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Review

Water, water everywhere, and its remarkable chemistry

Jim Barber*

Department of Biological Sciences, Wolfson Laboratoties, Biochemistry Building, South Kensington Campus, Imperial College London, Exhibition Road, London SW7 2AZ, UK

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Abstract

Photosystem II (PSII), the multisubunit pigment–protein complex localised in the thylakoid membranes of oxygenic photosynthetic organisms, uses light energy to drive a series of remarkable reactions leading to the oxidation of water. The products of this oxidation are dioxygen, which is released to the atmosphere, and reducing equivalents destined to reduce carbon dioxide to organic molecules. The water oxidation occurs at catalytic sites composed of four manganese atoms (Mn_4-cluster) and powered by the redox potential of an oxidised chlorophyll *a* molecule ($P680^{\bullet +}$). Gerald T (Jerry) Babcock and colleagues showed that electron/proton transfer processes from substrate water to $P680^{\bullet +}$ involved a tyrosine residue (Y_Z) and proposed an attractive reaction mechanism for the direct involvement of Y_Z in the chemistry of water oxidation. The 'hydrogen-atom abstract/metalloradical' mechanism he formulated is an expression of his genius and a highlight of his many other outstanding contributions to photosynthesis research. A structural basis for Jerry's model is now being revealed by X-ray crystallography.

Keywords: Photosynthesis; Photosystem II; Water oxidation; Hydrogen abstraction model; Babcock

1. Water, water everywhere and 'poop'-oxygen

Over the years, I was privileged to hear Jerry Babcock give many inspirational talks about his research on the water oxidation reactions of Photosystem II (PSII). Often during the opening preamble of his lectures my thoughts would turn to a verse from 'The Rime of the Ancient Mariner' by Samuel Taylor Coleridge (1772–1834).

> Water, water everywhere And all the boards did shrink
> Water, water everywhere Nor any drop to drink

Yes, there is water, water everywhere and Jerry would remind us that this is because water is a very stable molecule and therefore its oxidation by PSII involves difficult and thermodynamically demanding chemistry. "We have oceans of it. It's a thermodynamic sink," he would say. Perhaps Jerry would have approved of a modification to Coleridge's verse:

> Water, water everywhere Lots and lots of it Water, water everywhere But difficult to split

In fact, there are about 1.37×10^9 Gtonnes of water on our planet [1]. The invention of the water splitting reaction of PSII in photosynthetic organisms can therefore be considered as the 'Big Bang of Evolution' for it provided biology with an unlimited supply of hydrogen equivalents destined to reduce carbon dioxide to organic molecules. The by-product of the reaction is molecular dioxygen. Jerry Babcock often said that dioxygen is the 'poop' of the PSII-driven water-splitting process. However, the 'poop-oxygen' has also had enormous influence on the evolution of living organisms on our planet. It has given us an oxygenic atmosphere and allowed aerobic biology to prosper.

It seems that the first photosynthetic organisms able to oxidise water and evolve oxygen were cyanobacterial-like and date back about 2.5 billion years. They probably became quite widely spread throughout the sunlit surface waters of the Earth. At first, the oxygen they produced was used up in oxidising the then abundant Fe^{2+} and S^{2-} in the oceans and lithosphere. Once this great 'rust event' was complete about 2.2 billion years ago, the level

Abbreviations: EPR, electron paramagnetic resonance; PSII, photosystem II

* Tel.: +44-207-594-5266; fax: +44-207-594-5267.
E-mail address: j.barber@imperial.ac.uk (J. Barber).

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of oxygen in the atmosphere rapidly built up to its modern level. By the beginning of the Cambrian period (540 million years ago), the high oxygen content of the atmosphere and oceans allowed the evolution of a great variety of marine invertebrate animals deriving their energy from respiration. Air-breathing land animals appeared about 400 million years ago and 50 million years later land plants at last proliferated, leading the way to the Carboniferous Period (225–345 million years ago). During this time, the Earth's enormous deposits of fossil fuels were laid down.

Today about 100 Gtonnes of carbon is fixed annually by photosynthesis on a global scale resulting in the release of about 260 Gtonnes of oxygen into the atmosphere. Since the atmosphere contains 1.2×10^6 Gtonnes of oxygen, the cycling time of oxygen through the biosphere is approximately 4600 years [2]. If oxygenic photosynthesis were to suddenly stop, human beings would start to suffer from hypoxia as the partial pressure of oxygen in the atmosphere dropped from its current level of 21 kPa to anything much below 15 kPa. This, however, may not be the most urgent problem since we would quickly run short of food and suffer from the buildup of CO_2 levels in the atmosphere due to decaying trees and plants. We would also need to avoid the intense UV radiation resulting from the loss of the ozone layer, which would destroy terrestrial life as well as accelerate global warming.

