CHARACTERIZATION OF PROTEINS AND LIPIDS OF PHOTOSYSTEM I AND II PARTICLES FROM *CHLAMYDOMONAS REINHARDI*

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1. Introduction

The photochemical reactions (photosystem I and II) are localized at different sites on the photosynthetic membrane [1–3]. Therefore, on fractionation of thylakoid membranes by detergents or mechanical procedures, PS I and PS II are found to be concentrated in different fractions [4,5]. The components of the thylakoid membrane necessary for the functional integrity of PS I and PS II, have not yet been elucidated in detail. A prerequisite for further studies in this direction is information on the lipid and protein composition of PS I and PS II particles. Our investigations have been carried out with *Chlamydomonas reinhardi* in connection with studies on the biogenesis of the photosynthetic membranes of this alga [6,7].

2. Materials and methods

2.1. *Isolation of PS I and PS II*

Strain sr_3 [8] of *Chl. reinhardi* was cultivated mixotrophically in bottles containing 3.5 litre nutrient [9]. Fresh algae, 60 g, were washed with deionized water, mixed with 80 ml 0.05 M Tris–maleate buffer, pH 7.0, containing 0.015 M $MgCl_2$, 0.25 M sucrose and 0.55 M sorbitol (Medium I) to give a suspension of 10^9 cells/ml, and homogenized with a glass bead homogenizer [10]. After centrifugation for 20 min at 20 000 × *g* the pellet was stirred for 30 min with 50 ml 0.05 M Tris–maleate, pH 7.0, containing 0.015 M $MgCl_2$, 0.55 M sorbitol, 1% (w/v) bovine serum albumin (BSA) and 0.45% (v/v) Triton X-100 (Medium II), the concentration of chlorophyll was adjusted to 0.8 mg/ml. This membrane suspension was centrifuged for 2 h at 38 000 × *g* to give the supernatant s_{38} and the pellet p_{38}. s_{38} was centrifuged for 3 h at 248 000 × *g* (r_{av}) and the supernatant was diluted with 2 vol. water. PS I particles were obtained by centrifuging this solution for another 2 h at 248 000 × *g*. Fraction p_{38} was suspended by a Potter homogenizer and stirred for 30 min with 40 ml medium II, the chlorophyll content was 0.8 mg/ml. The suspension was centrifuged for 10 min at 5000 × *g*. PS II particles were obtained from the supernatant by centrifugation for 30 min at 20 000 × *g*.

2.2. *Separation and determination of lipids, total protein and chlorophyll*

Chilled samples, 0.5 ml, were extracted for 10 s with 3 ml hot MeOH containing 1% 2,6-di-*tert*-butyl-4-methylphenol as antioxidant. To the cooled extract 3 ml diethyl ether and 4 ml 1% NaCl were added, and the mixture was stirred and centrifuged. The residue of the ether phase was analysed by thin-layer chromatography (TCL) on silica gel G (layer 0.3 mm) with $CHCl_3$/MeOH/H_2O 65:25:4 (1st dimension) and $CHCl_3$/MeOH/isopropylamine/conc. NH_4OH 65:35:0.5:5 (2nd dimension) [11]. Spots were visualized using 2,7-dichlorofluorescein. Monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG) and sulfolipid (SL) were quantitated with anthrone [12], the phospholipids as phosphate [13] and lipid X (LX) with a hydroxamate method [14]. Chlorophyll was determined by the procedure [15] and protein by digestion and Nessler's reagent [16].

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2.3. *Separation and solubilization of membrane proteins*

Particle preparations were dialyzed overnight against 0.01 M Tris–HCl, pH 7.6, containing 0.002 M $MgCl_2$ and 0.005 M β-mercaptoethanol and centrifuged for 30 min at 345 000 × *g*. Lipid was removed from the pellet by extraction with acetone/water 9:1 (4 times) and the pellet was solubilized for 5 min at 90°C in a solution of 0.02 M Tris–HCl, pH 7.6, containing 0.014 M β-mercaptoethanol and 1% sodium dodecyl sulfate (SDS). After centrifugation, the supernatants were used for gel electrophoresis. Aliquots containing 120 μg protein in 10–50 μl of solution were layered on gels (8 × 0.6 cm) containing 8% acrylamide, 0.21% *N,N'*-methylene bisacrylamide, 0.05 M Tris, 0.05 M bicine, 0.2% SDS, 0.085% tetramethylethylenediamine and 0.05% ammonium persulfate, the pH of the gel was 8.3. Bromophenol blue was used as a front marker. The buffer solution used for electrophoresis contained 0.05 M Tris, 0.05 M bicine and 0.1% SDS, the pH was 8.3. Electrophoresis was carried out at room temperature at 5 mA/gel. Gels were stained with Coomassie brillant blue [17].

