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# Glycerolipids in various preparations of Photosystem II from spinach chloroplasts

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**The fatty acid and glycerolipid composition of three types of preparation of Photosystem II (PS II) from spinach thylakoids was studied, namely, the PS II membrane, the PS II core complex, and the PS II reaction center complex. The molecular ratios of lipid to photochemical reaction center II (P680) in these preparations were estimated to be about 150:1, 10:1 and 1:1, respectively. The only lipid molecule found in the PS II reaction center complex was monogalactosyl diacylglycerol (MGDG). This molecule was highly saturated as a result of the presence of hexadecanoic, octadecanoic and tetradecanoic acids; together they accounted for about 50% of the total fatty acids. This composition is in marked contrast to that of MGDG from thylakoid membranes, in which the trienoic fatty acids account for about 90% of the total. The PS II core complex contained MGDG, digalactosyl diacylglycerol and phosphatidylglycerol, but it lacked sulfoquinovosyl diacylglycerol, whereas the PS II membrane contained each of these four glycerolipids. The degree of saturation of the fatty acids in the PS II core complex was intermediate between that in the PS II reaction center complex and that in the PS II membrane. The results are discussed in terms of the structure and function of lipids in the thylakoid membrane.**

## Introduction

The thylakoid membrane, an intra-chloroplastic membrane, is the site of photosynthetic electron transport and energy conversion. This membrane is unique, as compared to the other membranes in the plant cell, in having a high proportion of glyceroglycolipids and a low proportion of phospholipids [1]. The glyceroglycolipids are MGDG, DGDG and SQDG, and they account for 40–50%, 20–30%, 5–10%, respectively, of the total glycerolipids, with variations depending on the species of plant [2]. The phospholipid in the thylakoid membrane is PG, which accounts for 10–20% of the total lipids [2].

The specific association of these lipids with the PS II complex has been studied in various types of preparation of PS II. Farineau et al. [3] reported that the lipid composition of PS II membranes prepared from spinach thylakoids with Triton X-100 was similar to that of thylakoid membranes with the exception of a decrease in the proportion of SQDG in PS II membrane. By contrast, Gounaris et al. [4] reported, for a similar preparation of PS II membranes, a lower proportion of DGDG and higher proportions of SQDG and PG than those in the thylakoid membrane. According to Gounaris and Barber [5], a preparation of PS II, which was prepared by treating the PS II membranes with Triton X-100 and which contained only a limited number of polypeptides, consisted of approx. 30 molecules of MGDG and approx. 10 molecules of SQDG per 40 molecules of Chl.

The specific binding of glycerolipids to protein complexes from the thylakoid membrane has also been studied, for example, the association of SQDG and DGDG with the ATP synthase [6], that of a phospholi-

Abbreviations: Chl, chlorophyll; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; Pheo, pheophytin; PS II, Photosystem II; SDS, sodium dodecylsulfate; SQDG, sulfoquinovosyl diacylglycerol; TLC, thin-layer chromatography, 14:0, tetradecanoic acid; 16:0, hexadecanoic acid; 16:1c, $\Delta^9$-*cis*-hexadecenoic acid; 16:1t, $\Delta^3$-*trans*-hexadecenoic acid; 16:3, $\Delta^{7,10,13}$-*cis*-hexadecatrienoic acid; 18:0, octadecanoic acid; 18:1, $\Delta^9$-*cis*-octadecenoic acid; 18:2, $\Delta^{9,12}$-*cis*-octadecadienoic acid; 18:3, $\Delta^{9,12,15}$-*cis*-octadecatrienoic acid.

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pid (presumably PG) with the cytochrome $b_6/f$ complex [7], and that of PG with the light-harvesting Chl-protein complex [8,9].

The role of the lipids in the function of the thylakoid membrane has been studied mainly by two methods, namely, by inactivation due to degradation of lipids by lipases and by reactivation by addition of exogenous lipids. Siegenthaler et al. [10,11] studied the effect of depletion of PG by digestion with phospholipase $A_2$ and concluded that this phospholipid, located in the inner monolayer of the thylakoid membrane, is essential for photosynthetic electron transport. Gounaris et al. [4] observed that the evolution of oxygen by PS II membranes was activated by the addition of exogenous DGDG and PC and inhibited by the addition of PG and SQDG. It has been shown that the activity of ATP synthase is stimulated by MGDG that contains polyunsaturated fatty acids [12]. The cytochrome $b_6/f$ activity, i.e., the plastoquinol/plastocyanin oxidoreductase activity, was stimulated by DGDG, PG and PC, but not by MGDG and SQDG [13]. The oligomeric form of the light-harvesting Chl-protein [14] and the system for the transfer of electronic excitation from the light-harvesting Chl to PS II core Chl can be reconstituted in the presence of PG [15]. However, such observations do not exclude the possibility that the exogenously added lipids produce artifactual and/or non-specific effects in the same way as do many detergents.

