Photosynthesis Research 7, 97–114 (1985).

Minireview

Polypeptides of photosystem II and their role in oxygen evolution*

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(Received: 1 October 1984; accepted: 16 November 1984)

Key words: oxygen evolution, photosynthesis, photosystem II, polypeptides, reaction center

Abstract. The linear, four-step oxidation of water to molecular oxygen by photosystem II requires cooperation between redox reactions driven by light and a set of redox reactions involving the S-states within the oxygen-evolving complex. The oxygen-evolving complex is a highly ordered structure in which a number of polypeptides interact with one another to provide the appropriate environment for productive binding of cofactors such as manganese, chloride and calcium, as well as for productive electron transfer within the photoact. A number of recent advances in the knowledge of the polypeptide structure of photosystem II has revealed a correlation between primary photochemical events and a 'core' complex of five hydrophobic polypeptides which provide binding sites for chlorophyll a, pheophytin a, the reaction center chlorophyll (P680), and its immediate donor, denoted Z. Although the 'core' complex of photosystem II is photochemically active, it does not possess the capacity to evolve oxygen. A second set of polypeptides, which are water-soluble, have been discovered to be associated with photosystem II; these polypeptides are now proposed to be the structural elements of a special domain which promotes the activities of the loosely-bound cofactors (manganese, chloride, calcium) that participate in oxygen evolution activity. Two of these proteins (whose molecular weights are 23 and 17 kDa) can be released from photosystem II without concurrent loss of functional manganese; studies on these proteins and on the membranes from which they have been removed indicate that the 23 and 17 kDa species form part of the structure which promotes retention of chloride and calcium within the oxygen-evolving complex. A third water-soluble polypeptide of molecular weight 33 kDa is held to the photosystem II 'core' complex by a series of forces which in some circumstances may include ligation to manganese. The 33 kDa protein has been studied in some detail and appears to promote the formation of the environment which is required for optimal participation by manganese in the oxygen evolving reaction. This minireview describes the polypeptides of photosystem II, places an emphasis on the current state of knowledge concerning these species, and discusses current areas of uncertainty concerning these important polypeptides.

Abbreviations

A 23187, ionophore that exchanges divalent cations with H^+; Chl, chlorophyll; cyt, cytochrome; DCPIP, dichlorophenolindophenol; DPC, diphenylcarbazide; EGTA, ethyleneglycoltetraacetic acid; P680, the chlorophyll a reaction center of photosystem II; pheo, pheophytin; PQ, plastoquinone; PS, photosystem; Q_A and Q_B, primary and secondary plastoquinone electron acceptors of photosystem II; Sn (n = 0, 1, 2, 3, 4), charge accumulating state of the

*The survey of literature for this review ended in September, 1984.

oxygen evolving system; Signals II_{vf}, II_f and II_s, epr-detectable free radicals associated with the oxidizing side of photosystem II; Z, primary electron donor to the photosystem II reaction center.

Introduction

The linear 4-step oxidation of water to molecular O_2 by photosystem II (PSII) of algae and higher plants is a unique and fascinating reaction whose properties have been revealed by the pioneering research of Kok and Joliot [1]. A number of reviews of PSII are available, and those of Radmer and Cheniae [2] and, more recently, of Renger and Govindjee [3] should be consulted for in-depth coverage of the oxygen-evolving reaction. The oxidizing side of PSII operates at unusually high redox potentials ($2H_2O \rightarrow O_2 + 4e^- + 4H^+$, $E_0' = +0.815V$) and this implies the presence of a structural environment which can contribute to the stabilization of the reactive chemical intermediates formed during the oxidation of water. Many, but most likely not all, of the catalytic components of PSII have been identified. As will be seen, these components can be segregated with respect to function (in either water oxidation or in the photochemistry/redox reactions which are coupled to the oxygen-evolving complex) as well as with respect to localization within the structural domains of PSII. A further segregation of components of PSII occurs when one inspects their mode of binding within the PSII complex: the cofactors required for O_2 evolution activity are more loosely bound to PSII than are the members of the photochemical/redox assembly. Extractable components of the oxygen-evolving complex include Mn, Cl^- and Ca^{2+}. The involvement of Mn in O_2 evolution is well-documented [2, 4–6]; 4 atoms of the metal, all essential for activity, are present per PSII reaction center [7]. Likewise, an extractable pool of Cl^- is an essential constituent of the oxygen-evolving complex [8, 9a, b); the presence of the anion stabilizes activity against inactivation by heat, elevated pH and amines [9–13]. The anion probably binds to a site on, or near, Mn [13, 14]. A Ca^{2+} requirement for PSII activity has been proposed [15, 16a, b]; recent work has established the existence of a high-affinity Ca^{2+} binding site within the oxygen-evolving complex [17–19a].

