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Photosystem II: evolutionary perspectives

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Based on the current model of its structure and function, photosystem II (PSII) seems to have evolved from an ancestor that was homodimeric in terms of its protein core and contained a special pair of chlorophylls as the photo-oxidizable cofactor. It is proposed that the key event in the evolution of PSII was a mutation that resulted in the separation of the two pigments that made up the special chlorophyll pair, making them into two chlorophylls that were neither special nor paired. These ordinary chlorophylls, along with the two adjacent monomeric chlorophylls, were very oxidizing: a property proposed to be intrinsic to monomeric chlorophylls in the environment provided by reaction centre (RC) proteins. It seems likely that other (mainly electrostatic) changes in the environments of the pigments probably tuned their redox potentials further but these changes would have been minor compared with the redox jump imposed by splitting of the special pair. This sudden increase in redox potential allowed the development of oxygen evolution. The highly oxidizing homodimeric RC would probably have been not only inefficient in terms of photochemistry and charge storage but also wasteful in terms of protein or pigments undergoing damage due to the oxidative chemistry. These problems would have constituted selective pressures in favour of the lop-sided, heterodimeric system that exists as PSII today, in which the highly oxidized species are limited to only one side of the heterodimer: the sacrificial, rapidly turned-over D1 protein. It is also suggested that one reason for maintaining an oxidizable tyrosine, TyrD, on the D2 side of the RC, is that the proton associated with its tyrosyl radical, has an electrostatic role in confining P^+ to the expendable D1 side.

Keywords: photosynthetic reaction centre; chlorophyll; redox potential; P_{680}; evolution

1. INTRODUCTION

In a previous publication Rutherford & Nitschke (1996) discussed the evolution of PSII, making several suggestions that were relevant to models of the structure and function of PSII existing at that time. In the current paper, some of the evolutionary ideas put forward at that time are reiterated and discussed in the context of recent advances in PSII research. This allows further suggestions to be made that are relevant not only to the evolution of PSII but also to the present understanding of the enzyme.

The PSII RC is an enzyme that is able to use visible light to drive a reaction in which electrons are taken from water on one side of the membrane and put onto plastoquinone on the other side of the membrane. The current state of knowledge of this enzyme has been extensively reviewed recently (e.g. Debus 2001; Peloquin & Britt 2001; Robblee *et al.* 2001; Rutherford & Krieger-Liszkay 2001; Diner & Rappaport 2002; Rutherford & Faller 2001; Goussias *et al.* 2002 and other articles in the same volume). While the photochemistry and the water chemistry seem to occur in the same central subunits of the complex, it is useful to think of PSII as being made up of two parts. These are a photochemical part, in which the ultrarapid and highly efficient charge separation and stabilization processes take place, and a catalytic part, made up of the charge accumulation device and the site of water oxidation.

The model of the photochemical part, in terms of the layout and function of cofactors, was put forward in the early- to mid-1980s (reviewed in Rutherford 1987, 1989; Michel & Deisenhofer 1988) and since then, although the quality of the evidence has improved and many details have accumulated, the basic model has not changed. This model was based on spectroscopy and comparisons with the better characterized B-RC. Later amino-acid sequence analysis and improvements in the biochemistry allowed a specific folding model for the heart of the RC, again based on comparisons with the B-RC and also on inhibitor-resistant mutant studies around the Q_B site (Trebst 1986; Barber & Marder 1986; Michel & Deisenhofer 1988). The idea that D1 and D2 made up the RC was verified through its biochemical isolation as a D1–D2–cytb_{599} particle still capable of charge separation (Nanba & Satoh 1987), while the folding model was verified through site-directed mutagenesis (Debus *et al.* 1988; Vermaas *et al.* 1988). Computer methods provided 3D versions of the folding model and allowed further insights (e.g. Ruffle *et al.* 1992: Svensson *et al.* 1996) and recently the model was verified and extended through crystallographic methods, firstly using electron diffraction (Rhee *et al.* 1998) and recently with improved resolution using X-ray diffraction (Zouni *et al.* 2001; see also Kamiya & Shen 2002). Throughout

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this time the predictions based on the original, comparative work were validated by many different lines of research but particularly by spectroscopy, which provided physical measurements of the distances between the cofactors, their geometries and in some cases their interactions with the nearby amino acids (see Van Mieghem *et al.* 1991; Koulougliotis *et al.* 1995; Hienerwadel & Berthomieu 1995; Zech *et al.* 1997; Deligiannakis *et al.* 1999; Dorlet *et al.* 2000; Diner *et al.* 2001; Kawamori *et al.* 2002). As far as the cofactors and their environments are concerned, it is still the case at the time of writing that the spectroscopic model of the cofactors and their environment has yet to be overtaken by that from crystallographic approaches.