Molecular weights were determined by comparison with co-electrophoresed:lysozyme (14 300 daltons), myoglobin (17 800 daltons), chymotrypsinogen A (25 000 daltons), ovalbumin (45 000 daltons) and BSA (67 000 daltons) as marker proteins.

2.4. *Determination of photosynthetic activity*

PS I activity was measured with an O_2 electrode of the Clark type [18] in the presence of dichlorophenyldimethylurea (DCMU) as an inhibitor of PS II. The reaction mixture contained 0.2 mM 2,6-dichlorophenolindophenol (DCPIP), 5.3 mM ascorbate, 15 μM DCMU, 1 mM methylviologen, 10 mM KCl, 30 mM Tris–HCl, pH 7.6, and particle suspension equivalent to 10–20 μg chlorophyll/ml. A Philips PF 13989 R lamp (1000 W) was used for illumination. PS II activity was assayed photometrically by measuring the reduction of DCPIP at 578 nm in the presence of 1,5-diphenylcarbazide (DPC) as electron donor [20]. The reaction mixture contained 0.06 M KH_2PO_4–Na_2HPO_4 buffer pH 7.4, 1 μM $MnCl_2$, 0.002% DCPIP, 0.5 mM DPC and particle suspension equivalent to 5 μg chlorophyll/ml. The reaction was stopped by the addition of SDS to a concentration of 0.63%.

3. Results and discussion

Properties of PS I and PS II particles are summarized in table 1. PS I and II activities are enriched in different particle fractions, but not completely separated from each other. The chl a/chl b ratio in PS I is about equal to that of the crude membrane fraction, but it is considerably lower in PS II particles. These values are similar to those found in other strains of *Chl. reinhardi* [21], whereas the chl a/ chl b ratio in PS I and PS II particles obtained from spinach by a similar procedure are much higher [22].

Densitometric tracings of the proteins separated by gel electrophoresis reveal 4 major peaks in the profile of crude membranes, as demonstrated in fig.1c. Molecular weights of 73 000, 65 000, 35 000 and 28 000 were determined by comparison with reference proteins. The 73 kD and 65 kD components are typical for PS I, the 35 kD and 28 kD components for PS II (fig.1a + 1b). A very similar pattern was also found in other strains of *Chlamydomonas* [24] in respect to the number and the molecular weight

Table 1
Photosynthetic activity and chl a/chl b ratio of cells and subcellular fractions from *Chlamydomonas reinhardi*

	Cells	Homogenate	Crude membranes	PS I fraction	PS II fraction
PS I activity[a]		1.49	1.0	1.98	0.54
PS II activity[a]		1.01	1.0	0.31	1.24
chl a/chl b	2.65	2.61	2.65	2.63	1.73

[a] Activity of crude membranes, 1.0

Values given are mean values from 2–6 assays

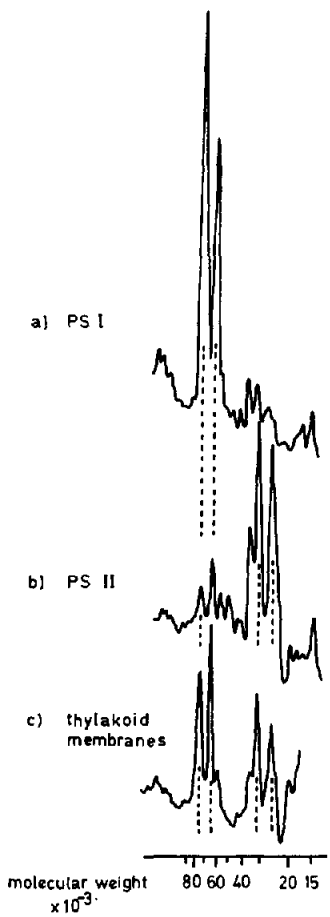

Fig.1. Gel scans of polypeptides from *Chlamydomonas reinhardi*. Conditions see text.