The tightly bound constituents of the PSII complex are those components of the reaction sequence which utilize photochemistry to generate the strongly oxidizing potentials which are required to produce O_2 from water. These components (Z, P680, pheo, Q_A, see list of abbreviations) and their function are reviewed in *Photosynthesis Research* by van Gorkom. (See also the review by Renger and Govindjee [3].) The components of the PSII complex are arranged as follows:

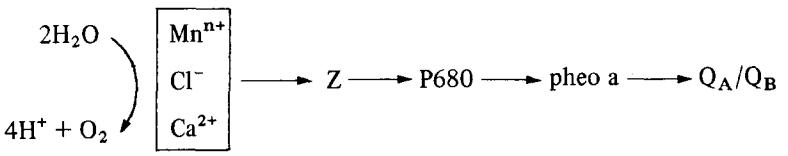

Table 1. Components of the isolated PSII core complex

Component	Stoichiometry	Function	Reference
Chl a	50 Chl/R.C.; 1P680/R.C.*	Antenna; R.C.	[22]
pheo a	2/50 Chl	Intermediate acceptor	[22]
Carotenoids	1/5 Chl	–	[22]
PQ-9	2/50 Chl	Z; Signal II_s; Q_A	[27]
Signal II_s; $Z^{\cdot+}$	1/115 Chl; 1/94 Chl	Z = primary donor to $P680^+$	[26, 28]
α-Tocopherol α-Tocopherolquinone	< 0.1/50 Chl	–	[27]
cyt b559	1/40–70 Chl	See ref. [31]	[22]

*RC = reaction center, other abbreviations are defined in the abbreviation list.

The representation shown above is an obvious oversimplification of the reaction sequence. One of the fundamental complexities of PSII is structural in nature; although catalysis of water oxidation, primary photochemistry, and other redox reactions require small inorganic ions and organic molecules, these species are ligated to a macromolecular superstructure comprised of a number of polypeptides. These polypeptides are organized within the thylakoid membrane bilayer so that reaction components are brought into proximity with one another, reactive intermediates are insulated from one another, and a vectorial pathway of electron transfer is established within the PSII complex. This architectural arrangement enables the process of water oxidation to be coupled to subsequent electron transfer reactions, and it facilitates the donation of protons from water oxidation, ultimately to the photophosphorylation reaction.

A recent period of intense research activity has enhanced our view of the polypeptide composition of PSII and has revealed the relationship between certain of these polypeptides and sites of catalytic activity within the PSII complex. It is now clear that the architecture of PSII is comprised of two domains. One of these, associated with the oxygen-evolving complex, is made up in part of water-soluble polypeptides. The second domain is hydrophobic in nature and contains components closely associated with the photochemistry of PSII.

The hydrophobic domain of PSII: the reaction center 'core' complex

Research initiated by Vernon and Shaw (20) showed that Triton X-100 could be used to isolate membrane fragments of the chloroplast thylakoid membranes (denoted TSF2 or TSF2a) which were capable of DCPIP reduction when DPC was supplied as an electron donor. Subsequent work by Satoh and Butler [21], Satoh [22], et al. [23] and Diner and Wollman [24] has produced highly refined preparations of a PSII 'core' complex which has made it possible to define rigorously the polypeptide composition of the PSII hydrophobic domain and to identify the components of PSII which are associated with this domain. The components of the PSII 'core' complex are

listed in Table 1. As can be seen from the Table, the components necessary for electron transfer from Z through P680 to Q_B (the exchangeable quinone/herbicide binding site) are associated with the isolated 'core' complex. It should also be noted that there is no firm consensus as yet on the quinone content of the complex. If Z and Signal II_s are both plastoquinone (PQ) molecules [25, 26], then along with Q_A the total quinone complement of the isolated complex should be 3 molecules of PQ per 50 or so Chl. The most recent work (Omata et al. [27]) places the number at 2, and estimates from a *Chlamydomonas* preparation [28] are even lower. There is no clear explanation for these discrepancies, but it may be that extraction techniques for quinones may not release all of the PQ bound to the core complex. The continuing interest in the roles of quinones in PSII, and the relationship between Z and Signals II_{vf} and II_f are topics which will certainly receive further investigation.

It is apparent that reaction center preparations of PSII contain about 50 molecules of Chl per complex, regardless of the biological origin or methods used for isolation [22, 28]. It is also of interest to note that the cyt b_{559} content of the reaction center complex (1 heme per reaction center) matches the stoichiometry detected in some oxygen-evolving PSII preparations [29, 30]. The functional role of cyt b_{559} remains enigmatic; the recent proposal of Butler and Matsuda [31] for cyt b_{559} function on the oxidizing side of PSII merits serious consideration by researchers interested in the mechanism of oxygen evolution.

