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Isolation and characterisation of a photosystem II reaction centre lipoprotein complex

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A photochemically active reaction centre complex has been isolated from photosystem II preparations of spinach chloroplasts by Triton X-100 solubilisation and sucrose gradient fractionation. Electrophoresis of the complex revealed 5 bands indicating polypeptides of apparent molecular masses of 47, 43, 33, 30 and 10 kDa. Lipid analyses showed that polar, as well as neutral, lipids are associated with the complex. For approx. 40 chlorophyll *a* molecules there were 3.4 plastoquinone-9, 3.3 pheophytin *a*, 2.9 β-carotene, 29.3 monogalactosyldiacylglycerol and 12.4 sulphoquinovosyldiacylglycerol molecules. These results suggest that this photosystem II reaction centre complex, which most likely represents a minimum photochemically active unit, is a lipoprotein complex. A striking feature of the associated polar lipids is their very low degree of unsaturation.

Photosystem II *Reaction center* *Lipoprotein* *Triton X-100 solubilisation* *Photosynthesis*

1. INTRODUCTION

Our understanding of the molecular organisation of PS II in the thylakoid membrane of higher plant chloroplasts has been greatly facilitated by the isolation of active PS II enriched membrane fractions [1,2]. Recently, the purification of PS II reaction centre complexes [3–5] has provided information on the protein composition and the identity of individual polypeptides involved in the functional activities of the intrinsic complex [6,7]. In addition, photochemically active preparations have been shown to contain a number of neutral lipids such as chlorophyll, pheophytin, quinone and carotenoids [8] and specific interactions are known to exist between the apoproteins of the complex and these neutral lipids [9–11]. The polar lipids of the thylakoid membrane consist mainly (~75%) of the 2 electroneutral galactolipids, MGDG and DGDG. The remainder is accounted for by the anionic glucolipid SQDG and the phospholipids, PG and PC [12]. These polar lipids are generally assumed to provide the bilayer matrix in which intrinsic pigment-protein complexes reside. It has been postulated [13] that the charged polar lipids, SQDG and PG, may be associated with protein complexes but the participation of polar lipids, especially the galactolipids, as integral parts of the pigment-protein complexes has also been questioned [14]. Although reconstitution experiments [15–18] have indicated the importance of polar lipids in optimising enzymatic activity of protein complexes, energy transfer processes and reassembly of supramolecular structures, direct evidence for specific associations of polar lipids with protein complexes have been scarcely

Abbreviations: PS II, photosystem II; PC, phosphatidylcholine; PG, phosphatidylglycerol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; Mes, 2-(*N*-morpholino)ethanesulphonic acid; DCPIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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reported [19,20]. Here we give the composition of a PS II reaction centre preparation isolated using Triton X-100. The results provide evidence on the lipoprotein nature of the complex and provide insight into its specific lipid-protein interactions within the thylakoid membrane.

2. MATERIALS AND METHODS

PS II preparations were obtained from spinach thylakoid membranes as previously described [1], except that the solutions were at pH 6.0. Reaction centre complexes were purified from the above preparations as follows: PS II preparations were suspended in 20 mM Mes/NaOH, pH 6.0, and centrifuged at 30000 × *g* for 45 min at 4°C. The resultant pellet was resuspended in the same buffer at a chlorophyll concentration of about 0.8 mg/ml. Triton X-100 was added to the solution at a ratio of detergent to chlorophyll of 12:1 (w/w). The sample was incubated on ice, in the dark, for 1 h and then loaded on 0.1–1 M linear sucrose gradients in 20 mM Mes/NaOH, pH 6.0, containing 0.1% Triton X-100. The gradients were centrifuged in an MSE Europa 65 ultracentrifuge at 180000 × *g* for 16 h at 4°C. The PS II reaction centre complex migrated as a sharp band at about 0.7 M sucrose and represented approx. 10% of the total chlorophyll loaded on the gradient. The complex catalysed the photoreduction of DCPIP with DPC as the electron donor at rates between 170 and 220 μmol/mg chlorophyll per h. It proved essential to keep the complex in the dark at all stages after isolation since in the absence of an electron acceptor the preparation became photoinhibited.

SDS-PAGE of the purified complex was carried out essentially as described in [21] on a 7–17% acrylamide gel in the absence of urea. Samples were applied to the gel without prior heating. Coomassie stained gels were scanned at 560 nm, using a Shimadzu CS-930 dual wavelength scanner and recorded with a Shimadzu DR-2 recorder.