The water-oxidizing activity is unique to PSII and thus the comparative approach has had a more limited contribution to understanding the catalytic part. Spectroscopic studies (Peloquin & Britt 2001; Robblee *et al.* 2001) have provided models that are consistent with what is, at present, relatively poorly resolved structural information obtained from X-ray crystallography (Zouni *et al.* 2001; Carrell *et al.* 2001; Rutherford & Faller 2001).

2. THE HETERODIMERIC CORE OF THE REACTION CENTRE: EVOLUTIONARY IMPLICATIONS

The structure of the heart of PSII can be summarized as being just like a B-RC but with a Mn cluster attached to the inner surface. The central structural motif of both RCs is a pseudo-symmetrical heterodimeric protein core. This feature was a surprise when it was discovered in the B-RC by X-ray crystallography (Michel & Deisenhofer 1988). This structure indicated that the RC had evolved from a homodimeric ancestor. Here, we consider the likely properties of this homodimeric RC (see also Rutherford & Nitschke 1996).

Let us assume that the homodimeric RC core was capable of reducing the quinone pool in the membrane. In a homodimer, electron transfer would occur up both sides of the RC. The quinones would have properties that would be a hybrid of those of the quinones (Q_A and Q_B) in existing PSII: i.e.

(i) the two quinones were Q_A-like in that they could accept electrons efficiently from an adjacent $Pheo^-$
(ii) they were both Q_B-like in that they were able to hold on to that electron (as a semiquinone) while waiting for the second electron to arrive, although it seems likely that charge recombination with P^+ (where P^+ is the cation form of the photoreactive pigment, also called P^+_{680}) would take place at rates similar to that occurring from Q_A^- rather than Q_B^-; and
(iii) like Q_A^-, they would be able to transfer an electron from one semiquinone to another to produce a fully reduced and protonated quinol, ready to exchange with a quinone from the pool (as per Q_BH_2).

This mode of function would have been lost when gene duplication and diversity led to the current heterodimeric RCs.

What were the pressures that favoured the heterodimeric RC over its homodimeric predecessor? The most obvious is inefficiency of light use.

(i) As mentioned in the previous paragraph, it seems likely that P^+Q^- charge recombination would occur as if from $P^+Q_A^-$, being less stable than the $P^+Q_B^-$ radical pair in the heterodimeric system.
(ii) Although the first photochemical reaction in a dark-adapted homodimeric RC would be as efficient as in the heterodimeric system, the subsequent photochemical reaction would have a 50% chance of occurring (forming a P^+Ph^- radical pair) on a side of the RC in which the quinone is already a semiquinone, Q^-. It seems fairly likely that forward electron transfer from Ph^- to Q^- would be inefficient. There are no examples of this kind of reaction occurring in existing RCs except at extremely low quantum yield (see Van Mieghem *et al.* 1989). This is ascribed, at least in part, to the protonation reactions associated with the further reduction of the semiquinone, which render the electron transfer reaction extremely slow. The most likely outcome of charge separation is that charge recombination from the P^+Ph^- radical pair would be enhanced and thus the energy would be lost.
(iii) Should the charge separation occur on the side with the unreduced quinone (a 50% chance or possibly greater if the existing semiquinone can act electrostatically to impede charge separation on its side), then the second quinone will be reduced, resulting in the RC containing two semiquinones.

After the formation of quinol, QH_2 (after undergoing Q^- to Q^- electron transfer (i.e. dismutation) and the associated protonation steps), it would leave the site (as per Q_BH_2). This would result in a period in which that quinone site was vacant. During this time any charge separation has a 50% chance of occurring on the side of the RC with an empty site, resulting in the recombination of the P^+Ph^- radical pair and hence the loss of the energy. Quinone exchange in a homodimer RC thus represents another major inefficiency.

It is clear then that in a RC in which the function was to perform lateral electron transfer to the quinone pool in the membrane, whenever light was limiting the homodimeric RC would have been inefficient compared with the present-day heterodimeric RCs. This inefficiency would have constituted a strong pressure favouring the evolution of a more efficient heterodimeric RC featuring (i) specialized Q_A and Q_B quinones, and (ii) charge separation on only one side of the RC (see Rutherford & Nitschke 1996).

