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P680, the primary electron donor of photosystem II

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Abstract

The primary electron donor of photosystem II is a special form of chlorophyll *a* known as P680. Its detection and subsequent biophysical characterisation has relied heavily on the technique of flash photolysis of Norrish and Porter [Nature 164 (1949) 658] and on the physical principles which emerged from photochemical studies of isolated chlorophyll *a* using this technique. When oxidised the P680 radical has a midpoint redox potential estimated to be 1.17 V or more which is needed to drive the oxidising reactions of the water-splitting process. Such a high oxidising potential dictates special properties of P680 which are discussed in terms of robustness and structural organisation of photosystem II. Of particular importance has been the recent finding that P680 is not a 'special pair' of chlorophyll molecules as is the case for the primary electron donors of other types of photosynthetic reaction centres. Instead P680 is composed of a cluster of four weakly coupled monomeric chlorophylls which together with the local protein environment enables this primary donor to generate a redox potential capable of oxidising water.

Keywords: P680; Primary electron donor; Photosystem II

1. Discovery

In the late 1940s, Norrish and Porter [1] introduced the technique of flash photolysis. In doing so they opened up the study of the light-induced excited state, which enabled many new physical principles of photochemistry to be established. Witt, working at the Technische Universität in Berlin, was the first to recognise the power of the Norrish–Porter flash technique for the study of photobiology and photosynthesis in particular [2,3]. Like Norrish and Porter, Witt et al. used xenon discharge lamps to generate microsecond flashes, and as a consequence revealed a number of transient absorption changes due to light-induced redox reactions within the photosynthetic electron transfer chain [4]. However, many signals were extremely small because of the overall background absorption arising from, for example, the large number of light-harvesting chlorophyll molecules present per electron transport chain. For this reason, Rüppel et al. developed and exploited the repetitive pulse technique coupled with signal averaging [5]. In this way μs transient absorption changes as small as ΔOD of 0.001 could be recorded and analysed. When Q-switched lasers became available in the mid 1960s, the possibility arose of extending the time scale of photochemistry and photobiology into the ns region. This opportunity was quickly grasped by Porter and Topp [6] and also by Witt and coworkers [7,8].

It was against this background of the application of the Norrish and Porter technique that Witt and colleagues were the first to measure the photooxidation and re-reduction of P680. In the early 1960s it had been assumed, based on the knowledge that there were two photosystems (PSI & PSII) operating in series [9] in oxygenic photosynthetic systems, that there would be two different primary electron donors. Indeed P700, the primary chlorophyll *a* donor of photosystem I (PSI), had been discovered in 1957 by Kok [10] and studied in terms of the two-light-reaction scheme by Duysens [11]. The time course of the P700 absorption change following a single-turnover flash was determined in 1963 by Witt et al. [12] and the transient difference spectrum reported by Döring et al. [13]. Although the formation of the $P700^+$ signal was extremely fast and within the 10 ns duration of the actinic flash, its decay due to re-reduction was relatively slow, with a time constant ranging from 10 to 20 ms depending on conditions. For this reason, the P700 signal was easily observed and could be separated from the chlorophyll fluorescence signal, which decayed in the ns time domain.

Detection of the P680 signal was far more difficult since it normally has a lifetime 100 times shorter than P700. Its signal was, therefore, masked by a noise level 10 times greater than that associated with P700 oxidation. Coupled

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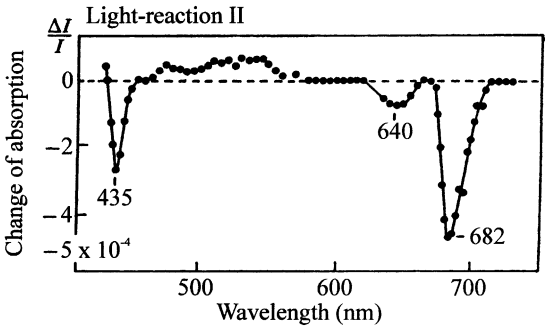

Fig. 1. The first ever transient optical difference spectrum due to P680 photoinduced oxidation measured by Döring et al. [14].

with this was the additional problem of chlorophyll fluorescence. Döring et al. overcame these problems by using the repetitive flash technique and discovered a very fast absorption signal which they attributed to P680 oxidation and re-reduction [14]. The transient difference spectrum of the P680 signal, recorded by Döring et al. [14] is shown in Fig. 1. It is characterised by absorption change maxima at about 680 and 435 nm, which contrast with those of P700 at 700 and 438 nm. Both, however, have red absorption maxima at longer wavelengths than the overall red absorption peak of the antenna chlorophylls, which are typically in the range 670–675 nm. Because of this red shift, both P680 and P700 act as exciton traps, although clearly P680 is 'shallow' compared to P700. However, to be noted is that these light-induced absorption spectra would have contained electrochromic shifts due to the electrical potential gradient generated by the charge separation across the membrane as well as the bleaching due to oxidation of the primary donors.

2. Redox properties

P680 is the primary electron donor of photosystem II (PSII). This photosystem uses photons in the visible region of the spectrum to oxidise water and reduce plastoquinone [15]. The oxidation of water is a complex and thermodynamically demanding reaction. An overall oxidising potential of almost 1 V versus standard hydrogen electrode (SHE) is required to extract electrons/protons from two molecules of water at physiological pH to generate dioxygen and four reducing equivalents. This very high oxidising potential is supplied by $P680^{\bullet+}$, the chlorophyll radical formed when light-induced primary charge separation occurs. The initial charge separation involves the reduction of a pheophytin (Pheo) molecule to form $P680^{\bullet+}Pheo^{\bullet-}$, a reaction which occurs in a few picoseconds. This is followed within 200 ps by electron transfer from $Pheo^{\bullet}$ to plastoquinone (Q_A) and thence to Q_B on a microsecond to millisecond time scale. Q_B is also a plastoquinone molecule but, unlike the Q_A, is able to accept two electrons and then two protons to form plastoquinol. In this fully reduced form, plastoquinol debinds from the Q_B site and diffuses into lipid bilayers where it is available for oxidation by the cytochrome b_6f complex. The empty Q_B site is then occupied by another plastoquinone molecule. Meanwhile, the $P680^{+}$ radical is reduced on a time scale of 10 ns by a redox-active tyrosine residue, which then oxidises a Mn ion contained within a cluster of four. Since four oxidising equivalents must be accumulated in order to oxidise two water molecules, the P680 $\rightleftarrows$ $P680^{\bullet+}$ cycle turns over four times in the course of generating one O_2 molecule. Not surprisingly the successive accumulation of oxidising equivalents within the $(Mn)_4$ cluster affects the kinetics of $P680^{\bullet+}$ reduction, which change from ns to μs with the advancement towards dioxygen formation [15].