The polypeptides of the PSII "core" listed in Table II are those found in higher plants. However, this same polypeptide assembly is present in a green alga [24] and cyanobacterium [32] and may thus have remained stable throughout a long period of evolution. As can be seen from the Table, the complex consists of 5 different polypeptides which range in size from 47 to 7–10 kDa (the molecular weights vary depending on the system used for electrophoresis; the molecular weights listed here are derived from determinations made by Satoh [22] using SDS-polyacrylamide gel electrophoresis in the presence of 6 M urea). These polypeptides serve as ligand sites for the various components of PSII and in many cases, tentative or firm assignments of component binding to a particular polypeptide have been made as shown in Table II. It seems clear that the 7–10 kDa polypeptide

Table 2. Polypeptide constituents of the PSII 'core' complex

Molecular weight	Tentative assignment of	Reference
(kDa)	binding site for:	–
47	P680 and pheo a; Chl *a*	(40–42)
43	Chl *a*	(40, 41)
32	Herbicides; Q_B	(23)
30 (or "34")	Mn (?)	(36–39)
10	cyt b559	(34, 35)

is the site for binding of the Cyt b_{559} heme moiety [33, 34], and the amino acid sequence of this polypeptide is known from gene-sequencing work (W.A. Cramer, personal communication). The 32 kDa polypeptide is the species responsible for herbicide and Q_B binding [23]; again, the amino acid sequence of this protein has been derived from gene sequences [35]. Herbicide binding to the 32 kDa species of the complex is weak (Bricker, T.A., personal communication) and this finding correlates with the observation that electron transport to DPIP is less sensitive to herbicide inhibition in this preparation than in intact thylakoid membranes [22]. The hydrophobic 30 ("34") kDa polypeptide of the 'core' complex may be involved in Mn binding, since this protein, also found in O_2 evolving PSII membranes from maize [36], has been proposed to share a common identity with the 34–36 kDa protein detected by Metz et al. [37] and Metz and Bishop [38] (see also Bishop [39] for an overview) in a *Scenedesmus* mutant which is defective in O_2 evolution activity and Mn binding capacity.

The 47 and 43 kDa subunits of the 'core' complex are observed in pigment-free forms on SDS-urea gels; however, both polypeptides have been partially [40] or completely [41a, b] purified with bound Chl. The 'impure' preparations carry out DCPIP reduction with DPC as the electron donor, and this activity correlates with fractions enriched in the 47 kDa protein [40]. Electrophoretically purified preparations of the 47 kDa protein show a 77 K fluorescence emission peak at 695 nm and may be enriched in pheo *a* [41]. More recent work by Tang and Satoh [42] on the isolated 47 kDa protein confirms the presence of a 695 nm fluorescence band. The polypeptide contains 3 Chl a, some pheo *a* (1 molecule per 3 molecules of 47 kDa protein), no PQ-9, and is inactive in the DPC → DCPIP assay. This preparation of the 47 kDa protein does not reduce pheophytin. As Tang and Satoh point out, the stoichiometries of bound pigments may have been altered during the isolation procedure, which includes substantial periods of exposure to detergent solutions, and primary photochemistry may also have been altered during isolation. Since the 43 kDa polypeptide has a 77 K fluorescence emission at 685 nm, but not at 695 nm [41], this polypeptide is currently viewed as a site for antenna pigment binding. Although most of the work in the literature concerning the 'core' complex has been performed with material from higher plants, Yamagishi and Katoh [32b] have obtained similar results on partially pure preparation of the 47 and 43 kDa proteins from the thermophilic cyanobacterium *Synechococcus*, where the properties and activities of the 47 kDa fraction are very similar to those of the polypeptide isolated from higher plant chloroplasts.

The hydrophilic domain of PSII: water-soluble 33, 23 and 17 kDa polypeptides

A recurrent theme in the literature concerning PSII prior to the early 1980's was that oxygen evolution activity was an incredibly complex reaction

which was very susceptible to partial damage or total destruction. This view of the process of oxygen evolution has been proven erroneous by two lines of biochemical investigation: (1) Pioneering experiments by Åkerlund et al. [43] and Sayre and Cheniae [44] have shown that the oxygen-evolving complex can be depleted of polypeptides by salt-washing or cholate extraction with loss of activity and that the extracted polypeptides can then be reconstituted onto depleted membranes, which results in partial restoration of the oxygen evolving reaction; and (2) several techniques involving the drastic use of detergents (Triton X-100, digitonin) have been developed for the isolation of highly active oxygen-evolving PSII membranes [45–47]. These two sets of experimental observations have generated an enormous amount of research on the polypeptide composition of the oxygen evolving reaction and have led to the partial elucidation of the structure and organization of its active site.

