Biochimica et Biophysica Acta, 767 (1984) 493–500
Elsevier

BBA 41653

QUANTITATIVE ANALYSIS OF MEMBRANE COMPONENTS IN A HIGHLY ACTIVE O_2-EVOLVING PHOTOSYSTEM II PREPARATION FROM SPINACH CHLOROPLASTS

YASUSI YAMAMOTO [a,*], KENICHI TABATA [a], YASUHIRO ISOGAI [a], MITSUO NISHIMURA [a], SHIGEKI OKAYAMA [b], KATSUMI MATSUURA [c] and SHIGERU ITOH [c]

[a] *Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812,* [b] *Laboratory of Biology, College of General Education, Kyushu University, Fukuoka 810 and* [c] *National Institute for Basic Biology, Okazaki 444 (Japan)*

(Received May 21st, 1984)

Key words: Oxygen evolution; Photosystem II; (Spinach chloroplast)

Stoichiometry of membrane components associated with Photosystem II was determined in a highly active O_2-evolving Photosystem II preparation isolated from spinach chloroplasts by the treatment with digitonin and Triton X-100. From the analysis with sodium dodecyl sulfate polyacrylamide gel electrophoresis and Triton X-114 phase partitioning, the preparation was shown to contain the reaction center protein (43 kDa), the light-harvesting chlorophyll-protein complex (the main band, 27 kDa), the herbicide-binding protein (32 kDa) and cytochrome *b*-559 (10 kDa) as hydrophobic proteins, and three proteins (33, 24 and 18 kDa) which probably constitute the O_2-evolution enzyme complex as hydrophilic proteins. These proteins were associated stoichiometrically with the Photosystem II reaction center: one Photosystem II reaction center, approx. 200 chlorophyll, one high-potential form of cytochrome *b*-559, one low-potential form of cytochrome *b*-559, one 33 kDa protein, one (to two) 24 kDa protein and one (to two) 18 kDa protein. Measurement of fluorescence induction showed the presence of three electron equivalents in the electron acceptor pool on the reducing side of Photosystem II in our preparation. Three molecules of plastoquinone A were detected per 200 chlorophyll molecules with high-performance liquid chromatography. The Photosystem II preparation contained four manganese atoms per 200 chlorophyll molecules.

Recent progress in the techniques of isolating active O_2-evolving Photosystem II (PS II) preparations from spinach chloroplasts has become a breakthrough for furthering the understanding of the molecular organization of the O_2-evolution enzyme complex [1–4]. These preparations were obtained by treatment of chloroplasts with non-ionic detergents (Triton X-100 and/or digitonin) under mild conditions. They were mostly devoid of Photosystem I (PS I) and the membrane proteins that are not essential for PS II. However, the amounts and the stoichiometry of the membrane components which are associated with PS II in these preparations have not been determined exactly. There are differences between the preparation methods which were employed for the isolation of the PS II preparations, and it might be reasonable to assume that even a small change in the amount or the organization of membrane components associated with PS II has considerable effects on the efficiencies of the primary processes

* To whom all correspondence should be addressed.

Abbreviations: Mes, 4-morpholineethanesulfonic acid; SDS, sodium dodecyl sulfate; cytochrome *b*-559_{HP}, high-potential form of cytochrome *b*-559; cytochrome *b*-559_{LP}, low-potential form of cytochrome *b*-559; HPLC, high-performance liquid chromatography; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Q, primary stable electron acceptor of Photosystem II; B, secondary electron acceptor of Photosystem II; Z, secondary electron donor of Photosystem II; PS, Photosystem.

of charge separation and the following water-oxidation process. In the present study, we estimated quantitatively the amount of several membrane components in a digitonin/Triton X-100 PS II preparation. We will discuss a possible organization of the actively O_2-evolving PS II complex.

