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Historical review

Unraveling the Photosystem I reaction center: a history, or the sum of many efforts

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Abstract

This article describes some aspects of the history of the discovery of the structure and function of Photosystem I (PS I). PS I is the largest and most complex membrane protein for which detailed structural and functional information is now available. This short historical review cannot cover all the work that has been carried out over more than 50 years, nor provide a deep insight into the structure and function of this protein complex. Instead, this review focuses on more personal views of some of the key discoveries, starting in the 1950s with the discovery of the existence of two photoreactions in oxygenic photosynthesis, and ending with the race towards an atomic structure of PS I.

Abbreviations: CD – circular dicroism; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPIP – 2,6-dichlorophenol-indophenol; EM – electron microscopy; EPR – electron paramagnetic resonance; FNR – ferredoxin:NADP$^+$ oxidoreductase; PC – plastocyanin; RC – reaction center

The search for a reasonable model

It has long been known that photosynthesis is the main process on earth that converts light energy from the sun into chemical energy. The primary processes of photosynthesis in plants are located in special organelles, the chloroplasts, and chlorophyll plays a key role in this process. However, the nature of the biomolecules involved in this process long remained a mystery (Rabinowitch 1956). Light was shed on the process in the middle of the last century, when the concept of two photosystems was introduced and confirmed by experimental evidence.

The idea of System 1 and System 2 as physical parts of the photosynthetic apparatus and the seats of light-induced charge separation, each with specific antenna pigments, was first introduced by Duysens in 1960 (Duysens 1960; Duysens et al. 1961; see also the historical reviews of Duysens 1989 and Witt, this issue). A few properties of Photosystem I (PS I) had already been discovered as separate observations made in oxygen-evolving organisms, but they were not yet integrated in a coherent scheme. These observations included the following:

- The specific action of far-red light, in the so-called Emerson effect.
- The existence of non-fluorescing chlorophyll *a*.
- The ability of chloroplasts to photo-reduce NADP$^+$ for the reduction of CO_2.

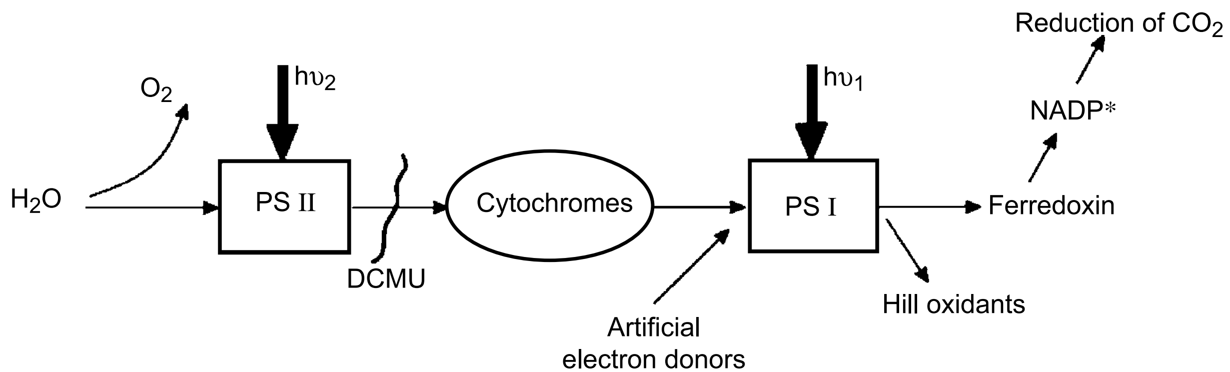


Figure 1. The pathway of electron transfer (thin arrows) from water to $NADP^+$ in oxygenic photosynthesis. Broad vertical arrows indicate transfer of absorbed excitation energy (hν) from the light-harvesting antennae to the reaction center of each of the two photosystems, PS I and PS II. The wavy line indicates the site of inhibition of electron transfer by DCMU (3(3,4-dichlorophenyl)-1,1′-dimethylurea).

- P700, discovered by Kok (1956) as a chlorophyll species giving rise to a bleaching at 705 nm under the effect of light.
- A light-induced EPR signal, discovered in 1956, and later named signal I (Commoner et al. 1956; Commoner 1961). It is apparently by coincidence that signal I and signal II of Commoner arise from species present in PS I and PS II, respectively.
- The oxidation of a *c*-type cytochrome, probably cytochrome *f*, but perhaps also cytochrome c_6 or c_8 in algae, in response to illumination.

Several possible models were advocated in attempts to integrate these and other data, as discussed by Duysens (1989). In 1960–1961, however, everything solidified and it became possible to integrate the separate observations in a coherent scheme. This started with the proposal by Hill and Bendall (1960) of two photo-acts functioning in series, a successful working hypothesis that, as recognized by the authors, had very little experimental support at that time. The first clear experimental evidence for the existence of two photosystems came from experiments of the Duysens and Witt groups (Duysens et al. 1961; Witt et al. 1961) which resolved fast electron transfer kinetics. Beinert et al. (1962) showed that the EPR signal I is that of oxidized P700. By 1962, the scheme of Figure 1, which is identical to one we use today, was widely accepted.

On these premises, further progress has consisted in the physical separation of PS I, in detailing electron transfer and energy transfer, and in determining the function and the topology of proteins. Finally, the 3-D structure brought many reliable answers, as well as a firm basis for new questions.

