated in the WOC. Another crucial result was the finding that the oscillation pattern is virtually independent of the number of intact PS II complexes. A consequence of this feature is the very important conclusion that the entity for charge separation and the WOC form an operational unit, i.e., the cooperation of four *strongly* oxidizing equivalents *as the* indispensable prerequisite for water oxidation to molecular oxygen is performed in each PS II complex. We emphasize that the Kok cycle (for a review, see Joliot and Kok 1975) is the basis of mechanistic considerations on this process. The characteristic period four oscillation of the  $S_i$  state population in the WOC provides the invaluable fingerprint for the analysis of all reactions of the WOC. In my opinion, the results of this work of Pierre Joliot and of late Bessel Kok and their coworkers is by far the greatest achievement for a deeper understanding of photosynthetic water oxidation. In order to emphasize the relevance of these findings, Govindjee and I published as guest editors a special issue of *Photosynthesis Research* on the occasion of 25th anniversary of discovery of the period four oscillation in flash induced oxygen evolution (Renger and Govindjee 1993).

### *The ADRY (acceleration of the deactivation reactions of the water splitting system Y) effect*

The Kok-cycle also offered a straightforward explanation for the effect of CCCP. Since several protonophoric uncouplers exert basically the same mode of destabilization of the oxygen evolution under repetitive flash excitation, this mode of action was designated as ADRY (acceleration of the deactivation reactions of the water splitting system Y) effect (Renger 1972).

The influence of ADRY-agents on the intermediates of the Kok-cycle was examined in cooperation with *the late* Bernadette Bouges-Bocquet in Pierre Joliot's laboratory in Paris. We found that ADRY agents are highly efficient in accelerating the decay of redox states  $S_2$  and  $S_3$  (via a cyclic reaction that does not comprise  $Q_A^-$  as electron source) *but do not affect*  $S_0$  and  $S_1$  (Renger et al. 1973). Bernadette made important contributions to our field, especially with respect to the *two electron gate leading to PQH<sub>2</sub> formation at the PS II acceptor side* (Bouges-Bocquet 1973; see also Velthuys and Amesz 1974) and kinetic and thermodynamic properties of the  $S_i$  states including the mode of reactions of the WOC with hydroxylamine in populating 'superreduced'  $S_i$ -states (for a review, see Bouges-Bocquet, 1980, and references therein). It was a tragic event that she left our world much too early.

### *Characterization of the photoactive pigment (P680) of Photosystem II*

The energetics of the Kok-cycle in the WOC requires the formation of a strong one-electron oxidant as driving force. This species that is formed by the light induced charge separation in PS II was identified as a chlorophyll (Chl) *a* by measuring its difference absorption spectrum and reaction properties (G. Döring et al. 1969), almost coincidentally with the period four oscillation of oxygen evolution (*vide supra*). The puzzle on the function of Chl<sub>II</sub> now generally designated as P680 (see Rabinowitch and Govindjee 1965; in the following, only the symbol P680 will be used) was resolved when we found that a cyclic electron flow exists between  $Q_A^-$  (at that time referred to as X320, see Stiehl and Witt 1969) and the PS II donor side (Renger and Wolff 1976) in thylakoids which are deprived of their oxygen evolving capacity by Tris-washing (Yamashita and Butler 1968). This reaction is characterized by half lifetimes of 100–200  $\mu$ s. Independently, and coincidentally, P680<sup>+</sup> was shown to be reduced with the same kinetics in this sample type (Havemann and Mathis 1976). Taking both results together, it became clear that in samples deprived of a functionally competent WOC, the back reaction of P680<sup>+</sup>  $Q_A^-$  takes place under repetitive flash excitation. Since in greening plants, the assembly of the WOC function lags behind that of the charge separation device (Strasser and Sironval 1972), we speculated already in our original paper that the back reaction might be of physiological relevance as a protective mechanism to photodamage (Renger and Wolff

1976). Decades later, this idea was substantiated at a much higher level of information (Keren et al. 2000; see also Adir et al., this issue).

#### *Information on cofactors and the organization scheme of water oxidation*

With respect to the nature of the WOC, it was known for a long time that manganese is an essential cofactor of oxygenic photosynthesis (Pirson 1937, 1994; Kessler et al. 1957). Detailed studies on the function of manganese and photoactivation by late George Cheniae (Cheniae and Martin 1969, 1970) led to the conclusion that each WOC contains 4–6 manganese. (see Frasc and Sayre, 2002, for an obituary of Cheniae). Likewise, chloride was proposed to act as a cofactor of photosynthetic oxygen evolution (Warburg and Lüttgens 1944; for a minireview, see Homann 2002). Bicarbonate has also been suggested to play a crucial role in PSII (for a discussion see Govindjee 2000 and references therein; and minireviews by Stemler (2002) and van Rensen 2002).

Most of the mechanistic details and all structural information were lacking when I finished my PhD thesis more than 30 years ago. *This missing information was a most exciting challenge that attracted many young scientists and it remained the most fascinating topic of my own research activities.*

In the following sections, the progress in this field achieved during the last three decades will be outlined by following our current knowledge on the functional and structural organization of water cleavage in PS II. It is absolutely impossible to provide an encyclopedic description here (for the state of the art 10 years ago, see the review of Debus (1992) with almost a thousand references). Thus, the description below will remain incomplete and somewhat biased by my views and my own research (for a more general description of milestones in photosynthesis research, see Govindjee 2000).

### **Mechanism of charge separation in Photosystem II**

#### *Formation of the radical pair P680<sup>+</sup> Q<sub>A</sub><sup>-</sup>*

Originally Q<sub>A</sub>(X320) was assumed to act as primary electron acceptor (Stiehl and Witt 1969). This idea turned out to be incorrect when the famous work of young scientists, particularly Slava Klimov and Vlad Shuvalov, in Krasnovsky's group (Krasnovsky 1992) in Pushchino (Russia) revealed that pheophytin

accepts the electron from the electronically excited singlet state of P680 (Klimov et al. 1977; see Klimov, this issue). It took almost one decade until the rate constants of Pheo<sup>-</sup> reoxidation (Nuijs et al. 1986) and Q<sub>A</sub><sup>-</sup> formation (Eckert et al. 1988; Bernarding et al. 1994) were resolved and shown to be virtually the same:  $k_{ET} = (300\text{--}400\text{ps})^{-1}$ . The kinetics of the preceding primary charge separation  $^1\text{P680}^* \text{PheoQ}_A \rightarrow \text{P680}^+ \text{Pheo}_A^- \text{Q}_A$  could only be addressed once the laser and detection technology had been significantly advanced to permit time-resolved measurements of absorption changes in the ps and sub-ps domain. However, the data obtained cannot directly be transformed into molecular rate constants owing to interference with excitation energy transfer processes among Chl *a* molecules with overlapping absorption of the various chlorins including that of P680. Therefore the details of the primary charge separation are still not fully resolved (see, e.g., Renger et al. 1995; Greenfield et al. 1997; Prokhorenko and Holzwarth (2000) and Diner et al. (2001) and references therein; for a historical minireview, see Seibert and Wasielewski, this issue).

### **Kinetics of P680<sup>+</sup> reduction**

With the discovery of the back reaction in PS II (vide supra), it was clear that an assignment of the originally measured 200  $\mu\text{s}$  relaxation of P680<sup>+</sup> to the linear electron transport (Döring et al. 1969) is in conflict with an efficient water oxidation because the dissipative recombination between P680<sup>+</sup> and Q<sub>A</sub><sup>-</sup> would compete to a large extent with a forward reaction of similar kinetics. It was therefore attractive to search for faster kinetics of P680<sup>+</sup> reduction. With Michael Gläser and Christoph Wolff, a thorough analysis was performed on P680<sup>+</sup> reduction. In spite of the rather limited time resolution ( $\sim 20 \mu\text{s}$ ) and poor signal to noise ratio (more than thousand signals had to be averaged), we arrived at two important conclusions (Gläser et al. 1976): a) in thylakoids with intact WOC a large fraction of P680<sup>+</sup> becomes reduced via a fast kinetics of  $\leq 1 \mu\text{s}$  and b) the reduction kinetics depend on the redox state S<sub>i</sub> of the WOC.

A real breakthrough in straightforward characterization of the fast kinetics of P680<sup>+</sup> reduction was achieved by Paul Mathis and coworkers revealing that the formation of P680<sup>+</sup> gives rise to positive absorption changes around 820 nm where high intensities of the measuring light beam can be used because there is no actinic effect on PS II. A 20 ns relaxation kinetics

was resolved in dark adapted thylakoids (van Best and Mathis 1978). This powerful method was later used to reach our original goal of the mid 1970s (vide supra), i.e. the characterization of the dependence of  $P680^{+}$  reduction kinetics on the redox states  $S_i$  (Brettel et al. 1984; Eckert and Renger 1988). After the destruction of the WOC, the reduction of  $P680^{+}$  is dominated by the electron transfer from a donor D (nowadays symbolized by  $Y_Z$ ) with a pH dependent kinetics in the range of 5–20  $\mu$ s (Conjeaud and Mathis 1980; Renger et al. 1984) and only when this donor stays oxidized the much slower ( $t_{1/2} = 100\text{--}200 \mu$ s) back reaction with  $Q_A^-$  dominates the  $P680^{+}$  reduction.

### Electron donors to $P680^{+}$

Data available 25 years ago unambiguously showed that the reduction of  $P680^{+}$  is orders of magnitudes faster than oxygen release in the range of 1 ms (Joliot et al. 1966). As a consequence, at least one redox component mediates the electron transfer from the WOC to  $P680^{+}$  and therefore the nature and properties of this component became the subject of intense research activities.

### EPR signals $II_{vf}$ , $II_f$ and $II_s$

The identification and characterization of the donor D is one of the great contributions of Jerry Babcock to our field. Jerry started his work on donor components of PS II when he performed EPR studies in the famous research group of Ken Sauer at Berkeley (California) at the end of the 1960s and the beginning of the 1970s. He analyzed the properties of the long known EPR-signal II (Commoner et al. 1956; Weaver and Bishop 1963) and proposed a model where the redox states  $S_2$  and  $S_3$  of the WOC are assumed to oxidize a species F into the radical state  $F^{+}$  that gives rise to EPR-signal  $II_s$  (Babcock and Sauer 1973). In subsequent studies, Jerry discovered the transient EPR signal II (Babcock and Sauer 1975a) and concluded that this is the physiological donor to  $P680^{+}$  (Babcock and Sauer 1975b).

The use of time resolved EPR measurements enabled Jerry and his colleagues to discover a flash-induced transient signal with a rise time of  $< 100 \mu$ s and a decay time of 400 – 900  $\mu$ s (Blankenship et al. 1975; Warden et al. 1976). It was designated as signal  $II_{vf}$ , where ‘vf’ stands for ‘very fast’ relaxation. This property markedly contrasted with signal  $II_f$  (

symbolizes fast) that has a 1000-fold slower decay. A very important result was the finding that the decay of signal  $II_{vf}$  varies with the flash number (Babcock et al. 1976) and that inactivation of the WOC by heat leads to decrease of the amplitude of flash induced signal  $II_{vf}$  and a mirror image increase of signal  $II_f$ . These data provided convincing evidence for the assignment of signal  $II_{vf}$  to the physiological donor D (now  $Y_Z$ ) of  $P680^{+}$  and that the oxidation steps induced by  $Y_Z^{OX}$  depend on the redox states  $S_i$  of the WOC with rate constants in the range of  $(1 \text{ ms})^{-1}$  up to  $\geq (100 \mu\text{s})^{-1}$  (Babcock et al. 1976). A very important result of this study was the observation that the rate of  $Y_Z^{OX}$  reduction by  $S_3$  coincides with that of  $O_2$  release. This phenomenon was one cornerstone in Jerry’s considerations on the mechanism of water oxidation (see section on hydrogen atom abstraction model). More than two decades later, the relaxation kinetics of signal  $II_{vf}$  were resolved (Razeghifard and Pace 1997) and shown to coincide with the rate of the oxidation steps in the WOC monitored by UV-absorption changes (see section on ‘Mechanism of the WOC’).

### Identification of tyrosine $Y_Z$ as a redox mediator between $P680^{+}$ and water oxidation complex

After the general pattern of the turnover of signals  $II_{vf}$  and  $II_f$  was resolved, attempts were made to substantiate the component(s) that give(s) rise to these EPR signals. The way of thinking to identify the species involved was somewhat biased by earlier proposals of an assignment to a plastoquinone radical (Kohl et al. 1969; Hales and Gupta 1981). To address the problem, EPR measurements were performed in frozen solutions of 2-methyl-5-isopropyl-p-benzosemiquinone in its protonated cation radical form. A comparison of the spectra with those of signal II led to the conclusion that the latter can be assigned to plastosemiquinone cation radicals (Ghanotakis et al. 1983). This suggestion was favored by complementary EPR-studies of another group (Brok et al. 1985) and also by the difference absorption spectrum of  $Y_Z^{OX}/Y_Z$  in the near UV region that was first measured in samples deprived of an intact WOC (Dekker et al. 1984b). Although the difference absorption spectra in the UV are not specific enough to permit unambiguous assignment to a particular species, analyses of the time course of these absorption changes provide an excellent tool for kinetic studies (for an analogous phenomenon of the redox transition of the WOC, see section on ‘Mechanism of photosynthetic water oxidation’). In spite

of the spectral similarities, the interpretation as a plastosemiquinone radical was shown to bear serious constraints on the protein environment in order to keep the radicals protonated (Renger and Govindjee 1985). In addition, we had earlier found that in Tris-washed inside-out-vesicles the oxidation of donor D is stoichiometrically coupled with the release of one proton (Renger and Völker 1982). Therefore, a highly structured protein matrix with very unusual properties had to be proposed to reconcile the experimental data with the idea that signals II ( $II_{vf}$ ,  $II_f$ ) represent a protonated  $PQH_2^+$  cation radical [for discussions, see Renger and Govindjee (1985) and Renger (1987b)]. It was a most impressive experience to me that Jerry in his open minded character not only considered the arguments disfavoring his idea but also made all efforts to solve the problem. In his first attempt, cyanobacteria were grown on isotropically labeled tyrosines and the observed spectral narrowing of signal II was used to identify  $Y_Z$  and  $Y_D$  as tyrosines (Barry and Babcock 1987). With the advancement of the techniques of molecular biology, a most suitable tool became available to find a straightforward answer. In this way, Babcock and his colleagues (Debus et al. 1988a; Babcock et al. 1989) and independently Metz et al. (1989) showed that in the cyanobacterium *Synechocystis* PCC 6803 the signals  $II_{vf}$  and  $II_f$  originate from tyrosine 161 of the D1 protein and that the signal  $II_s$  represents tyrosine 160 of the D2 protein (Debus et al. 1988b; see also Vermaas et al. 1989). The properties of the tyrosine radicals and their possible functional role became one of the main topics in Babcock's most successful research activities and led him to propose a very attractive mechanism of electron and proton coupled reactions in photosynthetic water oxidation (as will be outlined later under 'Mechanism of photosynthetic water oxidation'). In addition, Babcock and his coworkers (Boska et al. 1983) showed that the rise of signal  $II_f$  followed the decay of  $P680^{+}$  as a function of pH (from 5.2 to 6.9). We confirmed this finding by analyzing and covering a wider pH range (from 4 to 8), i.e., flash induced absorption changes in the near infrared (NIR) and ultra violet (UV) that are characteristic for  $P680^{+}$  reduction and  $Y_Z$  oxidation, respectively (Weiss and Renger 1986). The same approach of a combined measurement of NIR and UV absorption changes has been taken to show that kinetic coincidence also exists in systems with intact WOC (Gerken et al. 1988).

## Structure of Photosystem II

Structure analyses of the photosynthetic apparatus have a long tradition in Berlin where the first functional electron microscope had been constructed (a Nobel prize was awarded in Physics in 1986 to Ernst Ruska). Wilhelm Menke took the chance to use this new machine for pioneering work on electron microscopy of chloroplasts. In 1940, he showed the first pictures on the lamellar structure of chloroplasts from *Peperomia metallica* (Menke 1940). He also coined the now widely used term 'thylakoids' (see Staehelin, this issue). In addition, he performed seminal studies on essential constituents of chloroplasts, like RNA and lipids (for details of these great early studies, see Menke 1990). Although the general features of thylakoids were known, the membrane structure in general was still controversial in the 1960s. A real breakthrough was achieved when Singer and Nicolson (1972) proposed their fluid mosaic membrane model, where protein complexes are inserted as integral components into lipid bilayers. Based on this type of membrane organization and earlier successful attempts to solubilize thylakoids by detergents and to isolate chlorophyll-protein complexes with PS I and PS II characteristics (Anderson and Boardman 1966; Ogawa et al. 1966; see Anderson 2002 and Ogawa, this issue), the cofactors of the charge separation were assumed to be incorporated into integral membrane proteins. With respect to the arrangement of the functional components that are indispensable for a stable charge separation in PS I and PS II, an essential structural conclusion could be obtained from the measurements of electrochromic absorption changes. The data showed that these cofactors are anisotropically oriented within the thylakoid membrane so that the light induced charge separation leads to a vectorial electron transport from the inside to the outside of the thylakoid membrane (for reviews, see Junge 1975; Witt 1975). Later, it was shown that the back reaction between  $Q_A^-$  and  $P680^{+}$  is an electrogenic process (Conjeaud et al. 1979; Renger 1979). These functional studies led to a general concept for the arrangement of the cofactors  $Q_A$  and  $P680$  with respect to the normal of the thylakoid membrane but did not provide information on the nature of the matrix and the detailed structure. The methods available to identify the protein matrix were rather 'archaic' compared to modern techniques of membrane biochemistry and molecular biology.



*Figure 2.* From left to right: (*top row*) Günter Döring, Hans-Henrich Stiehl, Jochen Vater; (*middle row*) Wolfgang Junge, Bernd Rumberg and Ulrich Siggel; (*bottom row*) Christoph Wolf and Gernot Renger.

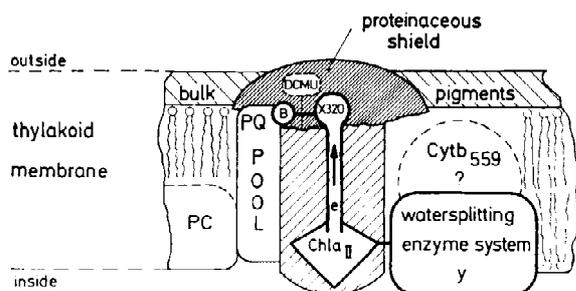


Figure 3. A simplified scheme of the Photosystem II complex reflecting the stage of knowledge 25 years ago (Renger 1976). The abbreviations Chla<sub>II</sub>, X320 and B have to be replaced by the now generally accepted symbols P680, Q<sub>A</sub> and Q<sub>B</sub>, respectively. The notation of plastocyanin as a membrane component is now known to be wrong; likewise the arrangement of bulk chlorophylls in the lipid phase is misleading. It is interesting to note that ~25 years ago it was not clear whether the bulk chlorophylls are anchored via the phytol chain into the lipid phase or bound to proteins. (A footnote on p. 297 in Renger (1976) had emphasized that the figure does not reflect the real structural organization of the bulk pigment system.) This question was clarified by late Phil Thornber and coworkers only a few years after publication of Figure 1 (see Markwell et al. 1979; for Thornber's obituary, see Cogdell 1996).

#### Identification of the herbicide binding site in Photosystem II

We attempted to approach the problem of the PS II protein matrix by using the proteolytic enzyme trypsin. The most interesting result of these studies was the finding that mild trypsin induced digestion of spinach thylakoids leads to interruptions of the linear electron transport pathway from water to the plastoquinone pool but simultaneously opens the road for oxygen evolution with K<sub>3</sub>[Fe(CN)<sub>6</sub>] as exogenous electron acceptor that is highly resistant to blockage by strong PS II inhibitors like diuron and atrazine. These studies led to the conclusion that there exists a protein that binds Q<sub>B</sub> and inhibitors and simultaneously covers up Q<sub>A</sub> thereby preventing rapid reoxidation of Q<sub>A</sub><sup>-</sup> by exogenous substances. Accordingly, this protein was referred to as 'proteinaceous shield' (Renger 1976). The model depicted in Figure 3 predicted some general features correctly but certainly failed in describing details, owing to limited information on the nature of these proteins.

A step forward in identifying the protein matrix of PS II was made at the beginning of the 1980s when the herbicide binding 'shielding protein' could be identified as a 32 kDa subunit (Mattoo et al. 1981; Pfister et al. 1981) and the nucleotide sequence of the encoding gene was resolved (Zurawski et al. 1982; see Bogorad, this issue).

#### Isolation of Photosystem II preparation with intact water oxidizing complex

Another approach to address questions on the structure of PS II was the attempt to isolate a PS II complex functionally fully competent in oxygen evolution. Earlier attempts (*vide supra*) were unsuccessful owing to the complete loss of activity. Ironically, the efforts to obtain isolated O<sub>2</sub> evolving PS II preparations were strongly pushed 'forward' by a very bad forgery event in our field. In 1980, a paper was published in the Proceedings of the National Academy of Science, USA, that described the isolation of a manganese containing protein of about 65kDa that was claimed to restore oxygen evolution in cholate treated PS II complexes incorporated into lipid vesicles (Spector and Winget 1980). It was like a short gold-rush: many people (including the editor Govindjee) went to Cincinnati/Ohio, where this work was done, in order to become familiar with this new 'magic' technique, but the 'nuggets' turned out to be worth nothing. Marc Spector was a talented scientist and very clever forger. It was interesting to observe the spectacular goings-on from outside. I will refrain from any comments on 'business' of science and only say that I felt sad for the honorable people that had to suffer from the action of Spector. He not only affected photosynthesis research but his action in the field of cancer research (kinase cascade) even had a disastrous effect. In this respect I highly recommend the book of William Broad and Nicholas Wade (1982) that contains further details on Marc Spector.

Although nobody was able to repeat the 'sensational' results of Spector, all efforts had been made to reach this goal even earlier by several means. Therefore, the action of detergents on the fractionation of the thylakoid membrane was thoroughly analyzed. As a result, procedures were developed for isolation of PS II enriched thylakoid membrane fragments. Following the early work of Leo Vernon (at the Kettering Research Lab in Yellow Springs, Ohio; see Vernon, this issue, and Jean-Marie Briantais (see Kouchkovsky 2002), the first successful attempt was reported by Deborah Berthold, Jerry Babcock and Charlie Yocum (1981) and therefore this sample type is often referred to as BBY preps (the term 'particles' is misleading and should not be used). This discovery was a great step forward in this field because it opened the road to analyze PS II without interference by PS I and a trigger for ongoing activity in isolation of protein subunits

and intensive research activities to identify the protein matrix of the cofactors.

*X-ray crystal structure of reaction centers from anoxygenic purple bacteria and implications for Photosystem II structure*

In sharp contrast to the rather limited information on PS II at that time, enormous progress was achieved through successful isolation and subunit characterization of the reaction centers from anoxygenic purple bacteria. The starting point was the important work of the late Dan Reed (killed on the way back home from the Third International Congress on Photosynthesis in Rehovot, Israel, 1974, by an aircraft bomb attack) and Roderick Clayton (Reed and Clayton 1968; see also the minireview of Roderick Clayton 2002). The highlight in this direction was the resolution of the crystal structure of the reaction center (RC) isolated from an anoxygenic photosynthetic bacterium *Rps. viridis* (Deisenhofer et al. 1984). The importance of this achievement was soon recognized by the award of the Nobel prize in Chemistry, in 1987, to Hartmut Michel, Johann Deisenhofer and Robert Huber of Munich (Germany). As a result of this cornerstone discovery in photosynthesis research, all cofactors of charge separation were shown to be incorporated into a heterodimeric protein matrix of polypeptides referred to as L- and M-subunits.

A first indirect step in elucidating the protein matrix of the cofactors of PS II was the finding that the polypeptides D1 and D2 of PS II (Rochaix et al. 1984) exhibit striking sequence similarities with subunits L and M of anoxygenic purple bacteria (Williams et al. 1983, 1984). Based on hydrophobicity analyses, it was inferred that both D1 and D2 have five transmembrane helices each in a similar way as known for the RCs of purple bacteria. As a consequence, the cofactors P680, Pheo,  $Q_A$  and  $Q_B$  were assumed to be bound to a D1/D2 heterodimer in analogy to the array of the corresponding components P, BPheo,  $Q_A$  and  $Q_B$  in the L/M heterodimer of purple bacteria RC (Trebst and Depka, 1985; Michel and Deisenhofer, 1988). In support of this idea, D1 and D2 were identified as Chl-binding proteins (Irrgang et al. 1986).

A real breakthrough was the report of O. Nanba and Kimiyuki Satoh (1987) on the isolation of D1/D2/Cyt b559 preparations that were able to perform light induced charge separation (see Satoh, this issue). Although these findings clearly showed that striking similarities in the functional and structural

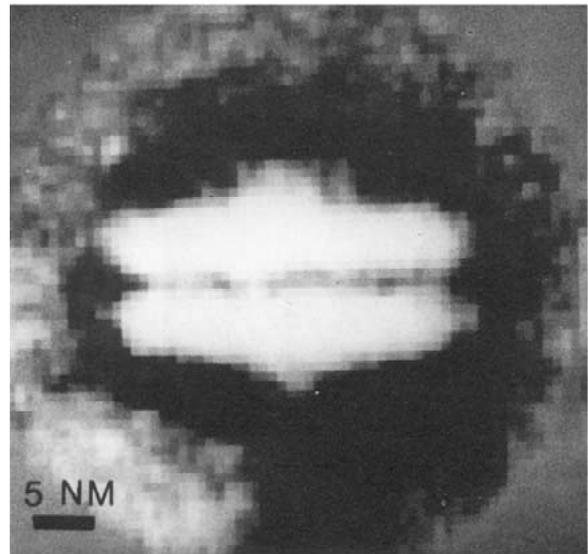


Figure 4. Averaged image of 14 aligned Photosystem II core complex dimers (the bulbs reflect the extrinsic 33 kDa protein; see Figure 8 of Haag et al. 1990).

organization of light induced charge separation exist between reaction centers of anoxygenic purple bacteria and PS II, it became also clear that the latter is much more complex. All attempts failed to isolate a PS II complex with full oxygen evolution capacity that is built up by a protein matrix of a similar small number of polypeptides as that of purple bacterial reaction centers. Preparations fully competent in oxygen evolution were always found to contain the Chl *a* binding polypeptides CP47 and CP43, the  $\alpha$ - and  $\beta$ -subunits of heme protein cytochrome b559 (cyt b559), smaller integral proteins and regulatory extrinsic subunits (Ikeuchi et al. 1985; Franzen et al. 1986; Ghanotakis et al. 1987; Haag et al. 1990). With this type of preparation and structural analysis by electron microscopy imaging, we were able to present for the first time a picture that revealed the binding of the extrinsic 33 kDa to the PS II complex and to provide a rough estimation of its dimension as illustrated in Figure 4 (Haag et al. 1990). Later, the resolution of electron microscopic analyses was improved and electron diffraction pattern analyses were performed successfully on two-dimensional crystals of PS II preparations. These data permitted an early insight into the structural arrangement of PS II core proteins and cofactors at a resolution of about 8 Å (see Rhee et al. 1998; Nield et al. 2000). Complementary information on distances between cofactors were obtained by applying pulsed EPR methods (Astashkin et al. 1997;

Dorlet et al. 1998; Lakshmi et al. 1999; Zech et al. 1997, 1999).

#### *Current state of structural information on Photosystem II*

The goal of seeing the PS II structure was finally achieved to a large extent when Athina Zouni, with several others, in Berlin, was successful in obtaining PS II crystals from the thermophilic cyanobacterium *Synechococcus elongatus* that enabled Peter Orth and Norbert Krauss to perform X-ray structure analysis at 3.8 Å resolution (Zouni et al. 2001; see H. Witt's paper, scheduled to appear in Part 3 of these history issues of *Photosynthesis Research*). This structure confirms the original idea (Trebst and Depka 1985; Michel and Deisenhofer 1988) of a close similarity of the arrangement of cofactors P680, Pheo, Q<sub>A</sub> and Q<sub>B</sub> in the heterodimeric D1/D2 protein matrix of PS II and P, BPheo, Q<sub>A</sub> and Q<sub>B</sub> in the heterodimer of L- and M-subunit of purple bacteria. At the present resolution, the details of its coordination sphere of the manganese centers are not yet clear. Strong efforts were made to identify via site directed mutagenesis amino acid residues that act as direct ligands to the manganese (for reviews, see Debus 2001; Diner 2001). As a result, His, Glu and Asp residues and the C-terminus carboxylic group were reported to be most likely candidates but so far any 'hard' proof of any of these is still lacking. Likewise, it is impossible to decide if amino acid residues from polypeptides other than D1 and D2 are also part of the ligand sphere of manganese. Another striking feature that remains to be resolved in its structure are the extended loops E on the luminal side between transmembrane helices V and VI of polypeptides CP43 and CP47. These loops play an essential role in the stability of the WOC in dark adapted cyanobacteria as illustrated by our studies of *Synechocystis* PCC 6803 mutants (Gleiter et al. 1994, 1995) where 5–8 amino acid residues were deleted at different positions (Haag et al. 1993). The underlying mechanism and possible effects owing to interaction with other polypeptides, especially the extrinsic 33 kDa protein are also topics of future research.

Complementary information on the structure of the tetranuclear manganese cluster of the WOC have been gathered from X-ray spectroscopy. This work was pioneered and performed by the Berkeley group of the late Mel Klein and Ken Sauer when they started their studies in the 1970s (see Britt et al. 2001). (For a discussion of Mn, see Vittal Yachandra's paper, sched-

uled to appear in Part 3 of these history issues.) As a result of extended X-ray fine structure (EXAFS) analyses, it was suggested that the manganese atoms are connected via  $\mu$ -oxo and  $\mu$ -carboxylato bridges at distances of about 2.7 and 3.3 Å and that the cluster is coordinated to the protein matrix via N and O atom containing ligands, i.e., His, Asp and Glu residues are most likely ligands (for a recent review, see Robblee et al. 2001 and references therein). In combination with X-ray crystallography and complementary spectroscopic techniques (EPR/ENDOR, FTIR) it can be expected that the exact geometry of the 'heart' of the WOC will be unraveled in the near future.

When considering structural characteristics, a unique feature of the WOC has to be taken into consideration, i.e., the complex is a nonequilibrium system that requires light driven activation reactions for its assembly (the process is called photoactivation) and the binding of extrinsic subunit(s) for the stabilization. Pioneering work to understand the mechanism of photoactivation was performed by the late George Cheniae and his coworkers (see Radmer and Cheniae 1977 and references therein). The process was shown to be a multistep reaction sequence (Tamura and Cheniae 1987) that requires Ca<sup>2+</sup> (Tamura et al. 1989; for a recent review, see Ananyev et al. 2001). Therefore, questions arise on the nature of the redox states that are involved. Since the early work of Bernadette Bouges-Bocquet (1980), it is known that the WOC can attain 'superreduced' states below S<sub>0</sub> when samples are treated with hydrophilic reductants like NH<sub>2</sub>NH<sub>2</sub> or NH<sub>2</sub>OH. In detailed studies, we have characterized the states S<sub>-1</sub>, S<sub>-2</sub> and S<sub>-3</sub> (Messinger and Renger 1994; Messinger et al. 1997). Recently Johannes Messinger and collaborators have obtained first evidence even for the existence of S<sub>-4</sub> and S<sub>-5</sub> (Messinger et al. 2001b). These states might be intermediates in the process of photoassembly of the WOC (Renger 2001a; Ono 2001).

#### **Mechanism of photosynthetic water oxidation**

An inspection of the Kok-cycle (see Joliot and Kok 1975) reveals that several questions have to be answered for a deeper understanding of photosynthetic water oxidation (for a list, see Renger 1987a, 1993). Among these, two key problems must be solved for the resolution of the mechanism: 1) What are the electronic configuration and nuclear geometry of each S<sub>i</sub> state in the Kok-cycle? and 2) What is the coupling

mode of electron and proton transfer and the energetic 'landscape' of the reaction coordinate of each individual redox step?

*Characterization of  $S_i$  states in the water oxidizing complex*

In principle, the first problem should be solved by using suitable spectroscopic methods. We have attempted to address this point by searching for flash induced absorption changes in the UV-VIS region that reflect redox transitions of the  $S_i$ -states in the WOC using the characteristic period four oscillation of the Kok cycle as a fingerprint. In our earlier studies with Christoph Wolff, we obtained first hints on the existence of absorption changes in the UV that exhibit period four oscillations when dark adapted samples are excited with a train of single turnover flashes (unpublished results). Unfortunately, due to the untimely death of Christoph (in 1975) this research was severely retarded and only in 1982, i.e., one year after the first report by Bruno Velthuys (1981), we published results unambiguously showing that the turnover of the WOC gives rise to difference spectra with bands peaking near 320 nm (Renger and Weiss 1982, 1983). Severe problems of the deconvolution of the measured difference spectra prevent an unambiguous assignment to individual redox transitions so that even the difference spectra  $\Delta\epsilon(S_{i+1}, S_i)$  gathered from highest quality experimental data exhibit significant differences (see Lavergne 1991 and van Leeuwen et al. 1993). Apart from these inherent complications, the difference spectra are rather broad and almost structureless so that no unambiguous conclusions can be drawn on the electronic configuration of the manganese cluster in the different  $S_i$  states as outlined by Renger (1999). Therefore, other methods like EPR and X-ray spectroscopy are more appropriate to address this problem.

An important discovery in the spectral  $S_i$ -state characterization was the observation of a  $g = 2$  multiline EPR signal that reflects the  $S_1 \rightarrow S_2$  transition in the WOC (Charles Dismukes and Yona Siderer 1981). This result not only provided direct experimental evidence for the oxidation of an at least dinuclear manganese cluster during the Kok-cycle but was also the starting point for a wealth of EPR-studies in order to characterize the different redox states of the WOC. At present, EPR-signals are known for all  $S_i$ -states with  $i = 0, 1, 2$  and  $3$  (see Peloquin and Britt 2001 and references therein). Likewise X-ray

absorption spectroscopy was used to gather, from K-edge measurements, information on the nature of the redox transition of the WOC (see Messinger et al. 2001a and references therein). Key conclusions from these spectroscopic studies are: the redox transitions  $S_0 \rightarrow S_1$  and  $S_1 \rightarrow S_2$  are manganese centered oxidation steps while discrepancies exist in whether or not the reaction  $S_2 \rightarrow S_3$  is also a manganese centered redox step or is rather a ligand centered process [for detailed studies and discussion, see Messinger et al. (2001a), and references therein]. The controversy on  $S_2 \rightarrow S_3$ , however, might be only a semantic problem if one assumes that a redox isomerism exists in  $S_3$  (Renger 1993). In this respect one problem should be briefly mentioned. Many sensitive spectroscopic techniques with high information content on the electronic configuration of the manganese cluster can be successfully performed only at very low (non-physiological) temperatures. Therefore it is indispensable to clarify whether or not the electron distribution in the different redox states  $S_i$  of the WOC change when the sample is cooled down to temperatures of liquid helium. This problem, originally outlined in a minireview (Renger 1987a), is not yet unambiguously solved.

*Reaction coordinates, hydrogen atom abstraction model*

Significant progress has been achieved during the last decade in unraveling reaction coordinates including the coupling of electron and proton transfer. The overall sequence comprises two essential steps: i) formation of  $Y_Z^{OX}$  by  $P680^{+}$  and ii) stepwise oxidation of the WOC by  $Y_Z^{OX}$ .

With respect to the role of  $Y_Z^{OX}$  as the unique oxidant of the WOC, it was assumed for a long time that this species acts as pure electron acceptor and that the deprotonation steps coupled with the  $S_i$ -state transitions are independent reactions (for a review, see Lavergne and Junge 1993). Jerry Babcock opened a new road for looking into this topic by his most stimulating model that he presented at the Xth International Photosynthesis Congress in Montpellier (Babcock 1995). Based on emerging data both from his own research group as well as of others, and combined with his broad knowledge and active research on oxygenases (especially cytochrome c oxidase) and other enzymes, he arrived at a very attractive hypothesis for the mechanism of water oxidation. He concluded that the neutral tyrosine radical  $Y_Z$ , formed by electron transfer to  $P680^{+}$  coupled with release

of the proton into the lumen, acts as abstractor of a hydrogen atom from substrate water coordinated to manganese centers of the WOC. In cooperation with Cecilia Tommos, Curt Hoganson and coworkers, he provided different lines of evidence that are in favor of the hydrogen atom abstraction model (Tommos and Babcock 1998; Hoganson and Babcock 1997). This model has highly stimulated research activities, and in my opinion it is a great step forward for a deeper understanding of photosynthetic water oxidation.

#### *Mechanism of P680<sup>+</sup> reduction by Y<sub>Z</sub>*

One crucial point in Babcock's model is the mode of coupling of proton and electron transport. The formation of Y<sub>Z</sub> by P680<sup>+</sup> occurs via multiphasic kinetics including at least three components referred to as 'fast' and 'slow' ns kinetics and  $\mu$ s-kinetics (Renger et al. 1983; Brettel et al. 1984; Eckert et al. 1984). Since P680 and Y<sub>Z</sub> are bound to the same protein matrix, questions arise on the origin of these complex kinetics. Based on the finding of a rather small activation energy of the order of 10 kJ/mol for the 'fast' ns component, we proposed that the reduction of P680<sup>+</sup> by Y<sub>Z</sub> is coupled with a proton shift to a nearby base (Eckert and Renger 1988). This idea is supported by numerous reports (see references in Christen et al. 1999) and the basic group X is most likely His 190 of polypeptide D1 (see references in Hays et al. 1999). An analysis of the 'fast' ns kinetics within the framework of the Marcus-theory of nonadiabatic electron transfer revealed that this process with a reorganization energy of about 0.5 eV (Renger et al. 1989) is kinetically limited by the electron transfer step and the van der Waals distance of about 10 Å gathered from this data (Renger et al. 1998) is in perfect agreement with the recent crystal structure at 3.8 Å resolution (Zouni et al. 2001). Detailed studies on kinetic isotope effects revealed that replacement of exchangeable protons by deuterons exerts virtually no effect on both 'fast' and 'slow' ns kinetics with  $k_H / k_D < 1.05$  (Karge et al. 1996). In contrast to the ns kinetics that were ascribed to PS II complexes with a functionally competent WOC the origin of the  $\mu$ s kinetics was a matter of controversial discussion (see Christen and Renger 1999 and references therein). In addition to a characteristic period four oscillation of the amplitudes (Gläser et al. 1976; Eckert and Renger 1988; Schilstra et al. 1998) the  $\mu$ s kinetics were shown to be prone to marked changes owing to H/D exchange, depending on the redox states S<sub>i</sub> of the WOC. Therefore the latter kinetics were

inferred to reflect a protein relaxation limited redox process that is coupled with proton shifts within a hydrogen bond network of PS II complexes with intact WOC (Christen et al. 1998; Schilstra et al. 1998).

Another interesting finding in this field was the observation from our laboratory that the extent of  $\mu$ s kinetics in mildly trypsinized PS II membrane fragments (from spinach) with intact WOC specifically depends on the presence of Ca<sup>2+</sup> (Völker et al. 1987; Renger et al. 1989). In a recent extension of this study to PS II core complexes with high oxygen evolution capacity, further evidence has been presented for a regulatory role of Ca<sup>2+</sup> in the process of P680<sup>+</sup> reduction by Y<sub>Z</sub> (Kühn et al. 2001). Based on the above mentioned studies, a scheme is presented for Y<sub>Z</sub> oxidation by P680<sup>+</sup> (Renger 2001b) that includes at least three types of rate limitations: i) electron transfer in the 'fast' ns kinetics, ii) short range protein relaxation without significant hydrogen bond rearrangement in the 'slow' ns kinetics and iii) 'large scale' changes of a hydrogen bond network at the PS II donor side are responsible for the  $\mu$ s kinetics.