Materials and Methods

Isolation of a highly active O_2-evolving PS II preparation. A highly active O_2-evolving PS II preparation was obtained as previously described [3] with several modifications. Broken chloroplasts prepared with a solution comprising 0.33 M sorbitol/10 mM Mes/120 mM NaCl (pH 6.5) (hereafter referred to as buffer A) according to the method previously described [3] were suspended in the same buffer solution at the chlorophyll concentration of 0.5 mg/ml. After the addition of digitonin (0.2%, w/v), the chloroplast suspension was stirred vigorously for 20 min on ice. The suspension was then centrifuged at $20\,000 \times g$ for 5 min, and the pellet was homogenized with a Teflon homogenizer and diluted to 0.5 mg chlorophyll/ml with the same buffer. To this suspension, Triton X-100 was added at the concentration of 0.85% (w/v) and the suspension was stood for 30 s on ice. For chloroplasts obtained from spinach grown in winter, the concentration of Triton X-100 was increased to 1.25%; the increase was usually necessary for removing the residual PS I complex from the PS II preparation. The Triton-treated subchloroplasts were then centrifuged at $35\,000 \times g$ for 10 min. The pellet obtained was washed once with a solution comprising 0.33 M sorbitol/10 mM Mes (pH 6.5) (buffer B) by centrifugation at $35\,000 \times g$ for 20 min and used as the PS II preparation.

Phase partitioning of the membrane proteins with Triton X-114 or butanol. Separation of hydrophilic and hydrophobic proteins in broken chloroplasts and the PS II preparation was carried out by phase partitioning with Triton X-114 [5,6] or with *n*-butanol [7]. In the *n*-butanol method, the hydrophilic proteins recovered in the aqueous phase after the phase separation was dialyzed overnight against 10 mM Mes (pH 6.5) and concentrated with an Amicon ultrafiltration cell model 52.

Alkaline treatment of the PS II preparation and purification of 33, 24 and 18 kDa proteins. The PS II preparation (0.1 mg chlorophyll/ml) was incubated with 20 mM Tris (pH 9.5) for 30 min at 0°C. After centrifugation at $35\,000 \times g$ for 30 min, the supernatant was dialyzed against 10 mM Mes (pH 6.5) overnight and concentrated with an Amicon ultrafiltration cell model 52. The concentrated protein fraction was then applied to a DEAE-Sepharose CL-6B column (4 cm × 10 cm) preequilibrated with 10 mM Mes (pH 6.5). The column was washed with 10 mM Mes (pH 6.5) and an 18 kDa protein not adsorbed to the DEAE-Sepharose under these conditions was collected in an eluent. A 24 kDa and a 33 kDa protein were eluted by the same buffer with a linear NaCl gradient from 0 to 0.5 M. Each protein fraction obtained after the chromatography was desalted and lyophilized. Concentration of protein was determined by the method of Lowry et al. [8].

SDS-polyacrylamide gel electrophoresis and heme staining. SDS-polyacrylamide gel electrophoresis was carried out as previously described [9]. The gels stained by Coomassie blue R-250 were analyzed at 565 nm by a Toyo digital densitometer DMU 33c. Heme staining was done after the electrophoresis according to the method of Thomas et al. [10] at 15°C and the stained bands in the gels were analyzed at 620 nm.

Measurement of O_2 evolution. O_2 evolution of broken chloroplasts and the PS II preparation was measured with a Rank oxygen electrode at 20°C. The reaction mixture (2.0 ml) contained either broken chloroplasts or the PS II preparation equivalent to 20 μg chlorophyll, 5 mM $MgCl_2$, 0.2 mM phenyl-*p*-quinone and 2 mM potassium ferricyanide in buffer B. NH_4Cl (5 mM) was added to the reaction mixture as an uncoupler when broken chloroplasts were used as a sample. Red actinic light was provided by a 650 W slide projector combined with a red glass filter (Toshiba VR 62).

Chemical analyses of P-700 and cytochromes. The content of P-700 in broken chloroplasts and the PS II preparation was determined by ascorbate minus ferricyanide difference spectra in the 700 nm region [11]. Cytochromes *f* and *b*-559_{HP} were determined by the standard procedure of Bendall et al. [12]. Cytochromes *b*-559_{LP} and *b*-563 were assayed by the method of Rich and Bendall [13]

where menadiol was used as a selective reductant of b-559$_{LP}$. All these determinations were carried out with a Shimadzu UV-240 spectrophotometer.

EPR measurements. Signal I and signal II at room temperature were measured with a Bruker ER200D EPR spectrometer operated in the X-band microwave region at 9.78 GHz. The microwave power was set at 20 mW and the field modulation intensity at 4 G. Samples were put in a glass capillary tube (1 mm diameter) and the depth of insertion of the tube into the cavity was the same in all measurements. The sample (60 μl) contained the PS II preparation equivalent to 0.11 mg chlorophyll, 10 mM Mes, 10 mM potassium ferricyanide and 1 mM EDTA (pH 6.5). To ensure oxidation of P-700, the sample was illuminated with saturating white light introduced by a light guide from a Xenon lamp.