Physical separation of PS I: from a valuable model to the real demonstration of the reaction center

The year 1966 saw a flowering of results on the separation of PS I by various methods of enrichment following the treatment of chloroplast membranes by sonication, or by detergents such as digitonin (Anderson and Boardman 1966; Wessels 1966), Triton X-100 (Vernon et al. 1966) and sodium dodecyl sulfate (Ogawa et al. 1966). During purification, enrichment was assayed by the content of P700 (detected by ΔA or by EPR), by an ability to photo-reduce $NADP^+$ with ascorbate/DPIP as electron donor (Katoh and San Pietro 1966), or from low-temperature fluorescence at 735 nm (Boardman et al. 1966; Cederstrand and Govindjee 1966). The preparations were also enriched in cytochromes *f* and b_6 – it took a long time to realize that this was a contamination. Later on, the presence of 4Fe–4S clusters was used as another safe criterion.

Physical separation of the reaction center in purple bacteria (Reed and Clayton 1968) had a considerable impact on the research on oxygenic photosynthesis. The concept of a PS I reaction center became dominant, although its effective isolation remained a long-standing problem: in isolated PS I preparations, what are the parts of the true RC, of an associated antenna, and of contaminants? The small number of chlorophylls in the RC of purple bacteria also led researchers on the wrong track of trying to decrease as much as possible the number of chlorophylls per P700. A rather good RC with about 100 chlorophyll *a* per P700 was prepared by Bengis and Nelson (1975) (see also Nelson and Ben-Shem 2002), and the peripheral antenna was well characterized by Mullet et al.

(1980). The separation of PS I turned out to be easier in cyanobacteria, as they do not have a membrane-integral PS I peripheral antenna. After attempts with several species (e.g., Ogawa et al. 1969; Dietrich and Thornber 1971; Nechushtai et al. 1983), *Synechocystis* and *Synechococcus* were revealed to include the most promising species (Newman and Sherman 1978; Takahashi and Katoh 1982; Wynn et al. 1989; Rögner et al. 1990), as the PS I from *Synechococcus elongatus* (now named *Thermosynechococcus elongatus*) provides a very stable RC appropriate for crystallization (see below).

Analysis of electron transfer. Or: how the number of electron transfer carriers increased over the years

What is P700? After the initial studies by the groups of Kok and Witt, leading to the idea that P700 is a special chlorophyll molecule that gets oxidized in response to light excitation, the couple P700/P700^{+} was shown to have an Em of +430 mV (later revised to about +490 mV). The next major result came from the work of Döring et al. (1968) who proposed, based on optical difference spectra, that P700 is made of two chlorophyll molecules. This result was later supported by the work of Norris et al. (1971, 1974), whose EPR and ENDOR studies on P700^{+} led them to the same conclusion, by showing that the unpaired electron is delocalized in the state P700^{+}. These were pioneering works, with important consequences for the study of all types of RC. Döring et al. (1968) and Norris et al. (1971, 1974) advocated the name 'special pair,' which is still a good way by which to designate the primary donor in RC. Absorption and CD spectra of P700 were also interpreted as originating from a chlorophyll dimer (Philipson et al. 1972).

What else do we want to know about P700? The more we know, the more we want to learn. Are these two molecules really chlorophyll *a*? This was the well-established model until 2001. In the mid-1980s the Watanabe group found striking evidence that chlorophyll a' (the C13 epimer of Chl a) is related to P700 (Watanabe et al. 1985). However, the group's ideas were not accepted at this time by most of their colleagues, who argued that epimerization might have taken place after extraction in the organic solvent used for analysis. More than 16 years after it was first proposed, the X-ray structure at 2.5 Å (Jordan et al. 2001) shows unambiguously that P700 consists of a heterodimer of Chl a and Chl a'. The question of how these molecules are positioned in the membrane was addressed by linear dichroism of oriented samples (Breton et al. 1975; Rutherford and Sétif 1990), and the question of how they are held by the protein was addressed by site-directed mutagenesis and answered by X-ray crystallography (see below). What is the reason for the rather low Em of P700/P700^{+}? What is the repartition of unpaired electron and spin densities in P700^{+} and in the triplet state ^{3}P700? These are still widely debated questions (see e.g., Breton et al. 1999), and are important because the special pair is at the heart of the photosynthetic primary reaction.

The ability to follow P700 oxidation by flash absorption spectroscopy at 700 or at 820 nm has led to many flash kinetic studies by the groups of H.T. Witt (Witt et al. 1961), B. Ke (Ke et al. 1973) and P. Mathis (Mathis et al. 1978). These studies established the basic model of charge separation in the PS I RC, in parallel with studies on purple bacteria. With respect to the sequence of electron acceptors, the general model contends that the excited primary donor sends an electron to the most proximal part of a sequence of electron acceptors. The historical direction, however, is the inverse: discoveries went from the most remote to the more proximal acceptors. The research started with the soluble terminal acceptors, NADP^{+} and ferredoxin, and with FNR, an enzyme that ensures their redox interaction (Shin and Arnon 1965; Shin, this issue). Several experiments then led to the concept of a soluble species called ferredoxin-reducing substance, but this path was wrong. An essential discovery occurred when a bound iron–sulfur center, first named bound ferredoxin, was photo-reduced at 77K, as shown by EPR spectroscopy (Malkin and Bearden 1971). Very soon afterwards, Hiyama and Ke (1971) obtained very convincing evidence by flash kinetic spectrophotometry for a primary acceptor that they named P430. Several years were devoted to deepening the understanding of bound ferredoxin and P430. They are probably the same species, but bound ferredoxin includes two 4Fe–4S centers, named centers A and B (Evans et al. 1974), with very low redox potentials of about −530 and −580 mV (Ke et al. 1973). It took nearly 10 years to show that both centers are carried on the same polypeptide, now named PsaC (Hayashida et al. 1987), and a further 10 years to establish that electrons were going from P700 first to center A and then to center B (Diaz-Quintana et al. 1998).

