Does functional photosytem II complex have an oxygen channel?

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Abstract Photosystem II complex (PSII) of thylakoid membranes uses light energy to oxidise extremely stable water and produce oxygen ($2H_2O \rightarrow O_2+4H^+ +4e^-$). PSII is compared with cytochrome *c* oxidase that catalyses the opposite reaction coupled to proton translocation. Cytochrome *c* oxidase has proton and water channels, and a tentative oxygen channel. I propose that *functional* PSII complexes also need a specific oxygen channel to direct O_2 from the water molecules bound to specific Mn atoms of the Mn cluster within PSII out to the membrane surface. The function of this channel will be to prevent oxygen being accessible to the radical pair $P680^+Pheo^-$, thereby preventing singlet oxygen generation from the triplet P680 state in functional PSII. The important role of singlet oxygen in structurally perturbed *non-functional* photosystem II is also discussed.

Key words: Cytochrome *c* oxidase; Oxygen channel; Photosystem II; $P680^+$; Singlet O_2; Structure–function relationship

1. Introduction

During photosynthesis, photosystem II (PSII), the multisubunit pigment–protein complex consisting of at least 25 different proteins catalyses the light-induced splitting of water to oxygen and reducing equivalents ($2H_2O \rightarrow O_2+4H^+ +4e^-$). Driven by sunlight, this vital reaction by PSII creates and maintains oxygen in the atmosphere, and together with PSI provides much of the global biomass. However, to oxidise extremely stable water, the strongest biological oxidant known, $P680^+$, is generated making PSII particularly vulnerable to light and oxidative stress.

At the heart of PSII, the reaction centre D1/D2 protein heterodimer binds all the redox factors necessary for stable light-induced charge separation across the thylakoid membrane [1] (Fig. 1). On illumination of the reaction centre P680, a special chlorophyll a (Chl a) pair donates energised electrons (4–20 ps) to a pheophytin molecule, Pheo, to form the radical pair, $P680^+Pheo^-$. Within 300 ps, $Pheo^-$ passes an electron to the primary plastoquinone electron acceptor, Q_A. $P680^+$ is rapidly reduced (ns to μs) by a redox-active tyrosine residue Y_Z (D1Tyr 161) that is successively reduced by the tetra-manganese cluster that stores the oxidation equivalents required to oxidise water to oxygen. The oxygen-evolving core PSII complex also includes inner antenna Chl a proteins, CP47 and CP43, the α and β subunits of cytochrome *b*559, several single helical, membrane-spanning proteins, a cluster of four manganese atoms probably all attached to D1 protein, as well as the extrinsic 33, 23 and 17 kDa lumenal proteins [2]. Associated with this core PSII complex is the large family of Chl a/b proteins (CP22, CP24, CP26, CP29 and LHCII) that regulate light-harvesting [3].

The PSII complex has a limited functional life as a dimer in the appressed granal membranes of plants. PSII photoinactivation is an inevitable consequence of its own photochemistry involving the highly reactive oxidised radical, $P680^+$, as well as damaging oxygen species, particularly singlet O_2, that mainly target D1 protein [4–8]. Up to growth light, the rate of repair of PSII via D1 protein synthesis is usually fast enough to prevent net photoinactivation. With increasing light intensity, particularly combined with other environmental stresses, the rate of damage to D1 protein exceeds the rate of de novo D1 protein synthesis and a loss of functional PSII occurs. Large dynamic structural changes are needed to restore PSII function. Non-functional dimeric PSII complex with damaged D1 protein is phosphorylated, peripheral LHCII Chl a/b proteins are detached, and the phosphorylated PSII core dimer is monomerized in the appressed granal domain [9]. The phosphorylated PSII monomers then laterally migrate to non-appressed stroma thylakoids where CP43 is first dephosphorylated and detached from the damaged PSII cores. Following dephosphorylation of D1 and D2 proteins, damaged D1 protein is degraded and simultaneously replaced by newly synthesized D1 protein in the stroma thylakoids [9,10].