Analysis of quinones. Extraction of prenylquinones from broken chloroplasts and the O_2-evolving PS II preparation was carried out as described by Okayama [14]. The quinones were analyzed by high-performance liquid chromatography (HPLC) with a Waters model 294 system having a Radical-PAK cartridge c-18 column. The mobile phase was a mixture of ethanol and methanol (1 : 3 or 1 : 4, v/v) containing 50 mM $NaClO_4$ and 2 mM $HClO_4$. The flow-rate was 2.0 ml/min. The separated quinones were monitored and determined optically by absorption at 254 nm and polarographically by a Yanaco VMD-101 electrochemical electrode. Other details are shown in Ref. 14.

Measurement of chlorophyll fluorescence induction. The transient of chlorophyll fluorescence was measured with a fluorescence spectrophotometer built in the National Institute for Basic Biology, Okazaki. The reaction mixture (3 ml) contained the PS II preparation equivalent to 30 μg chlorophyll, 0.33 M sorbitol, 10 mM Tricine and 10 mM $MgCl_2$ (pH 7.8). As an electron donor, 1 mM diphenylcarbazide was included in the reaction mixture. 5 μM DCMU was added where indicated.

Determination of manganese. The content of manganese in the preparations was determined with a Hitachi model 170-10 atomic absorption spectrophotometer equipped with an acetylene/air burner head. Drying and charring of the samples were carried out as previously described [15].

Results and Discussion

Previously we reported the isolation of an O_2-evolving PS II preparation from spinach chloroplasts by the treatments with low concentration of digitonin (0.25%) and Triton X-100 (0.2%) [1,3,9]. The preparation showed a high activity of O_2 evolution (150–400 μmol O_2/mg chlorophyll per h), but it contained a small contamination of PS I. To remove the residual PS I, we increased the concentration of Triton X-100 to 0.85–1.25% in the present study to make the detergent-to-chlorophyll ratio 17–25 (w/w). This simple modification led to a purer preparation of highly active O_2-evolving PS II subchloroplasts. O_2-evolution activity of the preparation was usually about 400 μmol O_2/mg chlorophyll per h with phenylquinone and potassium ferricyanide as the electron acceptor system. The densitograms of SDS-polyacrylamide gel electrophoresis showed that the number of polypeptides of the PS II preparation was significantly reduced compared with that of broken chloroplasts (Fig. 1). The polypeptides detected in the PS II preparation were the PS II reaction center complex (approx. 43 kDa, 41–42 kDa in Fig. 1) [16], the light-harvesting complex (a main band at 27 kDa, 26 kDa in Fig. 1) [17], cytochrome b-559 (10 kDa) [18], proteins probably associated with

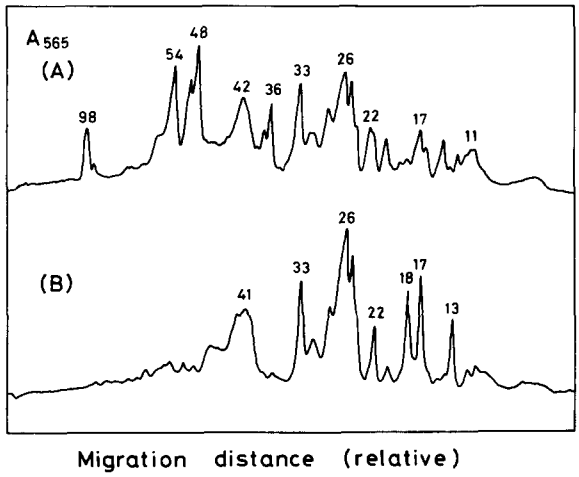


Fig. 1. Densitograms of SDS-polyacrylamide slab gel showing Coomassie blue-stained polypeptides. (A) Broken chloroplasts. (B) The PS II preparation. The figures show the molecular mass of polypeptide bands in kDa. Each sample contained 4 μg chlorophyll.

TABLE I

AMOUNTS OF MEMBRANE COMPONENTS IN BROKEN CHLOROPLASTS AND THE PS II PREPARATION

Data are the average of several measurements with different preparations (the number of the measurements shown in parentheses)± standard deviation.