Despite the similarities between PSII and the B-RCs in terms of the structure and function of the quinones, sequence comparisons have argued that the last common ancestor between these two types of RC was a homodimer, i.e. that the heterodimer and the Q_A and Q_B functions have evolved twice (Beanland 1990; see also Blankenship 1992 and Rutherford & Nitschke 1996). This is not too difficult to accept given the nature of quinone chemistry, the similar starting points and the similar pressures favouring the heterodimer. However, it does leave open the possibility that the uniquely oxidizing chemistry of PSII provided additional evolutionary pressures which had a role or even a key role in favouring the evolution of the heterodimer RC (see § 3).

3. A COMMON ANCESTOR FOR ALL REACTION CENTRES: REPERCUSSIONS FOR PSII STRUCTURE AND EVOLUTION

The RCIIs (i.e. those from PSII, purple bacterial and *Chloroflexus*) also seem to share a common ancestor with RCIs (those from PSI, heliobacteria and green sulphur bacteria). This was suggested based mainly on comparative spectroscopy of the cofactors (Nitschke & Rutherford 1991). The PSII RC has two symmetrical six membrane-spanning α-helical proteins associated with the RC core (the 43 kDa and 47 kDa proteins). These are related to the core antenna part of the RCI (Vermaas 1994; Rutherford & Nitschke 1996). So when the PSI crystal structure appeared, the organization of the peripheral antenna part could be used to predict structural features of the PSII RC with regard to the location of the 43 and 47 kDa polypeptides (Krauss *et al.* 1996; Schubert *et al.* 1998; Barber *et al.* 1999). A common ancestor for all RCs was suggested that was homodimeric both in terms of the core of the RC and the peripheral antenna (Vermaas 1994; Rutherford & Nitschke 1996; Krauss *et al.* 1996; Schubert *et al.* 1998). Two types of models have been discussed:

(i) those in which the more primitive system is seen as being the large RCI type and subsequent gene fission giving rise to RCIIs; and
(ii) those in which the small RCII is seen as the more primitive (because they are small) with the later fusion of the core genes with the peripheral antenna to give RCIs (see also Xiong *et al.* (1998) and Baymann *et al.* (2001) for a discussion of the potential evolutionary pathways).

Both of these models were discussed early on (Rutherford & Nitschke 1996) with the first model being favoured by certain authors (e.g. Rutherford & Nitschke 1996; Baymann *et al.* 2001) whereas others favoured the second view (Blankenship 1992; Nitschke *et al.* 1998).

One reason for favouring the 'RCI first' model is that the homodimer is the most primitive system and homodimeric RCIs still exist in the green sulphur bacteria and the heliobacteria (see Baymann *et al.* 2001). Indeed, PSI is a pseudo-homodimer in which charge separation seems to takes place on both sides of the RC (Gueros-Kuras *et al.* 2001). In addition, there are good phylogenetic arguments in favour of RCIIs evolving from RCIs based on sequence comparisons in the light of improved structural models (Baymann *et al.* 2001). Although option (ii) (i.e. RCII first) is not ruled out, for reasons of space (and personal prejudice), only option (i), the 'RCI first' model, will be dealt with here.

This model sees a homodimeric RCI, made up of two large, 11 trans-membrane α-helices subunits, undergoing a gene fission to form a tetrameric RCII, made up of two identical 5-helix subunits plus two identical 6-helix peripheral antenna proteins. The potential evolutionary pressures that would favour the proposed gene fission are not obvious. Nitschke & Rutherford (1991) suggested that the linked peripheral antennas (RCI) might hamper quinone exchange and this could represent a reason for gene fission resulting in the smaller RCII. The recent structural data (Zouni *et al.* 2001) indicate that this argument is not justified. The structural data do provide an alternative, though not terribly compelling, argument. Comparing PSII (Zouni *et al.* 2001) with PSI (Jordan *et al.* 2001), it seems that the gene fission has led to the loss of many chlorophylls, those located at the interfaces between the RC helices and the peripheral antenna helices. The less-dense packing of chlorophylls around the core could allow a specific excitation input route (or routes) that might be better regulated. Control of excitation, or perhaps just the economy associated with a decrease in the number of chlorophylls, could have favoured the split-gene RC.

There is a second argument that is ostensibly more convincing. The highly oxidizing chemistry associated with PSII damages the protein and this leads to a very fast turnover of the protein in which these reactions occur. Clearly, the fast turnover of a small, 5-helix, four pigment protein (i.e. a RCII subunit) is much more economical than that of a big, 11-helix protein containing anything from 20 to 40 pigments (i.e. a RCI subunit). This would appear to constitute a strong pressure to split the RC1 gene. Unfortunately, at present we do not favour this argument because it implies that the RCII was highly oxidizing before gene fission and this does not fit with the expectation that the B-RC (which also has the 5-alpha helix motif for its RC subunits), branched off from the evolutionary tree before development of the high oxidizing power (see § 4).