2. The molecular environment of $P680^+$

$P680^+$ has an enormous redox potential of about 1.17 V, in contrast to 0.4–0.6 V for the other oxidised primary donors in photosynthesis [7]. Clearly the environment around $P680^+$ must be extremely unusual to prevent it oxidising its near neighbours, either amino acids or pigments. Both chlorophylls and carotenoids in solution are normally oxidised by potentials of 0.6 V. In functional PSII, the protein matrix must maintain the structural hydrophobic domain around $P680^+$. In P680, the chlorin rings of the two Chl a molecules ligated to His198 on the D1 and D2 proteins may not be parallel to each other as in the BChl dimer, but tilted (30°) [11]. With less overlap between the chlorin rings, exciton coupling is much weaker than in photosynthetic bacteria, but charge separation across the plant thylakoid membrane is twice as efficient [1]. With weaker exciton coupling, P680 is a relatively shallow trap and primary charge separation is reversible. Due to this exciton–radical pair equilibrium [12], a photon trapped by

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P680 that causes primary charge separation (electron transfer from $P680^+$ to Pheo) may be followed by charge recombination between $P680^+$ and $Pheo^-$ to yield an exciton in the antenna:

$$(\text{PSII antenna})^* \rightleftarrows (\text{P680Pheo})^* \rightleftarrows P680^+Pheo^- \rightleftarrows P680^+PheoQ_A^- \quad (1)$$

Consequently a photon may visit the reaction centre several times before being trapped by electron transport or being dissipated from the antenna. Significantly, this exciton–primary radical pair equilibrium operates in both functional and non-functional PSIIs.

Carotenoids are essential components of all PSII and PSI Chl-proteins since they protect Chl from the potentially damaging effects of light and oxygen. Apart from the general energy dissipation by the xanthophyll cycle [13], carotenoids have two essential photoprotective roles by quenching excitation energy from triplet Chl (^{3}Chl) or singlet O_2 ($^1O_2$) [14]. (1) ^{3}Chl is rapidly quenched by carotenoids by triplet–triplet energy transfer, followed by triplet carotenoid (^{3}Car) returning to the ground state with heat dissipation. (2) Singlet O_2 formed by the reaction of oxygen with triplet P680 ($^3P680+{}^3O_2 \rightarrow P680+{}^1O_2$) also reacts with carotenoid to form ^{3}Car which is quenched as in reaction 1. The faster reaction 1 is predominant (90–95%) in leaves [14]. Yet in functional PSII during repeated $P680^+$ turnover when carotenoid quenching would seem to be essential, β-carotene could not directly quench ^{3}P680 when isolated PSII cores were bombarded with very high light [7]. Presumably the high potential of $P680^+$ would oxidise β-carotene were it close enough for direct carotenoid quenching. Without carotenoid quenching, charge recombination of the radical pair and spin dephasing would allow the triplet P680 state to generate singlet oxygen. However, in O_2-evolving PSII centres, the formation of lethal singlet oxygen via ^{3}P680 would be impossible, *if O_2 were excluded from the vicinity of $P680^+Pheo^-$*.

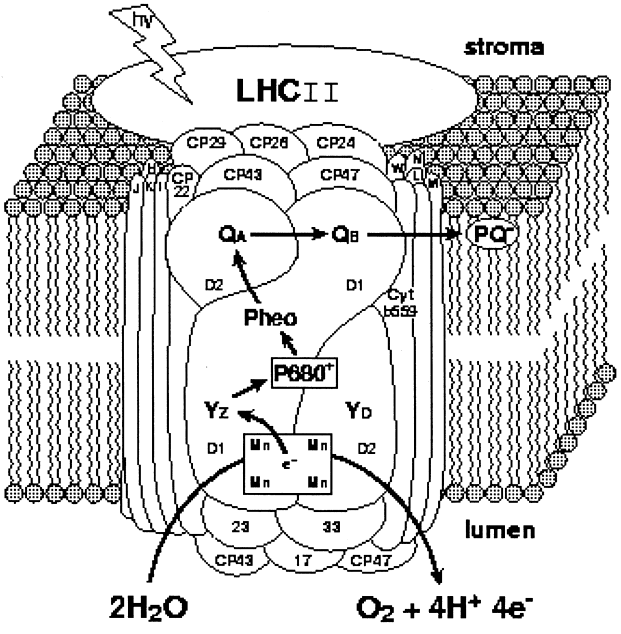

Fig. 1. Schema of PSII complex; see text for explanation.