Components	Broken chloroplasts		PS II preparation	
	nmol/mg chlorophyll	molecules per 370 chlorophyll molecules	nmol/mg chlorophyll	molecules per 200 chlorophyll molecules
P-700	2.2± 0.2 (4)	1	< 0.15 (4)	0
43 kDa protein [a]	100 ±11 (3)	n.d.	185 ±11 (3)	n.d.
Cytochrome *f*	2.4± 0.4 (4)	1	0 (4)	0
Cytochrome *b*-559$_{HP}$	4.2± 0.3 (4)	1.4	5.0± 1.6 (4)	1
Cytochrome *b*-559$_{LP}$	1.8± 1.0 (4)	0.6	5.4± 2.1 (4)	1
Cytochrome *b*-559$_{total}$	6.0± 0.9 (4)	2	10.7± 2.0 (4)	2
Cytochrome *b*-563	5.3± 1.0 (4)	2	0 (4)	0
33 kDa protein	n.d.	n.d.	6.6± 2.1 (6)	1
24 kDa protein	n.d.	n.d.	7.6± 3.0 (6)	1(–2)
18 kDa protein	n.d.	n.d.	8.5± 2.1 (6)	1(–2)
Plastoquinone A	81.5±23.1 (5)	27	16.6± 3.3 (5)	3
Plastoquinone B	0 (5)	0	0 (5)	0
Plastoquinone C_{1-4}	10.3± 3.0 (5)	3	1.3± 1.0 (5)	0
Plastoquinone $C_{5,6}$	0 (5)	0	0 (5)	0
Phylloquinone	5.8± 0.7 (5)	2	1.1± 0.5 (5)	0
α-Tocopherylquinone	4.2± 3.5 (5)	1	1.3± 0.8 (5)	0
Manganese	21.4± 3.9 (4)	7	22.2± 5.1 (4)	4

[a] For the 43 kDa protein, the relative amount of the protein was estimated from the densitograms of Coomassie blue-stained bands in the gels of SDS-polyacrylamide gel electrophoresis.

the O_2-evolution enzyme complex (33, 24 and 18 kDa) [1,4,9,19], the herbicide-binding protein (32 kDa) [20] and other minor polypeptides with smaller M_r. Although the 33 kDa protein responsible for O_2 evolution and the 32 kDa herbicide-binding protein were in the same M_r range, they were usually differentiated from each other on the electrophoresis gel. The 33 kDa protein was easily stained by Coomassie blue and showed a sharp band on the gel, whereas the diffuse band of the herbicide-binding protein was poorly stained by the dye as was shown in Ref. 21. The polypeptide that has M_r of about 90 kDa (98 kDa in Fig. 1) and is attributed to PS I complex [17] was absent in the PS II preparation.

Antenna size of the PS II preparation was estimated by comparing the amounts of cytochrome *b*-559 and PS II reaction center protein (43 kDa) between chloroplasts and the PS II preparation (Table I). It has been generally accepted that the total content of cytochrome *b*-559 in chloroplasts is two molecules per PS II reaction center [22]. Thus, the antenna size was estimated to be 370 chlorophyll molecules for broken chloroplasts on the basis of the amount of the cytochrome in broken chloroplasts. As both the amounts of the PS II reaction center protein and cytochrome *b*-559 in the PS II preparation were about 1.8-times

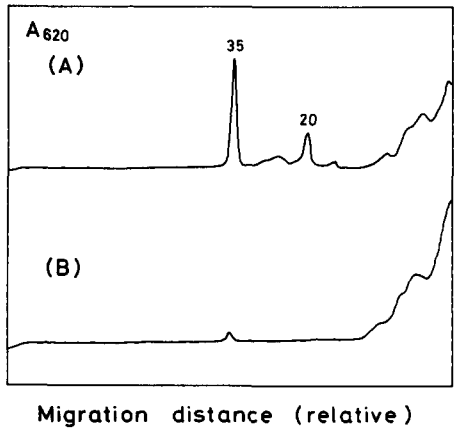


Fig. 2. Densitograms of SDS-polyacrylamide slab gel subjected to heme staining. (A) Broken chloroplasts. (B) The PS II preparation. Other conditions are the same as Fig. 1.