For lipid analyses, the preparation was extracted by the method described in [22]. Quinones were estimated by high-performance liquid chromatography (HPLC) as described in [23]. For estimation of pigments, samples were applied on Merck thin-layer chromatography silica gel-60 plates, and developed using petroleum ether:isopropanol (85:15, v/v) as the solvent. Pigment bands were scraped off the plates. Chlorophylls were eluted with acetone:water (80:20, v/v) and determined according to [24]. Pheophytin *a* and carotenoids were eluted with acetone and determined spectrophotometrically as described in [25,26], respectively. Polar lipids extracted as in [22] were separated on silica gel thin-layer chromatography plates using chloroform:methanol:water (65:25:4, v/v/v) as the solvent and identified by co-chromatography of purified lipids. Quantification of the lipid classes was carried out by gas-liquid chromatography according to [27]. Absorption spectra were obtained using a Perkin-Elmer absorption spectrophotometer (model 554), and emission spectra measured, using a Perkin-Elmer fluorescence spectrophotometer (model MPF44A). Cytochrome *b*-559 was determined from the dithionite-ferricyanide difference spectra according to [28] using a Perkin-Elmer spectrophotometer (model 557).

3. RESULTS

A photochemically active PS II reaction centre complex was isolated by Triton X-100 solubilisation of PS II preparations. The polypeptide composition of the purified complex is shown in fig.1.

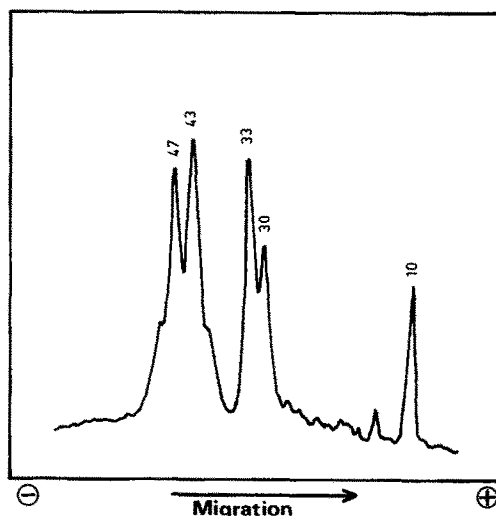

Fig.1. Densitometric tracing at 560 nm of SDS-polyacrylamide gel obtained from electrophoresis of the purified reaction centre complex. Numbers indicate molecular masses of the resolved polypeptides in kDa.

The apparent molecular masses of the 5 resolved polypeptides were estimated to be 47, 43, 33, 30 and 10 kDa. This polypeptide composition is practically identical to that of the reaction centre complex isolated by the action of digitonin using a combination of isoelectric focusing, sucrose gradient centrifugation and DEAE-cellulose column chromatography [11]. The latter preparation required the inclusion of urea in the SDS-gel to resolve the polypeptides and overcome anomalous migration behaviour observed in the absence of urea and attributed to residual digitonin bound to the complex [11]. The reaction centre complex isolated as reported here, however, showed no necessity for the inclusion of urea at any stage of the electrophoresis. The spectral characteristics at room temperature of the Triton X-100 derived reaction centre complex are shown in fig.2. The complex exhibits a fluorescence emission maximum at 682 nm and a red absorption maximum at 672 nm. As expected no absorbance due to chlorophyll *b* pigments was observed. Chemical analysis of the pigments verified the absence of chlorophyll *b* from the complex. The spectra shown here are very similar to those observed with the digitonin derived reaction centre complexes [6]. Components associated with the reaction centre complex isolated with Triton X-100 are shown in table 1. As determined spectrophotometrically this complex contained about 2 *b*-559 cytochromes for 40 chlorophyll *a* molecules. The cytochrome was present only as the dithionite reducible low potential form. Relative to the 40 chlorophyll *a* molecules the complex contained 3.4 plastoquinone-9, 3.3 pheophytin *a* and 2.9 β-carotene molecules. The association of the above

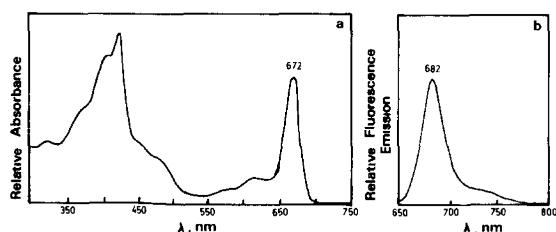

Fig.2. Room temperature absorption (a) and fluorescence emission (b) spectra of the purified reaction centre complex. In (b) the pigments were excited with 420 nm light with an excitation slit-width of 10 nm and emission slit-width of 2 nm.

Table 1

Components of the purified reaction centre complex

Component	Molar ratio (component/40/chlorophyll *a*)	
Cytochrome *b*-559	1.96 ± 0.2	(3)
Plastoquinone-9	3.4 ± 0.6	(5)
Pheophytin *a*	3.3 ± 0.2	(5)
β-Carotene	2.9 ± 0.1	(5)
MGDG	29.3 ± 0.8	(9)
SQDG	12.4 ± 0.4	(9)

Figures in parentheses represent numbers of preparations examined

neutral lipids with the reaction centre of PS II has been previously reported [8] although the stoichiometry differs from the one presented here. In addition to the above neutral lipids, the reaction centre complex isolated with Triton X-100 had polar lipids associated with it. The total amount of polar lipids found in the complex was approximately at a ratio of 1:1 with chlorophyll *a*. Only 2 polar lipid classes were present, namely MGDG and SQDG, the former being relatively more abundant. Analysis of the fatty acid composition of these lipid classes revealed quite unusual features. As shown in table 2, both lipid classes co-purifying with the reaction centre complex of PS II are characterised by an extremely low degree of unsaturation as compared with that observed in intact thylakoid membranes or indeed PS II membrane preparations [15]. Approximately half of the fatty acids of the molecules were found to be palmitic acid (16:0). Linolenic acid (18:3), which in normal thylakoids is the most commonly occurring fatty acid, was only present in very small amounts. The only significant contribution to unsaturation in these lipid classes was provided by oleic acid (18:1).

