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BIOCHEMICAL CHARACTERIZATION OF A HIGHLY ACTIVE O_2-EVOLVING PHOTOSYSTEM II PREPARATION FROM MAIZE

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An O_2-evolving Photosystem (PS) II preparation was isolated from maize by a Triton X-100 procedure (Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539). A highly active O_2-evolving preparation was obtained which evolved O_2 at 76% the rate of fresh chloroplasts ($H_2O \rightarrow$ 2,6-dichloro-*p*-benzoquinone) and was very sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea. There was no detectable PS I activity in the preparation (2,3,5,6-tetramethyl-*p*-phenylenediamine → methyl viologen). When analyzed by lithium dodecyl sulfate (LDS) polyacrylamide gel electrophoresis the O_2-evolving preparation was shown to be highly depleted in CP I, CF_1, and devoid of cytochromes *f* and *b*-563 (the absence of which was confirmed by difference spectroscopy). The preparation was enriched in the PS II reaction center polypeptides I and II, the 34 kDa polypeptide (Metz, J., Wong, J. and Bishop, N.I. (1980) FEBS Lett. 114, 61–66), the Coomassie blue-stainable 32 kDa polypeptide (Kuwabara, T. and Murata, N. (1979) Biochim. Biophys. Acta 581, 228–236), LHCP-associated polypeptides and cytochrome *b*-559. Polypeptides of unknown function at 40.5, 25, 24, 22, 16.6 and 14 kDa were also present in the O_2-evolving preparation. Triton X-114 phase partitioning (Bricker, T.M. and Sherman, L.A. (1982) FEBS Lett. 149, 197–202) indicated that the majority of these polypeptides were intrinsic. Only the polypeptides at 32, 25, 24 and 14 kDa were extrinsic. When examined by the octylglucoside procedure of Camm and Green (Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432) the PS II O_2-evolving preparation was shown to contain the chlorophyll-proteins CP 27, CP 29, CP II*, D, and CP a-1 and CP a-2. Chlorophyll-proteins associated with PS I were highly depleted. The visible absorption spectra indicated an enrichment of chlorophyll *b* and carotenoids in the preparation. The 77 K fluorescence emission spectrum (excitation wavelength = 435 nm) exhibits a strong F-686 with little F-695 shoulder and a broad, low-intensity F-735 emission.

Introduction

The proteins directly involved in electron transport within the thylakoid membranes of higher plants appear to be organized into at least three distinct functional and structurally separate complexes: the PS I complex which photochemically excites electrons accepted from reduced plastocyanin and reduces $NADP^+$, the plastoquinol-plastocyanin oxidoreductase (cytochrome *b*-563-*f* complex) which accepts electrons from plas-

Abbreviations: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LDS, lithium dodecyl sulfate; Mes, 2-(*N*-morpholino)ethanesulfonic acid; octylglucoside, octyl-D-glucopyranoside; PMSF, phenylmethylsulfonyl fluoride; PS, photosystem; Tricine, *N*-tris(hydroxymethyl)methylglycine; TMBZ, 3,3′,5,5′-tetramethylbenzidine; CP, chlorophyll-protein complex; LHCP, light-harvesting chlorophyll-protein complex; Chl, chlorophyll.

toquinol and reduces plastocyanin, and the PS II complex which extracts and photochemically excites electrons from water and reduces plastoquinone. The polypeptide compositions of functional PS I and cytochrome *b*-563-*f* complexes have been reasonably well characterized. Additionally, the functional characteristics of the isolated complexes as well as the function of many of the component polypeptides have been determined. The PS II complex, however, has not been as well characterized. While the polypeptide composition of the core of the PS II complex as well as those components located on the reducing side of the photosystem have been intensively studied, characterization of the components located on the oxidizing side has been severely hampered by the inability to isolate fully functional (i.e., O_2-evolving) PS II complex preparations.

Recently, a number of methods for the isolation of such O_2-evolving PS II preparations have been reported. These have included preparations generated by either mechanical disruption [1] or various detergent treatment protocols [2–4]. These preparations are also enriched in the light-harvesting chlorophyll-protein complex. Such preparations have been utilized in investigations of the polypeptide composition of the oxidizing side of PS II [2,4–8] as well as in the reconstitution of the entire photosynthetic electron-transport chain into lipid vesicles [9]. Additionally, these preparations may serve as excellent starting material for the isolation of the various polypeptide components of PS II. Unfortunately, the characteristics of these O_2-evolving PS II preparations have not been completely documented.