Another possible argument for evolutionary pressure on gene fission comes from the recent finding of Bibby *et al.* (2001) that under certain conditions some cyanobacteria have an antenna system that is made up multiple copies of a protein that is closely related to the 6-α-helix 43 kDa protein. In the model of RC evolution, which sees the fat RCI system as the common ancestor, the development of this antenna system was only possible after gene fission and the utility of this flexible antenna system could have constituted a selection pressure towards gene fission.

4. COMPARISONS BETWEEN PSII AND THE OTHER REACTION CENTRES: THE DIFFERENCES

The comparative approach has not only been concerned with confirming the predicted similarities between PSII and the B-RC. Indeed, the main interest in the comparative work has been focused on the differences because these are likely to be responsible for the different functions of the two systems. In a previous review on the evolution of PSII (Rutherford & Nitschke 1996), these differences were classified into several categories.

(i) Those directly associated with the special function of PSII: its uniquely high oxidizing power (P^+) and the components of the oxygen evolving enzyme (tyrosine Z, the Mn cluster and probably the Ca^{2+} ion that is essential for function) (reviewed in, for example, Peloquin & Britt 2001; Robblee *et al.* 2001; Diner & Rappaport 2002; Goussias *et al.* 2002).
(ii) Differences associated with the process by which the Mn cluster is assembled: so-called photoactivation (e.g. Ananyev *et al.* 2001).
(iii) Protection and regulation mechanisms required as a direct result of the first two points (e.g. Stewart &

Brudvig 1998; Rutherford & Krieger-Liszkay 2001; Telfer 2002).

(iv) Regulation mechanisms due to the fact that PSII is at the start of a linear electron transfer chain.

Although the study and understanding of the all of these classes of differences is required, not just for their intrinsic interest but also to avoid confusing the features of the various classes, this paper will focus only on the first class of differences: i.e. the heart of the matter.

(a) *Structure of P*

There is no special pair of chlorophylls in PSII. All other RCs have a so-called special pair of pigments as the photooxidizable pigment system but PSII does not. This has been clear for many years (Tetenkin *et al.* 1989; Braun *et al.* 1990; Hillmann *et al.* 1995; see also Dekker & Van Grondelle (2000) for a review) and was suggested even earlier than that (Davis *et al.* 1979; Rutherford *et al.* 1981). It was proposed that this was the fundamental difference between PSII and the other RCs and that the evolutionary step from the special pair to the ordinary monomer was the key event in the evolution of PSII (Rutherford & Nitschke 1996). When this was put forward several ambiguities remained over the actual organization of the pigments in the RC; however, the favoured model at that time (see e.g. Van Mieghem *et al.* 1991; Svensson *et al.* 1996) was the same as the current model. Crystallographic studies have now shown unambiguously that the major difference in the pigment organization in PSII compared with all the other types of RCs is the greater spatial separation between the two central chlorophylls in PSII (Rhee *et al.* 1998; Zouni *et al.* 2001). This point is elaborated below.

(b) *Charge separation*

The charge separation process in PSII (see figure 1) differs from that in the bacterial RC. This reflects key structural differences that are directly related to the capacity of PSII to generate a strong oxidant. The major difference is that charge separation takes place from the chlorophyll adjacent to the Ph, i.e. the $B_{D1}^{+}Ph^{-}$ is formed. This was first put forward in 1988 (Rutherford 1988) on the basis of the location of the RC chlorophyll triplet state and this was discussed in more detail in later papers (e.g. Van Mieghem *et al.* 1991; Rutherford & Nitschke 1996). This model has recently gained much stronger experimental support (Dekker & Van Grondelle 2000; Prokhorenko & Holzwarth 2000; Diner *et al.* 2001; Diner & Rappaport 2002; recent review: Barber & Archer 2001). The current view is that several different charge separation reactions can take place in a population of PSII RCs but that the dominant reaction is $B_{D1}^{+}Ph^{-}$ formation. Subsequent electron transfer from the nearest chlorophyll results in the cation being localized on the chlorophyll closest to the Tyr Z, the so-called P_{D1} chlorophyll (Diner *et al.* 2001). Earlier, the possibility was kept open that electron transfer could occur to the first Chl cation (B_{D1}^{+}) directly from the TyrZ (Rutherford & Nitschke 1996) but this option does not fit with recent spectroscopic evidence (Diner *et al.* 2001). A comparison of these reactions with those occurring in the bacterial RC (Woodbury & Allen 1995) shows that the first two reactions occur in inverted order. This change in roles, in which the chlorophyll that is the homologue of the primary electron acceptor in the bacterial RC acts as the primary electron donor in PSII, was seen as the key event in PSII evolution (Rutherford & Nitschke 1996).