The oxidised electron donor, $P680^{\bullet+}$, is among the strongest oxidants generated in biological systems and has a redox potential estimated to be 1.17 V versus SHE [16]. In other types of photosystems, the primary donors, such as P700 in PSI of oxygenic organisms, P870 in purple photosynthetic bacteria and P840 in green sulphur bacteria, are also chlorophyll molecules. But in all cases the redox potential of their oxidised form is 0.5 V versus SHE or less (see Fig. 2). These photosystems are therefore unable to oxidise water. With a redox potential of 1.17 V, $P680^{\bullet+}$ is potentially a very dangerous species, with the capacity to oxidise not only water, but also the very pigments and proteins that make up the PSII reaction centre. This property makes PSII unique and gives rise to a number of phenomena not found with other types of photosystems.

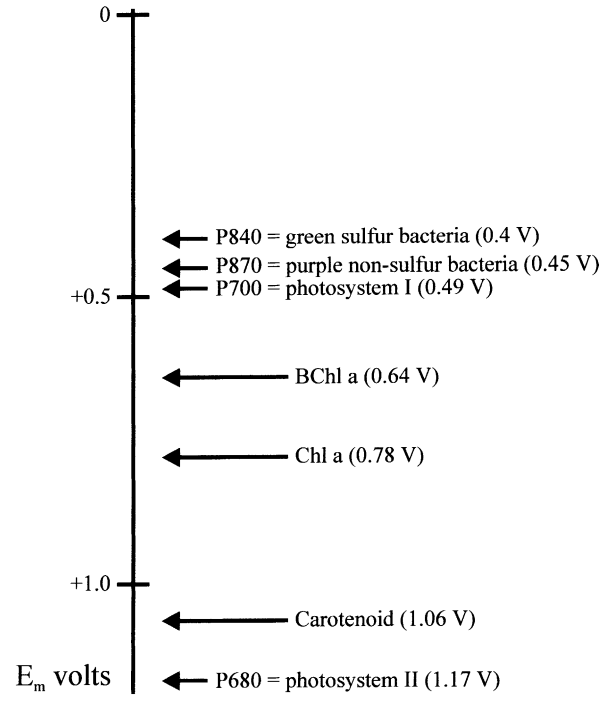

Fig. 2. Redox scale emphasising the oxidising potential of the $P680^{+}$ radical relative to that of photosynthetic pigments and the chlorophyll donors of other types of photosystems. The E_m values are on the SHE scale.

One of the important challenges of photosynthesis research is to gain an understanding of the factors that allow the chlorophyll *a* molecules that constitute P680 to have such a high redox potential and sustain this property within a delicate pigment/protein environment.

3. Comparison with other primary donors

Until recently our understanding of P680 has called heavily on the properties of the more intensely studied primary donors P870 of purple bacteria and P700 of PSI. Both spectroscopic and structural studies have indicated that P870 and P700 are composed of two closely interacting (bacterio)chlorophyll molecules, often termed a 'special pair'. It is also clear that the reaction centre of purple photosynthetic bacteria and that of PSII are very similar in many ways, so that P870 has been considered to be a model for P680 despite their very different redox potentials. The bacterial P870 is composed of two bacteriochlorophyll *a* molecules orientated perpendicular to the membrane plane with a centre-to-centre distance of 7.6 Å and an interplanar distance of 3.6 Å or less. The electronic coupling of this special pair is in the region of 1300–1900 cm^{-1} with an absorption maximum in the near infrared at 870 nm which is red-shifted from the absorption maximum of the antenna bacteriochlorophylls. This shift emphasises that P870 can be a relatively deep thermodynamic trap. In contrast, the excitonic coupling of P680 chlorophylls is 300 cm^{-1} [17] or less and thus the trap is shallow. Indeed, it is the spectral overlap between P680 and the antenna chlorophylls (the peak separation is only 200 cm^{-1} as compared to 1000 cm^{-1} in *R. sphaeroides*) which has made it difficult to perform rigorous optical analyses on this primary donor. An additional complexity is that the bacterial reaction centre contains two accessory chlorophylls, and until recently it was uncertain whether this feature was maintained within the PSII reaction centre. The accessory bacteriochlorophylls and the bacteriopheophytin acceptor within the bacterial reaction centre are optically distinct from P870, with splittings between their absorption maxima and that of P870 of about 1000 and 1500 cm^{-1}, respectively. No such spectral distinction occurs for the pigments within the PSII reaction centre although biochemical analyses indicated that there were other chlorophyll *a* present as well as those attributed to P680 [18].