The discovery that oxygen is released from plants is credited in part to Joseph Priestley (1733–1804) and in part to Jan Ingen-Housz (1730–1799). Priestley [3], in his publication of 1774, "Experiments and observation of a different kind of air", noted that 'The injury which is continually done to the atmosphere by the respiration of such a large number of animals……is, in part at least, repaired by the vegetable creation'. That is, according to him, plants produced 'dephlogisticated air'. However, it was probably Ingen-Housz [4] who realised that oxygen release from plants required the illumination of green parts. The work of these pioneers heralded the understanding of the oxygen/carbon cycles and the interplay of photosynthesis and respiration. This interplay is, of course, the perfect solution to the biological energy problem; water is split into oxygen and hydrogen by solar energy and recombines again to release energy to drive metabolism. Organic molecules act as the 'hydrogen' store. This cyclic process is totally nonpolluting, not even thermally polluting. Jerry Babcock worked on each end of the cycle: water oxidation to give dioxygen (PSII research) and the reverse reaction of dioxygen reduction to form water (cytochrome oxidase research).

The general idea, however, that light energy is captured and stored by plants seems to have emerged some years before the experiments of Priestley and Ingen-Housz from studies by the English botanist Stephen Hales (1677–1761), as recorded in his book, Vegetable Staticks, published in 1727. Indeed, Jonathan Swift (1667–1745), who probably knew of Hales' theories [5], seems to have picked up this theme when, in Gulliver's Travels (published in 1726), he describes the scientist at the Academy of Lagado (probably synonymous with the Royal Society) who was researching the possibility of storing light in cucumbers to be re-emitted at some later time. The academician was described as a 'man of a very meagre aspect, with sooty hands and face, his hair and beard long, ragged and singed in several places. His clothes, shirt and skin were all of the same colour. He had been eight years upon the project for extracting sunbeams out of cucumbers, which were to be put in phials, hermetically sealed, and let out to warm the air in raw, inclement summers.' He explained to Gulliver, 'that he did not doubt that in eight years more, he should be able to supply the governor's gardens with sunshine at a reasonable rate; but he complained that his stock was low and entreated me to give him something as an encouragement to ingenuity, especially since this had been a very dear season for cucumbers.' In response to this, Gulliver made a small donation since he had been warned that scientists at the Academy would beg for money to support their research. Nothing changes! Fig. 1 is a cartoon taken from the 1798 edition of Gulliver's Travels which I have on my book shelf at home that depicts Gulliver talking with the academician or 'projector', as Swift describes him.

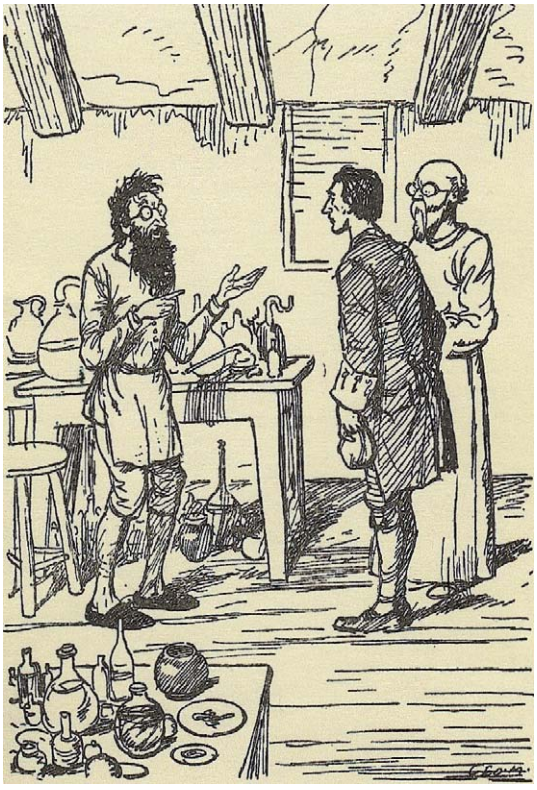

Fig. 1. A cartoon of Gulliver meeting the scientist (projector) working on the storage of light energy in cucumbers at the Academy of Logado. Gulliver notes 'The first man I saw was of a very meagre aspect'.

Despite Swift's perception of the storage of light energy by plants, it took another 120 years before the concept was given a firm scientific foundation by Julius Mayer, who in 1845 emphasised the remarkable property of plants in capturing light emitted from the sun and using it to do chemical work. He wrote 'Nature set herself the task of capturing the light flooding towards the earth and of storing this, the most elusive of all forces, by converting it into an immobile force... the plant world constitutes a reservoir in which the fleeting sun rays are fixed and ingeniously stored for future use, a providential measure to which the very existence of the human race is inescapably bound'.

2. The engine of life

PSII is truly the 'Engine of Life' and a major challenge of modern science is to understand exactly how this biological machine works. It was characteristic of Jerry Babcock to recognise and take up this challenge. Indeed, this may be reflected in a note written by him while he was in Berkeley around 1970 recently discovered in a desk Jerry occupied [6]. In a handwritten note he states 'It is very important to have a long range goal; once this is realised the steps necessary towards its attainment become logical progressions and not arbitrary plunges into the void'. Thanks to Jerry Babcock and many other talented scientists, we have progressed a long way with our understanding of photosynthetic water oxidation since Priestley initially described oxygen evolution from plants some 230 years ago. We now have the basic building blocks of physics, chemistry, protein and structural biochemistry and molecular biology to call on. Hill [7] and Ruben et al. [8] were the first to show experimentally that oxygen released by photosynthesis was derived from water. That the catalytic centre was associated with PSII stems from the Z-scheme hypothesis of Hill and Bendall [9] and from the demonstration by Boardman and Anderson [10] that PSII and photosystem I (PSI) can be separated biochemically into discrete protein complexes. This was followed by the discovery of the flash-induced oxygen evolution pattern by Joliot et al. [11] which was formulated into the S-state cycle by Kok et al. [12], as shown in Fig. 2. The idea that oxidising equivalents were stored at a single catalytic site was in line with the proposal as in Ref. [13] that Mn, with its ability to exist in several oxidation states, was involved in the formation of dioxygen. The careful studies of Cheniae [14], reviewed in 1970, indicated that there is a pool of Mn atoms associated with PSII and Jerry Babcock, together with Blankenship et al. [15], pioneered the use of electron paramagnetic resonance (EPR) spectroscopy to study this pool. It was this early work which paved the way for the discovery of the S_2-state multiline EPR signal by Dismukes and Siderer [16].