In this communication we wish to report the biochemical characterization of an O_2-evolving PS II preparation isolated from maize by the Triton X-100 procedure of Kuwabara and Murata [4]. This preparation has been examined with respect to photochemical activity, chlorophyll-protein and polypeptide composition, spectral characteristics, cytochrome composition and phase partitioning properties of the constituent polypeptides.

Materials and Methods

Isolation of the PS II O_2-evolving preparation

The PS II O_2-evolving preparation was prepared essentually according to the procedure of Kuwabara and Murata [4]. Maize thylakoid membranes were isolated from 2-week-old, greenhouse-grown plants by grinding the leaf material in a blender with 100 mM sucrose, 200 mM NaCl and 50 mM sodium/potassium phosphate buffer, pH 7.5. Except where indicated all of the buffers contained 1 mM each of the protease inhibitors, PMSF, ε-aminocaproic acid and benzamidine. The ground leaf material was filtered through two layers of cheesecloth and two layers of Miracloth (Calbiochemical Co.) and then centrifuged at 2000 $\times g$ for 5 min. The pellet was resuspended in 300 mM sucrose, 50 mM NaCl and 50 mM sodium/potassium phosphate buffer, pH 6.9, to a chlorophyll concentration of 2–3 mg/ml. Triton X-100 (20%, v/v) was added with vortex mixing (1 min) to yield a final detergent-to-chlorophyll ratio of 25 : 1. The suspension was immediately centrifuged at 2000 $\times g$ for 2 min and the supernatant was collected and centrifuged at 35 000 $\times g$ for 10 min. The pellet was resuspended in 50 mM sodium/potassium phosphate buffer, pH 6.9, and centrifuged at 10 000 $\times g$ for 2 min. The supernatant was collected and centrifuged at 35 000 $\times g$ for 10 min. The resultant pellet, which retains the ability to evolve O_2, was resuspended in 50 mM sodium/potassium phosphate buffer, pH 6.9.

Spectral analysis

Absorption spectra at 25°C were recorded on an Aminco DW-2 spectrophotometer and fluorescence emission spectra at 77 K were recorded on an SLM 8000 spectrofluorimeter. Cytochrome concentrations were determined from room-temperature, chemically induced difference spectra using an Aminco DW-2 spectrophotometer and procedures modified from those of Refs. 10–12. Chloroplasts and the PS II O_2-evolving preparation were diluted to 100 μg/ml Chl in 0.3 M sucrose, 10 mM NaCl and 25 mM Mes-NaOH, pH 6.5. Concentrations of other reagents were as follows: potassium ferricyanide, 0.06 mM; potassium ferrocyanide, 0.06 mM; hydroquinone, 0.2 mM; Triton X-100, 0.5%; dithionite was added as a few crystals.

Electron-transport assays

PS II activity was measured as O_2 evolution

with DCBQ/ferricyanide as an electron acceptor. The reaction mixture (3 ml) contained 40 mM Tricine-NaOH, pH 7.5, 67 mM KCl, 5 mM potassium ferricyanide, 400 μM DCBQ and 5 μg/ml Chl. No protease inhibitors were included in the reaction mixture.

PS I activity was measured as O_2 consumption via a modified Mehler reaction with ascorbate/DAD as the electron donor and methyl viologen as the electron acceptor. The reaction mixture (3 ml) contained 40 mM Tricine-NaOH, pH 7.5, 67 mM KCl, 1.7 mM methyl viologen, 1.7 mM KCN, 1 mM disodium ascorbate, 1 mM DAD, 10 μM DCMU and 5 μg/ml Chl. No protease inhibitors were included.

In both assays cool, saturating white light was supplied by a Labsource QH 150 fiber optics illuminator. O_2 evolution or uptake was monitored by a YSI Clark electrode.

Analysis of polypeptide and chlorophyll-protein composition

The polypeptides of both thylakoid membranes and the PS II O_2-evolving preparation were solubilized in 10 mM Tricine-NaOH, pH 7.5, 2% LDS, 7.5% 2-mercaptoethanol and 6% sucrose at 0–4°C. Samples were immediately analyzed by LDS-polyacrylamide gel electrophoresis in 10–20% gradient gels [13] which were run overnight at 1.5 W at 0°C. TMBZ staining for heme-dependent peroxidase activity was performed as described by Guikema and Sherman [14]. The gels were subsequently stained with Coomassie blue and destained by diffusion.