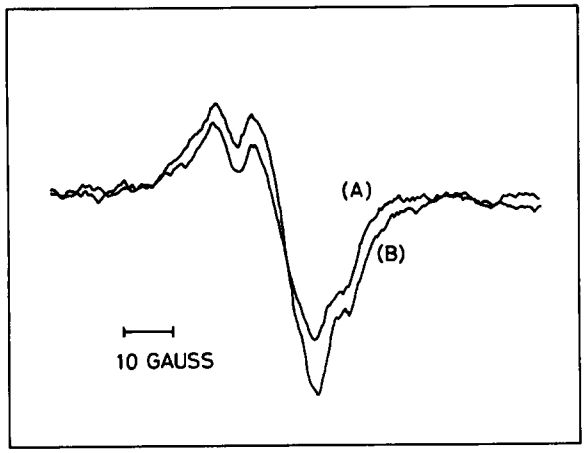
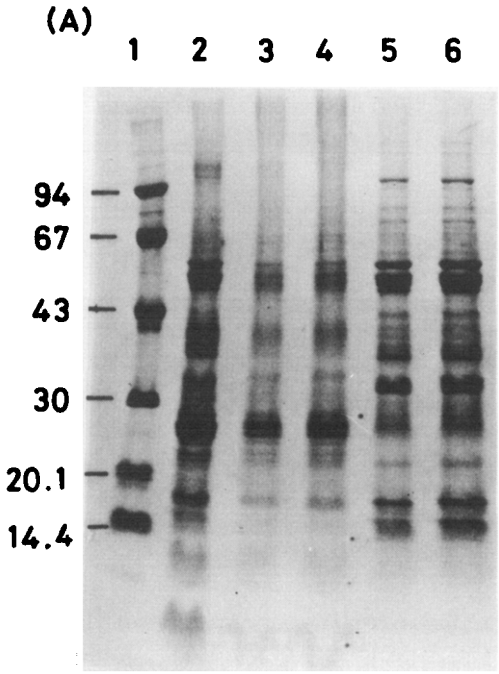


Fig. 3. EPR spectra of the PS II preparation at room temperature. (A) Dark. (B) Light. Details of the measurements are shown in Materials and Methods.

those in broken chloroplasts on the chlorophyll basis, the antenna size of the PS II preparation may be in the range of 200–210 chlorophyll molecules. As to the antenna size of chloroplasts, however, there are large variations depending on the materials used [23–25]. Thus, it should be noted that the antenna size of the PS II preparations might also be affected by physiological conditions of chloroplasts.



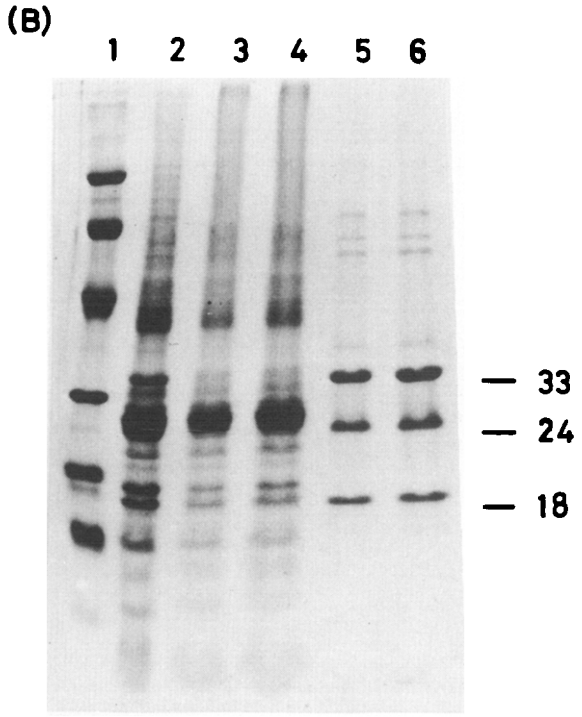


Fig. 4. A SDS-polyacrylamide slab gel showing the membrane proteins separated by phase partitioning with Triton X-114. (A) Broken chloroplasts. (B) The PS II preparation. Lane 1, marker proteins; lane 2, membranes before the phase partitioning; lanes 3 and 4, proteins partitioned into Triton X-114 phase; lanes 5 and 6, proteins partitioned into aqueous phase. The amounts of proteins applied were 20 μg for lanes 1, 2, 3 and 5, and 40 μg for lanes 4 and 6.

When the gels of SDS-polyacrylamide gel electrophoresis were subjected to heme staining, only a negligible amount of cytochrome *f* was detected in the 35 kDa region in the PS II preparation (Fig. 2). Cytochrome *b*-563, which was detected by heme staining as a 20 kDa band on a chromatogram of gel electrophoresis of broken chloroplasts, was not detected in the PS II preparation.