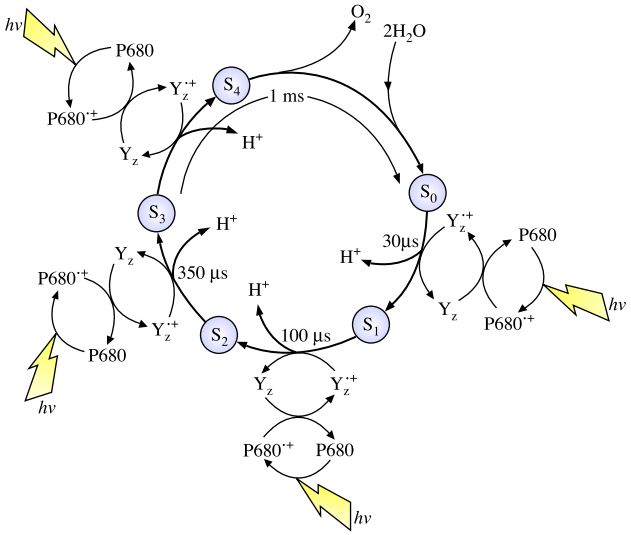

Fig. 2. The S-state cycle for the oxygen evolution reaction, as first suggested by Kok et al. [12], modified to accommodate the model proposed by Babcock and colleagues [44,45]. The scheme is based on the fact that, when a photosynthetic organism is exposed to a series of saturating flashes of light, the evolution of oxygen follows a period of four. This indicates that four oxidising equivalents must be utilized at a single catalytic centre before a dioxygen molecule is formed and released. Therefore, there must be four light-induced redox turnovers of P680 and tyrosine (Y_Z) to produce the four oxidising equivalents needed to oxidise two substrate water molecules. Dioxygen is produced on the S_4-to-S_0 transition. According to the hypothesis of Babcock and colleagues, each photo-induced step of the S-state cycle ($S_n \rightarrow S_{n+1}$) involves the removal of an electron and a proton from two bound water molecules. According to this model, the electron and proton are transferred to the neutral tyrosine radical ($Y_Z^{\bullet}$).

At about the same time Jerry, together with Yocum et al. [17], provided evidence for a stoichiometry of four Mn-atoms per PSII (Mn_4-cluster).

3. Signal II

It was the application of EPR and related resonance spectroscopies that would underlie Jerry Babcock's major contributions to our present day understanding of PSII. Horst Witt, Lou Duysens and Bessel Kok pioneered the application of optical absorption spectroscopy to photosynthesis research and in this way P680 was first described by Döring et al. [18] as the primary chlorophyll *a* electron donor of PSII. Later, Klimov et al. [19], also using optical spectroscopy, provided the evidence for pheophytin *a* (Pheo) acting as the primary electron acceptor of PSII. The same technique also established the role of plastoquinone as secondary electron acceptors of PSII [20,21]. However, it was EPR spectroscopy that would prove to be a powerful tool for investigating the donor side of PSII. The scene was set by the pioneering work of Commonor et al. [22]. Using chloroplasts isolated from spinach and intact Chlorella cells, they discovered light-induced EPR signals centred at $g = 2.005$, which became known as

Signal I and Signal II. It was Jerry Babcock, together with Babcock and Sauer [23], who dissected out different forms of Signal II and showed that they were derived from PSII. By using flash excitation, they defined three identical forms of Signal II, characterised by their lifetimes: Signal II_s, Signal II_f and Signal II_{vf} (s = slow, f = fast, vf = very fast). Signal II_{vf} was seen in untreated chloroplasts while Signal II_f was observed after inhibiting water oxidation. Signal II_s was observed in both oxygen evolving and inhibited samples and decayed very slowly in the dark. Fig. 3 shows Signal II_{vf} induced in dark-adapted chloroplasts and its inhibition by DCMU reported by Babcock et al. [25] in 1976.