In 1973, most if not all researchers were convinced that these bound ferredoxin(s) were the primary ac-

ceptors. However, in 1975 Evans et al. found strong EPR evidence for another Fe–S center, now named FX, which could be photo-reduced by PS I when centers A and B were already reduced. FX was shown to be a 4Fe–4S center, with a very low redox potential (about -0.8 V). It also had the unique property of being ligated by cysteine residues provided by both of the large protein subunits of PS I (Golbeck and Cornelius 1986). The discovery of FX was strongly considered as the final step in unraveling PS I electron acceptors: FX was thought to be the primary electron acceptor.

One of us (PM) was involved in experiments that led to the discovery of two electron acceptors that come before FX. While measuring the kinetics of electron return from acceptors to $P700^+$ under increasingly reducing conditions, and in PS I devoid of secondary acceptors, two kinetic phases were found, with half-times of 250 and 3 μs. The two phases were attributed to the return from FX and from an even earlier acceptor, named A_1 (Mathis et al. 1978; Sauer et al. 1978). These contributions were important because they opened new horizons; however, although the kinetic data were rich and correct, both hypotheses were wrong. The 250 μs phase actually comes from an acceptor named A_1, which preceeds FX, and which was later shown to be a phylloquinone molecule, while the 3 μs phase comes from a triplet state formed by back-reaction between $P700^+$ and the primary acceptor A_0 (Rutherford and Mullet 1981; Sétif et al. 1981).

In the 1980s the chemical nature of A_1, now known to be a phylloquinone molecule, was intensively investigated. Even if it was not initially expected that a quinone could have an Em low enough for that function (proteins or amino acid residues were favored instead), striking evidence for A_1 being a quinone was shown by different groups. Things were difficult because the spectroscopic properties of quinones are rather ambiguous, and also because there are so many of these molecules in photosynthetic membranes. In 1976 Thornber et al. showed that there is one phylloquinone per PS I reaction center, and speculated on a function analogous to that of quinones in purple bacteria. Many data then contributed to solving the problem, including flash absorption spectroscopy (Brettel and Golbeck 1995; Brettel 1997), EPR (Bonnerjea and Evans 1982; Gast et al. 1983; Heathcote et al. 1995, 1996), extraction-reconstitution (Schoeder and Lockau 1986; Itoh et al. 1987), and isotopic and photoaffinity labeling (Iwaki et al. 1992). See Golbeck and Bryant (1991) and Golbeck (1996) for further references. Intact PS I reaction centers contain two molecules of phylloquinone. Although one of them can be removed without significant functional damage, there is now a good indication that both participate in electron transfer, in parallel paths (see below). The first evidence for their location and orientation came from EPR investigations in solution (Dzuba et al. 1997) on oriented membranes (MacMillan et al. 1997) and single crystals (Bittl et al. 1997).

We finally reach the primary acceptor, A_0. A preliminary consensus, largely based on incorrectly interpreted or contradictory experiments, was rapidly reached for its chemical identity, chlorophyll *a*. There are multiple reasons that such a consensus may have been reached, including the fact that a pheophytin had been proved to be the primary acceptor in purple bacteria, that chlorophylls have distinctive features in differential absorption spectroscopy, and that their anion-radical is highly reducing (Fujita et al. 1978). A_0 was identified as chlorophyll *a* mainly because of a distinctive absorption bleaching around 690 nm associated with its reduction (Nuijs et al. 1986; Shuvalov 1976). Many EPR data are consistent with these findings. Let us add that the 3-D structure of PS I shows that two pairs of chlorophyll molecules are present on each branch, in addition to the Chl *a*/Chl *a′* heterodimer of P700. This structural arrangement implies that in fact electron transfer through A_0 may be a sequence of two steps, which were impossible to distinguish by spectroscopic methods.

Kinetic data are, of course, an essential part of the description of electron transfer. The kinetic processes in PS I were not as easy to analyze as those in reaction centers from purple bacteria. The sequence of electron acceptors is more complex, their absorption spectra are weak and overlap strongly, and there is no specific inhibitor comparable to DCMU in PS II. Moreover, the primary steps in PS I are very fast, and their rates are intertwined with those for energy transfer in the antenna. Electron transfer can be blocked at various points by a combination of methods: lowering the temperature, manipulation of the redox potential, removal of specific acceptors by treatment with solvents or by stripping away polypeptides (see Golbeck and Bryant 1991; Brettel 1997). Kinetics were measured by flash absorption spectrophotometry; photoelectric measurements were nicely resolved by picosecond electron transfer through A_0 and A_1 (Hecks et al. 1994; and see reviews by Brettel 1997 and by Brettel and Leibl 2001).

In a historical review, we cannot overlook the triplet state ^{3}P700 because its observation was a tool for the study of primary reactions, and since the properties of that state are a unifying factor in the realm of reaction centers. ^{3}P700 was first observed by one of us (Mathis et al. 1978; see also Shuvalov 1976), but was wrongly assigned, even though its kinetic properties were amazingly similar to those of its equivalent in carotenoid-less purple bacteria. Frank et al. (1979) were the first to make EPR measurements and the correct assignment. ^{3}P700 is populated with a high yield as a product of the primary radical pair ($P700^+A_0^-$) back reaction, but also as a product of the ($P700^+A_1^-$) back reaction. This property was recognized by Sétif and Brettel (1990) and is unique to PS I. The triplet state, together with other polarized EPR signals, has since been the object of multiple studies, often using refined physical methods (see e.g., Vrieze et al. 1996).