Octylglucoside solubilization of PS II-associated chlorophyll-protein complexes was performed by modification of the procedure of Camm and Green [15]. Thylakoids or PS II O_2-evolving preparation were suspended in 30 mM octylglucoside, 2 mM Tris-maleate, pH 7.0, to yield a detergent-to-chlorophyll ratio of 40. After 30 min dark incubation at room temperature the samples were brought to 10% glycerol and placed in the wells of an LDS-polyacrylamide gel (4.5% stacking gel, 10% separating gel). Electrophoresis was performed at 4°C at 15 mA constant current for 2.5 h.

The phase partitioning properties of the constituent polypeptides were analyzed by the Triton X-114 procedure of Bordier [16] as modified for chloroplast thylakoids by Bricker and Sherman [17].

Results and Discussion

Photosystem activities

The PS II O_2-evolving preparation isolated from maize by the method of Kuwabara and Murata [4] evolved O_2 at high rates. This activity was very sensitive to DCMU (Table I) and atrazine (data not shown). Additionally, the PS II O_2-evolving preparation was devoid of PS I activity (Table I) within the limits of detection by this method. These results are consistent with the findings of Kuwabara and Murata [4] using a PS II O_2-evolving preparation isolated from spinach. In our laboratories, PS II O_2-evolving preparations of similar characteristics have been isolated from a wide range of higher plant material including pea, fescue, spinach and the green alga *Scenedesmus* (unpublished data). We have not, however, been able to obtain analogous preparations from the cyanobacterium *Anacystis nidulans* R2 or the granaless maize mutant *Oy/yg*. This may suggest a requirement for grana stacking in the isolation of PS II O_2-evolving preparations from chloroplasts

TABLE I

LIGHT-DEPENDENT O_2 EVOLUTION (OR UPTAKE) BY MAIZE THYLAKOIDS AND THE PS II O_2-EVOLVING PREPARATION

Oxygen evolution (or uptake) was measured with a Clark electrode. Saturating white light was provided by a fiber optics illuminator. Final chlorophyll concentration was 5 μg/ml.

Preparation	Electron transport (μmol O_2/mg Chl per h)	
	Water → DCBQ/ferricyanide	Ascorbate/DAD → methyl vidogen
Thylakoids	304	1052
Thylakoids + 10 μM DCMU	9	–
PS II preparation	230	0 [a]
PS II preparation + 10 μM DCMU	4	–

[a] The addition of 1 or 5 μM plastocyanin to the reaction mixture of the PS II O_2-evolving preparation did not stimulate PS I-dependent O_2 uptake.

by this methiod. These results are similar to those reported by Henry and Moller [1] with PS II vesicles derived from mechanically disrupted spinach thylakoids and separated in a two-phase polymer system. This method also requires the presence of grana for the isolation of an O_2-evolving preparation.

Spectral characterization

Room-temperature absorption spectra of thylakoids and the PS II O_2-evolving preparation are shown in Fig. 1. The PS II O_2-evolving preparation exhibited absorption peaks at 677, 651 (shoulder), 468 and 437 nm. Thylakoids exhibited peaks at 679, 654 (shoulder), 471 and 438 nm. The PS II O_2-evolving preparation was enriched in Chl *b* (Chl *a*/*b* : O_2-evolving preparation = 2.0; thylakoids = 3.2) and in carotenoids absorbing at 470 nm. The blue shift in the major absorption peaks of this preparation has been consistently observed [1,2]. This shift may be the result of an enrichment in shorter wavelength absorbing forms of chlorophyll associated with the PS II antennae and reaction centers. The enrichment of carotenoids in this PS II O_2-evolving preparation is consistent with the findings of Lichtenthaler et al. [18] and of Braumann et al. [19] that two principal chloroplast carotenoids, neoxanthin and lutein, are associated with the light-harvesting chlorophyll-

