PHOTOSYSTEMS CATALYSE THE conversion of light energy, captured by chlorophyll, into forms that can be used by cyanobacteria, algae and higher plants. Within this process, photosystem 2 (PS2) is responsible for splitting water to form molecular oxygen, electrons and protons, a process assisted by photosystem 1 (PS1) and the cytochrome $b_6f$ complex. These events are vital for maintaining the present levels of biomass on our planet and for sustaining an oxygenic atmosphere.

Despite its importance, the reaction centre within PS2 that splits water is not fully understood at a molecular level. Most of our present knowledge on the structure-function relationship of this photosystem is drawn from analogies with reaction centres of photosynthetic purple bacteria (see Fig. 1), for which a high resolution three-dimensional structure is available. Although useful, this comparison is restricted, as phototrophic purple bacteria do not split water and their subunit composition is much simpler than that for PS2. In PS2, the chlorophyll-binding proteins CP43 ($PsbC\*$) and CP47 ($PsbB$) harvest light and transfer energy to a special electron acceptor, closely associated with this light-harvesting complex. These findings have given clues as to how these various light-harvesting systems operate. The PS2 core complex: the smallest unit that can split water is responsible for splitting water.

Although cross-linking experiments and studies with both site-directed and deletion mutants of cyanobacteria have shown that all these subunits are arranged in a dimeric structure of PS2, a more comprehensive understanding of the subunit organization of PS2 and the accompanying secondary antenna systems (phycobilisomes in cyanobacteria and the light-harvesting complexes in higher plants) and discuss possible physiological consequences of the proposed dimeric structure of PS2.

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Photosystem 2 (PS2) is the part of the photosynthetic apparatus that uses light energy to split water releasing oxygen, protons and electrons. Here, we present a model of the subunit organization of PS2 and the accompanying secondary antenna systems (phycobilisomes in cyanobacteria and the light-harvesting complexes in higher plants) and discuss possible physiological consequences of the proposed dimeric structure of PS2.

How does photosystem 2 split water? The structural basis of efficient energy conversion

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shape and molecular mass (about 450-500 kDa as determined by high-performance liquid chromatography (HPLC)-size exclusion and electron microscopy (EM) image analyses) confirm that it is composed of two monomeric core complexes.

Dimeric PS2 cores have also been isolated and characterized from higher plants. Image-analysis of negative-stained single particles show a similar size and electron density pattern to that of the oxygen-producing PS2 core dimers isolated from cyanobacteria. Contours obtained from the averaged top views of isolated dimeric particles in EM have been used for the model shown in Fig. 2; the two monomeric complexes show a rotational symmetry of towards each other with respect to the centre of the dimer, which is a low electron density area (see below).

Evidence for the existence of PS2 dimers in native membranes also comes from freeze-fracture analyses of thylakoids from both cyanobacteria and higher plants (Refs 8, 9), in which the stroma region shows exclusively the monomeric form and the grana region appears highly enriched for the dimer. The observed lateral separation of monomers and dimers is strongly supported by biochemical analysis of maize stroma and grana membranes.

This structural information also allows an observation made in 1964 of large tetrameric particles (185:155 nm) in thylakoid membranes from higher plants to be re-interpreted. These were postulated to be 'quantasomes' and to act as minimal photosynthetic units; they might well be made up of dimeric PS2 core complexes. The tetrameric particles disappear – concomitant with the loss of water-splitting activity – upon removal of the 33kDa extrinsic protein (PsbO) and two others, the 17kDa and 23kDa proteins (PsbQ and PsbE respectively) that are associated with the oxygen-evolving apparatus. The observed lateral separation of monomers and dimers is strongly supported by biochemical analysis of maize stroma and grana membranes.

Subunit arrangement in the PS2 core complex

Recent data obtained by negative staining of isolated particles and two-dimensional crystals of PS2 from both cyanobacteria and higher plants have led to a model of the possible subunit arrangements within the dimeric PS2 complex (Fig. 2).