Antennae and energy transfer: an unanticipated level of complexity

In the early days, the definition of PS I was mostly based on one property of its antenna; the fact that it absorbed and utilized light of longer wavelengths (above 700 nm) than PS II. For references, we refer the reader to a review (Gobets and van Grondelle 2001), and here we simply list several of the questions that had to be solved. We would also like to remind the reader that, as far as electron transfer goes, historical progress in biophysical and biochemical understanding has occurred in parallel.

What is the composition of the antenna? The number of cofactors in PS I was long a matter of debate. The determination of the structure of the RC of purple bacteria in 1985 was a milestone with respect to the understanding of the reaction center, but with respect to the pigment composition of PS I, research was delayed. Many groups chose to instead focus their attention to the separation of the core antenna from the RC of PS I – an impossible task – because, as we now know from the X-ray structure, PS I, in contrast to the type II reaction centers, is a joint photoreaction center and antenna system (Krauß et al. 1996; Schubert et al. 1998; Jordan et al. 2001). The X-ray structure shows that the PS I complex includes 96 chlorophyll a molecules and 22 carotenes. In plants and algae, a peripheral antenna named LHC1, with about 200 chlorophylls (*a* and *b*) consists of four polypeptides (Lhca1 to Lhca4) each of about 20 kDa (see Jansson et al. 1996 and references therein). In cyanobacteria, LHC1 is absent, and phycobiliproteins play the role of a peripheral antenna. However, the interaction of PS I with its external antenna systems is still not well understood, with many new discoveries occurring only recently. In 2001 it was discovered that, under iron deficiency, a ring of 18 subunits of a membrane intrinsic IsiA protein (showing homologies to the antenna protein CP43 of PS II) surrounds the trimeric PS I in cyanobacteria, serving as a membrane intrinsic external antenna (Bibby et al. 2001; Boekema et al. 2001).

The core antenna system in PS I is unique, containing 90 Chl *a* and 22 carotenoid molecules. The chlorophylls do not form a symmetrical ring structure, as in the antenna of purple bacteria RCs, but show a clustered network of Chls in different orientations, in which each chlorophyll is linked functionally to more than one neighbor (Jordan et al. 2001). Functionally, investigations using ultra fast spectroscopy showed that, after many steps of energy transfer, the excitation reaches P700 in about 20 to 35 ps, and charge separation then takes place very rapidly (1–2 ps). However, there are still many unsolved problems.

PS I was discovered in the early 1960s by its pool of chlorophylls, which absorb at wavelengths $>$700 nm. What are the origin and the function of these pools of chlorophylls, a subject of investigations for more than 40 years? Even now, when the complex structure and location of all 96 Chls have been discovered, this question is still unsolved and is an important subject for present research. In this respect many other questions related to the antenna system still await answers. What is the origin of the fluorescence emission at 735 nm at low temperature? Why is there no fluorescence induction in PS I? What is the path of energy transfer in the RC? Knowledge is progressing regarding the rate of energy transfer to P700, which is the trap for energy, but the problem is rendered very difficult by the large size and complicated structure of the unique core antenna in PS I.

The emerging story of the polypeptides: composition, topology, and functions

Discovery of the polypeptide subunits of PS I has resulted from improvement of isolation procedures and

discovery of their genes. Whereas the main protein subunits of PS II and the ATP-synthase were already known in the 1970s, knowledge of proteins constituting PS I was rare. Although a plant PS I preparation, containing most of the presently known protein subunits, was reported in 1977 by Bengis and Nelson (1977); Nelson and Ben-Shem 2002), even in the early 1980s PS I was widely thought to consist of one large polypeptide named CPIa. At the beginning of the 1980s, spectroscopic investigations showed the existence of a 'ferredoxin-like' protein, but the assignment of this protein to some lower MW bands seen on SDS-gels was still a matter of debate. In 1985 Fish et al. (1985) discovered the two genes of the major protein subunits in PS I, PsaA and PsaB. Soon later, PsaC (subunit VII) was identified as the subunit, which carries the terminal FeS clusters (Høj et al. 1987; Ohoka et al. 1988; Wynn et al. 1989) leading to a first structural model of PS I. The model consisted of a heterodimer with two large subunits forming the central core, and the small subunit PsaC, together with subunits PsaD (subunit II) and PsaE (subunit IV), attached to its stromal side. The identification of the genes of PS I in cyanobacteria was a major breakthrough (Golbeck and Bryant 1991; Herrmann et al. 1991; Mühlenhoff et al. 1993), because, therewith, the small subunits were identified: first at the level of the genes, and subsequently at the protein level. Site-directed mutagenesis unraveled the functions of many of the smaller polypeptides; see Chitnis (1996) for a review on their identification. Between 1990 and 2000, it became well established that cyanobacterial PS I consists of 11 protein subunits – PsaA to PsaF and PsaI to PsaM, whereas plants contain 13 subunits. Plants do not contain PsaM, but they do have three additional subunits: PsaG, PsaH and PsaN. However, the unraveling story of proteins does not end here, because the recent X-ray structure shows that (at least a thermophilic) cyanobacterial PS I contains a 12 subunit, PsaX, which had previously been identified by N-terminal sequencing in two different thermophilic cyanobacteria (Koike et al. 1989; Ikeuchi et al. 1991). However, the gene sequence of the gene *psaX* is still missing. In addition, plant PS I has been found to contain more protein subunits than previously suggested, for example, a 14th plant PS I subunit, PsaO has recently been identified (Knoetzel 2002).