The experiments of Åkerlund et al. [43] first showed that exposure of everted thylakoid vesicles to dilute (250 mM) NaCl solutions produced a loss of O_2 evolution activity concomitant with the extraction of water-soluble polypeptides. Of these polypeptides, a 23 kDa species produced strong reconstitution of oxygen evolution activity when it was rebound to salt-washed membranes; a 17 kDa species possessed much weaker reconstitution activity. Later research by Toyoshima et al. [48], using cholate extraction of thylakoid membranes, has shown that both the 23 and 17 kDa polypeptides are required for reconstitution of activity. The disparity between the results of Åkerlund et al. and Toyoshima et al. may arise from the differences in extraction techniques (dilute salt versus cholate) and the methods used for reconstitution. Other experiments utilizing PSII-enriched membranes have confirmed the results obtained with everted thylakoid vesicles, namely that salt-washing of PSII membranes will remove water-soluble 23 and 17 kDa proteins [29, 49a–52]. Some dispute has arisen as a result of these experiments regarding the necessity for the 23 kDa protein in oxygen evolution activity. Murata et al. [48, 50] show that loss of activity (50%) cannot be correlated precisely with loss (100%) of the 23 kDa protein. Part of this discrepancy may be due to the difficulty encountered in using densitometer scans of coomassie-stained polyacrylamide gels to quantitate release of a polypeptide (the 23 kDa) which electrophoreses in the same region as the diffuse light harvesting Chl *a*/Chl *b* complex polypeptides. The recent immunoelectrophoresis experiments by Larsson et al. [53] and Andersson et al. [54] suggest that there is indeed a close correlation between extraction of the 23 kDa protein and loss of O_2 evolution activity. Many of the differences among results relating to the activities of polypeptide-depleted membrane preparations have been resolved by recent work in Murata's laboratory (N. Murata, personal communication). These new data establish a critical relationship between ion (Ca^{2+}, Cl^-) concentrations and their

effects on the oxygen evolution activity of polypeptide-depleted PSII membranes.

The function of the 23 and 17 kDa proteins as components of the oxygen evolving complex has been studied by several groups. Åkerlund et al. [55] have found the same degree of inhibition and reconstitution of activity by removal and addition of the 23 kDa protein in everted vesicles using continuous and flashing light. They suggest that salt-washing causes a complete inhibition of activity rather than a slowing of the rate limiting step in the oxygen evolving reaction. Parallel studies [56] showed that salt extraction enhances the $P680^+ Q_A^- \rightarrow P680Q_A$ back reaction. Åkerlund [57] has used a platinum rate electrode to show that salt-washing of everted thylakoid vesicles exposes the oxidizing side of PSII to H_2O_2, and has proposed that salt washing exposes the S_2 state of the O_2 evolving complex (see ref. 1 for a descripition of the S-states) to reduction by H_2O_2. Ghanotakis et al. [30] have used EPR measurements of $Z^{\bullet+}$ decay to show that release of the 23 and 17 kDa polypeptides from PSII membranes blocks S_3 formation and exposes the pool of functional Mn to exogenous reductants. It is thus apparent that although release of the 17 and 23 kDa polypeptides by salt washing does not extract the pool of functional Mn, this treatment does generate an alteration in the operation of the oxygen evolving complex. This change is characterized by the loss of S_3 formation and a structural change which permits reductants to interact with the functional Mn pool.

An alternative approach to elucidation of the function of the 23 and 17 kDa polypeptides was reported by Ghanotakis et al. [18], who showed that addition of high, non-physiological concentrations (> 5 mM) of Ca^{2+} to salt-washed PSII membranes would restore high rates of oxygen evolution concomitant with an acceleration in the decay of $Z^{\bullet+}$. Murata and Miyao [58] and Nakatani [59] have also shown that high Ca^{2+} concentrations reconstitute activity in salt-washed PSII membranes. When the role of Ca^{2+} and the 23 and 17 kDa polypeptides was investigated further, it was shown [19a] that a dialysis procedure could be used to rebind the water-soluble 23 and 17 kDa polypeptides to salt-washed PSII membranes. This procedure, executed under conditions which excluded Ca^{2+}, did not reconstitute oxygen evolution activity. Subsequent addition of Ca^{2+} then reactivated the oxygen-evolving complex; a Ca^{2+} titration of the reactivation process showed that the presence of the 17 and 23 kDa polypeptides resulted in a substatially lowered Ca^{2+} requirement for maximal activity. In addition, the polypeptide-reconstituted membranes bound Ca^{2+} at a high affinity site; the combination of EGTA and A23187 were incapable of inhibiting the reconstituted activity. These results confirm earlier proposals [15–17] that Ca^{2+} is an essential constituent of the oxygen evolving complex, and show in addition that the 17 and 23 kDa polypeptides are essential for productive binding of Ca^{2+} at physiological ($<$ mM) levels. It has been implied (59) that since Ca^{2+}

by itself is capable of restoring O_2 evolution activity, the 17 and 23 kDa polypeptides are not required for activity of the oxygen-evolving complex. It is difficult to reconcile this proposal with the demonstrated effect of the polypeptides on Ca^{2+} binding to the oxidizing (electron donor) side of PSII.