of polypeptides and their distribution among PS I and PS II particles. According to Levin's nomenclature [23], they were named group I polypeptides (typical for PS I) and group II polypeptides (typical for PS II) consisting of components IIa, IIb and IIc. Based on this comparison, we conclude that the 73 kD and 65 kD components found in our experiments belong to the group I polypeptides, while the 35 kD and the 28 kD components represent the group II polypeptides, components IIb and IIc were not very well separated in our scans. Since group I is confined to PS I and group II concentrated in PS II, the peptide profile may be used as a criterium for purity of PS I and PS II particles, although there is also evidence for polypeptides common to both PS I and PS II [20]. As shown in fig.1a and 1b, PS I particles are almost free of group II polypeptides, while PS II particles contain only small amounts of group I polypeptides. The fact that PS I particles contain some PS II activity and, conversely, PS II particles also considerable amounts of PS I activity, raises the question, whether the dominant components of group I and group II polypeptides are necessary for electron transport. In fact, the role of thylakoid polypeptides in electron flow of PS I and PS II is a matter of controversy [20].

A role in membrane stacking rather than in electron flow has been ascribed to the components IIb and IIc since both of them are much reduced in or absent from unstacked chloroplast membranes (from higher plants [23,25] and from *Chl. reinhardi* [26]) possessing PS II activity. At least one polypeptide of group I is synthesized by the chloroplast itself [24,28], while all of the major polypeptides typical for PS II particles and visible SDS gels are translated in the cytoplasm [20,27]. There is, however, also evidence for a participation of both cytoplasm and chloroplast in producing polypeptides of PS I and PS II [20].

The lipid composition (table 2) is similar in the PS I and PS II fraction, and in whole membranes. Major components are glycolipids (MGDG, DGDG, SL) and phospholipids (PE, PG), both of which are generally present in chloroplast membranes [19] and have also been found in isolated PS I and PS II particles from spinach [29]. It should be noted that phosphatidyl choline was present generally in traces only and that the amount of phospholipids was much lower in the photosynthetic particle fractions than in crude membranes. All the cellular fractions contained in addition a phosphorus-free lipid component, which we called lipid X (LX) [9] and which appears to be identical with 1,2-diacylglyceryl-(3)-*O*-4′-(*N,N,N*-trimethyl)-homoserine found in *Ochromonas danica* [30]. The identification of LX will be the subject of a separate paper. The intracellular localization of LX, which is probably not confined to chloroplast structures, is under investigation.

According to Anderson [31], a mosaic-like structure of the thylakoid membrane is suggested with glycolipids and ionic phospholipids located in different areas of the membrane. This would implicate a different lipid pattern in different membrane

Table 2
Lipids in cells and subcellular fractions from *Chlamydomonas reinhardi*

	Lipids (mg/mg total chlorophyll)					
	MGDG	DGDG	SL	PE	PG	LX
Cells	0.69	0.24	0.26	0.03	0.15	0.36
Homogenate	0.70	0.22	0.26	0.03	0.11	0.41
Crude membranes	0.69	0.27	0.23	0.002	0.12	0.21
PS I fraction	0.68	0.27	0.32	tr	0.07	0.36
PS II fraction	0.67	0.20	0.25	tr	0.07	0.20

Values given are mean values from 2–6 assays

fragments. In fact, the lipid patterns of PS I and II particles from spinach are different from that of the whole membrane [29]. A similar inhomogeneity in *Chlamydomonas* particles is, however, not observed in our experiments. This may be due to the fact that the membrane is not fragmented into small enough pieces to allow the detection of an inhomogeneous distribution of lipids.

When PS I and PS II particles were isolated using sorbitol-free media, partial degradation of lipids was observed; yet the particles were photosynthetically active. This raises the question, if and to what extent lipids are necessary for the functional integrity of photosystems. Results obtained so far are controversial. On the one hand, in spinach chloroplast membranes 50–60% of the acyl lipids may be removed without affecting electron flow [31,32]. On the other hand, heptane extraction or lipase treatment of spinach chloroplasts causes a decrease in PS I activity [33], which is restored on addition of galactolipids. In membrane preparations from *Chl. reinhardi* y-1, on addition of egg lecithin PS I activity was slightly enhanced, but PS II activity greatly decreased [20]. The role of lipids in isolated PS I and PS II particles still remains to be elucidated.

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References

[1] Jacobi, G. and Lehmann, H. (1968) Z. Pflanzenphysiol. 59, 457.
[2] Sane, P. V., Goodchild, D. J. and Park, R. B. (1970) Biochim. Biophys. Acta 216, 162.
[3] Goodchild, D. J. and Park, R. B. (1971) Biochim. Biophys. Acta 226, 393.
[4] Brown, J. S. (1973) Separation of Photosynthetic Systems I and II, in: Photophysiology (Giese, A. C. ed) Vol. VIII, pp. 97–112, Academic Press, New York.
[5] Picaud, A. (1972) Biochim. Biophys. Acta 275, 414.
[6] Eichenberger, W. and Boschetti, A. (1975) FEBS Lett. 55, 117.
[7] Boschetti, A., Schaffner, J.-C. and Leuenberger, S. (1976) Chimia 30, 79.
[8] Boschetti, A. and Bogdanov, S. (1973) Eur. J. Biochem. 35, 482.
[9] Eichenberger, W. (1976) Phytochemistry 15, 459.
[10] Bolliger, W., Boschetti, A. and Grob, E. C. (1964) Experientia 20, 654.
[11] Allen, C. F. and Good, P. (1971) in: Meth. Enzymol. (Colowick, S. P. and Kaplan, N. O. eds) Vol. XXIII, pp. 523–547, Part A, Academic Press, New York.
[12] Heinz, W. (1968) Biochim. Biophys. Acta 144, 333.
[13] Donaldson, R. P., Tolbert, N. E. and Schnarrenberger, C. (1972) Arch. Biochem. Biophys. 152, 199.
[14] Kates, M. (1972) Techniques of Lipidology, p. 358, North-Holland Amsterdam.
[15] MacKinney, G. (1941) J. Biol. Chem. 140, 315.
[16] Grob, E. C. and Eichenberger, W. (1969) FEBS Lett. 5, 335.
[17] Maurer, H. R. (1971) in: Disc Electrophoresis, 2nd. edn, pp. 72–18, de Gruyter, Berlin.
[18] Eytan, G., Jennings, R. C., Forti, G. and Ohad, I. (1974) J. Biol. Chem. 249, 738.
[19] Hitchcock, C. and Nichols, B. W. (1971) Plant Lipid Biochemistry, Academic Press, New York.
[20] Bar-Nun, S. and Ohad, I. (1977) Plant Physiol. 59, 161.
[21] Steinback, K. E. and Goodenough, U. W. (1975) Plant Physiol. 55, 864.

[22] Ke, B., Sahu, S., Shaw, E. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36.
[23] Anderson, J. M. and Levine, R. P. (1974) Biochim. Biophys. Acta 333, 378.
[24] Levine, R. P., Burton, W. G. and Duram, H. A. (1972) Nature New Biol. 237, 176.
[25] Henriques, F. and Park, R. B. (1975) Plant Physiol. 55, 763.
[26] Levine, R. P. and Duram, H. A. (1973) Biochim. Biophys. Acta 325, 565.
[27] Hoober, J. K. (1970) J. Biol. Chem. 245, 4327.
[28] Jennings, R. C. and Eytan, G. (1973) Arch. Biochem. Biophys. 159, 813.
[29] Vernon, L. P. and Shaw, E. R. (1971) in: Meth. Enzymol (Colowick, S. P. and Kaplan, N. O. eds) Vol. XXIII, Part A, p. 277, Academic Press, New York.
[30] Brown, A. E. and Elovson, J. (1974) Biochemistry 13, 3476.
[31] Shaw, A. B., Anderson, M. M. and McCarthy, R. E. (1976) Plant Physiol. 57, 724.
[32] Anderson, M. M., McCarthy, R. E. and Zimmer, E. A. (1974) Plant Physiol. 53, 699.
[33] Krupa, Z. and Baszinski, T. (1975) Biochim. Biophys. Acta 408, 26.