We now briefly discuss the identification of the electron acceptors ferredoxin and flavodoxin, and the electron donors plastocyanin and cytochrome *c*6. Ferredoxin was identified early on as the primary electron acceptor of PS I. Later, it was shown that flavodoxin can substitute for ferredoxin under iron deficiency (Bottin and Lagoutte 1992). All three extrinsic subunits PsaC, PsaD and PsaE interact with ferredoxin (for review see Setif et al. 2002). The ferredoxin binding pocket has been tentatively identified in the 6 Å PS I structure. From electron microscopy, the binding pocket appears to be essentially the same for ferredoxin and flavodoxin. Recently, cocrystals have been reported from PS I with ferredoxin (Fromme et al. 2002), but we have still to wait for a detailed structure of the complex.

In plants, the Cu-containing plastocyanin (PC) serves as an electron donor to PS I (Baszynski et al. 1971), whereas cytochrome *c*6 can substitute for PC in cyanobacteria (Bohme and Kunert 1980). See Katoh (2003) for a discussion of early research on the role of plastocyanin. In plants, PsaF is involved in docking of PC (Farah et al. 1995; Hippler et al. 1996). In cyanobacteria, however, PsaA and PsaB may exclusively form the binding pocket, because deletion of PsaF has no influence on the binding kinetics of PC (Hatanaka et al. 1992, 1993).

A race towards the structure of PS I

Three methods contributed to the unraveling of the structure of PS I: electron microscopy on single molecules and membranes; electron crystallography based on 2-D crystals of PS I; and X-ray crystallography based on 3-D crystallization of PS I. Furthermore, experiments with atomic force microscopy also provided structural information on PS I (Fotiadis et al. 1998). The structural work on PS I was very much driven by investigation of the photosynthetic apparatus in cyanobacteria. The structure of cyanobacterial PS I is now known in great detail, whereas structural information unique to plant PS I was very limited for a long time. Very recently, the structure of a supercomplex of plant PS I with the LHC I complex from pea was determined at medium resolution of 4.4 Å by Nathan Nelson and coworkers (Ben-Shem et al. 2003) This is a large step forward in the understanding of plant PS I. It is the first insight into the structure of the supercomplex of plant PS I with its peripheral antenna system, the light-harvesting complex I (LHC I). The resolution is limited to 4.5 Å resolution; thus no side chains or loops of the light-harvesting complex could be assigned. However, it is the second milestone in the

understanding of PS I, after the discovery of the structure of PS I from cyanobacteria at atomic resolution (2.5 Å) 2 years ago.

One of the authors (PF) was directly involved in the first determination of atomic structure of PS I, and we discuss here the history of this discovery in a little more detail. The structural investigations of PS I with electron microscopy started in the late 1970s, when Newman and Sherman (1978) described the first rod-like particles assigned to PS I in the cyanobacterium *Synechococcus cedrorum*. Williams et al. (1983) characterized a PS I particle isolated from *Synechococcus* 6301, which contained ∼130 Chl/P700, and the centers FA, FB and FX. The particle was ellipsoidal, with a length of 18 nm and a height of 8 nm. The mass was estimated to be 300,000 to 400,000 Da. In retrospect, this characterization fits very well with the size, shape and molecular weight (356,000 Da) of a monomeric PS I unit. The plant PS I was also investigated at the beginning of the 1980s by the Mullet group (Mullet et al. 1980), revealing a more spherical structure with a diameter of 106 Å. Towards the end of the 1980s, two groups investigated PS I from cyanobacteria by EM and single particle analysis, detecting for the first time the co-existence of a monomeric and trimeric structure of PS I (Boekema et al. 1987, 1989; Ford 1987; Ford and Holzenburg 1988; Rögner et al. 1990). Today it is known that both the monomer and the trimer can exist in the natural membrane, depending on the environmental growth conditions. Through the use of electron microscopy, the trimeric organization of PS I has also been shown for *Prochlorotrix hollandica*, a cyanobacterium that contains Chl *b* (van der Staay et al. 1993). Prior to the high resolution X-ray structure, electron microscopy on deletion mutants was also helpful for the assignment of individual proteins in the PS I complex (Kruip et al. 1993). A step further toward the structure determination was provided by 2-D crystallization and electron crystallography. The first 2-D crystals of cyanobacterial PS I were observed and investigated by Ford et al. (1990). This work and further work on 2-D crystals of PS I from cyanobacteria unraveled the common molecular shape of the PS I complex (Böttcher et al. 1992; Hefti et al. 1992).