The PS II complex contained in PS II membranes prepared from spinach thylakoids with Triton X-100 [16] is composed of more than 20 different proteins [17,18], as follows: D1 (32 kDa) and D2 (34 kDa) proteins; two core antenna proteins (43 kDa and 47 kDa); light-harvesting Chl *a/b* proteins (24–29 kDa and 4 kDa); two subunits of cytochrome *b*-559 (9 kDa and 4 kDa); hydrophobic proteins of 24 kDa, 22 kDa and 10 kDa; several hydrophobic proteins with molecular masses of less than 10 kDa; and three extrinsic proteins of 33 kDa, 23 kDa and 18 kDa. By treating such PS II membranes with octyl glucoside, a PS II core complex can be prepared which is active in both photochemical charge separation and the evolution of oxygen. Such a preparation contains the D1 and D2 proteins, the core antenna proteins, the subunits of cytochrome *b*-559, the phosphoprotein, several hydrophobic proteins with molecular masses of less than 10 kDa, and only one extrinsic protein of 33 kDa [19].

Treatment of the PS II membranes with a high concentration of Triton X-100 followed by anion-exchange column chromatography results in purification of the reaction center II complex [20], which is composed of the D1 and D2 proteins, the subunits of cytochrome *b*-559 and, possibly, a hydrophobic protein of 5 kDa [21]. This complex is active in photochemical charge separation but inactive in terms of the evolution of oxygen.

Since three different preparations of PS II are available, each of which is clearly defined in terms of its component polypeptides and photosynthetic activity, it is appropriate to analyze the composition in terms of glycerolipids and fatty acids of these types of PS II preparation in order to elucidate the specific association of glycerolipid(s) and fatty acid(s) with the PS II complex and the PS II subcomplexes. We have found that one molecule of MGDG is associated with the PS II reaction center complex and that about ten lipid molecules (MGDG, DGDG and PG) are associated with the PS II core complex.

## Materials and Methods

*Preparation of PS II membranes, the PS II core complex and the PS II reaction center complex*

PS II membranes were prepared from spinach thylakoids by treatment with Triton X-100 as described by Kuwabara and Murata [16] and stored at −196 °C in the presence of 30% (v/v) ethylene glycol [22]. Before use, the membranes were washed three times with a medium of 10 mM NaCl, 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) by centrifugation and resuspension, and finally they were suspended in the same medium.

The PS II core complexes were prepared by treating PS II membranes with octyl glucoside and subsequent centrifugation by the method of Ikeuchi et al. [19]. The resultant sample was suspended in a solution of 1.0 mM digitonin, 500 mM sucrose, 10 mM NaCl and 25 mM Mes/NaOH (pH 6.5), and stored at −196 °C.

The PS II reaction center complexes were prepared by treatment of the PS II membranes again with Triton X-100, with subsequent anion exchange chromatography on a column of DEAE-Toyopearl 650M, by the method of Nanba and Satoh [20]. The chromatographic fraction that contained the purified PS II reaction center complex was brought to 40% saturation with $(NH_4)_2SO_4$, and, after incubation for 60 min at 0°C, the mixture was centrifuged at $1000 \times g$ for 60 min. The PS II reaction center complexes were collected as green floats.

*Extraction of lipids and removal of Triton X-100*

Lipids were extracted from the preparations of PS II by the method of Bligh and Dyer [23] with minor modifications. Each preparation of PS II, corresponding to approx. 500 μg of Chl, was dispersed in 10 ml of chloroform and methanol (1 : 2, v/v) on a vortex mixer. The dispersion was kept at room temperature for 5 min (standard conditions). A prolonged extraction, for 5 h, did not increase the amount of lipids extracted from any of the PS II preparations, suggesting that the extraction of lipids was complete within 5 min. Then, 3 ml

of chloroform and 5 ml of 500 mM KCl in $H_2O$ were added, and the lower chloroform layer was withdrawn. For complete recovery of lipids, 6 ml chloroform was mixed with the upper water/methanol layer, and the resultant lower chloroform layer was withdrawn. The chloroform fractions were combined and evaporated under reduced pressure.