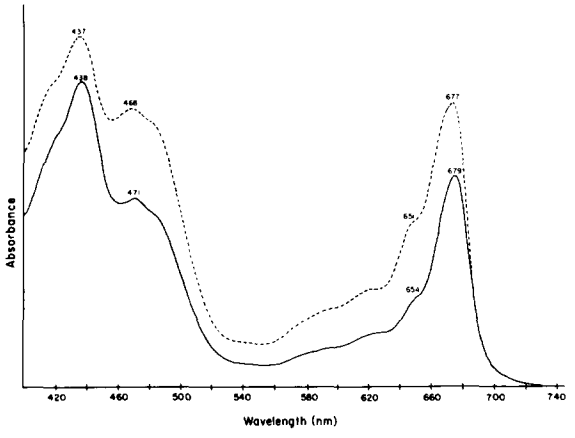

Fig. 1. Absorption spectra of maize chloroplast membranes (———) and the PS II O_2-evolving preparation (------). Samples were suspended in 10 mM Tris-HCl, pH 7.5. Absorbance is in relative units.

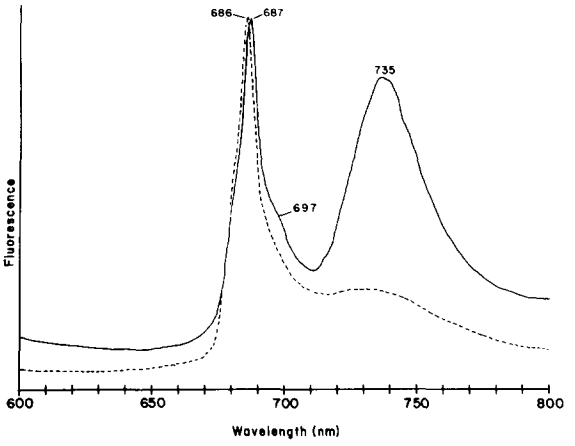

Fig. 2. Corrected 77 K fluorescence emission spectra of maize chloroplast membranes (———) and the PS II O_2-evolving preparation (------). The excitation wavelength was 435 nm. The samples were suspended on 10 mM Tris-HCl, pH 7.5, and 60% glycerol. Fluorescence is in relative units.

proteins, which are highly enriched in this preparation.

Fig. 2 illustrates the typical 77 K fluorescence emission spectra of isolated thylakoids and the PS II O_2-evolving preparation. The thylakoids exhibited strong major fluorescence peaks at 687 and 735 nm with a pronounced shoulder at 697 nm. The PS II O_2-evolving preparation exhibited a strong peak at 686 nm, a low-intensity peak at 735 nm and little F-697. The sharp F-686 indicates that LHCP is tightly coupled to the PS II reaction center with little or no free LHCP present [20].

These results differ from those of Henry et al. [1] who observed both F-687 and F-694 in their O_2-evolving vesicles. They attributed F-695 as arising from the PS II reaction center. Recent evidence, however, suggests that there is no one-to-one correspondence between F-695 and functional PS II reaction centers and that the absence of F-695 does not necessarily imply the absence of functional PS II reaction centers. Leto and Miles [11] have reported the presence of F-695 in the maize mutant *hcf*-3 which has been shown to be devoid of PS II reaction centers [21]. Mullet et al. [22] have reported the presence of a distinct F-695 in a highly purified PS I preparation treated with dithionite. Larkum and Anderson [20] have observed that seemingly similar preparations of PS II reac-

tion centers show a marked heterogeneity in the strength of F-695. Additionally, when PS II reaction centers are embedded in lipid vesicles, the strength of F-695 is completely dependent on the lipid-to-protein ratio of the vesicles. Finally, during the greening of iron-deprived *A. nidulans* R2, the increase of F-695 is directly related to the assembly of large PS I-associated chlorophyll-protein complexes. This suggests that F-695 may arise from an interaction between PS II and PS I in cyanobacteria (Pakrasi and Sherman, unpublished data).