4. DISCUSSION

Using a relatively simple and rapid isolation procedure employing the non-ionic detergent Triton X-100, we have prepared a PS II reaction centre complex similar to that isolated by Satoh [11] using digitonin and chromatographic techniques. Our preparation is completely devoid of the light-

Table 2

Fatty acid composition of the lipid classes co-purified with the reaction centre complex

Lipid class	% molar composition of fatty acids							Average double bond index
	16:0	16:1	16:3	18:0	18:1	18:2	18:3	
MGDG	41.9 ± 1.2	–	–	9.5 ± 0.5	25.4 ± 0.4	13.3 ± 0.7	9.9 ± 0.6	1.63
SQDG	48.9 ± 0.4	–	–	21.0 ± 0.4	20.9 ± 0.4	6.6 ± 0.4	2.6 ± 0.3	0.80

harvesting chlorophyll *a/b* polypeptide as determined by SDS-PAGE, spectral characteristics and chemical analysis of the pigments. The reaction centre complex resolved into 5 polypeptide bands by SDS-PAGE and visualised by Coomassie blue stain. Silver staining of the preparation revealed no additional polypeptides (not shown). These polypeptides have been previously identified and attributed to functional components of the reaction centre complex. The 47 kDa polypeptide has been attributed to the reaction centre polypeptide while that of 43 kDa has been identified as the main antenna chlorophyll protein of the PS II reaction centre [7]. It has been suggested that the low molecular mass polypeptide (10 kDa) is the apoprotein of cytochrome *b*-559. Spectrophotometric determinations in our preparation revealed the presence of 2 *b*-559 cytochromes for every 40 chlorophyll *a* molecules. The identity of the 2 polypeptides in the region of 30–33 kDa has not been established with certainty but it is believed that at least one represents the herbicide binding protein [11].

Although the polypeptide composition of this PS II preparation is similar to that previously reported [11], we have estimated different amounts of neutral lipids to be associated with the reaction centre complex. We find 3–4 plastoquinone-9 molecules for every 40 chlorophyll *a* molecules. Assuming that the secondary acceptor Q_B is present in our preparation, then this value is in line with the existence within the complex of the primary stable acceptor Q_A, and the secondary donor Z, which is thought to be plastoquinone [29]. We find higher levels of pheophytin *a* than those reported in [8]. Whether the difference is due to the different procedures used for the isolation of the complex remains to be investigated. The total amount of carotenoids associated with the reaction centre has been reported [8] to be 10 molecules for every 50 chlorophyll *a* molecules. In our preparation we detected β-carotene but it was found to be in a ratio of 3 to 40 chlorophyll *a* molecules.

An important finding of the present investigation is that the reaction centre complex co-purifies with small amounts of specific types of polar lipids. It is thus apparent that this reaction centre preparation is isolated as a lipoprotein-pigment complex. The levels of polar lipids associated with the complex are sufficiently low to assume that they are particles rather than membrane fragments. In agreement with this, it is shown that the lipid composition is very uncharacteristic of the bilayer since it contains only MGDG and SQDG with unusual levels of unsaturation. The degree of saturation of the acyl chains would indicate that both lipid classes are probably in the crystalline phase at room temperature but the low levels of lipid associated with the complex presumably do not impose constraints on the enzymatic activity of the complex. Moreover the degree of unsaturation of MGDG fatty acids affects not only the transition temperature of this lipid class but also its structural polymorphism [30], a property which may be important for its specific involvement with PS II.

In the case of SQDG, it normally occurs in the thylakoid with approximately half its fatty acids in the form of palmitic acid (16:0) while the remaining fatty acids contribute to an average double bond index of about 3.7. In the PS II reaction centre complex, however, it seems that only certain molecular species of this lipid class exist, since it appears that palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids make up most of the lipid present. The fatty acid content of MGDG associated with the particle is also very different to that found in the intact thylakoid with the total

absence of hexadecatrienoic acid (16:3) being noteworthy.

Whether specific types of bonds exist between these lipid molecular species and the protein complex is not known, but in any event they do seem to be an intimate part of the functional PS II unit which we have isolated. Yet to be established is the possibility that the presence of polar lipids is required for the normal PS II activity or for the correct organisation of the supramolecular complex.

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