Despite the differences in their optical absorption properties, P680 and P870 share a common and important feature: both form a spin-polarised triplet state at low-temperatures. Initially discovered in the purple bacterial reaction centre by Dutton et al. [19], this triplet signal was identified in P870 by EPR as a product of the recombination reaction between $P870^{\bullet+}$ and $BPheo^{\bullet-}$. This electron paramagnetic resonance (EPR) signal differs from that of a triplet formed by intersystem crossing. The spin-polarised triplet gives a complex low-temperature EPR spectrum characterised by a series of *g*-values. Rutherford [20] discovered the same spin-polarised triplet signal in PSII (see Fig. 3). By using orientated samples he showed that the *z*-peak of this triplet signal was tilted at approximately 30° to the membrane plane at low-temperatures unlike the 90° observed for the purple bacterial P870 spin-polarised triplet [21]. This orientation is reminiscent of that of the monomeric accessory bacteriochlorophyll within the purple bacterial reaction centre. The possible location of the triplet at low-temperature on a monomeric chlorophyll *a* of PSII was supported further by both EPR and angle-dependent magnetic resonance (ADMR) results [22]. At higher temperatures the triplet state is delocalised over two or more chlorophylls. An in-depth analysis of light-induced Fourier transform infra red (FTIR) difference spectra following formation of the triplet state has been conducted by Noguchi et al. [23]. From this work they suggested a model for P680 that consisted of two weakly interacting chlorophylls, one tilted at 90° and the other at 30° to the membrane plane.

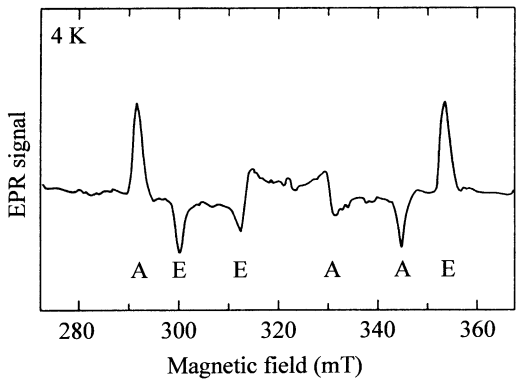

Fig. 3. Spin-polarised EPR triplet signal of P680 measured using isolated PSII reaction centres (taken from [30]).

4. Consequences of formation of the P680 triplet state

In certain organic solvents, chlorophyll readily forms a triplet state by intersystem crossing with yields approaching 70% [24]. In vivo, chlorophylls within the light-harvesting systems can also form triplets by intersystem crossing, especially when the photochemical trap is closed, for example, at high light intensities. The lifetime of the chlorophyll triplet state is typically about 1 ms but is considerably shortened if a triplet quencher, such as oxygen, is present. Oxygen normally exists in its triplet ground-state ($^3O_2$). When it quenches the chlorophyll triplet (^{3}Chl), it is converted to the excited singlet oxygen state ($^1O_2$).

$$^3\text{Chl} + {}^3O_2 \rightarrow {}^1\text{Chl} + {}^1O_2$$

Singlet oxygen is a powerful oxidant and will attack proteins and pigments in the vicinity of the site of its production, a property exploited by the technique of photodynamic therapy. To avoid this potential hazard, photosynthetic organisms

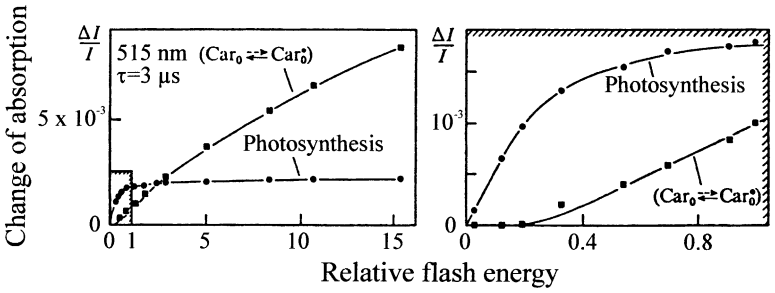

Fig. 4. Left: extent of the absorption change of a carotenoid reaction (associated with triplet state formation) and photosynthetic activity as a function of the intensity of the excitation flash. Right: enlargement of the lower flash energy region of the curve as indicated in the left-hand plot (taken from [25]).

use carotenoids to rapidly quench chlorophyll triplets and thus avoid singlet oxygen production. The triplet carotenoid ^{3}Car is harmless and decays by intersystem crossing to the ground-state singlet with evolution of heat.

$$^3\text{Chl} + {}^1\text{Car} \rightarrow {}^1\text{Chl} + {}^3\text{Car} \rightarrow {}^1\text{Chl} + {}^1\text{Car} + \text{heat}$$

This protective role of carotenoids has been termed the 'valve reaction' by Witt, who, with his colleagues, elegantly demonstrated [25] that carotenoid excited states accumulate at light intensities that saturate photosynthesis and thus maintain the reaction centre traps in a closed state (see Fig. 4). The quenching of ^{3}Chl by carotenoids occurs in all types of chlorophyll-binding light-harvesting complexes and in all reaction centres of oxygenic photosynthetic organisms except PSII. The triplet transfer process is assumed to occur by the Dexter mechanism [26] and requires the carotenoid to be located in van der Waals contact with the chlorophyll. Indeed, the high-resolution structures of the reaction centre [27] and outer light-harvesting system LH2 [28] of purple photosynthetic bacteria and the Chl *a*/*b* light-harvesting complex of PSII (LHCII) [29] have demonstrated this spatial requirement.