3. Hypothesis

I propose that in *functional PSII complexes*, oxygen will be directed out from the two water molecules bound to specific Mn atoms of the manganese cluster within PSII to the membrane surface by a specific oxygen channel, whose purpose is to prevent oxygen being accessible to the radical pair $P680^+Pheo^-$. If true, the generation of singlet oxygen via the triplet P680 state would be impossible in functional PSII. Compelling but indirect evidence supports this proposal.

4. Evidence supporting this hypothesis

1. PSII photoinactivation in leaves shows reciprocity between the irradiance level and time of illumination demonstrating that it depends only on the number of photons absorbed rather than the rate of absorption [15,16]. Being a light dosage effect, PSII inactivation in vivo occurs under all irradiances, even at low light. The probability for the photoinactivation of PSII is very low, however, as the quantum requirement ranges from 10^6 to 10^7 photons per PSII inactivated [8]. Given that reciprocity holds, a *single* mechanism is needed for the photoinactivation of PSII during steady-state photosynthesis. During steady-state photosynthesis, $P680^+$ most probably inadvertently inactivates PSII [8]. While in vitro studies of PSII photoinactivation demonstrated two mechanisms of photoinhibition, an acceptor side with triplet P680 combining with O_2 to form a highly reactive singlet O_2 and a donor side with $P680^+$ [4,5], we proposed that damage by singlet oxygen occurred only in non-functional PSII [8].
2. If the radical pair $P680^+Pheo^-$ was accessible to oxygen, singlet oxygen production should be proportional to the number of functional PSII. However, Hideg et al. [17] have convincingly shown that singlet O_2 is proportional to the number of *non-functional PSIIs*, demonstrating that singlet oxygen is produced by non-functional PSIIs.
3. One of the remarkable features of PSII is that the assembly of the Mn atoms within PSII requires minimally a two step light-driven process separated by a dark period, a Ca^{2+} and x Cl^- [18,19]. Magnuson et al. ([20] and references therein) have shown that without any bound Mn, electron transport from Q_A to Q_B is slow, but much faster when some Mn is bound, and finally with the binding of the remaining Mn, oxygen is evolved. Clearly during the photoactivation of PSII many dynamic conformational changes must occur within PSII.
4. Conversely, after the photoinactivation of PSII, marked structural changes take place at both donor and acceptor sides of the core PSII complex. Electrons can no longer be transferred from Q_A to Q_B, some Mn atoms, the Ca and Cl ions are liberated [20,21] and the 33 kDa protein and other extrinsic lumenal proteins may be removed. The structural integrity of the hydrophobic domain in the protein matrix surrounding $P680^+$ is disturbed ([21] and references therein). This suggests an 'opening up' of non-functional PSII structure with O_2 now being accessible to the enhanced concentration of $P680^+Pheo^-$ generated by charge recombination when electron transport is inhibited [8].
5. Oxygen as well as proton barriers have been demonstrated in very hydrophobic membrane protein complexes. Significantly, photostable LHCII trimers harbour highly pro-

tected pigment sites with limited access of O_2 and protons, thereby protecting chlorophyll and carotenoid molecules from damage by photooxidation and acid lability [22]. $P680^+$ may well be effectively insulated from O_2 within functional PSII.

6. A tentative oxygen channel exists in cytochrome *c* oxidase, the terminal protein complex of the respiratory chain of the inner mitochondrial membranes of eukaryotes and cell membranes of most bacteria. Cytochrome *c* oxidase is a redox-driven proton pump that couples the reduction of oxygen to water to the translocation of four protons across the membrane: it effectively catalyses the reverse reaction to that of PSII complex.

Following high resolution structures of cytochrome *c* oxidase [23,24], Tsukihara et al. [24] proposed that as well as proton and water channels, an oxygen channel also might be a prerequisite for cytochrome *c* oxidase functionality. Although an O_2 channel was not observed in their crystal structure (O_2 is a rod-shaped molecule with a 2.4 Å diameter), they proposed three possible pathways [25]. Molecular dynamic simulations of O_2 diffusion through cytochrome *c* oxidase revealed that oxygen appeared to be directed from the matrix to the oxygen-binding site heme a^3 of the binuclear redox centre [26]. The tentative oxygen channel starts at the hydrophobic cavity near the membrane-exposed surface of subunit I, close to the interface of subunit III where several lipid molecules are located, thus forming an effective hydrophobic O_2 reservoir [27].

