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Structural/functional dynamics of photosystem II

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Introduction

PSII complex has a limited life as a *functional* dimer in the appressed granal membranes of higher plants and more recently-evolved green algae. PSII photoinactivation is an inevitable consequence of its own photochemistry, involving $P680^+$ and singlet O_2 that target D1 protein (Anderson et al. 1998**)**. Up to normal growth light intensities, the rate of repair of PSII via D1 protein synthesis is usually fast enough to prevent net photoinactivation. With higher light intensity, particularly combined with other environmental stresses, the rate of damage to D1 protein exceeds the rate of its *de novo* synthesis and PSII is photoinactivated. Many dynamic structural and compositional changes are needed to restore PSII function. *Nonfunctional* dimeric PSII with damaged D1 protein is phosphorylated, peripheral LHCII Chl a/b-proteins are detached, and the phosphorylated PSII core dimer is monomerised in the appressed granal domain. The phosphorylated PSII monomers then migrate laterally to nonappressed stroma thylakoids where CP43 is first dephosphorylated and then detached from damaged PSII cores (Baena-González et al. 1999). Following dephosphorylation of D1 and D2 proteins, damaged D1 protein is degraded and simultaneously replaced by newly synthesized D1 protein in the stroma thylakoids (Baena-González et al. 1999). Thus, the highly regulated cycle between functional and non-functional PSII involves many dynamic structural and compositional changes of PSII.

1. The initial photoinactivation of PSII in vivo is probably caused by $P680^+$

Two candidates have been assumed to cause PSII photoinhibition (Aro et al. 1993):

(a) donor side photoinhibition by **$P680^+$** , and

(b) acceptor side photoinhibition due to **singlet O_2** generated from triplet P680:

$^3P680 + {}^3O_2 \rightarrow P680 + {}^1O_2$

Indirect evidence strongly suggests that the initial photoinactivation of PSII *in vivo* is caused by $P680^+$, the strongest known biological oxidising agent which is required to split otherwise stable water.

1. Since PSII photoinactivation in leaves shows reciprocity between irradiance level and time of illumination, it depends on photons absorbed and not the rate of photoinhibition. Being a light dosage effect, only one mechanism is required for the initial photoinactivation of PSII (Anderson et al. 1998).
2. Following light excitation and stable charge separation within the radical pair $P680^+Pheo^-$, $Pheo^-$ transfers an electron to Q_A very rapidly (10 psec), but the reduction of $P680^+$ by Tyr_Z

is much slower (20-200 ns). Hence $P680^+$ exists for quite a long time within the primary radical pair and could cause damage to its immediate surroundings.

3. O_2-evolving and Tris-treated PSII membrane fragments show nearly the same rate of stable charge separation determined by time-resolved measurements of 826 nm absorption changes: a mechanism involving double reduction and protonation of Q_A is most unlikely to occur *in vivo* (Napiwotzki et al. 1997).
4. If the radical pair $P680^+Pheo^-$ were accessible to oxygen, singlet oxygen production should be proportional to the number of functional PSII. Singlet O_2 production in leaves, however, is proportional to the number of *non-functional PSII*s (Hideg et al. 1998) demonstrating that singlet oxygen is produced by *non-functional* PSIIs.
5. β-carotene cannot directly quench $^3P680$ in isolated PSII cores, presumably because if it were close enough for quenching it would be oxidised by $P680^+$ (Barber 1998). In order to prevent the generation of singlet oxygen from the triplet P680 state *in vivo*, oxygen should not be accessible to the radical pair in *functional* PSII (Anderson 2000).

2. Is $P680^+$ protected from oxygen in functional photosytem II *in vivo*?

The above discussion strongly suggests that $P680^+$, rather than singlet oxygen, photoinactivates *functional* PSII. If true, there is an absolute requirement to prevent oxygen from reaching the radical pair $P680^+Pheo^-$ in *functional* PSII. Two main lines of reasoning suggest this requirement may be structurally possible.