Despite this general property of photosynthetic systems, the triplet state of P680 is not quenched by the β-carotene bound into the PSII reaction centre [30,31]. On the other hand, the room temperature lifetime of ^{3}P680 was shortened from 1 ms in the absence of O_2 to 25 μs under aerobic conditions (see Fig. 5). With 100% O_2, the ^{3}P680 lifetime shortened further to less than 10 μs (Telfer and Barber, unpublished). These results demonstrate that the following reaction can occur within the PSII reaction centre:

$$^3\text{P680} + {}^3\text{O}_2 \rightarrow {}^1\text{P680} + {}^1\text{O}_2$$

Using isolated PSII reaction centres, the above reaction was confirmed by the detection of flash-induced singlet oxygen production from its characteristic luminescence at 1270 nm [32]. Furthermore, under the same conditions an irreversible loss of P680 absorption was observed [33] and the PSII subunits, particularly the D1 protein, became oxidised by $^1O_2$ attack of specific amino acids [34]. Fig. 6 shows a typical spectrum derived by fast atom bombardment–mass spectrometry (FAB–MS) of a trypsin-derived fragment of the D1 protein (from residue 184 to residue 199). Over and above its main mass band of 1917.6, it was found to have additional satellite bands separated by increments of 16 mass units. The insert into Fig. 6 shows a folding diagram of the D1 protein indicating the region in which these multiple oxidations were detected. Of note is that considerable oxidations were found to be clustered around the site where P680 chlorophyll is thought to be ligated. The data shown in Fig. 6 were recorded with isolated reaction centres that lack Q_A and Q_B and thus have a high probability of generating ^{3}P680. Normally, and particularly in vivo, the frequency of the recombination reaction leading to ^{3}P680 is very much reduced by electron flow through the reaction centre, which prevents the accumulation of the $P680^{\bullet+}Pheo^{\bullet}$ charge-transfer state. Nevertheless, the possibility of charge recombination to form ^{3}P680 is never eliminated despite the existence of a wide range of protective mechanisms designed to avoid over-excitation of the reaction centre. Damage is reflected by the need to replace the D1 protein regularly [35]. This damage–repair cycle is unique to PSII and typically occurs for a particular PSII reaction centre every 60 min in leaves and algae illuminated at moderate light intensities. During this typical lifetime, well over 3 million photochemical turnovers can occur in the reaction centres, leading to substantial CO_2 fixation and O_2 evolution. Thus, it is a 'repair cycle' which gives

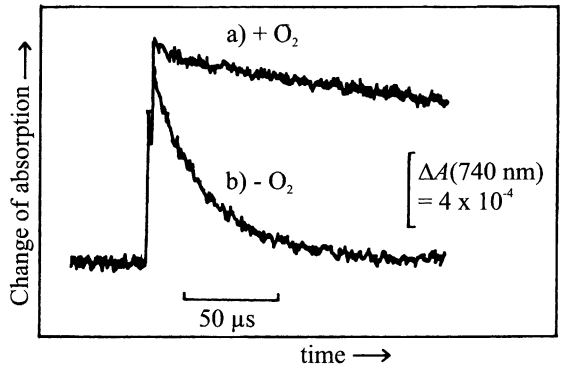

Fig. 5. Absorption transient due to the formation and decay of the flash-induced P680 triplet state measured at 740 nm in the absence and presence of atmospheric oxygen (taken from [31]).

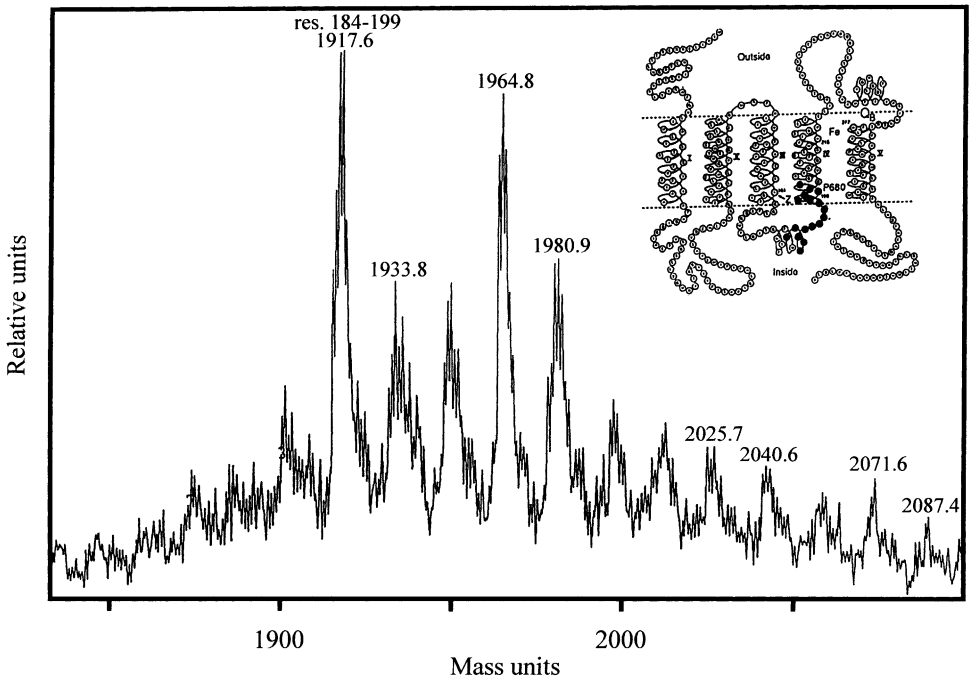

Fig. 6. A FAB–MS spectrum of a trypsin-derived fragment of the D1 protein (residues 184–199 as indicated in the insert, which is a folding diagram of the D1 protein). The mass spectrum is characterised by satellite bands that are spaced 16 mass units apart indicative of the formation of 'oxo-amino acids' (taken from [34]).

the robustness to PSII needed to overcome the consequences of conducting toxic oxidative chemistry. Perhaps the most detrimental property of PSII is the inability of β-carotene to quench the ^{3}P680. So why is it that the chlorophylls of P680 do not protect PSII from photochemical damage by using the carotenoid triplet quenching mechanism? The answer to this is obvious. If the β-carotene molecules within the PSII reaction centre were sufficiently close to P680 to facilitate triplet transfer, then they would be quickly oxidised by the $P680^+$ radical. Such a reaction would compete with the water oxidation process and generate an unstable β-carotene cation radical. Moreover, the problem cannot be overcome by transferring energy of the ^{3}P680 to a nearby chlorophyll, as happens in the purple bacterial reaction centre, because this chlorophyll would also have a high redox potential.

The special property of ^{3}P680 and its ability to generate $^1O_2$ is unique to PSII and is a consequence of the high oxidising potential of the $P680^{\bullet+}$/P680 couple. Of note is that although the β-carotene within the PSII reaction centre cannot 'defuse' the ^{3}P680 state, it does seem to act as a $^1O_2$ quencher [36]. Moreover, under some circumstances it may also act as a secondary electron donor to $P680^{\bullet+}$.

5. $P680^+$ as an oxidant

The primary function of $P680^{\bullet+}$ is to oxidise water. This it does by extracting electrons from a redox-active tyrosine, identified as residue 161 of the D1 protein. The characteristic time for oxidation of D1 Tyr161 ranges from 10 ns to several μs, depending on the oxidation state of the $(Mn)_4$ cluster and the particular stage of the water-splitting reaction. Since the electron transfer rate from $Pheo^{\bullet-}$ to Q_A is in the region of 200 ps^{-1}, the state $P680^{\bullet+}Pheo^{\bullet-}$ does not accumulate unless forward electron flow from Q_A is restricted, and even then there are dissipative pathways to reduce the probability of ^{3}P680 formation [37]. If on the other hand electron flow from H_2O is slowed or inhibited, the $P680^{\bullet+}$ state may be sufficiently long-lived to allow secondary oxidations to occur. One such reaction is the oxidation of Tyr161 of the D2 protein to create a species which does not seem to play a role in water oxidation [15]. $P680^{\bullet+}$ can also oxidise the β-carotene in the reaction centre with ms kinetics (see Fig. 7). The photooxidation of a carotenoid by PSII is unique and not observed in any other photosystem and is another clear demonstration of the high redox potential of $P680^{\bullet+}$. The photooxidation of β-carotene in PSII was first observed by Velthuys [38] and studied further by Schenck et al. [39] and Telfer et al. [36]. These early studies are now of special interest since the carotenoid may act as an 'electron-transfer wire', facilitating other secondary oxidation events within PSII [40–42]. These are the oxidation of cytochrome *b559* (cyt *b559*) and of peripheral chlorophylls known as Chl_Z/Chl_D bound into the reaction centre [43,44]. The rate of oxidation of these cofactors, when the water-splitting process is not operative, is in the ms time domain. Of importance is that, despite these slow rates, the secondary oxidations can occur at low-temperatures [45] although the two redox-active cofactors do not react in precisely the same manner to changes

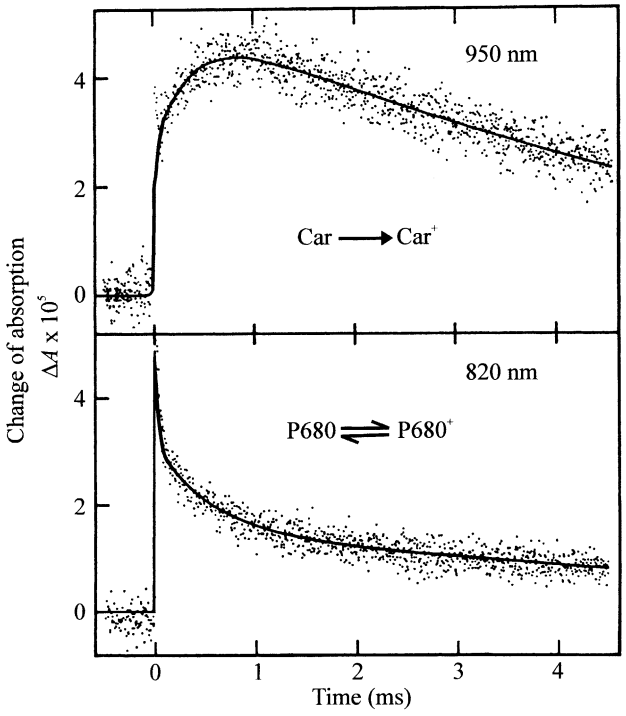

Fig. 7. Transient absorption spectra showing the oxidation of β-carotene in the PSII reaction centre by flash-induced $P680^{\bullet+}$ (taken from [36]).

in temperature [40,42]. There is considerable controversy as to whether the oxidation processes are linked linearly or involve parallel pathways to $P680^+$ [41].

6. Protein environment of P680

When the genes for the D1 and D2 proteins of PSII and for the L and M subunits of the reaction centre of purple photosynthetic bacteria were sequenced, it became very clear that there was a significant structural and functional homology between the two systems [46,47]. The determination of the structure of the bacterial reaction centre [48] allowed this homology to be exploited. In the purple bacterial reaction centre, the primary electron donor P870 was shown to be a special pair of bacteriochlorophyll molecules, each ligated to L His173 and M His200. The two bacteriochlorophyll molecules are both orientated perpendicular to the membrane with overlap at ring I. It is this arrangement that causes the strong exciton coupling and a large splitting of the far-red absorption bands. By analogy, the conserved residues D1 His198 and D2 His198 were identified as the potential ligands for a 'pair' of chlorophyll *a* molecules constituting P680. Mutations of D1 His198 do indeed shift the optical absorption spectrum of P680 [49] and reduce its potential [50]. Furthermore, D1 His198 mutations alter the quantum yield and free energy of the $P680^+Pheo^-$ radical pair [52]. Mutations of D2 His198 (197 in *Synechocystis*) gave less striking effects that probably represented subtle differences in the coordination geometry but, more importantly, indicated that the chlorophyll coordinated to this residue was not the long-lived P680 cation radical [51]. These studies, and others, indicate that there is little doubt that PSII binds two chlorophyll *a* molecules via ligands with D1 His198 and D2 His198 (197). But the weaker excitonic coupling observed for P680 as compared with P870 suggested that the chlorin rings are further apart or rotated so as to minimise the interactions between the two chlorophylls [53,54].