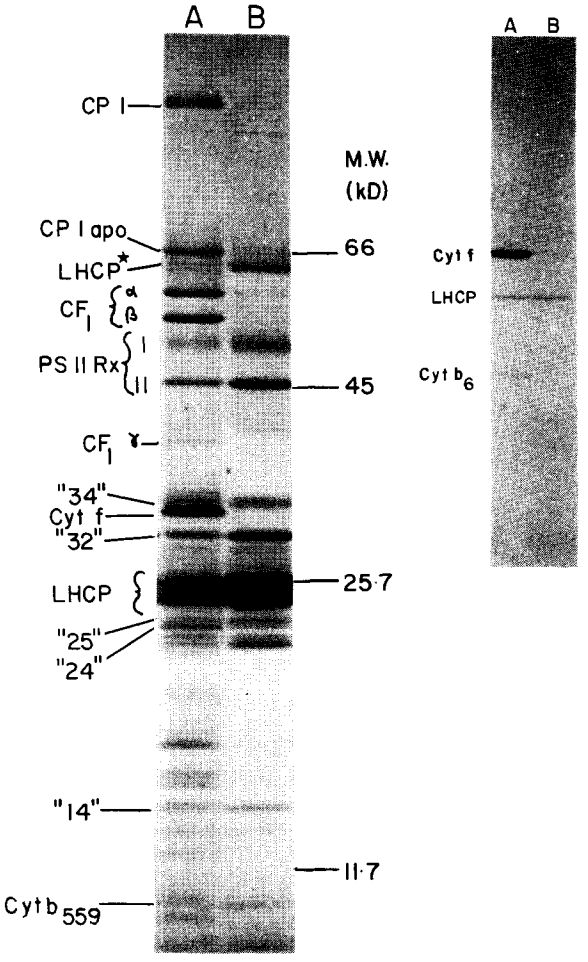

Fig. 3. Polypeptides of (A) chloroplast membranes and (B) the PS II O_2-evolving preparation analyzed in a 10–20% gradient LDS-polyacrylamide gel and stained with Coomassie blue. The gels were run overnight at 4°C. Known or tentatively identified polypeptides are indicated at the left of the gel. LHCP* is an oligomer of LHCP and corresponds to CP II* in the octylglucoside system (see Fig. 4). Insert: TMBZ staining of the heme-containing polypeptides of (A) thylakoid membranes and (B) the PS II preparation.

Polypeptide and chlorophyll-protein composition

The polypeptide composition of the PS II O_2-evolving preparation and maize thylakoid membranes is shown in Fig. 3. Identification of most of the major polypeptides of maize thylakoid membranes has been reported elsewhere [21]. The PS II O_2-evolving preparation was highly depleted of CP I and its apoprotein, the subunits of CF_1, as well as numerous other low molecular mass polypeptides. Heme-dependent peroxidase activity localized by TMBZ staining indicated the absence of cytochromes *f* and *b*-563 (Fig. 3, inset) in this preparation.

The components that were enriched in this preparation included the PS II reaction center polypeptides I (49 kDa) and II (45 kDa), and polypeptides at apparent molecular masses of 40.5, 34, 32, 25, 24, 16, 14 and 10 kDa. Additionally, at least four light-harvesting chlorophyll-protein polypeptides were present in the 25–26 kDa region. The PS II reaction center polypeptides I and II appear to be analogous to those described in *Chlamydomonas* [13]. These polypeptides are missing in the maize PS II mutant *hcf*-3 which lacks PS II reaction centers [21]. The presence of numerous light-harvesting chlorophyll-protein polypeptides has been previously reported for *Chlamydomonas* and spinach [23].

The 34 kDa polypeptide may be involved in water oxidation and/or the binding of manganese. Its absence has been correlated with the loss of O_2 evolution and a decrease in the amount of bound manganese in both *Scenedesmus* [24,25] and maize mutants [21]. Unfortunately, there has been much confusion in the literature with regard to this polypeptide. In particular, Kuwabara and Murata [4], Yamamoto et al. [2] and Akerlund and Jansson [8] have all suggested that an extrinsic polypeptide in this region, which is released by various treatments, is identical to the 34 kDa polypeptide described by Metz et al. [25]. As a point of clarification, there are two polypeptides in this region which appear to be associated with the oxidizing side of PS II: a 34 kDa polypeptide described by Metz et al. [25] which we show in this report to be

an intrinsic polypeptide (see below), and an extrinsic, 32 kDa polypeptide described by Kuwabara and Murata [4,26,27]. Both of these polypeptides are enriched in this PS II O_2-evolving preparation.