Andersson et al. [60a] have shown that the 23 kDa polypeptide also has an effect on the Cl^- requirement for O_2 evolution activity in mangrove thylakoids. Everted mangrove thylakoids, depleted at the 17 and 23 kDa proteins, could be fully reactivated by addition of high concentrations of Cl^-. When the 23 kDa protein (isolated from spinach) is rebound to these preparations, the amount of Cl^- required for activity is lowered substantially. Since the salt-washed spinach PSII preparations require Ca^{2+} for full activity, it is possible that both Cl^- and Ca^{2+} are necessary for activity in higher plants and the apparent requirement for only Cl^- but not Ca^{2+} could be a general phenomenon among photosynthetic membranes from halophytic plants. Very recent studies of Murata (described above) and of S. Izawa (personal communication) have addressed the question of the relationship between salt effects and oxygen evolution activity after removal of the 17 and 23 kDa proteins. These data implicate both polypeptides as structural elements which can affect the levels of Ca^{2+} and Cl^- necessary to elicit oxygen evolution activity in assay reaction mixture. This point is also addressed by Akabori et al. [60b], who have shown that under the appropriate conditions of reconstitution, the 17 kDa polypeptide can be shown to have a role in facilitating the action of Cl^- on oxygen evolution activity.

The third water-soluble protein which is a component of the oxygen-evolving complex was first isolated from thylakoid membranes by Kuwabara and Murata [61, 62], who showed that this water-soluble lysine-rich species was not related to the hydrophobic herbicide binding protein of the PSII 'core' complex. Research in several laboratories, using PSII-enriched membranes, has shown that the presence of the 33 kDa protein can be correlated with binding and stability of functional manganese within the oxygen-evolving complex. Yamamoto et al. [45] reported that Tris would solubilize the 33 kDa protein along with the 23 and 17 kDa species. These findings have been confirmed and expanded [25, 29, 30, 49a, b, 63, 64]. Briefly stated, a variety of treatments besides Tris (NH_2OH, Urea, high pH ± high ionic strength) will release the 33 kDa protein along with the 23 and 17 kDa proteins. Varying amounts of functional Mn are also released by these treatments. This partial Mn extraction is in agreement with an earlier report of heterogeneity in the functional pool of 4 Mn atoms associated with PSII in thylakoid membranes [7]. A report by Cammarata et al. [64] demonstrates nearly total extraction of Mn from PSII membranes by NH_2OH. This result may arise from the extraction procedure, which was carried out at pH 7.5 rather than at the acid pH values used by others. It should also be noted that although most conditions for extraction of the 33 kDa protein also release

Table 3. Extraction conditions for release of water-soluble polypeptides from PSII

Extraction condition	Material subjected to extraction*:	Release of:				Ref:
		33 kDa	23 kDa	17 kDa	Mn**	
Cholate	IT	?	?	?	0	(44)
Cholate	IT	−	+	+	0	(48)
Deoxycholate	IT	+	?	?	0	(67, 68)
0.25 M NaCl	ETV	−	+	+	0	(43)
1.0 M NaCl	PSIIM	−	+	+	0	(49, 51)
2.0 M NaCl	PSIIM	−	+	+	0	(29, 30)
0.8 M Tris	PSIIM	+	+	+	complete	(29, 45, 49)
NH_2OH, pH < 6.5	PSIIM	+	+	+	partial	(30, 49, 63)
NH_2OH, pH = 7.5	PSIIM	+	+	+	complete	(64)
2.5 M Urea	PSIIM	+	partial	partial	partial	(49)
0.7 M NaSCN	PSIIM	+	+	+	complete	(49)
pH 10	PSIIM	+	+	+	complete	(29)
100 μM NH_2OH + 2 M NaCl	PSIIM	+	+	+	complete	(62)
100 μM NH_2OH	PSIIM	−	−	−	0	(62)
1M $CaCl_2$ or $MgCl_2$	PSIIM	+	+	+	0	(65, 66)

*Abbreviations: IT = Intact thylakoids; ETV = everted thylakoid vesicles, PSIIM = purified PSII membranes.
**Complete = > 85% extraction.

the 23 and 17 kDa proteins, Murata et al. [49] have shown that urea produces strong extraction of the 33 kDa species while leaving behind substantial amounts of the 23 and 17 kDa proteins.