The bacterial reaction centre binds two further accessory BChls which are ligated to L His153 and M His180. These two histidines do not seem to be conserved in the D1/D2 proteins of PSII. However, the PSII proteins, unlike the L and M subunits, contain two other conserved histidines which are not found in the L and M subunits; namely D1 His118 and D2 His118 (117 in *Synechocystis*). Since the isolated PSII reaction centre consisting of the D1 and D2 proteins contains 6 chlorophylls, rather than 4 as in the purple reaction centre, it was speculated that these histidines bind two extra chlorophylls [55–57]. These have come to be known as Chl_Z (ligated to D1 His118) and Chl_D (ligated to D2 His118/117), and experimental evidence for their existence has been obtained from mutational studies [58,59]. By analogy with the bacterial reaction centre, D1 His118 and D2 His118/117 would be expected to be related by the same two-fold axis that relates the other cofactors, and to be located towards the lumenal side of helix B.

Mutational studies, e.g. D1 Glu130 [52], suggest that the active pheophytin acceptor of PSII is positioned and ligated in a similar way to the bacteriopheophytin bound to the L subunit in the bacterial reaction centre.

7. Direct structural information

Chemical, spectroscopic and mutational studies, coupled with analogy with the purple bacterial reaction centre, led to the conclusion that the PSII reaction centre contained a core of 6 chlorins (4 chlorophyll and 2 pheophytin) arranged around a two-fold axis in a similar arrangement to that found in the bacterial system. Indeed, molecular models have been proposed based on these properties [60,61]. As discussed above, whether the D1 His198/D2 His198 chlorophylls form a 'special pair' in a modified form remained uncertain until recent structural studies. Two-dimensional crystals of a PSII subcomplex composed of the D1 and D2 proteins, the chlorophyll-binding protein CP47 and other small proteins, including the α- and β-subunits of cyt *b559*, were analysed by electron cryomicroscopy [62,63]. This electron crystallographic study not only revealed the organisation of the transmembrane helices, but at 8 Å resolution provided densities in the three-dimensional map which could be assigned to the tetrapyrrole head groups of chlorophylls. This structural work showed that indeed the D1 and D2 proteins have five transmembrane helices, each arranged in the same way as those of the bacterial L and M subunits. Moreover, in the

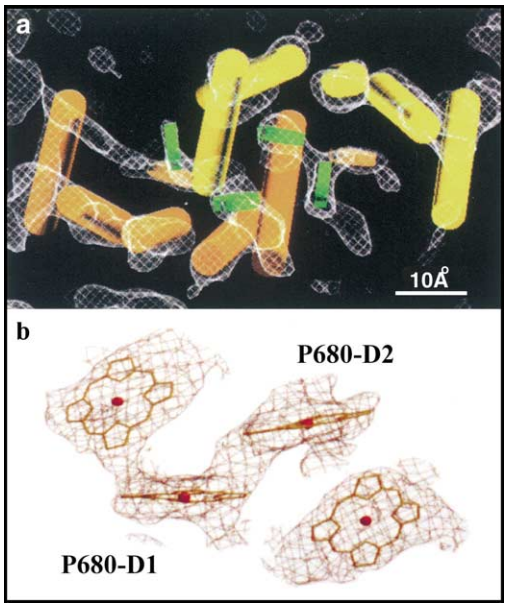

Fig. 8. (a) Positioning of the four 'core' chlorophylls (green) within the D1 (yellow) and D2 (orange) transmembrane helices showing the absence of a special pair. The data was obtained by electron crystallography and published in [63]. (b) Positioning of the four 'core' chlorophylls of P680 as determined by X-ray crystallography confirming the absence of a special pair in PSII [64].

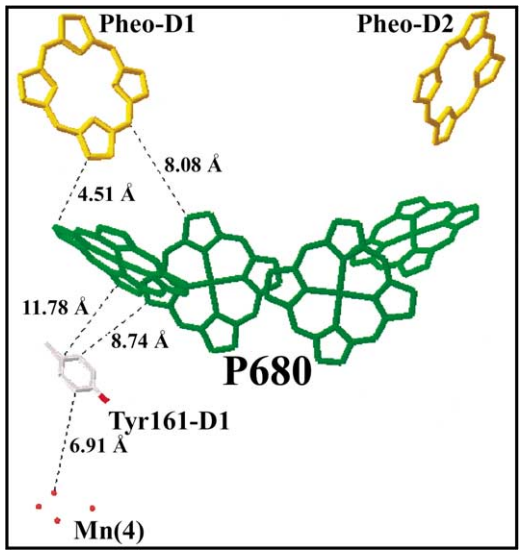

Fig. 9. Organisation of the redox centres in the core of the PSII reaction centre derived from X-ray crystallography [64] showing the edge-to-edge distances for the electron transport pathway from the Mn cluster to the Pheo-D1 electron acceptor. The figure was derived using coordinates obtained from Protein Data Bank website: http://www.resb.org./pdb/ under the PDB identifier 1FE1.

region of helices C and D of the D1 and D2 proteins, related by the two-fold axis, were densities in positions very similar to those of the bacteriochlorophyll/bacteriopheophytin of the bacterial reaction centres. However, there was no indication of a 'special pair', as can be seen in Figs. 8 and 9. Instead, the four densities attributed to chlorophyll *a* were approximately equidistant from each other, with a centre-to-centre distance of about 10–11 Å. Two further densities assigned to pheophytin were located in approximately the same position as the bacteriopheophytin in the bacterial reaction centre, in agreement with the conclusions drawn from mutational studies [52]. Because of the relatively low resolution of the map, especially in the *z*-direction, the precise orientation of the porphyrin rings could not be determined. Nevertheless, this work indicated for the first time that 'P680' was a tetramer of four chlorophylls. Confirmation of this has recently come from X-ray diffraction analysis of three-dimensional crystals of the PSII core dimer of *Synechococcus elongatus*, where the centre-to-centre distance between the four chlorophylls was shown to be about 10 Å [64]. Moreover, the X-ray crystallography showed that the orientation of the chlorophylls was similar to that of the bacteriochlorophylls within