It was from these studies that Jerry and colleagues were able to assign the species giving rise to Signal II_f/II_{vf} as a redox active intermediate between the oxygen evolving reaction and P680 [24,25]. This species became known as 'Z' and the initial idea was that it was a quinone [26]. However, with the solving of the structure of the reaction centre of purple photosynthetic bacterium *Rhodospeudomonas viridis* [27] and the realisation that there were striking homologies between this system and the D1 and D2 proteins of PSII [28,29], the scene was set to elucidate the chemical identity of Z. It was noted that in the structure of the reaction centre of *R. viridis* a tyrosine residue (LTyr162) was strategically placed between P960 and the heme which reduces this primary donor when oxidised. It was therefore speculated that Z may be a tyrosine residue [29,30] and elegantly shown to be the case by the isotopic labelling studies of Barry and Babcock [31]. They fed deuterated tyrosine to cells of the cyanobacterium *Synechocystis* sp. PCC 6803 and found that the EPR properties of 'Z' were dramatically modified. The idea that Signal II was due to a tyrosyl radical was confirmed by site-directed mutagenesis and, moreover, Signal $II_{f/fv}$ corresponded to Tyr 161 on the D1 protein. This discovery resulted from collaboration between Jerry Babcock, Lee McIntosh, Rick Debus, Bridgette Barry and coworkers [32,33]. Complementing their mutational studies were those conducted by Diner et al. [34]. I distinctly remember when the conclusions of these seminal studies were published and the joy I had in telling my undergraduate students that we now know that Z is a tyrosine residue. It was Vermaas et al. [35] as well as Babcock and his team [32] who showed that Signal II_s was derived from the related tyrosine of the D2 protein, positioned at 160 [35].

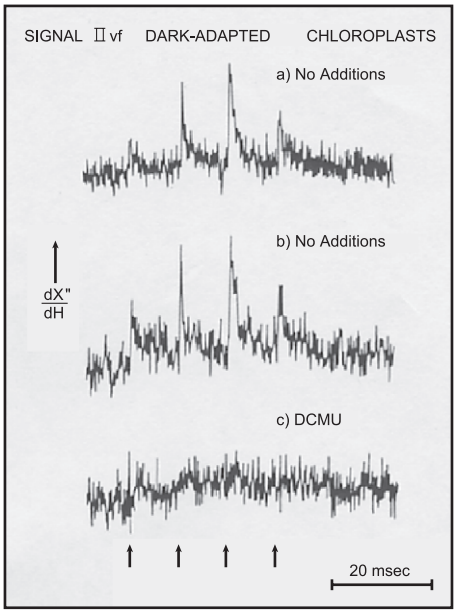

Fig. 3. Signal II_{vf} formation in dark-adapted spinach chloroplasts saturating, 10-μs flashes given as indicated by upward arrows (a) and (b) no additions: (s) + 10^{-4} M DCMU measured by time-resolved EPR using microwave power, 100 mW and a 4-G modulation amplitude. Time constant was 100 μs and each curve is the average of 1024 events. Taken from Babcock et al. [25].

4. The role of Y_Z/Y_D in water oxidation

The structure of the purple bacterial reaction centre [27], the homology between the L and M subunits and the D1 and D2 proteins [28,29,36] and isolation of the D1/D2/cyt b_{559} complex [37,38] provided a basis for a molecular model of PSII and therefore opened up discussions about the role of D1 Tyr161 (Y_Z) and D2 Tyr160 (Y_D) in water oxidation chemistry. It was clear that Y_D was not directly involved in water splitting. However, it has been suggested by Styring and Rutherford [39] that perhaps the function of oxidised Y_D is the resetting of the S-state clock in the dark (converting S_0 to S_1) and/or perturbing the delocalisation of the oxidising equivalent of $P680^{\bullet +}$ towards the D1 protein side of the reaction centre [40] and therefore increasing the probability of the radical residing on the chlorophyll ligated to D1 His198 [41,42]. The pseudo-twofold symmetry of the PSII structure is clearly broken by the fact that the Mn_4-cluster is located on the D1 side of the reaction centre and this therefore gives rise to the functional differences between Y_Z and Y_D.

5. Remarkable chemistry

A question that occupied Jerry's thoughts in the years just prior to his death was whether Y_Z could play an active role in the chemistry of water oxidation? It was clear to all that no single step of the water oxidation reaction process could involve a redox potential greater than the 1 V provided by the photo-oxidation of P680 [43]. Moreover, the overall free energy drop available to drive water oxidation on the electron donor side of PSII is small, less than 200 mV per hydrogen atom stripped from water. As Jerry would say, "This PSII machine is working on the thermodynamic edge". It therefore seemed inconceivable to Jerry that successive electron extractions from the Mn_4-cluster during the S-state cycle could lead to the accumulation of positive

charges within the water oxidising site. Jerry pointed out that such an accumulation may be damaging and most certainly would make electron extraction more and more difficult and therefore thermodynamically very demanding. "PSII needs to avoid the coulombic explosion" he would say. With similar thoughts about thermodynamic limitations, he considered it unlikely that large conformational changes would occur in the Mn_4-cluster during the S-state cycle, such as the breakage of structural oxo-bridges. It was from these considerations that he proposed an important direct functional role for Y_Z in the chemistry of water oxidation. He raised the possibility that the oxidation of Y_Z by $P680^{\bullet +}$ to a neutral tyrosyl radical ($Y_Z^{\bullet}$) could allow a novel metalloradical enzyme mechanism to catalyse water splitting. In its deprotonated oxidised state, the tyrosine could possibly receive an electron and a proton from substrate water molecules undergoing oxidation. This 'hydrogen-atom abstraction model' therefore solved the problem of the 'coulombic explosion' and gave a basis for an electroneutral reaction scheme for the S-state cycle as elegantly described in his Science paper of 1997 with Hoganson and Babcock [44] and his article with Tommos and Babcock in 1998 [45]. He proposed a hydrogen-atom abstraction on each step of the S-state cycle and suggested, as shown in Fig. 4, that the dioxygen was formed from the oxygen atoms of the two bound water substrate molecules without any ligand exchange within the oxo-bridges of the Mn_4-cluster (note that he assumed the Mn_4-cluster to be the dimer-of-dimer model of Yachandra et al. [46]). Each hydrogen abstraction would need to be linked with an oxidation step of about 1 V available from $P680^{\bullet +}$. This therefore meant that the redox potential for oxidised tyrosine must also be close to 1 V. The arguments for this being the case are rigorously discussed in the review by Tommos and Babcock [47] in which they concluded that deprotonation of the phenolic group of Y_Z is closely coupled to its oxidation, thus yielding the neutral tyrosine radical, having a redox potential which drops to about 0.97 V as the phenolic proton migrates through the protein matrix by domino deprotonation, finally leading to hydrogen ion release at the thylakoid lumenal surface. This time-dependent proton diffusion would also explain the complex kinetics of $P680^{\bullet +}$ reduction by Y_Z, ranging from nanosecond to microsecond [48 49]. Together with Charles Yocum and Demetrious Ghanotakis, Jerry had provided strong evidence that Ca^{2+} is a cofactor for the water oxidation reaction [50,51], a concept which was reinforced by EPR and related studies, particularly those of Bill Rutherford and the Berkeley group [52,53]. For this reason, Jerry included Ca^{2+} in his reaction scheme, playing a role as an anchor for Cl^- as shown in Fig. 4. He assumed that the role of Cl^-, the other known inorganic cofactor involved in water oxidation, was to stabilise the high valency states of the Mn-cluster generated at the S_2-state and beyond.

6. Predicted structure

Clearly, Y_Z must be located between the Mn_4-cluster and P680. The small free energy change associated with Y_Z oxidation ($Y^{\bullet}/P680^{\bullet +}$ of about 0.15 V) and the speed of the fastest electron transfer leading to is oxidation would place it 10 Å or less from $P680^{\bullet +}$ [47]. Moreover, modelling of the D1/D2 protein heterodimer based on the X-ray structure of the related L and M-subunits of purple

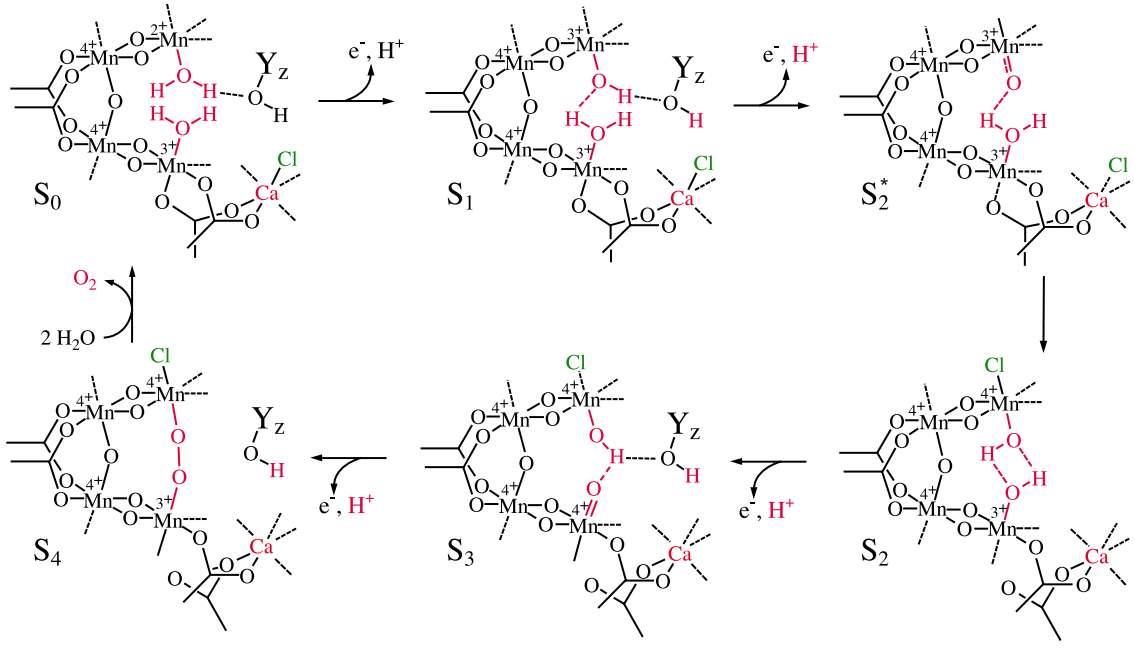

Fig. 4. The Babcock 'hydrogen-atom abstraction' scheme for water oxidation taken from Tommos and Babcock [45].

bacteria had indicated that D1 His190 could be the base close to Y_Z [54]. Similarly, D2 His189 was modelled to be close to Y_D. A wide range of studies has given support to this modelling as thoroughly reviewed by Debus [55]. Thus, Jerry assumed that D1 His190 played the important role of accepting the proton released as Y_Z was oxidised by $P680^{\bullet +}$. Indeed, site-directed mutagenesis and other studies supported this assignment [56–59]. Also it has been suggested that the other nitrogen of the imidazole group of D1 His190 may hydrogen bond to D1-E189 [57]. According to his model, the proton would be passed to a neighbouring proton acceptor and ultimately be released into the thylakoid lumen. This path would therefore be a channel for all the water protons.

The centre-to-centre distance between the Mn_4-cluster and Y_Z has been predicted to be 8–10 Å based on resonance spectroscopy [60–63]. However, as Jerry pointed out, a distance of less than 8 Å would be compatible with this hydrogen abstraction model and emphasised that it is the edge-to-edge distance which was important and that factors other than electron tunnelling controlled each step of the S-state cycle.

7. Actual structure

In 1997, an 8-Å projection map of PSII was obtained by electron cryomicroscopy [64] and a year later an 8-Å 3D structure was reported [65]. At this resolution, we were able to assign the transmembrane helices of the D1 and D2 proteins as well as those of CP47 and other low molecular weight proteins. As expected, the arrangement of the10-transmembrane helices of the D1/D2 heterodimer was very similar to that of the L and M subunits of the purple bacterial reaction centre. Moreover, at this resolution we were able to assign densities to the tetrapyrrole headgroups of the chlorine bound to the D1/D2 heterodimer and to CP47. This confirmed a belief that P680 was not a 'special pair' of closely interacting chlorophyll molecules as in the case of its counterpart in the bacterial reaction centre [54]. Instead, based on Mg–Mg distances, it seemed that P680 is composed of four equally spaced chlorophylls related by the same pseudo-twofold axis which related the transmembrane helices of the D1 and D2 proteins. The 2D crystals used for this study were grown in my laboratory and the electron crystallography carried out in Werner Kühlbrandt's group. As our 3D map was being calculated, I visited Werner's laboratory in Frankfurt regularly to be actively involved in its interpretation and assignment of densities. During one of these visits, Jerry Babcock also visited Werner's laboratory and we were able to show him our map and discuss the possible assignment of the heme of cytochrome b_{559}. It was of course Jerry who provided the experimental evidence that the heme of this cytochrome is bound to the α- and β-subunits via axial histidine ligands [66]. "Its just like spectroscopy", he said, "you are battling with signal to noise just as we do". That afternoon, with Jerry's input, we did tentatively assign the position of cytochrome b_{559} but did not include it in our paper [65] because there was some uncertainty although the assignment was later included in Rhee's PhD thesis [67]. From then on, Jerry took considerable interest in our work and would regularly ask me if we had resolved the density for the Mn_4-cluster. Fortunately he was at the Gordon Conference on Photosynthesis held in the summer of 2000 when the group from Berlin reported their 3.8-Å structural model of PSII derived from X-ray crystallography. Unlike electron crystallography, X-ray diffraction analysis readily locates metal centres and at long last the density due to the Mn_4-cluster was visualised.

In other respects, there was considerable agreement between our structure of the higher plant PSII reaction centre determined by electron crystallography [68,69] and the cyanobacterial PSII structure derived by X-ray crystallography [70], particularly in the positioning of transmembrane helices and organisation of the P680 chlorophylls [71–73]. However, the X-ray study gave us the first direct look at the structure of the Mn_4-cluster and its environment as well as providing some distance parameters. The resolution of the X-ray map (3.8 Å) was not sufficient to give a precise structure of the cluster but suggested that three Mn atoms are positioned at the corners of an isosceles triangle with the fourth Mn atom centrally located in the plane. A density was tentatively assigned to Y_Z, which was about 7 Å from the Mn-density (centre to centre). The X-ray derived map also confirmed our data [64] that P680 was not a special pair but that the four Chl molecules corresponding to the four bacteriochlorophylls of the purple bacteria are more or less equally spaced at about 10 Å centre to centre. The Chls corresponding to the special pair and almost certainly ligated to D1 His198 (P_{D1}) and D2 His197 (P_{D2}) were shown to be orientated in parallel and at right angles to the plane of the membrane. The two Chls (Chl_{D1} and Chl_{D2}) corresponding to the accessory bacteriochlorophylls were positioned and orientated (about 30° to the membrane plane) rather like their bacterial counterparts. Because of the absence of a 'special pair', it is assumed that the excited P680 state (P680*) is delocalized over all four chlorophylls [74]. Electron donation to pheophytin could therefore occur from any one of these chlorophylls but probably from the Chl closest to the active pheophytin acceptors of (Chl_{D1}) (see Fig. 5). However, there is convincing evidence [42] that the long-lived state $P680^{\bullet +}$ is located on the chlorophyll ligated to D1 His198 (P_{D1}), suggesting redistribution of the oxidising equivalents from Chl_{D1} to P_{D1}. According to the 3.8-Å X-ray model, P_{D1} chlorophyll is about 10 Å from Y_Z (centre to centre).