Since this PS II O_2-evolving preparation is sensitive to DCMU and atrazine (Table I), it presumably contains the herbicide-binding protein. Gardner [28] and Pfister et al. [29], using the photoaffinity probe azidoatrazine, have identified a 32 kDa component which binds atrazine. The same 32 kDa component has been shown to bind photoaffinity analogs of plastoquinone [30] and has been suggested to be the component 'B', the primary reducer of the plastoquinone pool. Steinback et al. [31] have shown that this 32 kDa component is identical to the product of the chloroplast genome encoded by 'photogene 32', a rapidly labeled, photoinduced polypeptide [32]. This polypeptide (the 32 kDa 'herbicide-binding' polypeptide) has also been confused with the 32 kDa component described by Kuwabara and Murata [26]. The polypeptide described by Kuwabara and Murata [26] is stained with Coomassie blue, released by high pH treatment [4], and contains lysine [27]. The herbicide-binding polypeptide apparently does not stain well with Coomassie blue and is not released by treatments which release the polypeptide described by Kuwabara and Murata [33]. Additionally, nucleotide sequence analysis of photogene 32 from higher plants does not reveal any lysine codons [34].

When thylakoids or the PS II O_2-evolving preparation are treated with high pH [4,35] or 0.8 M Tris [2,5,36] they lose their ability to evolve O_2 and release bound manganese. There is a concomitant release from the PS II preparations of polypeptides of apparent molecular masses of 32, 25 and 14 kDa. Additionally, in maize, a 24 kDa polypeptide is released (data not shown). These polypeptides may be involved on the oxidizing side of PS II and they are present in this maize PS II O_2-evolving preparation. Akerlund et al. [5] has demonstrated the reconstitution of O_2 evolution in salt-washed, inside-out vesicles with a 23 kDa component. A 17 kDa polypeptide has been utilized to reconstitute O_2 evolution in cholate-extracted spinach thylakoids [37].

While the function of cytochrome *b*-559 has not been determined, it appears to be intimately associated with the PS II reaction center and is enriched in this (see below) and other PS II preparations [1,3,4]. The apoprotein of cytochrome *b*-559 has an apparent molecular mass of 10 kDa and comigrates with the 10 kDa polypeptide enriched in this PS II O_2-evolving preparation.

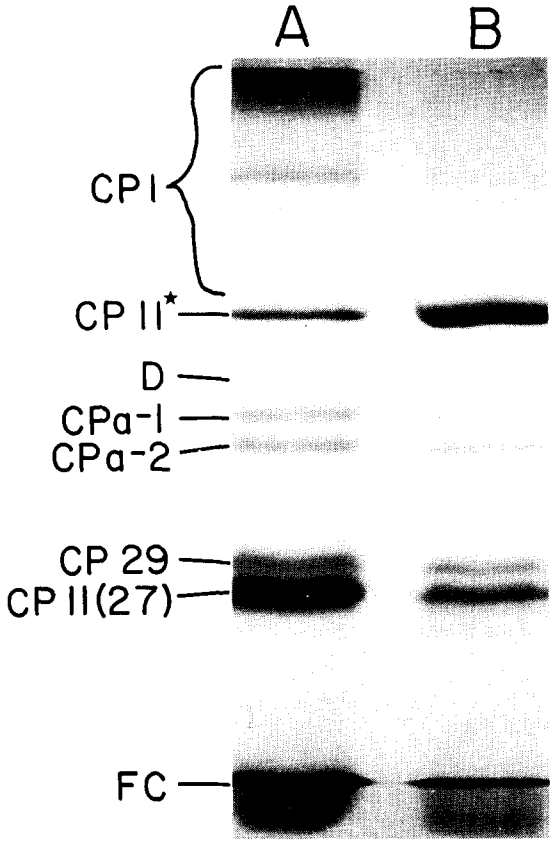

Fig. 4. Chlorophyll-protein complexes of (A) chloroplast membranes and (B) the PS II O_2-evolving preparation separated on an unstained, 10% LDS-polyacrylamide gel. The gels were run for 2.5 h at 4°C. Presumptive identifications of the various chlorophyll-proteins are indicated at the left of the gel. FC, free chlorophyll.

The chlorophyll-proteins of this PS II preparation were examined by the octylglucoside extraction procedure of Camm and Green [15]. Only a faint trace of PS I-associated chlorophyll-proteins was observed, while the PS II chlorophyll-proteins were enriched in the preparation (Fig. 4). Particularly prominent is CP II* which was highly enriched in this preparation. Also present were CP a-1 and CP a-2, CP II(27) CP 29 and its presump-

tive oligomer D. Additionally, two chlorophyll-proteins of slightly higher mobility than CP II(27) were observed. The origin of these high-mobility chlorophyll-proteins is unknown.