Of considerable interest is the apparent correlation between extraction of Mn and the 33 kDa protein. This correlation exists for a number of conditions discussed above. There are important exceptions to the correlation, however. Ono and Inoue [65a, b] report that 1M $CaCl_2$ or $MgCl_2$ extracts the 33, 23 and 17 kDa proteins from PSII membranes and inactivates O_2 evolution without a substantial release of Mn. Some activity can be restored to these membranes by addition of the 33 kDa protein [66]; Franzen and Andreasson [67, 68] have used deoxycholate to extract polypeptides from intact thylakoid membranes. An important result of these studies is that the 33 kDa protein is released by deoxycholate and mild heat shock without extraction of functional Mn. Ghanotakis et al. [69] have found that several reductants (H_2O_2, hydroquinone, benzidine) will extract up to 80% of the functional Mn from salt-washed PSII membranes (lacking 23 and 17 kDa proteins) without release of the 33 kDa protein. Although all of these experiments challenge the correlation between the binding of Mn to the oxygen-evolving complex and the presence of the 33 kDa protein, it is clear that without the water-soluble 33 kDa protein, the oxygen-evolving complex is either partially or completely inactivated. It therefore seems reasonable to conclude that although it cannot be shown that the 33 kDa protein binds Mn, this species is required to form a structurally active environment which promotes oxygen evolution, as has been suggesged by Ono and Ioue (66b). It is also possible that special extraction conditions may permit retention of Mn on the 33 kDa protein [70].

Table 3 summarizes for the reader the conditions which have been used

Table 4. Properties of Water-soluble 17, 23 and 33 kDa polypeptides

	33 kDa	23 kda	17 kDa	Reference
M.W. (kDa)	32–34	23–24	16–18	[43, 45, 49, 70, 71, 72]
pI	5	6.5	9.2	[69]
UV Abs. Max. (nm)	275–276	264–277	266–276	[71, 73, 74, 75]
Polarity Index	47%	49%	48%	[71]
Polar Amino Acids (%)				[49, 71, 75]
Lysine	9–10	10–12	9–11	
Arginine	3	2–8	5–6	
Histidine	0	0–0.7	0–1	
Asparagine	9	9–12	10–11	
Glycine	12–14	9–10	11–12	
Cystein	N.D.	N.D.	N.D.	
Methionine	0.5–0.7	0–0.6	0–0.2	
Tyrosine	3	2–4	3–6	

N.D. = not detectable

to extract the water soluble polypeptides of the oxygen-envolving complex from thylakoids and PSII membranes. Data on Mn release are also included. Table 4 collects various properties of the three polypeptides, and includes the contents of selected polar amino acids. Further work is required to clarify the contents of cystein, methionine and histidine in these proteins. It will be interesting to learn the results of gene sequencing experiments, now in progress in laboratories such as that of R. Hermann, which will yield the amino acid sequences of each of these proteins.

Controversies, unresolved questions and speculative models for the polypeptide composition of PSII

As the preceding sections indicate, research on PSII is producing a body of results which may be broadly construed as a consensus regarding the polypeptide composition of PSII and the roles of these polypeptides in the activity of the oxygen evolving complex of this photoact. As with any fast-moving field of research, there are areas of disagreement and uncertainty regarding PSII, and we will address some of those issues here. One uncertainty which concerns the 'core' complex is that the actual polypeptide binding sites for P680, Z and Q_A have not yet been rigorously identified. The detection of pheo a in a preparation of the 47 kDa polypeptide would suggest that perhaps P680 and Q_A may also bind to this protein [41a, b]. Since several methods [32b, 40, 41a, b, 42] now exist for enrichment or purification of the 47 kDa species, it seems likely that more information on its pigment and quinone content will be available soon. The question of Mn binding site(s) on hydrophobic polypeptides of the 'core' complex should also be addressed here. The *Scenedesmus* mutant isolated by Metz and Bishop [37, 38] shows a correlation between loss of Mn binding, presence of low potential cyt b559 and an increase in molecular weight of a 34 kDa protein. Is this altered polypeptide a hydrophobic constituent of the 'core' complex, or is it the water-soluble protein whose release affects the state of functional Mn? Recent work by J. G. Metz and M. Seibert (J. Metz, personal communication) has shown that the 'core' complex isolated from the mutant contains the altered 34 kDa protein, so the mutation has affected a hydrophobic, rather than hydrophilic polypeptide. What must now be determined is whether this increase in molecular weight of the 34 kDa polypeptide has a direct effect on its ability to bind Mn or whether the increase in molecular weight instead causes structural alterations in the 'core' complex which affect the actual Mn binding sites residing on other polypeptides. Another important question requiring further research concerns the sites of interaction between proteins of the 'core' complex and the water-soluble 33, 23, and 17 kDa polypeptides. Results of preliminary immunoprecipitation experiments, reported in [52], indicate that the water-soluble polypeptides are bound to, or interact with, species whose molecular weights are 43, 27, 22, and 9 kDa. These

findings would indicate that other proteins (apparently hydrophobic 27 and 22 kDa species) found in oxygen-evolving PSII membranes, but not in the 5-polypeptide 'core' complex, may be essential structural elements of the oxygen-evolving complex. Further information on these proteins is necessary for a complete understanding of the role of PSII polypeptides in oxygen evolution activity.