Almost complete absence of PS I in the PS II preparation was also demonstrated by chemical determination of P-700 (Table I) and EPR spectra of the PS II preparation at room temperature (Fig. 3). The EPR spectra observed in the dark-adapted PS II preparation in the *g*-2.00 region in the presence of ferricyanide had the typical signal II. It was almost free from the contribution of signal I ($P\text{-}700^+$). Signal I was not detected even when the

sample was illuminated by saturating light. The same result was also obtained at liquid helium temperature (data not shown).

Of the proteins in the PS II preparation, only the three proteins with M_r 33, 24 and 18 kDa were partitioned into the aqueous phase after the phase separation with Triton X-114 (Fig. 4). All other proteins, including the PS II reaction center complex, the light-harvesting complex, the herbicide-binding protein and cytochrome *b*-559 were partitioned into the Triton X-114 phase, which reflected their hydrophobic nature. The proteins in the PS II preparation showed almost the same behavior in the phase separation with *n*-butanol. Preliminary data were shown in Ref. 26.

The amounts of the 33, 24 and 18 kDa proteins associated with the PS II preparation were estimated by measuring absorbance of the Coomassie-stained protein bands in the gels of SDS-polyacrylamide gel electrophoresis. As the degree of staining by Coomassie blue varies with proteins, simple comparison of the densities of protein bands may be misleading. In the present study, we used the freshly-purified 33, 24 and 18 kDa proteins as standards and carried out electrophoresis with the protein fraction obtained after the alkaline treatment of a given amount of PS II preparation and the standard proteins of several different concentrations on the same gel. The amounts of the 33, 24 and 18 kDa proteins obtained by this procedure in the PS II preparation were one 33 kDa, one (to two) 24 kDa and one (to two) 18 kDa proteins per 200 chlorophyll molecules (Table I). Alkaline treatment (pH 9.5) employed here was the most effective in removing the three proteins from the membranes among several treatments. Other treatments such as that with 0.8 M Tris (pH 8.5) or *n*-butanol sometimes did not completely remove the proteins from the membranes and were not suitable for the quantitative determination of these proteins in our preparation.

Cytochrome *b*-559 is a component tightly associated with PS II, but the ratio of high- and low-potential forms of the cytochrome are known to vary with preparation [27]. In our PS II preparation, two molecules of cytochrome *b*-559 were detected per 200 chlorophyll molecules, one being a high-potential form, the other a low-potential form (Table I). At room temperature, these cytochromes were neither reduced nor oxidized upon illumination of the PS II preparation with saturating flashes (data not shown). Cytochromes *f* and *b*-563 were not detected in the PS II preparation, which is compatible with the results of SDS-polyacrylamide gel electrophoresis (Figs. 1 and 2).

The size of the electron acceptor pool in the PS II preparation was estimated from the measurements of induction in chlorophyll fluorescence at room temperature (Fig. 5) [28]. The integrated area over the fluorescence-induction curve in the presence of DCMU gave a pool size of one electron equivalent which is ascribed to the primary stable electron acceptor Q. The total pool size of the reducing side of PS II determined by the integrated area over the induction curve obtained in the absence of DCMU was about three electron equivalents in the PS II preparation.

Quinones in broken chloroplasts and the PS II preparation were determined with HPLC (Table I). The major quinone species detected in the PS II preparation was plastoquinone A, and the number of plastoquinone A molecules per 200 chlorophyll molecules was about three. Plastoquinone C_{1-4}, phylloquinone and α-tocopherylquinone were detected only as minor components in the PS II preparation. As the measurement of chlorophyll

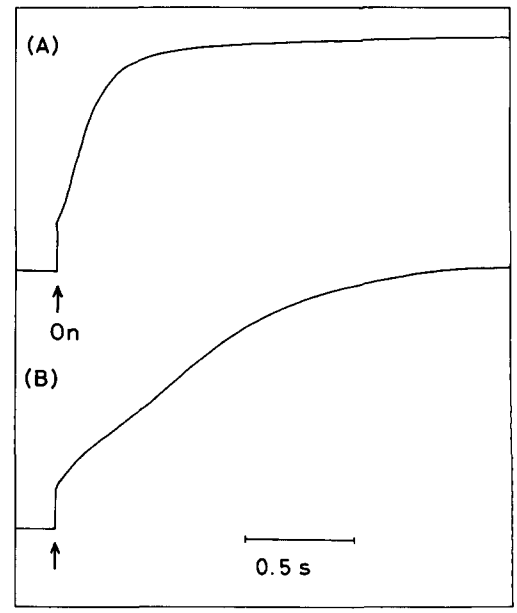


Fig. 5. Time courses of fluorescence induction in the PS II preparation. (A) With DCMU (5 μM). (B) Without DCMU.