The chlorophyll-proteins CP II(27) and its oligomer CP II* appear to serve as the primary light-harvesting antennae for PS II [15]. The reaction center for PS II is thought to be associated with either CP a-1 or CP a-2, since mutants which lack functional PS II reaction centers also lack these chlorophyll-proteins. The functions of the other chlorophyll-proteins have not been determined.

Cytochrome determinations

Examination of chemically induced difference spectra indicated that cytochrome *b*-559 is the only cytochrome present in this PS II O_2-evolving preparation (Table II). Cytochrome *f* could not be detected in a hydroquinone (plus Triton X-100) versus ferricyanide (plus Triton X-100) difference spectrum. In a hydroquinone or ferrocyanide versus ferricyanide spectrum, only a symmetrical peak at 559 nm was observed. Addition of dithionite to the sample cuvette, while increasing the height of the 559 nm peak, did not shift the peak to a longer wavelength. This indicated that cytochrome *b*-563 was not present. These results are consistent with our observation that no TMBZ-stainable polypeptides are present in this PS II preparation. Determination of total cytochrome *b*-559 was facilitated by the absence of other cytochromes. The data indicated that more than one redox species of cytochrome *b*-559 was present. Hydroquinone reduction demonstrated that 80% of the cytochrome was in the high-potential form.

Phase parititioning in Triton X-114

It has been shown that only proteins with extensive hydrophobic domains (i.e., intrinsic pro-

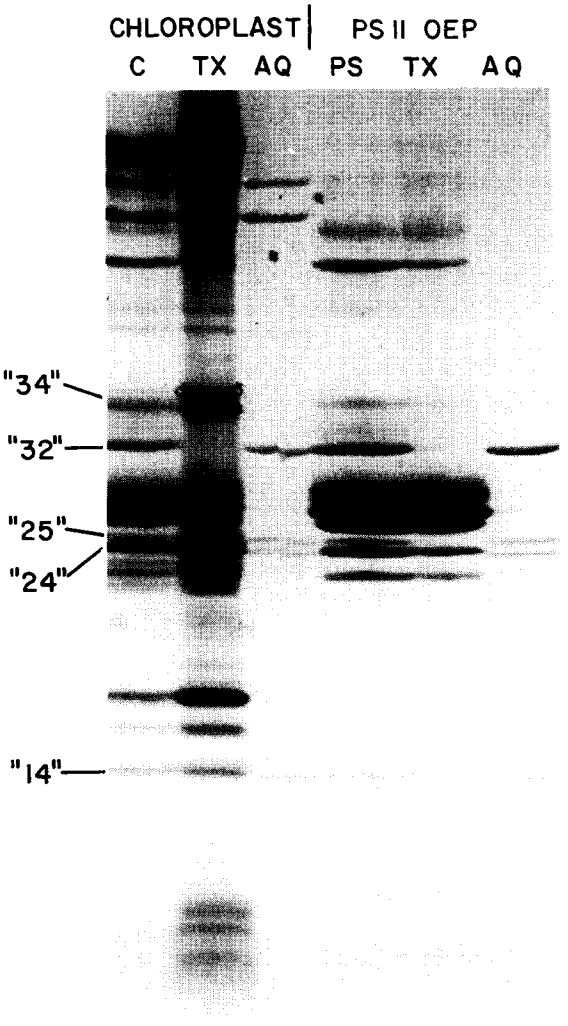

Fig. 5. Triton X-114-mediated phase partitioning of chloroplast membranes and the PS II O_2-evolving preparation (OEP). (C) Chloroplast membranes, (PS) PS II preparation, (TX) Triton X-114 phase which contains intrinsic polypeptides, (AQ) the aqueous phase which contains extrinsic polypeptides. The polypeptides were separated on a 10–20% gradient, LDS-polyacrylamide gel which was electrophoresed overnight at 4°C. The five polypeptides which appear to be involved on the oxidizing side of PS II are labeled to the left of the gel.

TABLE II

ESTIMATED CYTOCHROME CONTENT OF MAIZE THYLAKOIDS AND THE PS II O_2-EVOLVING PREPARATION

The amounts of cytochromes were determined from reduced minus oxidized difference spectra at room temperature. Final chlorophyll concentration was 100 μg/ml. Value in parentheses gives the number of cytochromes per 600 Chl. n.d., not detectable.