A number of questions remain with regard to the polypeptide composition and function of oxygen-evolving PSII membranes. One area of continuing uncertainty is that of stoichiometries of the water-soluble polypeptides. A possible origin of the differences we will describe may be technical; densitometer scanning of coomassie blue-stained proteins on SDS gels, immunoelectrophoresis, amino acid analyses, and protein concentrations required for reconstitution have all been used to estimate the stoichiometries of the 33, 23, and 17 kDa species in thylakoids, everted vesicles and in PSII membranes. Differences in estimations of reaction center concentrations may also contribute to further divergences in results. Here, we will briefly summarize results of (a) Murata et al [76], who have employed densitometry, amino acid analysis, and immunology to analyze PSII membranes; (b) Cammarata, et al. [64], who have used densitometry with proteins from PSII membranes of spinach and wheat; and (c) of Larsson et al. [53] and Åkerlund et al. [43], who have used rocket immunoelectrophoresis and the protein concentrations required for reconstitution of O_2 evolution activity to analyze the situation in intact thylakoids and everted thylakoid vesicles. Rocket immunoelectrophoresis indicates that intact thylakoid membranes contain one 23 kDa polypeptide per 170 chl and one 17 kDa species per 205 Chl, which would imply the presence of 2 each of these proteins per PSII reaction center if there were one reaction center per 400 chl in these preparations. The reconstitution-protein concentration technique produces a value of 1.3 of the 23 kDa polypeptide per reaction center in everted thylakoid vesicles. This estimate depends on the assumption that the everted preparations have one PSII reaction center per 300 Chl and on the assumption that all binding is productive (i.e., that each polypeptide reactivates an oxygen-evolving center). Since complete reconstitution was not achieved in these experiments, the value of 1.3 may be a minimum estimate. In Murata et al.'s recent experiments [76], 1 each of the 33, 23, and 17 kDa proteins were found (by densitometry, immunology (for the 33 kDa species) and by amino acid analysis) for every PSII reaction center (1 per 220 Chl). Cammarata et al. [64], working with PSII membranes of about the same reaction center enrichment as those used by Murata et al., have found by densitometer scanning that their preparations contain one 33 kDa protein per 1.7 molecules of the 23 kDa species. They suggest that these values may underestimate the true concentrations of the 33 and 23 kDa proteins, and indicate that the true values may be as high as 2 each of the 33 and 23 kDa species per reaction center. Until further experimental work is reported, we cannot draw a firm

conclusion as the actual stoichiometries of any of the water soluble proteins, except to note that the limits are at present a minimum of one each per reaction center with a maximum that approaches two. In this same vein, we point out a similar discrepancy among several groups regarding the content of cyt b559 in PSII membranes. Murata et al. [76] and Lam et al. [77] report 2 cyt b559 hemes per PSII reaction center, whereas Cammarata et al. [64], C. Yerkes-Crofts (personal communication), Sandusky et al. [29] and Ghanotakis et al. [30] find one heme. Ford and Evans [78] report one high-potential cyt b559 per P680 and another low-potential population (about 40% of the total heme). Most groups find equal amounts of both the low-potential and high potential forms of cyt b559; so, there is one area of agreement with regard to the status of cyt b559 in the oxygen-evolving PSII preparations.

Another unsettled problem is that of the relationship between the 33 kDa polypeptide, Mn binding to the PSII membrane and oxygen evolution activity. As we noted in the preceding section of this review, any of 3 conditions can be produced in PSII membranes by selective extraction procedures: release of both the 33 kDa protein and Mn; release of the 33 kDa protein without release of Mn; and release of Mn without substantial release of the 33 kDa protein. Where the 33 kDa protein has been extracted without removal of Mn from the PSII membrane, Murato and Miyao [49b] and Ono Inoue [65b] have both shown that Mn retention on the membrane requires elevated ionic strength (200 mM NaCl or $CaCl_2$ at an even higher concentration, 1M; it appears that Cl^- is the stabilizing factor from the results presented by Miyao and Murata [49b]). The observation that some oxygen evolution activity can be restored to these extracted membranes would suggest that 33 kDa protein is necessary for structural stability rather than catalytic efficiency of the Mn-complex. Further work is necessary, however, to strengthen this hypothesis. Membranes lacking the 33 kDa protein have not been reconstituted to recover the high levels of oxygen evolution activity achieved with the 23 kDa water-soluble polypeptide or with the Ca^{2+}-dependent reactivation process. It is also unclear whether the stabilizing influence of added anions (or perhaps cations) which results in Mn retention by the extracted membranes reflects a role for these ions in the oxygen-evolving complex or whether their presence mimics a charge environment normally provided by the 33 kDa protein.

Finally, the precise organization of hydrophobic and water-soluble polypeptides which comprise the PSII complex is unknown. One can, however, devise an approximate scheme based on the available data concerning the PSII 'core' complex and the water-soluble proteins of the oxygen evolving complex. It is logical to assume that the five polypeptides of the reaction center are organized so as to span the thylakoid bilayer and establish the conditions necessary for vectorial electron transport (also see van Gorkom [19b]). Beyond this observation, one cannot ascertain the actual organization

of each polypeptide with respect to the others, except to suggest that the two Chl-binding proteins (47 and 43 kDa) may be in contact with one another. Until cross-linking experiments and x-ray diffraction analyses have been carried out, intimate details of the 3-dimensional structure of the 'core' complex will remain obscure.