The extracted lipids were dissolved in a mixture of methanol and $H_2O$ (85 : 15, v/v) and subjected to reversed-phase HPLC on an octadecylsilane column (YMC, Kyoto, Japan; A-302, 0.46 cm i.d. × 15 cm) in order to remove Triton X-100 from the lipid extract. Upon elution with the same mixture of methanol and $H_2O$ (85 : 15, v/v) at a flow rate of 1.0 ml · $min^{-1}$ for 10 min, Triton X-100 passed through the column while all the lipids remained adsorbed to the column. Then all the lipids were eluted with methanol at the same flow rate over the course of 60 min. This reversed-phase HPLC step was necessary because the large amounts of Triton X-100 in the preparations of PS II interfered with the subsequent chromatographic separation of the various classes of lipids.

*Separation of lipid classes and analysis of fatty acids*

The total lipids were separated into lipid classes by HPLC on silica gel by the method of Demandre et al. [24] with minor modifications. The lipids were dissolved in a mixture of 2-propanol and hexane (4 : 3, v/v) and subjected to HPLC on a column of silica gel (Tosoh, Tokyo, Japan; Silica-60, 0.78 cm i.d. × 30 cm), which had been equilibrated with the same mixture. Pigments and quinones were removed by elution with the same mixture for 10 min. Lipids were then eluted with a linear gradient from 2-propanol/hexane (4 : 3, v/v) to 2-propanol/hexane/$H_2O$ (4 : 3 : 0.75, v/v) for 20 min, and then with 2-propanol/hexane/$H_2O$ (4 : 3 : 0.75, v/v) for 30 min. The flow rate for each solvent system was 2.0 ml · $min^{-1}$. The elution of lipids was monitored at 208 nm. The lipid classes in the eluted fractions were identified by comparisons of their retention times with those of the following authentic classes of lipids: MGDG, DGDG, SQDG and PG, all isolated in our laboratory from *Synechocystis* PCC6803 grown at 34°C [25]; and PC and phosphatidylethanolamine from pig liver, PG from egg yolk, phosphatidylinositol from soybeans and from beef brain, all purchased from Serdary Research Laboratories, (London, Ontario, Canada). The identification of the various classes of lipids by HPLC was confirmed by TLC and staining with the molybdenum blue reagent for phospholipids [26], with Dragendorff's reagent for PC [27], with the anthrone reagent for glycolipids [28], and with ninhydrin for aminolipids [29]. The HPLC described above successfully fractionated all the lipid classes from the preparations of thylakoid and PS II. TLC on silica gel plates could not be used for the fractionation of lipid classes in place of the column chromatography on silica gel because the contamination by 16 : 0 and 18 : 0 from the thin-layer plates produced significant errors in the determination of the fatty acid compositions when the amounts of lipids were very small, as was the case in the present study.

The total lipids before the reversed-phase HPLC and the separated classes of lipids were subjected to methanolysis with 5% HCl in methanol at 85°C for 2.5 h. The resultant methyl esters were analyzed with a gas-liquid chromatograph (Shimadzu, Kyoto, Japan; GC-14A) equipped with a hydrogen flame-ionization detector. The fatty acid methyl esters were separated on a capillary column (Quadrex, New Haven, CT, U.S.A.; CPS-1, 0.25 mm i.d. × 50 m) coated with cyanopropylmethyl silicone at a thickness of 0.25 μm. The temperatures of the column and flame-ionization detector were 180°C and 200°C, respectively. Analysis of chromatographic data was performed with a data processor (Shimadzu, Chromatopack C-R3A). The fatty acid methyl esters were identified with a gas chromatograph-mass spectrometer (JEOL, Tokyo, Japan; JMS-DX 300), equipped with a mass data analysis system (JEOL, JMA-3100), and the same column as that used for gas-liquid chromatography. For quantitative determinations of fatty acids, a known amount of methyl pentadecanoate was added to the samples of lipids before transmethylation.

*Biochemical analysis*

Evolution of oxygen by preparations of PS II was monitored with a Clark-type oxygen electrode, as described previously [16]. The reaction medium for PS II membranes contained 300 mM sucrose, 10 mM NaCl, 25 mM Mes-NaOH (pH 6.5), 0.3 mM phenyl-*p*-benzoquinone, and PS II membranes corresponding to 8.0 μg Chl · $ml^{-1}$; the medium for the PS II core complex contained 400 mM sucrose, 100 mM NaCl, 10 mM $CaCl_2$, 40 mM Mes-NaOH (pH 6.5), 1.0 mM digitonin, 1.0 mM $K_3Fe(CN)_6$ and PS II core complexes corresponding to 2.0 μg Chl · $ml^{-1}$. Polypeptides in preparations of PS II were analyzed by SDS-polyacrylamide gel electrophoresis, in the presence of 6.0 M urea, in the buffer system described by Laemmli [30]. Amounts of Chl were determined by the method of Arnon [31]. The molar absorption coefficient at 663 nm of Chl *a* was used for that of Pheo *a*.