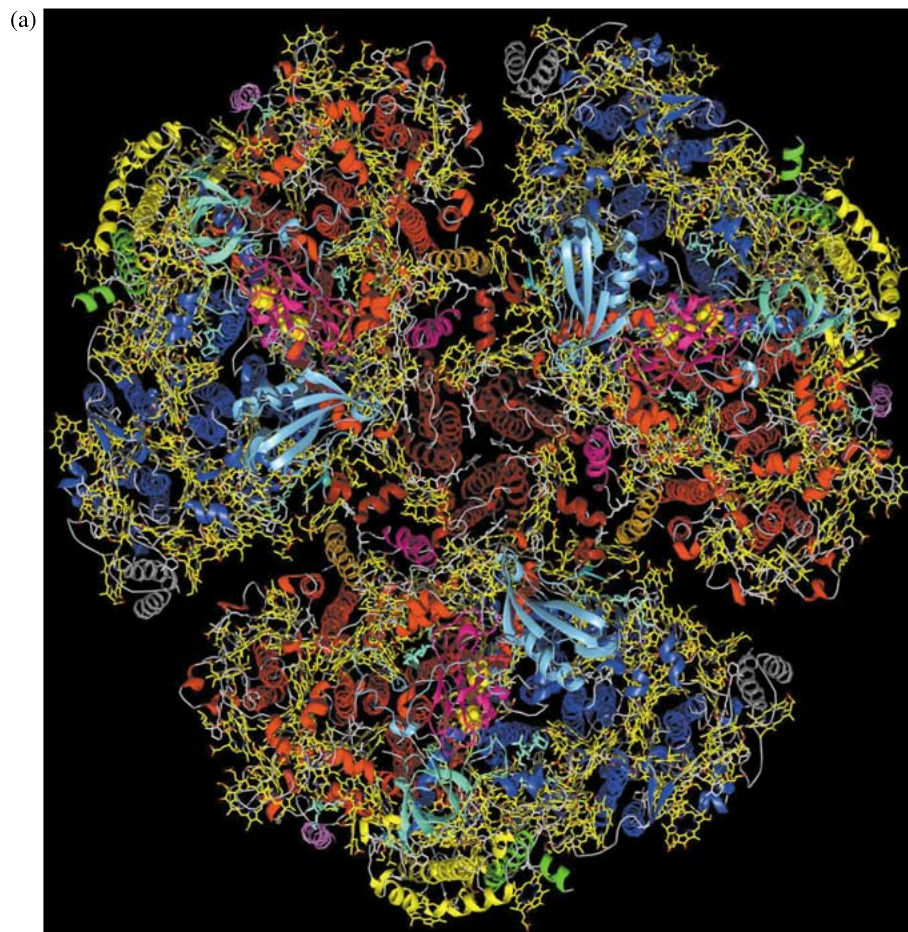
Even today, important structural information for the plant system is provided by single particle analysis and image processing (Boekema et al. 1990) or by electron microscopy/crystallography on 2-D crystals. In 1997 and 1998 Robert Ford's group reported the first successful 2-D crystallization of plant PS I (Kitmitto et al. 1997, 1998).

Very recently the complex isolated from green algae was also investigated by electron microscopy, revealing clues about the arrangement of the external antenna system and the localization of individual subunits that are unique to plants (Germano et al. 2002; Kargul et al. 2003). In addition, Ben-Shem et al. (2003) have recently published a structure at 4.4 Å for PS I from pea (*Pisum sativum*). This structure demonstrates that plant PS I is monomeric, with trimer formation forbidden by the small subunit psaH. Four plant Lhca polypeptides are linked in dimer of dimers, forming a half-moon shaped antenna on one side of the reaction center, otherwise substantially similar to cyanobacteria PS I. The electron microscopy results provided structural information at low resolution; however, the major and most exciting goal for the determination of the structure was to determine the structure of PS I at atomic resolution. In 1987, the first crystals of cyanobacterial PS I were reported by two groups: Robert Ford and his collaborators reported the crystallization of PS I from the thermophilic cyanobacterium *Phormidium laminosum* (Ford 1987) and the group of Ingrid and Horst Tobias Witt (Witt et al. 1987) reported the crystallization of PS I from the thermophilic cyanobacterium *Synechococcus* sp. (now re-named *Thermosynechococcus elongatus*). Further improvements in and characterizations of the crystals were reported one year later from both groups (Ford et al. 1988; Witt et al. 1988). For pictures of H.T. Witt and Ingrid Witt, his wife, see article of H.T. Witt in this issue (Witt, this issue).

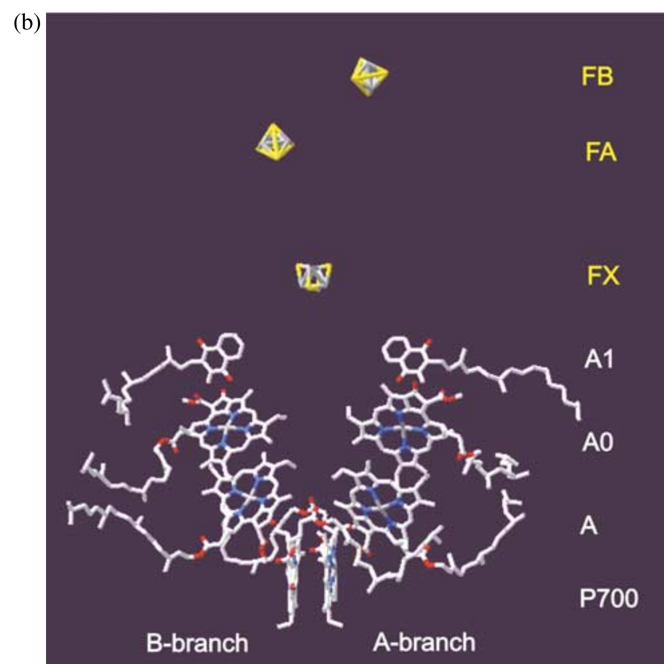
In 1988, many investigators became excited by these results, anticipating a low-resolution structure of PS I within a year. However, it actually took four more years for the first X-ray structure of the trimeric PS I from a *Synechococcus* sp. (now named *T. elongatus*) to be determined at 6 Å resolution (Krauß et al. 1993). Considerable effort was made to improve the reproducibility and quality of the crystals, work which included variation and optimization of the growth conditions of cells, isolation procedure, and crystallization conditions (Witt et al. 1992; Witt, this issue).