fluorescence transient in our preparation showed the presence of an electron acceptor pool of three electron equivalents on the reducing side of PS II, it may be reasonable to assume that two of the three plastoquinone A molecules detected in our photosystem-II preparation are ascribed to the primary stable electron acceptor Q and the secondary electron acceptor B. Q and B are known as a one- and a two-electron carrier, respectively [29]. The third candidate ascribed to plastoquinone A is Z, the secondary electron donor of PS II. Z has been characterized by EPR [30,31], and the lineshape of the signal was shown to be similar to that of semiquinone radicals [32].

The amount of manganese associated with PS II has been a matter of debate [33]. In the present study, we detected four manganese atoms per 200 chlorophyll molecules in the PS II preparation (Table I), which is larger than the value obtained previously [15]. Several reasons may be given for this discrepancy. Firstly, the PS II preparation used for the assay of manganese in the previous study had relatively low activity of O_2 evolution (approx. 200 μmol O_2/mg chlorophyll per h) probably due to partial removal of manganese from the membranes. Secondly, the previously used PS II preparation contained contamination of PS I, and the assumption that 200 chlorophyll molecules comprise the antenna of PS II may not be valid.

Considering all these results, we suggest the organization of PS II as follows. The 43 and 47 kDa proteins are the reaction center proteins that bind chlorophyll *a* [34]. The herbicide-binding protein and cytochrome *b*-559 are the hydrophobic proteins tightly associated with the reaction center complex. From the data on the kinetics of P-680 absorption change and signal II in EPR measurement, it was suggested that the secondary electron donor Z and P-680 are located on the same polypeptide [35,36]. Probably, the primary electron acceptor Q is also located on the same polypeptide and the charge separation at PS II occurs efficiently in the highly organized structure. The 33, 24 and 18 kDa proteins are the hydrophilic proteins assumed to participate in O_2 evolution [1,4,9,19]. Although partial reconstitution of O_2 evolution with these proteins was reported by several authors [37–39], their exact roles in O_2 evolution are still not clear. The site of manganese binding is also under debate. The manganese binding was attributed to the 33 kDa protein [40], but a recent result suggests that the manganese-binding sites are located on the core complex of PS II reaction center [41]. More lately, however, a report appeared showing that a manganese-containing 33 kDa protein can be isolated from chloroplasts under an appropriate condition [42]. In the present study, we tentatively assume the manganese-binding site to be at the interface of the 33 kDa protein and the PS II core complex.

Quite recently, organization of O_2-evolving PS II preparations from spinach chloroplasts was studied by several authors. Dunahay et al. [43] investigated the membrane structure of several PS II preparations and showed that the detergents employed for the isolation of the PS II preparation had considerable effects on the organization of the membranes. Stoichiometry of two cytochrome *b*-559 molecules per PS II reaction center detected in our preparation is consistent with that obtained in a Triton PS II preparation by Lam et al. [44]. However, a smaller stoichiometric ratio, i.e., one cytochrome *b*-559 per PS II reaction center, was also reported with a similar Triton PS II preparation [45]. The size of the quinone pool on the reducing side of PS II reported in Ref. 44 is 3–4 times larger than that estimated in our preparation. Lavorel et al. [46] showed that the pool size tended to be larger in the Triton PS II preparation than in the digitonin/Triton PS II preparation. As the efficiency of the primary steps of charge separation and O_2-evolution process in PS II is regulated by specific interactions of the membrane components associated with PS II, further studies on the details of the organization of these components in the thylakoid membranes at molecular and supramolecular level are required for unveiling the exact mechanism of O_2 evolution in chloroplasts.

Acknowledgements

This work was supported by a grant 58540429 from the Ministry of Education, Science and Culture of Japan. We gratefully acknowledge Professor Y. Fujita of the National Institute for Basic Biology for critical discussion, and Dr. T. Hiyama

of Saitama University for valuable advices in EPR measurement. Thanks are also due to Dr. H. Hattori of the Center for Analytical Instruments, National Institute for Basic Biology for his help in EPR and atomic-absorption measurements.