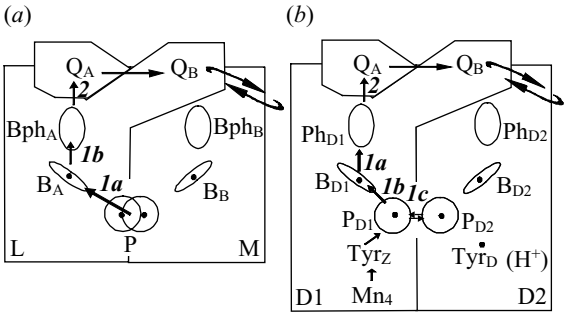

Figure 1. Comparison of the schematic presentations of (*a*) B-RC with (*b*) PSII. Only the core of the two RCs are shown, which are made up of two heterodimeric proteins called L and M or D1 and D2, respectively. These cores contain all the important cofactors involved in the photochemistry. For the B-RC the cofactors are as follows: four bacteriochlorophylls P_A, P_B, B_A and B_B (where the P_A and P_B make up the dimeric special pair), two bacteriopheophytin Bph_A and Bph_B and the two quinones Q_A and Q_B. In PSII the cofactors are as follows: four monomeric chlorophylls P_{D1}, P_{D2}, B_{D1} and B_{D2}, two pheophytins Ph_{D1} and Ph_{D2} and the two quinones Q_A and Q_B. In addition to these cofactors, PSII contains also the two redox-active tyrosines TyrZ and TyrD and the manganese cluster (Mn_4). The H^+ next to TyrD indicates the proposition that upon TyrD oxidation its phenolic proton is trapped on a nearby base (B). This accumulation of a charge near TyrD has an electrostatic effect on P_{D2} and influences the localization of P^+ (see § 4d). The arrows indicate the pathway of the individual electron transfer reactions.

(c) *P_{680}, P_{680}, what makes your cation so hot?*

The central question has remained unanswered concerning what exactly has occurred in PSII to make the P cation so oxidizing. A range of suggestions has been made and some of these suggestions are briefly summarized here as follows.

(i) Chlorophyll is intrinsically more difficult to oxidize than bacteriochlorophyll and being at a shorter wavelength has more energy available in its excited state (see Blankenship 2002). There must, however, be more to it than that since a special pair of chlorophylls exist as P_{700} in PSI and P_{700}^{+} is no more oxidizing than the special pair in bacterial RCs (Brettel 1997; Jordan *et al.* 2001).

(ii) The electrostatic environment around the chlorophyll could be very positive due to charged amino acids (see Mulkidjanian 1999), the presence of uncompensated metal ions, or to H-bonds to the pigment (Kalman *et al.* 1999); thus making the cation very high potential. Evidence for such a effects have been reported using Fourier transform infrared (Noguchi *et al.* 1993) and solid state nuclear magnetic resonance (Matysik *et al.* 2000). In the latter work, it was suggested that a positive charge next to

the C=O of ring E (i.e. hydrogen bond(s), metal or even protonation) resulted in specific localization of the charge over a restricted part of the chlorophyll molecule making the Chl more oxidizing (Matysik *et al.* 2000).

(iii) The influence of the dielectric of the protein is often discussed as an influence on the potential of the cofactors. However, this is constituted of the multiple specific molecular interactions between the protein and the pigment (H-bonds, aromatic overlap, ligands and local charges). The non-specific dielectric of the protein remains relevant, however, in terms of the mediation of additional electrostatic effects from relatively distant charges. However, in this regard, there is no reason to think that this is especially different in PSII compared to any other RC.

(iv) Ring torsions are known to affect redox properties of chlorin molecules. According to theoretical predictions a perfectly flat chlorophyll will give rise to the most oxidizing cation (see Fajer 2000). (The same line of argument leads us to predict that ChlZ, the PSII chlorophyll monomer that acts as a side-path electron donor at low temperature and that appears to have a relatively low potential, is likely to have a distorted tetrapyrrol ring.)

(v) As mentioned in § 4a, the pigments are monomers in PSII, not a special pair. A special chlorophyll pair is considered to be more easily oxidized (and thus a lower potential) because the electron hole can be shared out over two molecules instead of one. Thus, monomers are intrinsically more oxidizing.

These factors have been the subject of a good deal of consideration and specific suggestions have been made. Before we address a specific model another factor should be addressed which is important in our thinking. There are six pigments, all in rather close proximity, within the PSII RC core (four chlorophylls and two pheophytins): all of these species need to be at a very high potential. If any one of these species were less oxidizing than the P cation then that species would be oxidized and the oxidizing potential diminished. So specific models in which one or two chlorophylls are tuned to unusually high potentials, due to a special local environment, are rather unattractive. To maintain the central motif of pigments, as seen in the other RCs, as strongly indicated not least from evolutionary arguments, it was necessary to propose that all of the central pigments were at a high potential (Rutherford & Nitschke 1996; see also Durrant *et al.* 1995). How this was achieved was not directly addressed, mainly because of the existing structural and mechanistic ambiguities. The removal of such ambiguities in recent years (Rhee *et al.* 1998; Zouni *et al.* 2001; Diner *et al.* 2001) allows solutions to be proposed.

The solution to the problem may be simple: that monomeric chlorophylls (or bacteriochlorophylls) in the dielectric environment provided by a RC protein are intrinsically extremely oxidizing. There is good reason to think that this is the case. First, in B-RCs elegant studies have been done in which the bacteriochlorophyll special pair has been engineered, through changes in its electrostatic environment (mainly through H-bonds), to increase its potential to more than 940 mV; high enough to oxidize tyrosine (Kalman *et al.* 1999). In this RC there is no evidence that the so-called B_L and B_M monomeric bacteriochlorophylls are oxidized by the adjacent, souped-up special pair (see Kalman *et al.* 1999 and references therein), thus the monomeric bacteriochlorophylls must be at a significantly higher potential. Second, electrochemical redox titrations of the monomeric bacteriochlorophylls in isolated RCs gave E_m values (*ca.* 800 mV) that are higher than those obtained for isolated chlorophyll even in low dielectric solvents and much higher than those obtained in other solvents (Kropacheva & Hoff 1998). Third, electrochemical redox titrations of PSII show very little chlorophyll oxidation until very high potentials are applied (R. Edge, E. Anxolabehere-Mallart and A. W. Rutherford, unpublished data).

Given this solution, it seems possible that the oxidizing power of PSII was generated by a mutation (or mutations) that simply split the ancestral special chlorophyll pair. The resulting RC contained only a set of ordinary chlorophylls that were neither special nor paired but this was the main feature that was required because ordinary monomers are intrinsically very oxidizing. Some or all of the other factors influencing the redox potential listed above probably do exist in PSII (Noguchi *et al.* 1993, see Mulkidjanian 1999 for a specific electrostatic model), but only to tune up the system, not only in terms of gaining the additional oxidizing power for optimum function but also in terms of the relative potentials of the chlorophylls to allow the appropriate localization of the cation. It thus seems that PSII could have evolved its very high redox potential by a single event and not by the gradual step-wise process that has been put forward in other evolutionary theories (e.g. Olson & Pierson 1987; Blankenship & Hartman 1998).

The sudden jump theory, with the key being the transition to monomeric chlorophylls, was suggested earlier in a less specific form (Rutherford & Nitschke 1996). The elegant work in bacterial RCs, with the incremental, H-bond by H-bond, increase of the potential of the bacteriochlorophyll special pair to obtain oxidizing power sufficient to oxidize tyrosine (Kalman *et al.* 1999), may not mimic steps that actually occurred in evolution. In fact, another engineering strategy, namely the splitting of the special pair, may be a better way to generate oxidizing power in a single step.

(d) *The oxidizable tyrosines, manganese and water oxidation*

Rutherford & Nitschke (1996) pointed out that the presence of the oxidizable tyrosines in symmetrical positions on D1 and D2 in PSII indicated that an ancestral homodimer existed that was able to oxidize tyrosine on both sides of the RC. It was suggested that it may have been possible that Mn oxidation could have existed, perhaps even doing primitive water oxidation, on both sides of the RC. This hypothetical protein, although somewhat outlandish, is, however, worthy of consideration because its properties may allow us to understand features of the current PSII.

Our first reflection concerning a homodimeric oxygen-evolving PSII-type RC is that the two charge accumulation systems would compete against each other, resulting in inefficiencies in light-limiting conditions. This then

would constitute an additional pressure favouring a heterodimer. Second, as mentioned above, PSII has adopted an unusual strategy to deal with the apparently inevitable protein damage associated with the highly oxidizing chemistry occurring in PSII: it simply throws away the damaged protein (D1) and plugs in another. In the hypothetical oxygen-evolving homodimer, it seems clear that fast turnover of both sides of the RC would be highly inefficient. Therefore for reasons of economy, a strong pressure would favour the move to the heterodimeric RC with the water oxidizing reactions, and consequently the protein damage, limited to one side.

The idea that Tyr D evolved from a RC in which it played a TyrZ-like role as a rapid electron donor has recently obtained support from the demonstration that under some circumstances it can donate electrons to P^+ in the sub-microsecond time-scale: tens of thousands times faster than was previously thought (Faller *et al.* 2001). It may be both possible and interesting to engineer the D2 side of the RC to turn on TyrD as a rapid electron donor under conditions of enzyme function. Taking this idea further, it may be possible to find evidence for a residual metal- (Mn?) binding site on D2 and if so, a reverse evolution genetic engineering project could be attempted to resurrect the putative metal binding site to determine what the minimal requirements are for water oxidation.

A question that is often asked is why should Tyr D have been maintained if it plays no role in electron transfer. There are two proposals in the literature for the role of TyrD. The first was triggered by the demonstration that Tyr D can oxidize the Mn cluster in the S0 state up to the S1 valence (Styring & Rutherford 1987). It was thus suggested that it can play a role in oxidizing the low valence states of Mn even in the dark and this may be important for the stability of the cluster in the dark and during photoactivation (Stryring & Rutherford 1987; Vermaas *et al.* 1988). A second role is that, by maintaining its proton close by upon oxidation, it can have an electrostatic effect on the potential and more importantly the location of the chlorophyll cation, P^+ (Boerner *et al.* 1993; Nugent *et al.* 1994; Faller *et al.* 2001; Ananyev *et al.* 2002). It has recently been shown experimentally that the absence of TyrD does indeed affect photoactivation (and indeed the yield of oxygen evolution) (Ananyev *et al.* 2002). This indirect electrostatic effect of $TyrD^{\bullet}$ could restrict the chlorophyll cation to the D1 side chlorophyll and this could be important in terms of D1 turnover, making sure that the most oxidizing species (and hence oxidizing damage) is limited to D1. It would be interesting to test if the D2 protein was more susceptible to photodamage in TyrD-less mutants as predicted by the present suggestion.

This role for TyrD, as a redox device for shifting the pK_a of an amino acid (itself or more likely a neighbouring group) in order to generate an electrostatic influence on the adjacent chlorophylls, seems a rather complex way of generating a banal electrostatic effect. This could be done in principle by the presence of a positively charged amino acid through a mutation. However, the use of TyrD in this way makes good sense when it is considered that (i) the region close to the chlorophylls is hydrophobic and it is not trivial to maintain a charged amino acid in this environment, and (ii) such a charged amino acid could greatly perturb the assembly of the membrane protein. The TyrD redox-mediated charge group is only generated when it is needed, i.e. after the protein is assembled and when PSII is active.

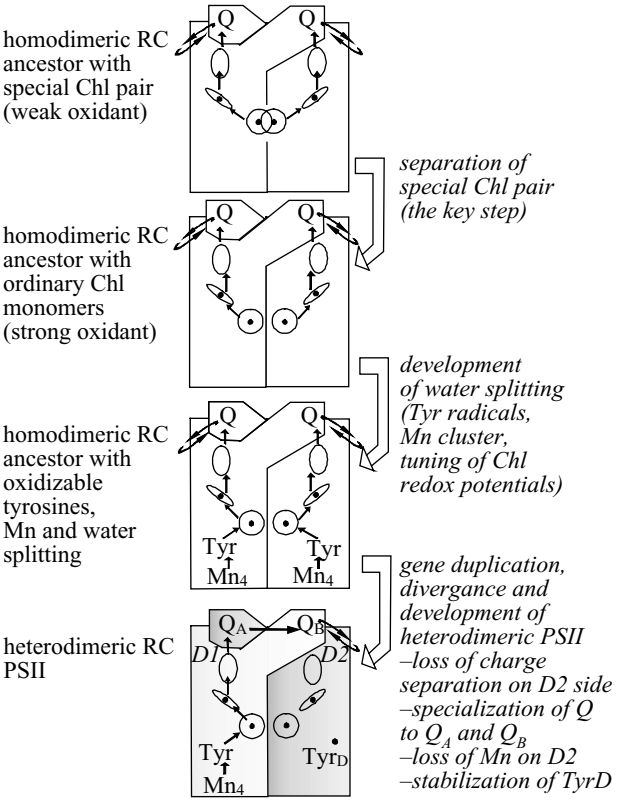

Figure 2. A simplified summary of the most important steps in the evolution of PSII according to the view outlined in this review. The same schematic representation as in figure 1 was used (for details see legend of figure 1). For clarity, the tetrapyrroles are represented as circles without specific labels, the pheophytins being represented by circles while chlorophylls with their central Mg^{2+} is represented by circles with central dots.