The CP43 subunit. By comparison with electron micrographs of isolated PS2 complexes that lack the CP43 subunit, we place CP43 towards the periphery in our model (Fig. 2a); this is in line with the finding that this subunit is the last one to be incorporated during PS2 core assembly, and that the deletion of the psbC gene does not prevent the assembly of a PS2 complex in vivo. Moreover, CP43 can be removed biochemically from isolated PS2 cores to yield a CP47-D1-D2-cyt b complex obtained with spinach cores indicate that the dimeric state is more stable and functionally active compared to isolated monomers (B. Hankamer et al., unpublished).

Figure 1
Scheme comparing the central electron transport components of (a) photosynthetic purple bacteria and (b) photosystem 2 (PS2), both of which are quinone-type reaction centres. The related primary electron donors (chlorophyll P680 in PS2 and P870 chlorophyll in purple bacteria), primary electron acceptors (pheophytin, Phe and bacterio- pheophytin, BPhe), and quinones, which act as secondary and tertiary electron acceptors (Q_A and Q_B), are shown. These redox components are sequentially arranged within two homologous reaction centre proteins, the D1-D2 subunit in PS2 and the L-M subunit in purple bacteria.

Figure 2
(a) Top and (b) side view of the model for a dimeric photosystem 2 (PS2) complex both in higher plants and cyanobacteria. The model is based on averaged views of electron micrographs with areas showing the largest differences being contoured. Colours indicating the suggested positions for PS2 subunits are superimposed on the top and side view. In the top view the position of the extrinsic 33 kDa subunit (PsbO) has been superimposed.
Model for state transitions in a cyanobacterial thylakoid membrane (modified from Ref. 29); (a) state 1 (favouring linear electron flow, with dimeric PS2, dimeric b_{6f} and monomeric PS1 complexes) and (b) state 2 (favouring cyclic electron flow, with monomeric cyt b_{6f} and trimeric PS1 complexes; monomeric PS2 is not involved). Components depicted in the figure are photosystems 1 (PS1) and 2 (PS2) in green, cytochrome b_{6f} complex (orange), plastoquinone (black circles), plastocyanin (dark blue), ferredoxin (red) and the ferredoxin–NADP oxidoreductase (yellow). The phyceobilisome light-harvesting system (PBS) is light blue. For simplicity, other parts of this thylakoid membrane besides the components of the photosynthetic electron transport chain have been omitted. Adapted with permission from Ref. 29.
to the D1-D2 heterodimer of the reaction centre. In addition it seems possible that energy can be distributed between the two reaction centres of the dimer via the CP47 subunits; this could be important for ensuring optimal usage of excitation energy. Fluorescence induction measurements support this idea: monomeric complexes show an exponential increase of fluorescence intensity with time, while dimers show a sigmoidal increase in fluorescence intensity and a positive connectivity. This finding is consistent with the idea of cooperativity existing between the two connected monomers.

In cyanobacteria, PS2 complexes also serve as binding sites for an additional hydrophilic light-harvesting system, the phycobilisome (PBS). Freeze-fracture electron micrographs show rows of hemi-discoidal PBSs on the surface of cyanobacterial thylakoid membranes, with a periodicity that matches that of dimeric PS2 particles in the membrane underneath. This finding suggests that the PBS-PS2 supercomplexes are composed of two PS2 complexes and one PBS; an arrangement that is also confirmed by biochemical analyses and energy transfer studies. Also, the two basal PBS core cylinders that are responsible for the PBS-PS2 connection have a compatible size to the oxygen-evolving PS2 core dimer. The organization of the PBS-PS2 supercomplexes into tightly arranged rows not only allows for a high packing density of PBSs, but also potentially for high-efficiency excitation-energy transfer between adjacent PS2 units. This would result in an energy-conducting fibre system that facilitates an efficient energy distribution along the plane of the thylakoid, and thus help to optimize light harvesting.

However, the association of PBS and the dimerization of PS2 seems to be dynamic, as more-or-less distributed PBSs have also been observed on the surface of cyanobacterial thylakoid membranes: the extent to which PBSs are organized into rows seems to be related to the light condition during growth. Uncoupling or partial dissociation of PS2 and PBS apparently results in their lateral redistribution, which in turn alters the energy distribution among the photosystems. By these so-called 'state transitions' cyanobacteria respond to preferential excitation of PS2 or PS1 by selective enhancement of excitation energy transfer to the less active photosystem.

A model has recently been proposed that tries to combine structural and physiological data. Figure 3 illustrates the most important features of this model. Under light conditions that favour the photochemistry of PS1 – so called 'state 1' – PBSs are attached to dimeric PS2, while under conditions that light preferentially excites PS2 ('state 2') PBSs become functionally connected to trimeric PS1. The redistribution of PBSs might be caused by the dissociation of dimeric PS2 into monomers and a concomitant trimerization of the PSI complex. Alternatively, in state 2, PBSs might still be connected to one monomer of the previous PS2 dimer, while trimeric PS1 replaces the other PS2 monomer to form a close association with PS2.

Direct energy transfer from PBSs to PS1 under 'state 2' has been shown by spectrometric measurements of cyanobacterial thylakoid membranes, and the maximal quantum efficiency of this transfer is enhanced considerably in mutants that lack PS2, suggesting a specific PBS-PS1 complex. As the cyt b/f complex has also been reported to exist in both highly active dimeric and low-active monomeric forms, the above discussion could herald a general principle of energy distribution within the thylakoid membrane as a result of a dynamic equilibrium of mono- and oligomeric forms of all three membrane protein complexes of the photosynthetic electron transport chain, i.e. PSI, PS2 and the cyt b/f complex. A dynamic clustering of photosystems caused by different light regimes was also reported for rhodophyta and reflects changes in functional domains that result in enhancement of the quantum yield owing to maximized cooperativity between the photosystems.

Higher plants. By contrast, green algae and higher plants have their thylakoid membranes structurally organized into stacked grana and unstacked stroma regions, with the stroma membranes containing most of the PSI and the grana regions highly enriched in PS2. The cyt b/f complex is more or less evenly distributed among both membrane regions.

Furthermore, PS2s of green algae and higher plants do not possess PBSs, but
instead have intramembranous antenna protein complexes that bind both chlorophyll \(a\) and chlorophyll \(b\) for additional light harvesting. These proteins are predominantly CP29 (Lhcb4), CP26 (Lhcb5), CP24 (Lhcb6) and the light-harvesting complex 2 (LHC2 or Lhcbl-Lhcb2). Biochemical and structural studies have shown that they are closely associated with PS2, but the level of association differs between grana- and stroma-located PS2 complexes: stromal PS2 complexes occur exclusively in the monomeric form and have few chlorophyll \(a\)- and chlorophyll \(b\)-binding proteins associated with them (for summary, see Refs 28, 32), while a close association of the various light-harvesting complexes with the oxygen producing dimeric PS2 cores, occurring exclusively in the grana, has been suggested\(^{29,32}\). Biochemical fractionation by gel electrophoresis failed to reveal any association of LHC2 with monomeric PS2, and was only seen with dimeric PS2 (Ref. 33). Obviously, the conversion between monomeric and dimeric forms of PS2 modulates its function and plays a role in the degradation-repair cycle associated with D1 turnover\(^{31}\), which in turn might involve reversible phosphorylation\(^{31}\). In more detail, EM studies of wild-type PS2 and mutants that lack chlorophyll \(b\) and all chlorophyll \(a\)- and chlorophyll \(b\)-binding proteins\(^{13,19}\) suggest that the small chlorophyll-binding proteins CP29, CP26 and CP24 connect the dimeric core complex to the trimeric LHC2 (Refs 5, 9, 18, 36); this has resulted in the model presented in Fig. 4.

A comparison with Fig. 2a clearly shows the dimeric core complex in the central domain. If the side view of this model is compared with that presented in Fig. 2, it can be seen that the two extrinsic 33kDa subunits are overlapping and therefore appear as a single (red) subunit with a maximal height of 9 nm in the central area. Figure 4 also indicates (by comparison with Fig. 2) the likely position of the various chlorophyll \(a/b\)-containing LHC subunits on either side of the dimeric core. By contrast to most other models of the PS2 antenna complex (summarized in Ref. 32), which are based on biochemical data and cannot indicate the precise position of the antennae, the structure given in Fig. 4 suggests a perfect rotational symmetry of the dimeric state, including all antenna systems. The assignment of protein densities with specific chlorophyll \(a\)- and chlorophyll \(b\)-binding proteins in Fig. 4 is consistent with several pieces of biochemical data (for review, see Ref. 32) and especially prominent for the trimeric LHC2 (Ref. 36).

Functionally, the monomeric proteins CP29, CP26 and CP24 have a relatively small contribution to the PS2 antenna system, as together they bind only 15% of the PS2 chlorophyll. However, the inner localization of this antenna system is consistent with its proposed role in regulating the efficiency of excitation energy transfer from the outer antenna LHC2, to the core\(^{21}\). By contrast, the trimeric LHC2 complexes (Lhcb1-Lhcb2) contain about 63% of the PS2 chlorophyll and, therefore, represent the major antenna system for PS2.

The light-harvesting antenna of the dimeric PS2 of the grana region was suggested to consist of one copy each of CP29, CP26 and CP24, and two to four trimers of LHC2, resulting in an antenna size of 230-250 chlorophyll molecules per reaction centre\(^{31}\). As the antenna size of the LHC2-PS2 complex in Fig. 4 was determined to contain only about 100 chlorophyll molecules, this difference could be accounted for by a ratio of two or three additional trimeric LHC2 per reaction centre. These additional LHCs might be responsible for forming contacts between the dimeric LHC2-PS2 complexes in the membrane, thus building an antenna network that spreads across the whole grana region. Indeed, two distinct subpopulations of LHC2 with slight differences have been shown to exist\(^{17}\). Therefore, it is possible that these subpopulations of LHC2 could be flexible under different light conditions and might migrate—depending on their degree of protein phosphorylation—to the stroma-exposed thylakoid regions to associate with PS1. This arrangement is comparable to the PBSs in cyanobacteria and red algae.

The equidistant arrangement of PS2 in grana as seen in freeze-fracture sections indicates that PS2, surrounded by their antenna systems, form random networks\(^{17}\). The idea of a network of pigment proteins covering the membrane is supported by the high protein density of the grana membrane, implying the possibility of an efficient excitation energy transfer over long distances. This suggestion is supported by fluorescence induction measurements with thylakoids of higher plants giving data that are consistent with energy transfer between PS2 dimers in the grana region\(^{15}\).

In summary, it seems that the dimerization of PS2 core complexes in higher plants, as well as in cyanobacteria, is an important organizational and functional state, playing a key role in the interaction with secondary antenna systems, i.e. PBSs and chlorophyll \(a/b\)-binding proteins, respectively.

**Evolutionary significance**

Cyanobacteria might serve as a model system for higher plants in an evolutionary sense. In cyanobacteria, PS2s are clustered in rows of PS2 dimers, to which PBSs are attached. In higher plants, a very similar dimeric organization of PS2 is seen in the grana regions, which would allow efficient photosynthesis. By analogy to the cyanobacterial PBSs, higher plants exhibit several (between six and eight) trimeric LHC2 complexes associated to varying degrees with a dimeric core complex. Therefore, a kind of PS2 network is created in both cases.

In higher plants, this PS2 network gives rise to grana\(^3\), while the stroma regions contain only monomeric PS2 (Ref. 15). By contrast, the cyanobacterial thylakoid membrane not only contains all components of photosynthetic electron-transfer machinery, but also the respiratory electron-transfer chain. These interconnected PS2s in cyanobacterial thylakoid membranes under 'state 1' conditions might be regarded as a precursor of the specialized 'grana networks' of higher plants, while the cyanobacterial 'state 2' [i.e. PBSs attached to PS1 (Ref. 29)] might be the precursor of the stroma region. In both cases, the clustering of dimeric PS2 also results in an effective separation from PS1, which is necessary according to the significant difference in the kinetics of their trapping reactions (PS2 is slow compared with PS1)\(^{39}\). A clear evolutionary link between organisms containing PBSs and chlorophyll \(b\)-based LHCs is further indicated by the coexistence of both types of antenna system in several rhodophytes\(^9\) and the recent finding of domains with highly enriched PS1 or PS2 in thylakoid membranes of cyanobacteria\(^11\).

It appears that a dimeric complex is preferred for the basic water-splitting function of PS2, irrespective of being prokaryotic or eukaryotic. As bacterial reaction centres are not arranged as dimers and they do not split water and evolve oxygen, the dimeric arrangement in PS2 might have emerged.
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with the transition to an oxygenic atmosphere, and thus, might be vital to optimize and sustain the water-splitting function.

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