1. Oxygen as well as proton barriers have been demonstrated in very hydrophobic membrane proteins. Significantly, isolated LHCII trimers permit only limited access of either oxygen or protons to pigment sites (Siefermann-Harms and Angerhofer 1998). The oxygen barrier is likely to be preserved *in situ* in thylakoid membranes and prevent oxidation of chlorophylls and carotenoids, vital for the regulation of light harvesting and energy dissipation.

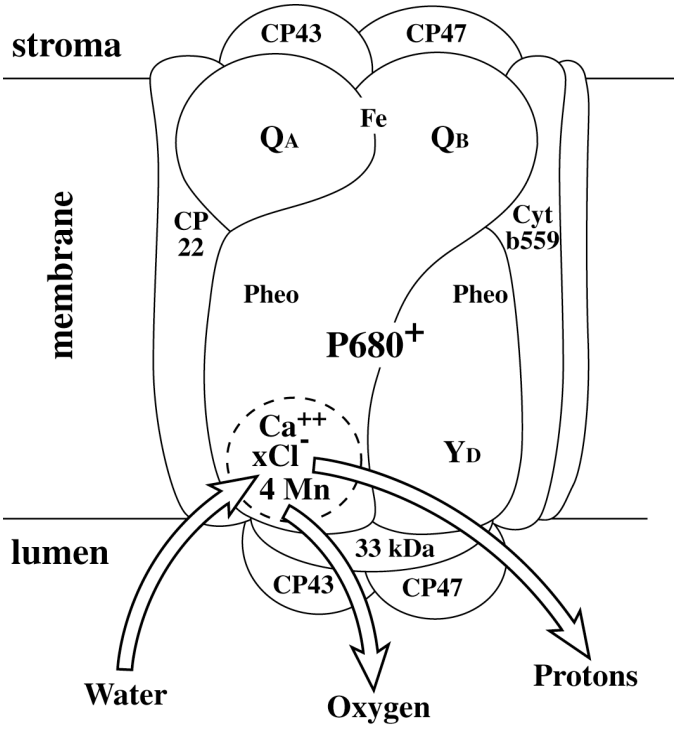

Fig 1. Proposed channels in functional PSII.

2. Cytochrome c oxidase is a mitochondrial 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 the PSII complex. From their high resolution structure of cytochrome c oxidase, Tsukihara et al. (1995) proposed that as well as proton and water channels, an oxygen channel might also be needed for cytochrome c oxidase to function. Dynamic simulations of oxygen molecules passing from the matrix to its binding site in cytochrome c oxidase reveal a possible oxygen pathway (Hofacher and Schulten 1998).

Although the catalytic mechanism for O_2 binding and reduction in cytochrome c oxidase does not generate highly reactive oxygen species, the successful coupling of oxygen reduction (electron transport) to proton pumping by PSII must be achieved by the separation of more hydrophobic and more polar domains within protein complexes. Recently, I proposed that the oxygen evolved from the two water molecules bound to specific manganese within the manganese cluster on functional PSII will be directed out to the membrane surface by a specific oxygen channel (Fig. 1). The purpose would be to ensure that oxygen is inaccessible to ^{3}P680: if true, singlet oxygen production will not occur in functional PSII (Anderson 2000). In functional PSII, water also needs direct access from the lumen to its binding site on the specific Mn atoms of the manganese cluster as proposed by Wydrzynski et al. (1996)

Lipids are also important in the separation of more hydrophobic from more polar domains within membrane protein complexes: a paradigm emerges that most membrane protein complexes contain lipids that have structural as well as functional roles. Phosphatidylglycerol is involved in the stabilization of PSII dimers (Kruse et al. 2000), while diphosphatidylglycerol is needed for stabilisation of cytochrome c oxidase dimers. Indeed, the proposed oxygen and water channels may well be more protected in PSII dimers than PSII monomers, partly accounting for the preferential formation of PSII dimers in vivo, although PSII monomers are also functionally active.