## Results

The characteristics of PS II membranes, PS II core complexes, and PS II reaction center complexes, prepared as described in Materials and Methods, were the same as those reported previously [16,19,20]. For example, the photosynthetic oxygen-evolving activities of the PS II membranes and PS II core complex were as high as 400 and 1200 μmol/mg Chl per h, respectively. By

TABLE I

*Glycerolipid compositions and molecular ratios of glycerolipid to Chl in thylakoid membranes and three types of preparation of PS II*

Numbers in parentheses represent numbers of experiments.

| Preparation | Lipid (mol%) | | | | | Molar ratio lipid/(Chl (+Pheo)) |
|---|---|---|---|---|---|---|
| | MGDG | DGDG | PG | SQDG | PC | |
| Thylakoid membrane (1) | 56 | 29 | 5 | 3 | 7 | 2.2 |
| PS II membrane (1) | 59 | 27 | 10 | 3 | 1 | 0.71 |
| PS II core complex (3) | 32±1 | 34±5 | 34±4 | 0 | 0 | 0.20±0.02 |
| PS II reaction center complex (3) | 91±4 | 0 | 9±4 | 0 | 0 | 0.18±0.01 |

contrast, the PS II reaction center complexes were inactive in terms of the evolution of oxygen, but they were able to photoreduce Pheo *a* in the presence of sodium dithionite. SDS-polyacrylamide gel electrophoresis revealed that the polypeptide compositions of these preparations were essentially the same as those originally reported [16,19,20]. The ratio of Chl *a* to Chl *b* was 1.9–2.0 in PS II membranes and higher than 15 in both PS II core complexes and PS II reaction center complexes.

All the preparations of PS II contained high levels of Triton X-100, which was found to interfere with the separation of extracted lipids into classes during subsequent HPLC on silica gel. The reversed-phase HPLC removed all the Triton X-100 from the lipid extracts and subsequent HPLC on silica gel separated the lipids into five classes: MGDG, DGDG, SQDG, PG and PC. Fig. 1 shows an example of the fractionation of lipids from the PS II core complex.

The glycerolipid composition and the ratio of glycerolipid to Chl of preparations of thylakoids and PS II are presented in Table I. The PS II membrane was similar to the thylakoid membrane in terms of lipid composition with the exception that the level of PC was negligible in PS II membranes. In both types of membrane, MGDG accounted for more than 50% of the total glycerolipids, and PG and SQDG each accounted for less than 10%. The ratio of lipid to Chl was reduced to 2 : 3 in PS II membranes, as compared to the ratio of 2 : 1 in thylakoid membranes.

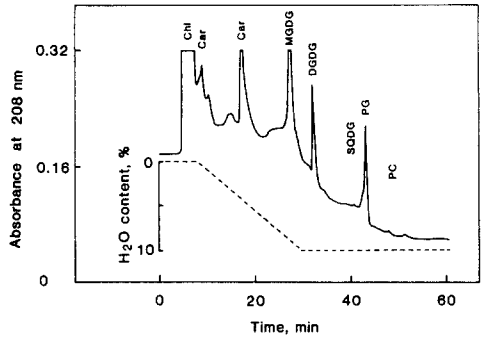


Fig. 1. Elution profile after HPLC of total lipids from the PS II core complex. Conditions for HPLC and for removal of Triton X-100 from the total lipids by reversed-phase HPLC are described in Materials and Methods. Car, carotenoids.

In the PS II core complex, MGDG, DGDG and PG were present at about the same levels, whilst SQDG and PC were absent. The proportion of lipid relative to Chl was very much lower than that in the PS II membrane. This result differs from that of Gounaris and Barber [5] who detected MGDG and SQDG, but no DGDG and PG, in a preparation of PS II similar to the preparation of PS II core complex analyzed in the present study.

In the PS II reaction center complex, MGDG accounted for 90% of the total glycerolipids and PG accounted for only 10%. The other classes of lipids, namely, DGDG, SQDG and PC, were all absent from this preparation. The ratio of lipid to Chl (+Pheo) was about the same as that in the PS II core complex.

TABLE II

*Fatty acid compositions of total lipids from thylakoid membranes and preparations of PS II*

Numbers in parentheses represent numbers of experiments. t, trace (less than 0.5%).

| Preparation | Fatty acid (mol%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1c | 16:1t | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 | Total satd. | 16:3+18:3 |
| Thylakoid membrane (1) | t | 5 | t | 2 | 13 | t | 1 | 4 | 75 | 5 | 88 |
| PS II membrane (1) | t | 9 | 1 | 4 | 9 | 1 | 1 | 6 | 70 | 10 | 79 |
| PS II core complex (3) | 3±1 | 17±2 | 4±1 | 13±1 | 6±1 | 4±1 | 4±1 | 5±1 | 44±2 | 24 | 50 |
| PS II reaction center complex (3) | 6±1 | 32±2 | 7±1 | 3±1 | 3±1 | 12±1 | 9±1 | 4±1 | 24±2 | 50 | 27 |

TABLE III

*Fatty acid compositions of MGDG from thylakoid membranes and preparations of PS II*

Numbers in parentheses represent numbers of experiments. t, trace (less than 0.5%).

| Preparation | Fatty acid (mol%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1c | 16:1t | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 | Total sat. | 16:3+ 18:3 |
| Thylakoid membrane (1) | t | 2 | t | 0 | 20 | t | 1 | 2 | 74 | 2 | 94 |
| PS II membrane (1) | 2 | 4 | 1 | 0 | 18 | 1 | 1 | 2 | 72 | 7 | 90 |
| PS II core complex (3) | 4±1 | 20±2 | 3±1 | 0 | 8±2 | 12±2 | 4±1 | 4±1 | 45±5 | 36 | 53 |
| PS II reaction center complex (3) | 5±1 | 27±2 | 7±1 | 0 | 4±1 | 18±1 | 9±1 | 5±1 | 25±2 | 50 | 29 |

TABLE IV

*Fatty acid compositions of PG from thylakoid membranes and preparations of PS II*

Numbers in parentheses represent numbers of experiments. t, trace (less than 0.5%).

| Preparation | Fatty acid (mol%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1c | 16:1t | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 |
| Thylakoid membrane (1) | t | 15 | 0 | 41 | t | 1 | t | 4 | 40 |
| PS II membrane (1) | 1 | 17 | 0 | 36 | t | 2 | 1 | 3 | 40 |
| PS II core complex (3) | t | 18±1 | 0 | 44±2 | t | 1±1 | t | 5±1 | 31±2 |
| PS II reaction center complex (3) | 4±1 | 35±3 | 0 | 27±3 | 0 | 15±1' | 5±1 | 6±1 | 8±1 |

In Table II, the fatty acid composition of total lipids from thylakoid membranes and from three types of preparation of PS II are compared. The PS II membrane and the thylakoid membrane were similar in terms of fatty acid composition. They were rich in trienoic acids, 18:3 and 16:3. In the PS II core complex, the relative level of trienoic acids was lower than that in the PS II membrane, and that of the saturated acids, 14:0, 16:0 and 18:0, was higher. The relative levels of 16:1c, 16:1t and 18:1 were also higher. Such increases in the relative levels of the saturated and monounsaturated fatty acids and the corresponding decreases in the trienoic fatty acids were the most prominent features of the PS II reaction center complex. The

TABLE V

*Fatty acid compositions of DGDG from thylakoid membranes and preparations of PS II*

Numbers in parentheses represent numbers of experiments. t, trace (less than 0.5%).

| Preparation | Fatty acid (mol%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1c | 16:1t | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 |
| Thylakoid membrane (1) | t | 4 | t | 0 | 6 | t | t | 2 | 88 |
| PS II membrane (1) | t | 7 | t | 0 | 4 | 1 | 1 | 2 | 85 |
| PS II core complex (3) | 1 | 15±2 | 1 | 0 | 3±1 | 5±2 | 1 | 2±1 | 72±4 |

TABLE VI

*Fatty acid compositions of SQDG from thylakoid membranes and PS II membranes*

t, trace (less than 0.5%).

| Preparation | Fatty acid (mol%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1c | 16:1t | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 |
| Thylakoid membrane | 1 | 27 | 3 | 0 | 2 | 2 | 4 | 17 | 45 |
| PS II membrane | 3 | 41 | 7 | 0 | 2 | 5 | 3 | 8 | 41 |

saturated acids together accounted for 53% of the total fatty acids, whereas the trienoic acids together accounted for only 24% (Table II).

In Table III, the fatty acid composition of MGDG from the thylakoid membranes and from the three types of preparation of PS II are compared. The changes in the fatty acid composition were similar to those in the total lipids. The relative level of saturated fatty acids increased and that of trienoic fatty acids were somewhat lower in the PS II core complex and even lower in the PS II reaction center complex.

Table IV shows the fatty acid composition of PG from the preparations of thylakoid membrane and PS II. In this class of lipids, no marked difference in the fatty acid composition was found between the thylakoid and the PS II membranes. The fatty acids in the PS II core complex were more saturated than those in the PS II membrane. The most striking change was that observed in the fatty acids in PG from the PS II reaction center complex, in which the level of 18 : 3 decreased to only 8%, while levels of 16 : 0 and 18 : 0 increased to 32% and 15%, respectively. A single *trans*-unsaturated fatty acid, 16 : 1t, was found at high levels in all four preparations.

Unlike the fatty acid compositions of MGDG and PG, that of DGDG was relatively constant in the thylakoid membrane, the PS II membrane and the PS II core complex. However, there were slight increases in relative levels of 16 : 0 at the expense of 18 : 3 (Table V). Similarly, the fatty acid composition of SQDG from the PS II membranes, as compared to that of thylakoid membranes, showed an increase in the relative level of 16 : 0 at the expense of 18 : 2 (Table VI).

## Discussion

Prior to the separation of the various classes of lipids from the preparations of PS II that contained Triton X-100, we used reversed-phase HPLC to remove the detergent from the lipid extracts. This chromatographic step was essential because all the preparations of PS II contained large amounts of Triton X-100, which interfered with the subsequent fractionation of lipid classes by HPLC on silica gel. This detergent was found to exert a significant effect on the $R_F$ values of lipids during TLC on silica gel. For separation of the various classes of lipids the second HPLC step could not be replaced by TLC on silica gel plates. The requirement for a second HPLC step was especially conspicuous in the case of the PS II core complex and PS II reaction center complex, where only small amounts of lipid samples were available for analysis. The amounts of fatty acids, with 16 : 0 and 18 : 0 as the major components, that were present as contaminants in the thin-layer plates far exceeded the amounts of fatty acids from the lipid samples, thereby increasing the apparent degree of saturation in the analysis of the fatty acids. With the use of HPLC on silica gel, no such contamination occurred.

In the present study, we demonstrated that the composition of lipids in PS II membranes was similar to that in thylakoid membranes with the exception of the absence of PC in the PS II membranes. This result is consistent with the result reported by Farineau et al. [3] but differs from that reported by Gounaris et al. [4], who found that SQDG, PG and an unidentified phospholipid were present at higher relative levels in PS II membranes than in thylakoid membranes. Our analysis of the lipid composition of the PS II core complex indicates that this complex contains MGDG, DGDG and PG. This result is not in agreement with the result reported by Gounaris and Barber [5] who found, in a very similar type of preparation of PS II core complex, the presence of MGDG and SQDG that were composed of highly saturated fatty acids. The difference between our result and that of Barber's group is most likely due to the misidentification of DGDG and PG on the TLC plates that originated from the effect of Triton X-100. Not only did Triton X-100 probably cause a change in the $R_F$ values, but it is also likely that the lipid samples were contaminated by saturated fatty acids from the TLC plates.

In the present study, we made a detailed analysis of glycerolipids from four well-defined preparations: the thylakoid membrane; the PS II membrane, which contains the PS II complex composed of more than 20 different polypeptides; the PS II core complex of 10 polypeptides; and the PS II reaction center complex of

TABLE VII

*Estimated molecular ratios of glycerolipid to reaction center II (P680) in preparations of PS II*

The calculations were made on the assumption that the ratios of Chl (+Pheo) to P680 were 220 : 1, 50 : 1 and 6 : 1 in the PS II membrane, the PS II core complex and PS II reaction center complex, respectively.

| Preparation | Ratio of lipid to P680 (mol/mol) | | | | |
|---|---|---|---|---|---|
| | Total lipid | MGDG | DGDG | PG | SQDG |
| PS II membrane | 150 | 90 | 40 | 15 | 5 |
| PS II core complex | 11 ±1 | 3 ±1 | 4±1 | 4±1 | 0 |
| PS II reaction center complex | 1.1±0.1 | 1.0±0.1 | 0 | 0.1 | 0 |

five polypeptides. The molecular ratios of Chl (+Pheo) to the reaction center II, P680, in these preparations have been estimated by others and ourselves to be 220:1 [32], 50:1 [33,34] and 6:1 [20], respectively. From these values and the ratios of lipid to Chl in Table I, it is possible to estimate the molecular ratios of lipid to P680 (Table VII). The molecular ratio was 150:1 for the PS II membrane and 11:1 for the PS II core complex. The significant decrease in the PS II core complex suggests that the treatment with octylglucoside extracted the PS II core complex from the PS II membrane.

The PS II reaction center complex contained only one molecule of MGDG per P680 and one-tenth of a molecule of PG (Table VII). The small amount of PG in the PS II reaction center complex might be explained by contamination of the preparation by the light-harvesting Chl-protein complexes, which are known to bind PG [9]. However, such contamination is unlikely because the fatty acids in PG from the reaction center complex were highly saturated and differed from those in the other preparations. Moreover, the PG associated with the light-harvesting Chl-protein is more unsaturated than the PG extracted from leaves [8,14]. It is possible that most of the PG in the reaction center complex was released during the harsh treatment with concentrated Triton X-100. Thus, it is suggested that this MGDG molecule, and possibly, the PG molecule, are necessary for maintenance of the structural organization of the PS II reaction center complex and for the photochemical charge separation. Furthermore, it is noteworthy that the fatty acids in MGDG and PG are much more saturated than the fatty acids in the bulk lipids in thylakoid and PS II membranes. Since increased saturation of fatty acids results in a more rigid structure of the lipid molecules, the occurrence of saturated fatty acids in the lipids in the reaction center complex suggests that they stabilize the conformation of this complex. In order to test this hypothesis, further studies are necessary in which one species of lipid in the reaction center complex is replaced by a member of the same lipid class with a different degree of fatty-acid unsaturation. Such replacement can be carried out by extraction and consecutive reconstitution or by introduction of a mutation that affects the degree of unsaturation of fatty acids.

The PS II core complex contained about three molecules each of MGDG, DGDG and PG per P680. Since this complex is highly active in photochemical charge separation and the photosynthetic evolution of oxygen, this result suggests that either some or all of these classes of lipids are necessary for the proper conformation of the PS II core complex. In this regard, it is noteworthy that the fatty acids of lipids from the PS II core complex are more saturated than those of the thylakoid and PS II membranes. This result is in agreement with the suggestion of Siegenthaler et al. [10,11,36] that the transport of electrons through PS II depends essentially on those PG and MGDG molecules that are located in the inner monolayer of the thylakoid membrane.

Gounaris et al. [4] reported that exogenously added DGDG and PC activate the system for the evolution of oxygen in PS II membranes, and they suggested that these classes of lipids are essential for this activity. Hirayama and Nishida [35] observed a similar effect of PC in a preparation of thylakoid membranes. However, in the present analysis we found that PC was absent from the PS II membrane and from the PS II core complex, both of which were highly active in terms of the evolution of oxygen. Thus, it appears that exogenously added PC may have non-specific or biologically irrelevant effects on the oxygen-evolving activity. In addition, our results suggest that SQDG is unlikely to be necessary for the activity of PS II since this lipid was absent from the PS II core complex which was fully active in terms of both photochemical charge separation and the evolution of oxygen.

It should be emphasized that the various preparations examined in this study were obtained by treating the thylakoid membranes with Triton X-100, and then by treating the resultant PS II membranes with octylglucoside or concentrated Triton X-100. These detergents may displace lipid molecules at specific sites without loss of photosynthetic activities. The data presented in this study relate to the lipids that remained in situ after various treatments with detergent. It seems likely, therefore, that more saturated and, thus, more rigid, lipid molecules would remain bound to the protein complex than the more unsaturated and, therefore more fluid, lipid molecules under these conditions. It is also possible that the treatments with detergent result in the more efficient extraction of polyunsaturated lipid molecules than of saturated and monounsaturated ones, leaving saturated and monounsaturated molecules bound to the PS II core complex and the PS II reaction center complex.

## Conclusion

(1) One MGDG molecule, containing highly-saturated fatty acids, is associated with the PS II reaction center complex, which is composed of about five polypeptides and which is active in photochemical charge separation. It is suggested that this lipid molecule is essential for the proper conformation of the PS II reaction center complex.

(2) About ten lipid molecules, including MGDG, DGDG and PG, are associated with the PS II core complex, which is composed of about ten polypeptides and is active both in photochemical charge separation and in the photosynthetic evolution of oxygen. It is

suggested that either some or all of these lipid molecules participate in the photosynthetic evolution of oxygen, and that neither PC nor SQDG contributes to the activities of PS II.