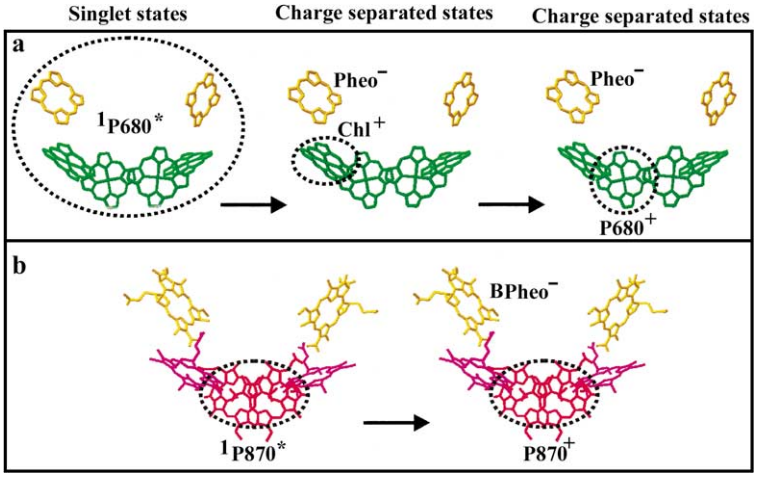

Fig. 10. Schemes for primary charge separation in PSII (Panel a) and purple bacteria (Panel b) emphasising the distinct differences between the two systems. The initial oxidant Chl^+ is thought to be the accessory chlorophyll bound to the D1-branch of the reaction centre while $P680^+$ is assumed to be the chlorophyll ligated to D1 His198.

the bacterial reaction centre. It should be noted that the separation of the D1 His198 and D2 His198 chlorophylls in PSII was also predicted from molecular modelling using the carbon backbone of the L and M subunits as a template [61]. In addition, the X-ray work identified densities assigned to Chl_Z and Chl_D associated with helix B of the D1 and D2 proteins.

8. Consequences of the structural analyses

Because of the close proximity of four 'P680' chlorophyll molecules, they must all have a high redox potential (in excess of 1 V). If this were not the case then the $P680^+$ radical would oxidise its neighbouring low potential chlorophyll molecules rather than driving the oxidation of water. Moreover, the absence of a 'special pair' means that all chlorophylls have similar absorption properties, which suggests that the excited P680* state is delocalised over all the chlorins, including the two pheophytin molecules since they have overlapping absorption spectra. This feature is the basis of the multimer model of Durrant et al. [53]. The absence of an 'exciton trap' means that charge separation may occur in principle from any one of the chlorophyll molecules forming $Chl^{\bullet+}Pheo^{\bullet-}$. Since van Mieghem et al. [21] identified the low-temperature P680 triplet state as localised on a chlorophyll orientated at 30° to the membrane plane, it seems highly likely that the initial electron donor to Pheo is an 'accessory' chlorophyll, which is orientated at this angle. This possibility has been reinforced by recent photon-echo [65] and mutational [51] studies and discussed in a recent review by Dekker and van Grondelle [54]. By analogy with the bacterial reaction centre, the 'accessory' chlorophyll donor and the pheophytin acceptor would be located on the D1 side of the reaction centre. This would mean that the oxidising equivalent or 'hole' delocalises and that, if the D1 His198 chlorophyll has a lower redox potential than the other oxidised 'accessory' chlorophyll, the oxidising equivalent would tend to be preferentially associated with this chlorophyll. This series of events is supported by the recent work of Diner et al. [51] who identified the $P680^{\bullet+}$ as being located on the D1 His198 chlorophyll which has a low-temperature, long wavelength absorption maximum at 672.5 nm. On the other hand the D1 accessory chlorophyll absorbs at 684 nm at 80 K and therefore would act as the initial trap. The concept of 'hole' migration is shown diagrammatically in Fig. 10 and contrasts with the primary charge separation process in purple bacteria, where the primary donor is the special pair. Alternatively, there remains the possibility that at room temperature the 'hole' delocalisation in PSII is spread evenly over all four chlorins and oxidation of D1 Tyr161 is a kinetically favoured electron-transfer event. The centre-to-centre (C/C) and edge-to-edge (E/E) distances between the cofactors in the reaction centre are compatible with this mechanism. According to the X-ray derived model of the PSII reaction centre [64] the distance between the D1 accessory Chl and Pheo is 10.8 Å (C/C) or 4.5 Å (E/E), which contrasts with the longer D1 His198 Chl to Pheo distances of 15.3 Å (C/C) and 8.1 Å (E/E). On the other hand, the estimated distance between D1 Tyr161 (Y_Z) and D1 His198 Chl is 8.7 Å (E/E) compared with 11.8 Å (E/E) for accessory Chl and D1 Tyr161.

9. Why does PSII have monomeric chlorophyll as a primary donor?

There are at least three possible reasons for the absence of a special pair in PSII:

1. With no special pair, there is no significant absorption shift to the red and therefore no deep exciton trap. Excitations therefore not only delocalise among the chlorophylls within the reaction centre but also are sufficiently energetic to return to the antenna system. It is for this reason that PSII has an unusually high fluorescence yield when its photochemical trap is closed. Since PSII is highly vulnerable to damage by over-excitation [35], the shallow trap allows regulatory mechanisms, such as in the xanthophyll cycle [66], to operate within the antenna to quench excess excitons.
2. The lack of a significant red shift means that the P680* is kept as strong a reductant as possible and therefore able to maximise on the energy of a red photon for bridging the large energy gap between P680 oxidation and Pheo reduction (~1.6 eV).
3. Monomeric chlorophyll is probably required to produce redox potentials in the order of 1.2 V. As long ago as 1979, Davis et al. [67] predicted from the redox and other properties of the model compound magnesium tetraphenylchlorin and on the properties of Chl *a* in solution that P680 would turn out to be a ligated Chl *a* monomer rather than a dimeric special pair. In special pairs such as P700 and P870 of PSI and purple bacteria, respectively, the interplanar distance between the two cofacial (bacterio)chlorophyll macrocycles is sufficiently small that there is significant exciton coupling. This allows the high-energy "hole" in $P700^{\bullet+}$ or $P870^{\bullet+}$ to be delocalised over two macrocycles rather than one, lowering its energy by a "particle in a box" size effect. In P680, there is no special pair so the hole in $P680^{\bullet+}$ must remain localised on a single Chl *a* molecule (even if it hops from one to the other in the course of the primary electron transfer). Several pieces of evidence suggest that the confinement of the "hole" of $P680^+$ on a Chl *a* monomer causes the midpoint redox potential E_m of the $P680^+$/P680 couple to be ~0.25–0.3 V higher than it would be if P680 were dimeric: (a) the INDO calculations of Datta et al. [68] of the E_m of various dimeric and monomeric P680 model systems indicate that the increase in E_m for monomeric rather than dimeric P680 is ~0.2–0.3 V. (b) The calculated lowering of the ionisation potential of BChl by 5.9 kcal mol^{-1} [69] and the