3. After the initial photoinactivation of PSII a cascade of conformational changes occur: D1 protein in non-functional PSII will be attacked by singlet oxygen

The temporal sequence of the many dynamic compositional changes that take place when PSII is photoinactivated is not fully defined. Electrons can no longer be transferred from Q_A to Q_B and at least two Mn atoms, Ca and Cl ions, and the 33kDa protein are removed. Such dramatic changes will cause dynamic conformational changes in photoinactivated PSII at both the donor and acceptor sides of core PSII. Boekma et al. (1999) have shown that removal of the 33 kDa protein induces an inward shift in the strongly bound trimeric LHCII, as well as destabilizing the monomer-monomer interaction in the central core dimer, leading to structural rearrangements of the core monomers. Clearly when PSII is photoinactivated, the structural integrity of the hydrophobic domain in the protein matrix surrounding $P680^+$ is rapidly disturbed. This suggests an "opening up" of *non-functional* PSII structure with O_2 now becoming accessible to the enhanced concentration of $P680^+Pheo^-$ generated by charge recombination when electron transport is inhibited. If the proposed oxygen and water channels are destroyed in *non-functional* PSII, oxygen will have direct access to $P680^+Pheo^-$ and singlet oxygen will be produced.

Structural peturbation of the macrostructure of *functional* PSII will also occur above light-limiting conditions: when more light is absorbed by the photosynthetic pigments than can be utilised in photosynthetic electron transport, nonphotochemical dissipation (NPQ) of excitation energy is induced as one mechanism for the photoprotection of PSII. NPQ depends on several processes, but mainly on the energization of thylakoids by the transthylakoid pH

gradient (Horton et al. 1999). Probably, changes in the macrostructure of *non-functional PSII* will also aid NPQ.

4. Non-functional PSII containing D1 protein will be attacked by singlet oxygen in appressed granal membranes, prior to lateral migration for repair

Oxidation by singlet oxygen of specific amino acids of D1 and to a lesser extent of D2 protein in isolated PSII cores occurs under very high irradiance (Barber 1998). Although not demonstrated *in vivo*, this oxidation by singlet oxygen of many amino acids of D1 and D2 proteins probably occurs in the *non-functional* phosphorylated PSII dimers that are confined to appressed grana regions (Anderson 1999). Since no PSI complexes are located in the appressed membrane domains, singlet oxygen will react locally with the *non-functional* PSII and not damage PSI. Singlet oxygen successively oxidises specific amino acids of D1 and D2 proteins without disassembly of the *non-functional* PSII located in stacked granal membrane domains. Then the co-ordinated processes of D1 protein degradation and *de novo* synthesis occurs in non-appressed domains, the region accessible to D1 proteases and chloroplast ribosomes.

Conclusion

The highly oxidising potential of $P680^+$ means that PSII function is always at risk, despite the many photoprotective strategies that regulate light absorption mainly by the conversion of excess energy into heat. $P680^+$ most probably is the agent that causes PSII photoinactivation *in vivo* rather than triplet P680 reacting with oxygen to generate singlet oxygen. We hypothesise that $P680^+$ is shielded from oxygen in *functional* PSII, and any triplet P680 formed is rapidly quenched and unable to act with oxygen. Following the initial photoinactivation of PSII, *non-functional* PSII undergoes marked conformational changes allowing the oxygen and water channels to open up so that triplet P680 may now react with oxygen to generate singlet oxygen. Under sustained high light, singlet oxygen plays an important role in successively oxidising certain amino acids of D1 and D2 proteins without disassembly of PSII cores as the *non-functional* PSII containing "oxidised" D1 and D2 proteins accumulate in appressed membranes. Hence grana stacking regulates both the rate of D1 protein degradation and turnover. This exquisitely regulated cycle which balances *functional* PSII and phosphorylated *non-functional* PSII still containing damaged D1 protein in appressed membrane domains with, on the other hand, D1.D2.CP47 units in non-appressed membrane domains where D1 protein turnover restores PSII function, clearly involves many structural changes within PSII.

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