In the meantime, PS I was crystallized from other cyanobacteria. Rachel Nechustai's group reported the crystallization of PS I from the thermophilic cyanobacterium *Mastigocladus laminosus* (Shoham et al. 1990; Almog et al. 1991), while Hartmut Michel's group reported crystallization of PS I from the mesophilic cyanobacterium *Synechococcus* PCC

(a)



(b)



7002 (Tsiotis et al. 1993). In the same year, crystallization of pea PS I was reported in the thesis of B. Andersen (Andersen and Scheller 1993; Andersen 1994).

Further attempts were made to crystallize the monomeric PS I (Jekow et al. 1995, 1996), however, the crystals never reached the quality of the trimeric PS I crystals, probably because of the lower stability of the monomeric complex.

The big question was whether the quality of the crystals could be improved, so that they might diffract X-rays to higher resolution leading to an improved 3-D structure. Because of the loose packing behavior of PS I in the unit cell, most researchers were not optimistic that the resolution of the crystals of the trimeric PS I complex from *S. elongatus* could ever be improved. The packing behavior was consistent with a solvent content of 78%, and with less than 5% of the surface of the protein being involved in crystal contacts (i.e., form the contact sites between the PS I complexes within the unit cell). As we now know, only four salt bridges per monomer form the contact sites within the crystals, despite the fact that a single PS I trimer consists of more than 100,000 atoms.

Despite these objections and reservations, we persisted, and were able to improve the quality of the crystals. A completely new isolation procedure was developed, one which uses only a single detergent for all purification and crystallization steps, and which keeps all proteins and cofactors within the complex. Based on these improved crystals, the structure of PS I was improved to 4 Å resolution (Krauß et al. 1996; Schubert et al. 1997). There, we saw for the first time the general arrangement of the 36 alpha helices in the transmembrane region of the PS I reaction center; 78 chlorophylls had been identified, with 6 of them assigned to the electron transfer chain. Although there could be no assignment of the extrinsic subunits PsaC, PsaE and PsaD, we had finally identified the main body of PsaC and the terminal FeS clusters, FA and FB, of the electron acceptors it contains.

This structure showed that, in contrast to the type II reaction centers, PS I represents a joint antenna and reaction center system, and was a big step forward in understanding of the structure and function of PS I. However, the amino acids and major cofactors, such as the carotenoids, could not be assigned at this resolution and therefore the location of the individual subunits and details of the protein–cofactor interaction, were still unknown. Would it be possible to further improve the resolution of the crystals? One major problem of the crystallization process lies in the weak interaction between the trimeric units in the crystals. Therefore, the crystals suffer from all kinds of disturbances induced by the gravity of the earth, including interaction with the walls of the reaction vessel during growth, sedimentation and convection. One way to avoid this problem is by leaving the gravity field and crystallizing PS I under microgravity in space.

We participated in four missions of the Space Shuttle Columbia in which we crystallized PS I under microgravity. The missions were 1995 USML-2, 1997 LMS, 1998 STS-95, and the tragic last mission, STS-107, in which the Shuttle exploded during reentry over Texas on the 1st of February 2003, with the loss of all seven crew members. No results could be rescued from the last mission, but very promising results were obtained in the previous missions. The crystals grew under microgravity to a size, which was 20 times greater than any PS I crystal grown before, on earth. The largest crystal from the USML-2 mission was 4 mm long and had a diameter of 1.5 mm. At that time, in 1995, earth-grown PS I crystals diffracted only to 5 Å, and had a mosaicity[1] of $>1°$, these crystals diffracted to 3.3 Å. Because of the large size of the crystal from USML-2, a complete native data set could be collected at room temperature on one single crystal. This is the best data set ever collected from PS I crystals without freezing. The derivative data sets had to be measured from earth-grown crystals, limiting the resolution, still, to 4 Å. However, because of the very good quality of the native data set from the

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Figure 2. (a) Structure of the trimeric PS I from *T. elongatus* as determined by X-ray structure analysis at 2.5 Å resolution (Jordan et al. 2001). The direction of view is perpendicular to the membrane plane, and from its stromal side. The 12 different protein subunits are shown in a ribbon representation in different colors. Color coding of the membrane intrinsic subunits: PsaA, blue; PsaB, red; PsaC, light pink; PsaD, light green; PsaE, cyan; PsaF, yellow; PsaI, dark pink; PsaJ, green; PsaK, gray; PsaL, brown; PsaM, orange; and PsaX, violet. Chlorophylls are represented by their chlorin-headgroups (yellow), carotenoids are depicted in light gray, and the lipids are shown in turquoise. (b) The pathway of electron transfer. The view is parallel to the membrane plane. The organic cofactors of the electron transfer chain are arranged in two branches. The left branch is called the B-branch, whereas right branch is the A-branch. The names indicate that most (but not all cofactors of the A/B-branch are coordinated by PsaA/PsaB. The three [4Fe–4S]-clusters FX, FA and FB are located at the stromal side of the membrane (on top of the figure). At right margin the names of the cofactors are given as derived from spectroscopic investigations.

crystal that was grown under microgravity, the electron density map was improved dramatically, so that, for the first time, all extrinsic subunits PsaC, PsaE and PsaD were assigned, and all cofactors of the electron transport chain became visible (Klukas et al. 1999a, b) (for the PS I team see Figure 5). This was a great improvement, but the major goal – the structure of PS I at atomic resolution – was still not achieved. The space shuttle experiments gave the first indication that a lamellar phase of the detergent is involved in nucleation. To increase further the quality of the crystals on earth and to grow well-ordered single crystals, avoiding the nucleation problems, we determined the full phase diagram for the solubility of PS I, which depended on important physical and chemical parameters such as ionic strength, the nature of the salt, temperature, pH, etc., as well as on the presence of cryo-protectants. Detailed knowledge of the physical chemistry behind the solubility, stability and crystallization behavior of PS I allows us to implement sophisticated seeding techniques which lead to a dramatic improvement of the crystals and to a structure at 2.5 Å resolution, and to find proper conditions in which to

Figure 3. Top: Petra Fromme. *Middle*: Photo of Hill Prize award ceremony, Petra Fromme (left) and Nobert Krauß (middle). Also seen is Jan Anderson (right) who presented the award to Fromme and Krauß in Brisbane, Australia, in 2001. *Bottom*: Athina Zouni, Norbert Krauß, Petra Fromme, Govindjee and Horst T. Witt at the Hill Prize award ceremony.

Figure 4. Paul Mathis (author). *Top*: 7 April 1994; *bottom*: working on his first flash absorption apparatus in March 1966.

Figure 5. The Berlin PS I crew. This photograph was taken during data collection at the beamline in Tsukuba in 1998, where we collected data from PS I crystals using the Weissenberg technique. *Back row from left to right*: Wolf-Dieter Schubert, Norbert Krauß, Patrick Jordan and Petra Fromme. In front: Olaf Klukas.

Figure 6. Hervé Bottin and Paul Mathis discussing results with Alex Péronnard and with American visitors Devens Gust and Tom Moore, in 1983. *From left to right*: Tom Moore, Paul Mathis, Alex Péronnard, Devens Gust and Hervé Bottin.

freeze the crystals without increasing their mosaicity (Fromme 1998, 2002; Jordan et al. 2001). The structure of the trimeric PS I at 2.5 Å resolution is shown in Figure 2a. Figure 2b shows the electron transport chain of PS I, as revealed by the crystal structure.

This structure (Figure 2) showed for the first time all cofactors present in one monomeric unit. All 127 cofactors have been assigned; 96 chlorophylls, 22 carotenoids, 3 4Fe–4S clusters, 2 phylloquinones, and 4 lipids. All protein–cofactor interactions are therefore now known. These interactions will form the basis for a deeper understanding of the function of PS I, and for answering many exciting questions that still remain concerning its function.

Conclusions: unsolved problems and prospects for the future

History is unpredictable. In the case of PS I, for which knowledge at beginning of the 1980s was much less refined than for PS II, much more is now known concerning its structure and function. In the 1960s, PS I was considered to resemble most closely the RC of purple non-sulfur bacteria, because of the similarity of P700 and P870, and the fact that both oxidize a c-type cytochrome. However, as more became known about the system, more evidence accrued that PS I belongs to a different class of photoreaction centers, together with the heliobacteria and green sulfur bacteria. These type-I reaction centers share the common feature of having Fe–S clusters as the terminal Fe–S centers. The close relationship between these type-I reaction centers has been a hot topic for more than 10 years (Nitschke and Rutherford 1991; Blankenship 1992; Vermaas 1994; Schubert et al. 1998). The determination of the structure of the cyanobacterial PS I at 2.5 Å has now opened the way for investigation of many further exciting questions concerning the structure and function of PS I. Some of these are listed as follows:

- Are both branches active in electron transport in PS I? This is a controversial topic (Guergova-Kuras et al. 2001; Hastings and Sivakumar 2001; Rigby et al. 2002; Xu et al. 2003a, b).
- How does the excitation energy transfer from the bulk antenna to P700 occur?
- Why does P700 have a heterodimeric structure, consisting of one Chl *a* and the C13 epimer of Chl *a*?
- How are the thermodynamic properties of the 127 cofactors influenced by the protein?
- What is the function of the 5 *cis* carotenoids (among the 22 carotenoids in total) and the four lipids in PS I?
- How does PS I interact with the external antenna, both in cyanobacteria and in plants?
- How is PS I assembled?

With the determination of the structure, the race concerning the understanding of the structure–function relationship of all the cofactors and their functions has just begun. This race will keep us busy in the coming decades.

Figure 7. Gallery of photographs of the PS I researchers at Commissariat & l'Énergie Atomique (CEA) in Saclay, France. *Top left*: Bill Rutherford (left) and Jonathan Hanley (right), April 2000. *Top right*: Bill Rutherford, Klaus Brette, Bernard Lagoutte and Pierre Setif. *Bottom left*: Pierre Sétif studying PS I electron acceptors by EPR, in 1983. *Bottom right*: Klaus Brettel (center) teaching the method of flash absorption during a European Science Foundation (ESF) school in Saclay, in 1994.

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Figures 3 and 5 show photographs of one of the authors (Fromme) with her collaborators. Figures 4, 6 and 7 show photographs of the other author (Mathis) along with his collaborators.

Note

1. The mosaicity is indicative for the disorder of the crystals. In an ideal crystal (which does not exist), the reflections would have a infinitesimal small width, so during data collection and rotation of 0.5°/image each reflection would be only visible on one image at the time. Local disorder leads to a broadening of the reflections. A mosaicity of 1° indicates that the reflections are disordered over a width of 1°.

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