The recent publication of a 3.7-Å structural model of the cyanobacterial PSII by Kamiya and Shen [75] gives a more extensive tracing of the C_α -backbone and for some subunits amino acid assignments have been made based on sequences derived from genes of *Synechococcus elongatus/vul-*

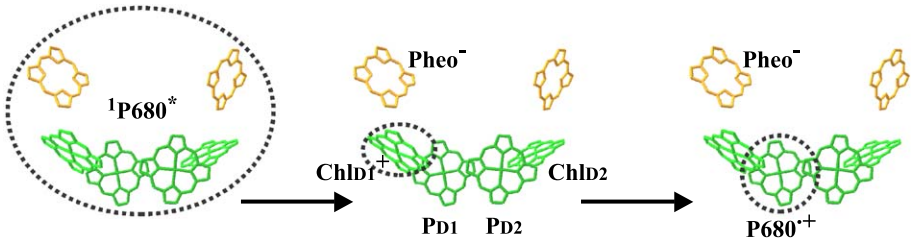

Fig. 5. A scheme for primary charge separation in PSII suggesting that the initial event involves electron donation from the Chl_{D1} which is closest to the active pheophytin (Pheo) acceptor followed by delocalisation of the oxidising equivalent to the P_{D1} chlorophyll to form the stable $P680^{\bullet+}$ state [41]. The positioning of the four chlorophylls (P_{D1}, P_{D2}, Chl_{D1}, Chl_{D2}) and the two Pheos taken from the X-ray structure of Zouni et al. [70].

canus. This new X-ray data offers an opportunity to look further into the structural predictions that underlie the Babcock hydrogen abstraction model.

Data from Kamiya and Shen has been used to construct the structural model for the Mn_4-cluster/Y_Z/P680 site as shown in Fig. 6. In the absence of higher-resolution X-ray data and with no direct access to the electron density map, the Kamiya and Shen data deposited in the RCSB protein data bank, 1ILZ.pdb, suggests that the closest distances from the Mn_4-cluster to Y_Z and from Y_Z to P_{D1} are 6.5 Å (Mn atom centre to phenolic oxygen of Y_Z) and 8.8 Å (edge to edge), respectively. According to the available model and the application of the SwissPdpViewer, version 3.7 (Ref. [76]), a nitrogen of the D1 His190 is only 2.4 Å from the phenolic group of Y_Z while the other nitrogen of D1 His190 is 3.4 Å from the carboxyl oxygen of D1 Glu189. Interestingly, according to this structural model, His190 also closely approaches the Mn_4-cluster with the shortest distance estimated to be 3.8 Å to Mn atom number 4 in 1ILZ.pdb.

It should be emphasised that the model for the Mn_4-cluster/Y_Z/P680 site presented in Fig. 6 has been produced

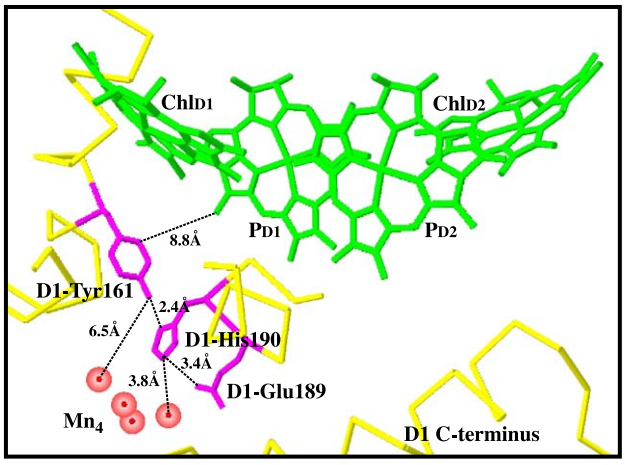

Fig. 6. A structural model of the Mn_4-cluster/Y_D/P680 site based on the data of Kamiya and Shen [75] deposited in RCSB protein data bank, 1ILZ.pdb, and the application of version 3.7 of Swiss-PdbViewer for reconstruction of the D1 His190 and D1 Glu189 side chains based on the assignments of Kamiya and Shen in 1ILZ.Pdb.

simply by taking the data of Kamiya and Shen deposited in the RCSB protein data bank and by applying version 3.7 of the SwissPdpViewer to construct the side chains of D1 His190 and D1 Glu189 according to Kimiya and Shen's assigned positions for these amino acids. It is therefore highly speculative. Nevertheless, it supports the general expectations that Y_Z and D1 His190 are sufficiently close to allow proton exchange (actually unrealistically close given that the H-bonding distance between phenolic oxygen and a imidazole nitrogen is more like 2.9 Å) and places D1 Glu189 in H-bonding distance to D1 His190 via the second imidazole nitrogen. In principle, therefore, this could facilitate deprotonation of D1 His190 as suggested by Jerry Babcock and supported by his in-depth computational analysis with Blomberg et al. [77]. It should be noted, however, that the Berlin group concluded from their X-ray data that D1 His190 is not in close proximity to Y_Z [78]. The 6.5 Å estimated for the Mn (defined as Mn atom 3 in 1ILZ.pdb) to Y_Z distance can be shortened to 5.8 Å allowing for an ionic radius for Mn of 0.7 Å (Mn^{III}/Mn^{IV} six-coordinate octahedral). Since the substrate water molecules may be bound to the peripheral of the Mn_4-cluster, the proton/electron transfer distance to Y_Z could be even shorter than 5.8 Å and thus compatible with Babcock's metalloradical hypothesis. The 8.8 Å estimated for the distance between Y_Z and $P680^{\bullet+}$(assuming the radical cation is localised on P_{D1}) is also in line with the fastest electron transfer event between these two cofactors.