Preparation	Cytochrome (nmol/μmol Chl)		
	f	*b*-563	*b*-559 (total)
Thylakoids	1.5 (0.9)	2.6 (1.6)	3.4 (2.1)
PS II preparation	n.d.	n.d.	4.8 (3.0)

teins) can form mixed micelles with nonionic detergents [39,40]. Bordier [16] has demonstrated that intrinsic membrane proteins can be separated from extrinsic membrane proteins by solubilizing the membranes in Triton X-114 solutions followed by temperature-mediated precipitation of the Triton X/protein mixed micelles. Intrinsic polypeptides precipitate with the Triton X-114 while extrinsic polypeptides remain in an aqueous phase. This technique has been utilized by Bricker and Sherman [17] to investigate the topological arrangement of maize thylakoid membrane proteins and has helped us to clarify the relationships of polypeptides in the 30–35 kDa region.

The Triton X-114 phase partitioning properties of maize thylakoids and the PS II O_2-evolving preparation are shown in Fig. 5. A majority of the major thylakoid polypeptides partitioned into the Triton phase. These included the apoprotein of CP I, cytochromes *f* and *b*-563 (see Ref. 17), the light-harvesting chlorophyll-protein polypeptides, the reaction center polypeptides of PS II, and many others. Those polypeptides which partitioned into the aqueous phase included the subunits of CF_1, and polypeptides at 32, 25, 24 and 14 kDa. A majority of the polypeptides of the PS II O_2-evolving preparation also partitioned into the Triton phase including the PS II reaction center polypeptides and the light-harvesting chlorophyll-protein polypeptides. Additionally, the 34 kDa polypeptide described by Metz and Miles [21] partitions into this phase, indicating that it is an intrinsic polypeptide.

While this PS II preparation was depleted in CF_1, it was enriched in the other major aqueous partitioning polypeptides of 32, 25, 24 and 14 kDa. These polypeptides are identical to the polypeptides released by high pH treatment [4] and Tris washing [2].

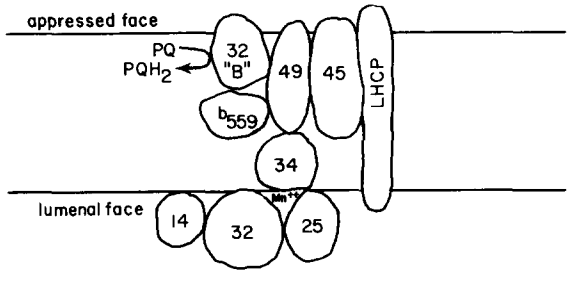

Fig. 6. Diagrammatic representation of the organization of the PS II complex in higher plants. The complex consists of at least six intrinsic polypeptides, three extrinsic polypeptides, and contains bound manganese.

Utilizing this information in conjuction with data obtained from topological studies [17,31,41], investigations with PS II-deficient mutants [11,21], and results obtained from other PS II complex preparations, it is possible to present a tentative model for the organization of the PS II complex in the thylakoid membranes of higher plants (Fig. 6). The intrinsic polypeptides of the complex include the light-harvesting chlorophyll-protein polypeptides, the 49 and 45 kDa PS II reaction center polypeptides, the 34 kDa polypeptide [21,25], cytochrome *b*-559 and presumably the 32 kDa herbicide-binding polypeptide. All of the these possibly cytochrome *b*-559 possess surface-exposed regions which are either sensitive to trypsin treatment or are enzymatically radioiodinated [17,31].

The extrinsic polypeptides of the complex include the 32 kDa polypeptide described by Kuwabara and Murata [26], and polypeptides of 25 and 14 kDa. These polypeptides are confined to the luminal face of the thylakoid. Manganese may interact with both the 34 kDa, intrinsic polypeptide and one or more of the extrinsic components. Mutants which lack the intrinsic 34 kDa component also lack bound manganese [21,25], while treatments which release bound manganese such as high pH and Tris washing release the three extrinsic polypeptides without disturbing the intrinsic 34 kDa component. Experiments with higher plant material have indicated that manganese is not associated with any of these components in vitro [5].

O_2-evolving PS II preparations, such as the one of Kuwabara and Murata which is characterized in this communication, are extremely useful in the investigation of PS II organization. Such complexes posses a much simpler polypeptide profile than do intact chloroplasts while maintaining high rates of PS II activity. They additionally provide investigators with the opportunity to study polypeptide components involved on the oxidizing side of PS II and may prove to be instrumental as a tool in the elucidation of the structural nature of the O_2-evolving site.

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