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## References

1 Douce, R., Holz, R.B. and Benson, A.A. (1973) J. Biol. Chem. 248, 7215–7222.
2 Harwood, J.L. (1980) in The Biochemistry of Plants. Vol. 4. Lipids: Structure and Function (Stumpf, P.K., ed.), pp. 1–55, Academic Press, New York.
3 Farineau, N., Gurillot-Salomon, T., Tuquet, C. and Farineau, J. (1984) Photochem. Photobiol. 40, 387–390.
4 Gounaris, K., Whitford, D. and Barber, J. (1983) FEBS Lett. 163, 230–234.
5 Gounaris, K. and Barber, J. (1985) FEBS Lett. 188, 68–72.
6 Pick, U., Gounaris, K., Weiss, M. and Barber, J. (1985) Biochim. Biophys. Acta 808, 415–420.
7 Doyle, M.F. and Yu, C.A. (1985) Biochem. Biophys. Res. Commun. 131, 700–706.
8 Tremolieres, A., Dubacq, J.P., Ambard-Bretteville, F. and Remy, R. (1981) FEBS Lett. 130, 27–31.
9 Dubacq, J.C. and Tremolieres, A. (1983) Physiol. Veg. 21, 293–312.
10 Siegenthaler, P.-A., Smutny, J. and Rawyler, A. (1987) Biochim. Biophys. Acta 891, 89–93.
11 Siegenthaler, P.-A., Rawyler, A. and Smutny, J. (1989) Biochim. Biophys. Acta 975, 104–111.
12 Pick, U., Weiss, M., Gounaris, K. and Barber, J. (1987) Biochim. Biophys. Acta 891, 28–39.
13 Chain, R.K. (1985) FEBS Lett. 180, 321–325.
14 Remy, R., Tremolieres, A., Duval, J.C., Ambard-Bretteville, F. and Dubacq, J.-P. (1982) FEBS Lett. 137, 271–275.
15 Siefermann-Harms, D., Ross, J.W., Kaneshiro, K.H. and Yamamoto, H.Y. (1982) FEBS Lett. 149, 191–196.
16 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539.
17 Murata, N. and Miyao, M. (1987) in Progress in Photosynthesis Research, Vol. I. (Biggins, J., ed.), pp. 453–462, Martinus Nijhoff, Dordrecht.
18 Miyao, M. and Murata, N. (1987) in Topics in Photosynthesis, Vol. 9, Photoinhibition (Kyle, D.J., Osmond, C.B. and Arntzen, C.J., eds.), pp. 289–307, Elsevier, Amsterdam.
19 Ikeuchi, M., Yuasa, M. and Inoue, Y. (1985) FEBS Lett. 185, 316–322.
20 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
21 Ikeuchi, M. and Inoue, Y. (1988) Plant Cell Physiol. 29, 1233–1239.
22 Miyao, M. and Murata, N. (1983) Biochim. Biophys. Acta 725, 87–93.
23 Bligh, E.G. and Dyer, N.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
24 Demandre, C., Tremolieres, A., Justin, A.-M. and Mazliak, P. (1985) Phytochemistry 24, 481–485.
25 Wada, H. and Murata, N. (1989) Plant Cell Physiol. 30, 971–978.
26 Dittmer, J.C. and Lester, R.L. (1964) J. Lipid Res. 5, 126–127.
27 Beiss, U. (1964) J. Chromatogr. 13, 104–110.
28 Yamakawa, T., Irie, R. and Iwanaga, M. (1960) J. Biochem. 48, 490–507.
29 Fahmy, A.R., Niederwieser, A., Pataki, G. and Brenner, M. (1961) Helv. Chim. Acta 44, 2022–2026.
30 Laemmli, U.K. (1970) Nature 227, 680–685.
31 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
32 Murata, N., Miyao, M., Omata, T., Matsunami, H. and Kuwabara, T. (1984) Biochim. Biophys. Acta 765, 363–365.
33 Satoh, K. and Mathis, P. (1981) Photobiochem. Photobiophys. 2, 189–198.
34 Omata, T., Murata, N. and Satoh, K. (1984) Biochim. Biophys. Acta 765, 403–405.
35 Hirayama, O. and Nishida, T. (1987) Agric. Biol. Chem. 42, 141–146.
36 Rawyler, A. and Siegenthaler, P.A. (1989) Biochim. Biophys. Acta 975, 283–292.