5. EVOLUTION OF PSII

In summary (figure 2), it is suggested that P^+ and the neighbouring chlorophylls are all very oxidizing because they are monomers and that chlorophyll monomers in the environment provided by the RC protein are intrinsically very oxidizing. The key event in the evolution of PSII is suggested to be the mutation(s), which resulted in the appearance of this oxidizing capacity, and that this could have occurred by a single event in which the special pair of chlorophylls (which is common to all other reactions centres) was split apart resulting in the formation of ordinary monomers. All four monomeric chlorophylls in the centre of PSII are thus highly oxidizing. Charge separation seems to take place mainly between the pheophytin and the adjacent chlorophyll ($B^+_{D1}Ph^-$) followed by electron donation from the next nearest chlorophyll (P_{D1}). It is suggested that the monomerization of the special pair occurred in a homodimer and that a homodimeric RC evolved that underwent tyrosine oxidation, Mn oxidation and possibly water oxidation on both sides of the RC. The

heterodimeric RC was favoured for reasons of efficiency in light usage and economy, particularly with regard to protein damage (re: D1 turnover) as the ambient oxygen concentration built up. It is suggested that an electrostatic influence of $TyrD^{\bullet}(H^{+})$ could be important in confining P^{+} to the D1 side of the RC thereby restricting damage to D1.

6. OTHER POINTS

(a) *Special pair first*

A corollary of the key hypothesis here (i.e. that P evolved through a splitting of the special pair) is that the special pair was present in existing RCs before the evolution of PSII. Some schemes of RC evolution see the earliest RC as a monomeric protein, equivalent to half a current RC (e.g. Van Gorkom 1987; Blankenship 1992). Linking such a scenario with the present hypothesis leads to a situation in which a monomeric chlorophyll (in a monomeric RC) evolves to a special pair (in a dimeric RC) then to a monomeric chlorophyll (in a dimeric RC). This would seem a somewhat tortuous route. However, given that there are no existing monomeric RCs, it seems possible that there never was such a thing (see Mulkidjanian & Junge 1997; Nitschke *et al.* 1998). Instead, the first charge separating photochemical RC would have been a homodimer and that the reason for that was precisely because the special pair of pigments came with dimerization of the protein. The special pair was needed for the first photosynthetic RC because at the same time it was an energy trap (i.e. at long wavelength) and an easily oxidized species. The pre-existing monomer protein could have had a different, non-RC role, such as a u.v. screen as suggested earlier (Mulkidjanian & Junge 1997).

(b) *Special pairs and PSII*

The special pair is not only easier to oxidize than a monomer but its absorption is also shifted to longer wavelengths. The longer wavelength absorption works well as an energy trap but at the expense of having less intrinsic energy in its excited state. For a chlorophyll special pair this shift is perhaps not very big, as we see by the energy available in P^{*}_{700} (1.75 eV) compared with P^{*}_{680} (1.80 eV). If, however, PSII is limited thermodynamically as some researchers think, then this small increase in the energy available in a chlorophyll monomer (compared with that in special chlorophyll pair) may be functionally important. Although it makes good sense then that PSII abandoned the special pair to shorten the wavelength and thus increase the energy available for water oxidation, the increase in oxidation power would appear to be the more important consequence of the transition from special pair to ordinary chlorophyll monomers.

(c) *On the oxidizing potential prior to heterodimerization*

It is suggested that water-splitting activity in PSII, or at least the high oxidizing potential, evolved in a homodimer and that the transition to the heterodimer took place subsequently (see figure 2). Given the advantages conferred by heterodimerization thanks to the specialized Q_A/Q_B system in an RC that performs electron transfer into a membrane quinone pool (see § 2), the question of why heterodimerization did not occur prior to the appearance of the high oxidizing potential (and water splitting?) arises. One small rationalization of this is the idea that PSII evolved under conditions where the efficiency of light use in the homodimeric PSII ancestor was not limiting for its function nor for the growth of the organism. One can speculate on what circumstances could result in such a situation. However, here we limit ourselves to pointing out two of the factors that could be of potential relevance to such a situation. (i) PSII may have evolved in the same membrane as PSI, and ancestral pre-PSII RCs may not have been required for growth (see Rutherford & Nitschke 1996). (ii) It is possible that prior to water oxidation, electron-donating substrates were in low abundance and thus rate limitations may have been at the level of substrate binding. When water oxidation occurred, the donor-side rate limitation was alleviated and only then did the selection pressures to develop the heterodimer (i.e. efficiency gains from Q_A/Q_B specialization, a single Mn complex and from one-side-limited protein damage) become significant.

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GLOSSARY

B-RC: purple bacterial reaction centre
PSI: photosystem I
PSII: photosystem II
RC: reaction centre
RCI: type I reaction centre
RCII: type II reaction centre