8. Conclusions

The genius of Babcock's 'hydrogen-atom abstraction/metalloradical' reaction scheme for oxygen evolution, involving a neutral tyrosyl radical directly driving the chemistry of water oxidation on each step of the S-state cycle, is its relative simplicity compared with other postulated reaction mechanisms. As emphasised above, the most recent structural information available to date [75] seems to be consistent with his ideas. However, although it is unlikely that Jerry Babcock's precise scheme will be correct in all its detail, as emphasised by a number of compelling arguments and alternative reaction schemes presented by other workers (see for example Refs. [78–85]), he will be remembered as

a giant in PSII research by his provision of a conceptional framework by which, I am sure, the 'remarkable chemistry' of water oxidation will ultimately be elucidated.

9. Addendum

As my work on the structure of PSII using electron crystallography had been superseded by the X-ray studies of Zouni et al. [7] and Kamiya and Shen [75], I decided to continue my studies of PSII by exploring the use of X-ray crystallography. In collaboration with my colleagues, Kristina Ferreira, Tina Iverson, Karim Maghlaoui and So Iwata, a complete structure of PSII from *Thermosynechococcus elongatus* has been refined to 3.5 Å resolution since writing the bulk of this paper dedicated to Jerry Babcock [86]. It is now clear from our work that D1 His190 is in hydrogen bonding distance of Y_Z and D2 His189 to Y_D, as predicted by Jerry. We have concluded that the metal cluster is a cubane-like Mn_3CaO_4 cluster bridged to a fourth Mn via a mono-μ-oxo bond. The protein ligands for three of the Mn ions within the cubane-like cluster are D1 Asp342, CP43 Glu354, D1 His332 and D1 Glu189. D1 His337 also interacts with one of the oxygens of the cubane. The fourth Mn has two protein ligands, D1 Asp170 and D1 Glu333. D1 Asp61 is close by and is probably linked to the fourth Mn ion by an intervening water molecule. The Ca^{2+} within the cubane-like structure has three μ-oxo ligands but no protein ligands, although the carboxylate of D1 Ala344 is close by. Within our structure, Ca^{2+} also has two non-protein ligands which may be provided by a bicarbonate ion. This non-protein density provides a third ligand to the fourth Mn. This putative bicarbonate may be replaced by substrate water molecules. The distances between Mn ions within the cubane-like structure is 2.7 Å, and for Mn- Ca^{2+}, the distance is 3.4 Å. These distances together with the 3.3 Å for mono-μ-oxo bridge are consistent with EXAFS data [53]. The distance between Y_Z and P_{D1} is about the same as given previously [70,75], but the distance between the metal cluster and Y_Z is about 5 Å. Indeed, the proposed water substrate molecule ligated to Ca^{2+} is almost in hydrogen bonding distance to Y_Z. However, unlike the prediction in Fig. 6, D1 Glu189 is not in H-bonding distance of D1 His190 as Jerry Babcock had hoped. Indeed, we detected no obvious proton pathway from D1 His190 to the surface. Instead, we have identified a proton/water channel leading to/from the OEC. This channel is composed of D1 Asp61, D1 Glu65, D2 Lys317 and D2 Glu312 link to the catalytic centre by water molecules and possibly D1 Glu189. D2 Glu312 is close to the lumenal surface where the opening is formed by the extrinsic loops and domains of the D1, D2 and PsbO proteins. Unless there are conformational changes in higher S-states, our 3D structure of PSII does not seem to favour the hydrogen atom abstraction model proposed by Jerry. It is likely that while electrons derived from water oxidation proceed to $P680^+$ via Y_Z, protons leave from the other side of the catalytic centre through the hydrophilic channel.

Finally, a cubane-like $Mn_3Ca^{2+}O_4$ cluster bridged to a nearby fourth Mn ion as suggested by our structural analyses is in line with mechanisms of water oxidation proposed by Siegbahn et al. [87,88], Vrettos et al. [81] and Pecoraro et al. [89]. Although different in detail, they all argue for the water oxidation chemistry to occur on a single Mn ion. During the S-state cycle, only one of the water substrate molecules binds to this Mn ion, and after protonation, its oxygen is converted to a highly reactive electrophilic intermediate, either an Mn(IV) oxyl radical [87,88] or a Mn(V) oxo [81,89]. The formation of the other O–O bond is suggested to occur by a nucleophilic attack from a second substrate water molecule ligated to Ca^{2+}. We propose that the fourth Mn ion and the adjacent Ca^{2+} bind the substrate water molecules as required by the above schemes. Although the cubane-like $Mn_3Ca^{2+}O_4$ cluster with a mono-μ-oxo bridge to a fourth Mn ion offers a robust structure for the water oxidation catalytic centre, higher resolution data is needed to confirm this organisation and to investigate any structural changes that occur in higher S-states.

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