More detailed information is available on the organization of the water-soluble 33, 23 and 17 kDa proteins. As the reader can see from the earlier discussion of these proteins, the 23 and 17 kDa polypeptides are bound by electrostatic forces to the PSII membranes [29, 30, 43, 49a, b, 51]. The binding site(s) are organized so that these polypeptides comprise part, but not all, of a structural shield around the tetra-Mn cluster of the oxygen-evolving complex [30, 57, 69]. Binding of the 33 kDa protein to the PSII complex involves a more complex set of factors, including ionic and hydrophobic forces [29, 45, 49a, b, 63, 65a, b, 66, 67, 68] as well as a possible contribution from the presence of Mn [63, 69, 70], perhaps in the form of ligand-field stabilization energies from Mn (III) or Mn (IV) [79] It is very likely that the 33 kDa protein contains some, but probably not all, of the binding sites for the 23 kDa protein [54]. The 17 kDa protein binds to the 23 kDa species [53]. Lastly, as shown by the immunoprecipitation experiments of Ljungberg et al. [52], the 33 kDa protein appears to have binding sites associated with one or more of a collection of polypeptides which include the 43 and 10 kDa species of the 'core' complex.

A number of models for the organization of the polypeptides of PSII have appeared [35, 39, 48, 53, 64, 80, 81]. We summarize the salient features of these models in Figure 1, which shows the current view of the polypeptide organization of PSII as synthesized from work in a number of laboratories. The approximate location of the 33, 23, and 17 kDa with respect to one another and the hydrophobic core complex may be reasonably accurate but, as shown, this arrangement ignores the possible roles of proteins other than those of the 'core' complex found in oxygen-evolving PSII membrane preparations. Likewise, binding sites for Z, P680, pheo a and Q_A are not known with certainty, as reflected in the model of Figure 1. Perhaps the most important question, the precise location of Mn binding sites within the active PSII complex, is also an unresolved question. From the data at hand, we would suggest that these metal binding sites are shared among several polypeptides, both water-soluble and hydrophobic, of the PSII complex. We show Mn residing at an interface between the 'core' complex and the water soluble polypeptides to indicate the probable involvement of several polypeptide species in Mn binding.

While all models for the organization of PSII are at present subject to varying degrees of uncertainty, the reader should bear in mind that no such models existed prior to 1983. Photosynthesis research is a novel field in which biochemistry, biophysics, and now molecular genetics have been brought to bear on a variety of problems. In the case of PSII and the oxygen-

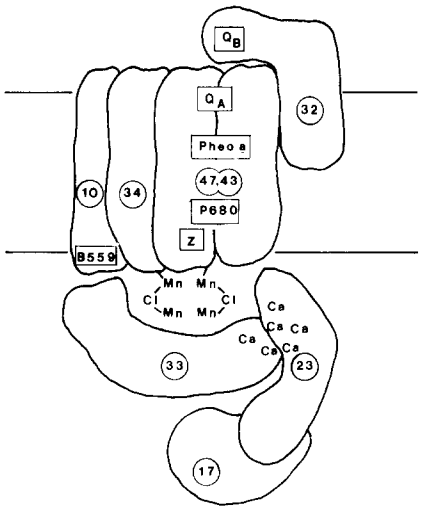

Figure 1. Two-dimensional schematic diagram of the polypeptides of photosystem II (PSII) which have been discussed in this review. 0 = approximate molecular weights (in kDa) for these polypeptides; □ = actual or speculative sites on these polypeptides where various catalytic components of PSII are bound. Four Mn atoms are shown in the catalytic cluster; this number is based on quantitations carried out by investigators working with highly active preparations of PSII membranes and thylakoids. Actual sites of Mn ligation have not been determined so the ligand sites shown are speculative. Chloride and Ca^{2+} binding involve Mn and water-soluble polypeptides; Ca^{2+} binding sites may also exist on hydrophobic polypeptides. B559 refers to cytochrome b-559; see list of abbreviations for other symbols.

evolving complex it is reasonable to suggest that the current pace of research will refine and update present models of PSII structure in a very short time and that in the future, speculative models will be replaced with facts.

Acknowledgements

A number of investigators have generously shared their results with us prior to publication. We thank G.T. Babcock, G. Renger, Govindjee, Ki. Satoh, L.E. Andersson, N.I. Bishop, Y. Inoue, N. Murata, G.C. Dismukes, J. Metz, T.A. Bricker and B. Diner. The author's research on PSII and the oxygen evolving reaction has been funded by grants to C.F.Y. from the National Science Foundation and the Competitive Research Grants Office of USDA.

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