In cytochrome *c* oxidase the reduction of oxygen liberates water to the matrix *via* a specific water channel. The catalytic mechanism for O_2 binding and reduction does not lead to highly reactive oxygen species as happens within PSII complex. However, the success of the intricate coupling of oxygen reduction to proton pumping by cytochrome *c* oxidase may be achieved by specific ways to separate more hydrophobic from more polar domains within the protein complex.

This separation of more polar and more hydrophobic domains within functional PSII is even more important. The environment around $P680^+$ is almost certainly shielded from bulk water, as protonic relaxation at the P680 site is slow [28]. In functional PSII, water also needs directed access from the lumen to the specific water-binding Mn atoms of the Mn cluster, probably via a channel as proposed by Wydrzynski et al. [29]. Thus, in functional PSII, neither O_2 nor water should have direct access to $P680^+Pheo^-$. Rather water probably enters via a specific pathway and O_2 exits by a distinct hydrophobic channel, formed in part by the association of the 33 kDa protein at the lumenal surface and the required positioning of the large lumenal loop E of CP47. Lipids also are probably very important in the separation of more hydrophobic from more polar domains within membrane protein complexes. A new paradigm is emerging that most membrane protein complexes contain lipids that have structural as well as functional roles. Phosphatidylglycerol is involved in the stabilization of PSII dimers [30], while cardiolipin (diphosphatidylglycerol) is needed for the stabilisation of cytochrome *c* oxidase dimers [31]. Indeed the proposed oxygen and water channels may well be more protected in PSII dimers than monomers, partly accounting for the preferential formation of PSII dimers in vivo, although PSII monomers are functionally active.

5. The role of singlet O_2 in non-functional PSII complexes

Although the temporal sequence of the dynamic compositional changes that take place when PSII is photoinactivated is not yet defined, clearly many compositional changes occur. In non-O_2-evolving PSII, the Mn cluster, Yz and $P680^+$ are no longer protected by the 33 kDa extrinsic protein and the lumenal loop E of CP47, and at least some Mn atoms, a Ca^{2+} and x Cl^- and probably some of the extrinsic lumenal proteins are lost. Such compositional changes will cause dynamic conformational changes in photoinactivated PSII and the proposed oxygen and water [29] channels will be destroyed. Boekema et al. [32] demonstrated by electron microscopy and single particle image analysis that the removal of extrinsic proteins and inorganic cofactors caused profound conformational changes in non-functional PSII. Removal of the 33 kDa protein induces an inward shift that strongly binds trimeric LHCII, as well as destabilizes the monomer–monomer interaction in the central core dimer, leading to structural rearrangements of the core monomers [32]. Barber and colleagues' [7] have shown oxidation by singlet oxygen of many specific amino acids in the D1 protein, and to a lesser extent in D2 protein, in isolated PSII cores under very high irradiance. Although not demonstrated in vivo, this oxidation by singlet oxygen of many specific amino acids of D1 protein probably occurs in the non-functional phosphorylated PSII dimers that are confined to appressed grana regions, prior to migration of PSII monomers out to non-appressed domains for PSII repair. It allows the singlet oxygen to react locally and damage D1 protein without disassembly of the PSII cores.

6. Conclusions

Given the unique problem of the high potential of oxidant $P680^+$ that is required to oxidise water to oxygen, the photoinactivation of PSII is inevitable. This leads to a highly dynamic and complex structural heterogeneity of the cycle between functional and non-functional PSII complexes, which involves regulated degradation and de novo D1 protein synthesis [4–10]. Compelling though indirect evidence suggests that the radical pair $P680^+Pheo^-$ in functional PSII should be protected from oxygen. By analogy to the tentative oxygen channel of cytochrome *c* oxidase, I propose that oxygen will be liberated from the water bound to specific Mn atoms of the Mn cluster via a specific channel to the membrane surface (lumen or stroma?). The function of the proposed oxygen channel is to prevent O_2 having direct access to $P680^+Pheo^-$, and singlet O_2 generated via the triplet P680 state in functional PSIIs. High-resolution studies of PSII crystals nearing completion [33] may test the hypothesis. Only when $P680^+$ with a fateful first oxidative step destroys oxygen evolution, will the ensuing cascade of structural perturbations of PSII destroy the proposed oxygen and water channels. Then oxygen will have direct access to $P680^+Pheo^-$, singlet O_2 will be produced and successively oxidise specific amino acids of D1 protein, thereby targeting D1 protein for eventual degradation and replacement.

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