peak shifts in its optical spectrum [70] consequent on BChl dimer formation suggest that dimerisation lowers the redox potential of bacterial special pairs by ~0.25 V. (c) The red shift of 2200 cm^{-1} in the lowest energy absorption band of the BChl *b* special pair as compared with monomeric BChl *b* in solution [71,72] is equivalent to a lowering of the midpoint potential by 0.27 V as a consequence of dimerisation.

The monomeric nature of P680 may therefore be the single biggest factor in determining its very high oxidation potential. However, other possible influences include the local protein microenvironment (nearby amino acid residues and their charges, the nature of the axial ligation of the chlorophyllic Mg^{2+} ion and the extent of hydrogen bonding) and general dielectric effects. Those factors that destabilise the oxidised radical relative to its reduced state will raise the midpoint potential of the couple and render the radical a stronger oxidant. An upshift in redox potential is expected if the protein environment of the primary donor becomes less polar or less charged (with fewer charged peptide residues) since the positive charge on the donor is then less stabilised by solvation or electrochromic effects. This effect is probably modest: for example, the oxidation potentials of P870 in mutants of *R. sphaeroides* with less polar microenvironments are uplifted by ~30 mV compared with the wild type [73]. A positive shift in oxidation potential is also to be expected if the primary donor forms extra or unusually strong hydrogen bonds (via the acetyl oxygen atom of the macrocycle), since this interaction will be weaker for the positively charged donor than its neutral form, or if axial ligation of the central Mg^{2+} is weaker in the oxidised compared with reduced state. Indeed, site directed mutations in *R. sphaeroides* have shown this to be the case. There is also the general dielectric effect of the protein environment. The formation of small charged species is generally disfavoured in such low-dielectric media. The dielectric stabilisation of the dimer cation relative to the neutral dimer is therefore normally greater than that of the monomer cation relative to the neutral monomer, and this is a further factor that will tend to raise the oxidation potential of P680. Finally, small non-planarities or other geometric particularities in the ground-state conformation of porphyrinic macrocycles can affect E_m. The cumulative effect of these secondary factors on E_m of P680 cannot be assessed until more precise structural information about the reaction centre becomes available. At this stage the only approach is to predict the protein environment of the four chlorophylls which make up P680. The best model to date, which is consistent with the 3.8 Å X-ray structure [64], is that of Svensson et al. [61]. It predicted that the two Chl *a* ligated to His198 of D1 and D2 would be separated by about 10 Å centre-to-centre and that there would be new specific protein–chromophore interactions. Indeed, the model predicted H-binding between D1 Thr286 and the carbomethoxy group of ring V of D1 Chl198 and D2 Thr283 with the corresponding group of ring V of D2 Chl198. In the case of the other two chlorophylls the situation is more complicated since the equivalent bacterial ligating histidines are missing. The protein backbone could contribute the ligands and there may be hydrogen bonds to the main chain oxygen atom of Met199 (which is observed in D1 and D2) to the keto oxygen of ring V of the chlorophylls.

The importance of H-bonding in determining the redox potential of the primary donor has been investigated in some depth in the reaction centre of the purple bacterium *R. sphaeroides*. Allen and coworkers have systematically introduced hydrogen bonds to the conjugated carbonyl groups of the special pair and shifted the midpoint potential from 0.5 to 0.8 V or more [74,75]. The potential generated in the most oxidising mutant was sufficient to oxidise a Tyr engineered into the structure close to the special pair [76]. This impressive work gives a hint of how substantial redox shifts can be imposed on a primary donor by the local protein environment of the porphyrins even when they exist as a 'special pair'. In the case of P680 chlorophylls it seems likely that a combination of its monomeric state and the interaction of Chls with their protein environment give rise to their high oxidising potential.

10. Conclusions

Spectroscopic and structural studies have shown that P680 is not a special pair. Rather it is composed of four weakly interacting high-potential chlorophylls. This suggests that the mechanism of primary charge separation within PSII is very different to that in other types of reaction centre, which contain a red-shifted trap due to electron delocalisation within the dimer. The lack of such a trap in the PSII reaction centre means that primary charge transfer could occur from the chlorophyll closest to the Pheo acceptor and that the primary oxidising equivalent either migrates to, or delocalises among, the other chlorophylls within the reaction centre. This series of events seems to be aided by differences in the energetics of the singlet excited states of the chlorophylls. However, as in the case of the purple bacterial reaction centre, and perhaps PSI, there remains the preferred directionality of the charge separation along the 'active' arm. Why this arm is preferred is not yet known, but hopefully this will become more apparent with improved spectroscopic analyses of the reaction centre chlorophylls and the elucidation of a high-resolution structure sufficient to reveal the exact location of the four chlorophylls within their individual protein environments. This structural information will provide the details needed to understand how redox potentials of 1 V or more can be generated by the oxidation of the P680 chlorophylls.

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