#### *Electron/proton pathway(s) and O–O bond formation in the water oxidation complex*

Transient UV absorption changes provide an excellent tool to study the kinetics of the redox steps in the WOC (Dekker et al. 1984a; Renger and Weiss 1986). We started our work on the reaction coordinate of water oxidation in the mid-1980s in cooperation with Yorinao Inoue's group (in Japan) when Horiike Koike came to work for three months in my laboratory in Berlin. At first, the temperature dependence of the redox transitions in the WOC of PS II complexes, isolated from thermophilic cyanobacteria, was analyzed (Koike et al. 1987). In the following years, we continued to determine the activation energies and the kinetic isotope effect owing to replacement of exchangeable protons by deuterons in PS II preparations from higher plants (Renger and Hanssum 1992; Renger et al. 1994; Karge et al. 1997). The results obtained led to three important conclusions: i) the activation energies of the redox transitions in the WOC are comparatively small and dependent on the S<sub>i</sub>-state, ii) kinetic H/D isotope exchange effects are small and iii) the reaction coordinates of the WOC exhibit a striking invariance during the evolutionary development from cyanobacteria to higher plants (Renger 2001a).

One essential postulate of the original hydrogen atom abstraction model is the stoichiometric release of one proton into the lumen when  $Y_Z$  becomes oxidized. This phenomenon has been observed in samples deprived of a functional competent WOC (Renger and Völker 1982) but is not unambiguously confirmed in preparations with fully intact WOC. Furthermore, the stoichiometry of proton release coupled with individual  $S_i$  oxidation steps is pH-dependent except for the  $S_2 \rightarrow S_3$  transition where one proton is always released per transferred electron (Lavergne and Junge 1993). It is therefore attractive to speculate that the hydrogen atom abstractor function of radical  $Y_Z^{\cdot}$  as proposed by Jerry Babcock is restricted to  $S_2$  and  $S_3$  oxidation whereas the other two oxidation steps might involve a different pathway of electron and proton transfer (Renger 2001a).

The key step in the process of water oxidation is the O–O bond formation. The mechanism of this reaction is still not yet clear. The vast majority of experts favour the idea that this step occurs only in  $S_4$ . For several reasons, I propose that there exist already in oxidation state  $S_3$ , two rapid equilibria with respect to i) electronic configuration between manganese and substrate (redox isomerisms) and ii) nuclear array of protons in coordinated substrate (oxywater  $\rightleftharpoons$  hydrogen peroxide tautomerism). These equilibria most likely comprise different intermediates, including a particular  $S_3$  state with an electronic configuration and nuclear geometry corresponding to a complexed peroxide (Renger 1993, 2001a, b). In this way,  $S_3$  can be considered as entatic state of dioxygen formation. This idea will certainly dominate my thinking and future research on oxygen evolution.

#### *Substrate/product transport in the water oxidizing complex*

Of mechanistic relevance for each enzymatically catalyzed reaction are the processes of substrate entry and product release. Questions on water substrate entry into the Kok-cycle can be successfully addressed by using  $H_2^{18}O$  as substrate. The first studies performed more than 15 years ago by Radmer and Ollinger (1986) showed that the substrate water is exchangeable in all  $S_i$ -states including  $S_3$ . When reading this paper it became readily clear that the time resolution was insufficient for any straightforward conclusion (Renger 1987a). In cooperation with Klaus Bader and Georg Schmid in Bielefeld, we attempted to address this problem by mass spectroscopic measurements

in samples illuminated with groups of light-flashes. Based on the results obtained, the substrate water was inferred to be most likely exchangeable in all  $S_i$ -states (Bader et al. 1993). Unfortunately the flash group experiments did not provide the tool to circumvent the key problem of rate limitation by  $H_2^{18}O / H_2^{16}O_2$  exchange. This problem was solved by Johannes Messinger in cooperation with Tom Wydrzynski and Murray Badger, in Australia: the two substrate water molecules were found to exhibit markedly different exchange kinetics in  $S_3$  (Messinger et al. 1995). Hillier and Wydrzynski (2000) have extended these studies to all the  $S_i$ -states. The details of the pathway of the substrate water from the bulk phase of the lumen into the catalytic site of the WOC, the entry of water and the possible control by regulatory elements remain to be discovered in future studies

With respect to product release I proposed that a definite transport pathway exists in the protein matrix in order to assure a directed reaction (Renger 1999). A similar conclusion was reported recently by Jan Anderson (2001). At present, no sound information is available on this problem.

#### **Retrospective and future outlook**

This personal minireview presents reflections on the progress of our knowledge in understanding the structural and functional organization of photosynthetic water cleavage during the last three to four decades. I am extremely grateful for having the chance to contribute to the field to some extent. It is not only a stimulating intellectual pleasure but – even more importantly – a great chance to work with ambitious students, with colleagues all over the world and – at best – to find friendship.

When considering all facets of our field, I must say that real new concepts are rare. One of these is Jerry Babcock's hydrogen atom abstraction model. In my opinion, the WOC is a supramolecular device tailored by nature for the bioenergetically fundamental step of solar energy exploitation by water cleavage. As a consequence, I would like to propose that the protein matrix of the manganese cluster is a key functional element of the WOC acting as proton transfer 'director' via hydrogen bond fine tuning of the system. It is more than simply the sum of individual molecules. Therefore, it can be even misleading when we rely on oversimplified model systems. I look forward with



Figure 5. G. Renger (left, author) and Govindjee (right, editor) at the former's 60th birthday celebrations in Berlin in 1997.

great interest to seeing the future development in our field of research.

I end this perspective of WOC with a happy memory: a photograph of myself with Govindjee at my 60th birthday celebration in 1997 in Berlin (Figure 5).

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