References

1 Yamamoto, Y., Doi, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 133, 265–268
2 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234
3 Yamamoto, Y., Ueda, T., Shinkai, H. and Nishimura, M. (1982) Biochim. Biophys. Acta 679, 347–350
4 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539
5 Bordier, C. (1981) J. Biol. Chem. 256, 1604–1607
6 Bricker, T.M. and Sherman, L.A. (1982) FEBS Lett. 149, 197–202
7 Maddy, A.H. (1966) Biochim. Biophys. Acta 117, 193–200
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
9 Yamamoto, Y., Shimada, S. and Nishimura, M. (1983) FEBS Lett. 151, 49–53
10 Thomas, P.E., Ryan, D. and Levin, W. (1976) Anal. Biochem. 75, 168–176
11 Marsho, T.V. and Kok, B. (1971) Methods Enzymol. 23, 515–522
12 Bendall, D.S., Davenport, H.E. and Hill, R. (1971) Methods Enzymol. 23, 327–344
13 Rich, P.R. and Bendall, D.S. (1980) Biochim. Biophys. Acta 591, 153–161
14 Okayama, S. (1985) Plant Cell Physiol., in the press
15 Yamamoto, Y. and Nishimura, M. (1983) Biochim. Biophys. Acta 724, 294–297
16 Satoh, K. (1979) Biochim. Biophys. Acta 546, 84–92
17 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127–158
18 Wasserman, A.R. (1980) Methods Enzymol. 69, 181–202
19 Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232
20 Pfister, K., Steinback, K.E., Gardner, G. and Arntzen, C.J. (1981) Proc. Natl. Acad. Sci. USA 78, 981–985
21 Kyle, D.J., Ohad, I., Guy, R. and Arntzen, C.J. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.) pp. 401–410, Academic Press, Tokyo
22 Cramer, W.A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133–137
23 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. USA 77, 4712–4716
24 Melis, A. and Harvey, G.W. (1981) Biochim. Biophys. Acta 637, 138–145
25 Andersson, B. and Haehnel, W. (1982) FEBS Lett. 146, 13–17
26 Yamamoto, Y. and Nishimura, M. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 333–336, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
27 Bendall, D.S. (1982) Biochim. Biophys. Acta 683, 119–151
28 Murata, N., Nishimura, M. and Takamiya, A. (1966) Biochim. Biophys. Acta 120, 23–33
29 Bouges-Bocquet, B. (1980) Biochim. Biophys. Acta 594, 85–103
30 Blankenship, R.E., Babcock, G.T., Warden, J.T. and Sauer, K. (1975) FEBS Lett. 51, 287–293
31 Babcock, G.T., Blankenship, R.E. and Sauer, K. (1976) FEBS Lett. 61, 286–289
32 Hales, B.J. and Gupta, A.D. (1981) Biochim. Biophys. Acta 637, 303–311
33 Cheniae, G.M. (1970) Annu. Rev. Plant Physiol. 21, 467–498
34 Satoh, K., Nakatani, H.Y., Steinback, K.E., Watson, J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150
35 Satoh, K. and Mathis, P. (1981) Photobiochem. Photobiophys. 2, 189–198
36 Babcock, G.T., Ghanotakis, D.F., Ke, B. and Diner, B.A. (1983) Biochim. Biophys. Acta 723, 276–286
37 Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10
38 Toyoshima, Y. and Fukutaka, E. (1982) FEBS Lett. 150, 223–227
39 Miyao, M. and Murata, N. (1983) Biochim. Biophys. Acta 725, 87–93
40 Yamamoto, Y. and Nishimura, M. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 229–238, Academic Press, Tokyo
41 Ono, T. and Inoue, Y. (1984) FEBS Lett. 166, 381–384
42 Abramowicz, D.A. and Dismukes, G.C. (1984) Biochim. Biophys. Acta 765, 318–328
43 Dunahay, T.G., Staehlin, L.A., Seibert, M., Ogilvie, P.D. and Berg, S.P. (1984) Biochim. Biophys. Acta 764, 179–193
44 Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211
45 Sandusky, P.O., Selivius DeRoo, C.L., Hicks, D.B., Yocum, C.F., Ghanotakis, D.F. and Babcock, G.T. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 189–199, Academic Press, Tokyo
46 Lavorel, J., Seibert, M., Maison-Peteri, B. and Briantais, J.-M. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I. pp. 199–202, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague