Do Glycerolipids Display Lateral Heterogeneity in the Thylakoid Membrane?

Sylvie Duchêne and Paul-André Siegenthaler*
Laboratoire de Physiologie Végétale, Université de Neuchâtel, CH-2007 Neuchâtel, Switzerland

ABSTRACT: The lateral heterogeneity of lipids in the thylakoid membrane has been questioned for over 20 yrs. It is generally believed that glycerolipids are asymmetrically distributed within the plane of the membrane. In the present investigation, we isolated several thylakoid membrane domains by using sonication followed by separation in an aqueous dextran–polyethylene glycol two-phase system. This technique, which avoids detergent treatments, allowed us to obtain stroma and grana lamellae vesicles as well as grana central core and grana margin vesicles from thylakoids. The relative distribution of the four lipid classes, i.e., monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and phosphatidylglycerol, was found to be statistically identical in all four thylakoid fractions and in whole thylakoids. Similarly, the relative amount of fatty acids in each individual lipid and the eight main phosphatidylglycerol molecular species was identical in all thylakoid membrane fractions tested as well as in the intact thylakoid membrane. Based on presently available procedures for obtaining thylakoid subfractions that are unable to discriminate microdomains within the membrane, it is concluded that glycerolipids are evenly distributed within the plane of the thylakoid membrane. These data are discussed in terms of "bulk" and "specific" lipids.

Paper no. L8426 in *Lipids 35,* 739–744 (July 2000).

Glycerolipids are major components of the thylakoid membrane. They consist of four classes: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG). These lipids are characteristic of photosynthetic membranes. Galactolipids (MGDG and DGDG) represent about 80 mol% of total lipids and are therefore considered to be the most abundant membrane lipids in the world. In addition, they are characterized by an exceptionally high content of trienoic acids, mainly, α-linolenic acid; additionally, in the so-called 16:3-plants, hexadecatrienoic acid is found in MGDG. SQDG is enriched in palmitic acid (35 mol%), whereas PG contains a unique fatty acid, *trans*-Δ^3-hexadecenoic acid (e.g., 1,2). The fatty acid composition of these glycerolipids is unique and gives rise to a great number of molecular species (3,4). It is surprising that, up to now, none or only a few of these lipid molecular species have been assigned to a specific location in the membrane or to a specific role in the photosynthetic function.

During the past years, several attempts have been made to determine whether, in a manner similar to proteins, acyl lipids are also asymmetrically distributed in the plane of the thylakoid membrane (TM). Among them, the following approaches have been used: (i) mild solvent extraction of freeze-dried membranes (e.g., 5); (ii) fractionation of subchloroplast particles enriched in photosystem I (PSI) or photosystem II (PSII) activities (e.g., 6); (iii) separation of appressed (granal) and nonappressed (stromal) regions of thylakoids (e.g., 7–10); (iv) purification of the membrane protein complexes (e.g., 11–19); (v) detection of glycerolipids by using antibodies directed to individual lipids bound to the surface of the TM, to subchloroplast particles, or to individual proteins (e.g., 20–22, and references therein); (vi) reconstitution of photosynthetic structures and activities with lipids (for a review, see Ref. 23). The general conclusions of these studies are that glycerolipids are asymmetrically distributed within the plane of the TM, although the lateral heterogeneity of thylakoid proteins is much more pronounced than that of lipids (for a review, see Ref. 1).

Several studies argue for this conclusion. For instance, one molecule of MGDG (and possibly one molecule of PG) was found to bind one PSII reaction center complex (18). Furthermore, the fatty acids of these two lipids are much more saturated (50% of the total fatty acids) than those in the bulk lipids of thylakoid and PSII membranes (10% of the total fatty acids). The removal of DGDG from isolated light-harvesting chlorophyll *a/b* protein complex (LHCII) renders the complex unable to form two- or three-dimensional crystals. The ability to crystallize is completely restored by the addition of DGDG at a ratio of about four molecules of DGDG per polypeptide for three-dimensional crystallization, suggesting the existence of several binding sites at the periphery of the trimeric complexes (24). The sulfolipid SQDG was found to be associated with the coupling factor complex (CF_0–CF_1) of spinach (16). This suggests that this acidic glycolipid is

*To whom correspondence should be addressed at Laboratoire de Physiologie Végétale, Université de Neuchâtel, Rue Emile-Argand 13, CH-2007 Neuchâtel, Switzerland. E-mail: Paul-Andre.Siegenthaler@bota.unine.ch

Abbreviations: B3, grana lamellae vesicles; B3-420S, grana central core vesicles (or central core of the appressed region); Chl, chlorophyll; DGDG, digalactosyldiacylglycerol; F695, fluorescence at 695 nm; F740, fluorescence at 740 nm; LHCII, light harvesting chlorophyll *a/b* protein complex; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; 420S, grana margin vesicles; SQDG, sulfoquinovosyldiacylglycerol; TM, thylakoid membranes; T3, stroma lamellae vesicles.

firmly bound to the ATP-synthetase complex and may play a special role in the mechanism of energy coupling. Several examples show the involvement of PG molecular species in the maintenance of the structure and function of the TM. In lipid-depleted LHCII, only PG containing 16:1 (3t), but not PG containing 16:0 fatty acids, induced reoligomerization from monomer forms of LHCII (25). In addition, PG is involved in the stacking of thylakoids (23).

Because most of the above methods, especially those for obtaining subchloroplast particles, involved the use of detergents, which are known to partially extract and/or displace lipids (8), in the present investigation we isolated the TM in several domains by using sonication followed by separation in an aqueous dextran–polyethylene glycol two-phase system (26). When comparing the content and the characteristics of lipids in TM, stroma lamellae vesicles (T3) and grana lamellae vesicles (B3), grana central core vesicles (B3-420S) and grana margin vesicles (420S), our results indicate that, considering the domains tested in this investigation, no lateral heterogeneity occurred in the TM.

EXPERIMENTAL PROCEDURES

Preparation of thylakoid vesicles. Spinach plants (*Spinacia oleracea* L.) were grown in a growth chamber at 20°C with a light period of 12 h and incident light intensity of 400 μE/m^2/s. Thylakoids were prepared from leaves according to Andreasson *et al.* (27). After the final centrifugation, the thylakoid preparation was suspended for 45 min in 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl, and 100 mM sucrose supplemented with 1 mM $MgCl_2$ to allow a complete stacking of the membranes before fragmentation, then adjusted to 4 mg chlorophyll (Chl)/mL. T3, B3, B3-420S, and 420S were obtained by essentially following the procedure described by Wollenberger *et al.*, (28): (i) thylakoids were mixed with two polymers (5.6 dextran and 5.6% polyethylene glycol), then sonicated on ice (6 × 30 s, with 1-min resting intervals); the mixture was submitted to a series of three partition steps in the same aqueous dextran–polyethylene glycol two-phase system allowing the separation of T3 and B3; (ii) polymers were added to the B3 fraction, and the mixture was sonicated (14 × 30 s, with 1-min resting intervals); then a series of three partition steps in the same aqueous dextran–polyethylene glycol two-phase system allowed the separation of B3-420S and 420S. The four vesicle preparations were diluted 4 to 5 times with the initial suspension medium and centrifuged at 100,000 × *g* for 30 min to remove polymers. The pellets were resuspended in 10 mM sodium phosphate buffer (pH 7.4), 5 mM NaCl, 1 mM $MgCl_2$, and 100 mM sucrose, then adjusted to 1 mg Chl/mL.

Chemical analyses. Total lipids were extracted by adding 4 mL chloroform/methanol (53:37, vol/vol) and 2 mL 0.5 M KCl to the thylakoid suspension (150 μL). This resulted in a two-phase system. The lipids of the lower phase were separated by thin-layer chromatography on silica gel plates (precoated silica gel plates, no. 5626; Merck, Darmstadt, Germany) in two dimensions. Then, MGDG, DGDG, SQDG, and PG were methylated and the resulting fatty acid methyl esters separated and identified by gas–liquid chromatography (29). Molecular species of PG were identified in the TM fraction, T3, and B3-420S by high-performance liquid chromatography following the procedure described by Kito *et al.*, (30), modified by Xu and Siegenthaler, (4). Chl concentration was determined according to Bruinsma (31). The relative content of Chl *a* and *b* was estimated (32).

Photosynthetic activities. Fluorescence emission spectra at 77 K of thylakoid preparations were determined (33).

RESULTS

Characterization of thylakoid domains. Table 1 shows a few biochemical characteristics of the TM and the derived thylakoid domains, i.e., T3, B3, B3-420S, and 420S. When total glycerolipids, i.e., the sum of MGDG, DGDG, SQDG, and PG, were expressed in nmol/mg Chl, μg/mg Chl, or nmol/nmol Chl, the data did not display significant differences between TM and the different fractions. Though there are no statistical values available for expressing the content of total glycerolipids as a function of proteins (nmol/mg protein or μg/mg protein), Table 1 shows that the difference between the values characterizing the TM, grana, and stroma lamellae did not exceed 13.5%. On the basis of the standard deviations calculated for the level of total glycerolipids/Chl, this difference cannot be considered as significant.

As shown by other authors (28), the Chl *a/b* ratio was the highest in stroma lamellae and the lowest in the grana central core. The low-temperature emission of fluorescence at 695 and 740 nm reflects the relative amount of PSII and PSI in the different thylakoid fraction. In Table 1, we see that the thylakoid membrane was slightly enriched in PSII (F_{695}/F_{740} = 1.2, where F_{695} is the fluorescence at 695 nm and F_{740} is the fluorescence at 740 nm). When TM was fractionated in distinct domains, the two photosystems were not distributed uniformly. B3 and B3-420S, which are enriched in PSII, displayed the highest F_{695}/F_{740} ratio values, whereas T3 exhibited a very low value. The 420S had intermediate F_{695}/F_{740} ratio values. Altogether, these results confirmed that the foregoing thylakoid fractions, which originate from different TM domains, are distinct and well-defined, as described in the literature (34).

Composition of lipids, fatty acids, and PG molecular species. The composition in lipid classes (MGDG, DGDG, SQDG, and PG) of four TM domains compared to that of the whole membrane is shown in Table 2. The relative amount of each lipid, expressed as mole percentage, in TM was similar to the values published in the literature (1). The relative distribution of the four lipid classes in T3 and B3, as well as in B3-420S and 420S, was statistically identical. This shows that these domains displayed the same relative lipid distribution as that observed in the whole TM. In a similar fashion, the relative amount of fatty acids in each individual lipid was identical in the four TM domains as well as in the whole TM (Table 3).

TABLE 1
Biochemical Characteristics of Thylakoid Membrane Domains

Membrane characteristic	Thylakoid membrane domain[a]				
	TM	T3	B3	B3-420S	420S
Total glycerolipids					
nmol/mg Chl	1641 ± 162 (n = 7)	1788 ± 272 (n = 10)	1586 ± 40 (n = 4)	1601 ± 151 (n = 11)	1806 ± 116 (n = 4)
μg/mg Chl[b,c]	1319 ± 130	1437 ± 219	1275 ± 32	1287 ± 121	1452 ± 93
nmol/nmol Chl[c,d]	1.48 ± 0.15	1.61 ± 0.24	1.43 ± 0.04	1.45 ± 0.13	1.63 ± 0.10
nmol/mg protein	560	520	590	ND[e]	ND
μg/mg protein	450	420	470	ND	ND
Protein[f]					
μg/mg Chl	2900	3400	2700	ND	ND
Chl *a*/*b* (n = 12)	2.8 ± 0.1	4.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.6 ± 0.2
F_{695}/F_{740} ratio	1.2 ± 0.2 (n = 12)	0.3 ± 0.1 (n = 12)	3.9 ± 0.3 (n = 7)	4.6 ± 1.5 (n = 10)	2.4 ± 0.9 (n = 4)

[a]TM, thylakoid membranes; T3, stroma lamellae vesicles; B3, grana lamellae vesicles; B3-420S, grana central core vesicles; 420S, grana margin vesicles; F695/F740, ratio of fluorescence at 695 nm compared to fluoresence at 740 nm; Chl, chlorophyll.
[b]Total glycerolipids expressed in μg/mg Chl were calculated by taking into consideration the molecular weight of each lipid class and the relative amount of each lipid class in the thylakoid membrane.
[c]The number of experiments (n) for the calculation of the standard deviation is the same as that indicated in the second line of this table.
[d]Total glycerolipids expressed in nmol/nmol Chl was calculated by taking into consideration the molecular weights of chlorophyll *a* and *b* and the Chl *a*/*b* ratio.
[e]ND, not determined.
[f]Protein content is from Albertsson *et al.* (26).

It is well established that in spinach plants, a chilling-resistant species, thylakoids contain 10 molecular species of PG, three of them, 18:3/16:1(3t), 18:3/16:0 and 16:0/16:1(3t), being prominent (3,4). PG molecular species were determined only in two thylakoid domains, i.e., T3 and B3-420S, because of the lack of material in the other fractions. Concerning the eight main PG molecular species, there were no differences between the two fractions and the TM (Table 4).

DISCUSSION

The four TM domains (T3, B3, B3-420S, and 420S) used in this study were obtained after a series of sonication and repeated partition steps in an aqueous dextran–polyethylene glycol two-phase system. Compared to other techniques using detergents to solubilize the membrane, the two-phase system technique offers real advantages, namely, in avoiding unverifiable displacement and selective extraction of lipids, which generally occurs in the presence of detergents and therefore may generate biased conclusions.

According to the pioneering work of Murphy and Woodrow, (9) and Gounaris *et al.*, (7), who used a similar experimental approach to isolate B3 and T3, TM was found to display considerable lateral heterogeneities in the distribution of all major membrane components, including lipids. On the contrary, the present results show quite clearly that the relative distribution of the four lipid classes (MGDG, DGDG, SQDG, and PG), as well as the relative amount of fatty acids in each individual lipid and the main PG molecular species, was statistically identical in all four thylakoid fractions and in intact thylakoids. This finding is quite important for understanding the molecular organization of lipids in the TM and argues in favor of the existence of two types of lipid molecules, i.e., the bulk lipids and the specific lipid molecules. This hypothesis was first proposed in 1980 (35) and subsequently refined (36,37).

TABLE 2
Composition in Lipid Classes of Four Thylakoid Membrane Domains[a]

Thylakoid fractions	Lipid class[b] (mol%)			
	MGDG	DGDG	SQDG	PG
TM (n = 7)	50 ± 1	28 ± 2	9 ± 1	13 ± 2
T3 (n = 10)	51 ± 1	28 ± 3	9 ± 1	12 ± 2
B3 (n = 4)	47 ± 4	27 ± 2	12 ± 3	14 ± 3
B3-420S (n = 11)	49 ± 6	29 ± 3	10 ± 3	12 ± 2
420S (n = 4)	52 ± 1	28 ± 2	10 ± 2	10 ± 1

[a]MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; for other abbreviations see Table 1.
[b]The mol% values are calculated from the data shown in Table 1. For each thylakoid fraction, the 100% values corresponded to the sum of the four acyl lipids.

TABLE 3
Fatty Acid Composition of the Four Lipid Classes in Various Thylakoid Membrane Domains

Acyl lipid Thylakoid fraction	Fatty acid[a] (mol%)						
	16:0	16:1(3t)	16:3	18:0	18:1	18:2	18:3
MGDG							
TM (n = 7)	Tr	Tr	17.6 ± 0.6	Tr	Tr	2.3 ± 0.4	80.1 ± 0.9
T3 (n = 10)	Tr	Tr	17.8 ± 0.6	Tr	Tr	2.4 ± 0.5	79.8 ± 0.9
B3 (n = 4)	Tr	Tr	16.7 ± 1.0	Tr	Tr	2.3 ± 0.1	81.0 ± 0.8
B3-420S (n = 11)	Tr	Tr	17.8 ± 0.5	Tr	Tr	2.5 ± 0.5	79.7 ± 1.3
420S (n = 4)	Tr	Tr	17.7 ± 1.4	Tr	Tr	2.9 ± 0.3	79.4 ± 3.8
DGDG							
TM (n = 8)	5.9 ± 0.6	Tr	2.8 ± 0.2	Tr	Tr	2.5 ± 0.3	88.8 ± 1.5
T3 (n = 10)	5.9 ± 0.7	Tr	2.9 ± 0.2	Tr	Tr	2.5 ± 0.4	88.7 ± 1.6
B3 (n = 4)	8.6 ± 3.4	Tr	2.6 ± 0.1	Tr	Tr	2.5 ± 0.3	86.3 ± 2.0
B3-420S (n = 11)	5.5 ± 1.2	Tr	2.8 ± 0.2	Tr	Tr	2.7 ± 0.4	89.0 ± 2.1
420S (n = 4)	5.5 ± 1.6	Tr	2.7 ± 0.1	Tr	Tr	3.0 ± 0.2	88.8 ± 1.9
SQDG							
TM (n = 8)	41.1 ± 1.6	Tr	1.7 ± 0.3	Tr	Tr	9.0 ± 0.8	48.2 ± 1.8
T3 (n = 10)	41.6 ± 0.2	Tr	1.5 ± 0.6	Tr	Tr	9.1 ± 1.0	47.8 ± 1.3
B3 (n = 4)	44.5 ± 1.9	Tr	1.3 ± 0.1	Tr	Tr	7.5 ± 2.0	46.6 ± 3.5
B3-420S (n = 11)	41.9 ± 0.9	Tr	1.6 ± 0.2	Tr	Tr	9.6 ± 1.1	46.9 ± 2.4
420S (n = 4)	41.4 ± 2.9	Tr	1.6 ± 0.3	Tr	Tr	10.5 ± 1.6	46.5 ± 6.6
PG							
TM (n = 8)	16.0 ± 0.5	38.0 ± 1.1	0	Tr	Tr	6.4 ± 0.7	39.6 ± 0.9
T3 (n = 11)	16.1 ± 0.6	37.7 ± 0.9	0	Tr	Tr	7.3 ± 1.0	38.9 ± 1.2
B3 (n = 4)	15.2 ± 2.2	37.4 ± 2.0	0	Tr	Tr	7.0 ± 1.3	40.4 ± 2.2
B3-420S (n = 11)	15.3 ± 0.4	40.0 ± 1.5	0	Tr	Tr	6.6 ± 0.6	38.1 ± 1.3
420S (n = 4)	14.1 ± 2.3	39.1 ± 1.2	0	Tr	Tr	7.1 ± 0.9	39.7 ± 1.3

[a]Tr, traces (less than 1 mol%). Results are expressed in mol% for each lipid class. For TM, 100% values corresponded in nmol/mg Chl to 825 ± 89 for MGDG, 450 ± 57 for DGDG, 140 ± 12 for SQDG, and 214 ± 45 for PG; for T3 fraction, to 924 ± 143, 501 ± 103, 158 ± 20, and 214 ± 43; for B3 fraction, to 745 ± 38, 428 ± 13, 190 ± 20, and 222 ± 64; for B3-420S, to 774 ± 101, 474 ± 77, 163 ± 62, and 190 ± 43; for 420S fraction, to 944 ± 53, 510 ± 47, 181 ± 26, and 171 ± 11. For other abbreviations see Tables 1 and 2.

The bulk lipids predominate in the TM and fill the spaces between the various proteins. They can be considered as having a structural role. For instance, they offer a hydrophobic matrix to the proteins and pigments and, owing to their high degree of unsaturation, confer an appropriate fluidity to the membrane. In addition, MGDG, which is the major lipid in the TM, can form, under certain conditions, nonbilayer configurations and therefore influence the structure and the photosynthetic function of the membrane (38). The analyses of lipids in the different domains of the membrane (Tables 2–4) concern the bulk lipids and reveal that the level of the four lipid classes (MGDG, DGDG, SQDG, and PG), the nature of their acyl chains, and the main molecular species of PG are identical in B3 and T3, as well as in B3-420S and 420S.

By contrast, the specific lipids are by nature less abundant than the bulk lipids, but especially much more saturated than the bulk lipids (15,18,19). These specific lipid molecules can be considered as functional or strategic lipids. They are involved in specific interactions with proteins, resulting in an appropriate maintenance of the conformation and/or orientation of protein molecules in the membrane and high photosynthetic performances. These lipids are encountered in the four lipid classes and display specific functions. For instance, MGDG sustains charge separation (18), DGDG induces three-dimensional crystallization of the LHCII (24), and SQDG is associated with the structure and function of the coupling factor complex (16). In addition, specific molecules of PG have been reported to be involved in oligomerization of LHCII (25), the stacking of thylakoids (23), and the support of the electron flow activity (39,40). The specific lipid molecules are, of course,

TABLE 4
Composition in PG Molecular Species of Four TM Domains[a]

Thylakoid fraction	Molecular species (mol%)							
	18:3/16:1(3t)	18:3/16:0	18:2/16:1(3t)	18:2/16:0	18:1/16:1(3t)	16:0/16:1(3t)	18:1/16:0	16:0/16:0
TM (n = 8)	61.8 ± 4.3	10.6 ± 1.8	5.2 ± 2.7	6.0 ± 1.6	2.8 ± 1.4	9.6 ± 1.1	1.2 ± 0.7	2.8 ± 1.9
T3 (n = 11)	63.2 ± 4.8	11.1 ± 1.3	4.5 ± 1.6	6.0 ± 1.7	2.4 ± 1.1	9.7 ± 2.1	1.6 ± 1.5	1.5 ± 1.4
B3-420S (n = 11)	64.2 ± 4.9	9.7 ± 1.1	5.3 ± 2.1	5.7 ± 1.7	2.8 ± 1.7	9.5 ± 1.3	1.4 ± 0.8	1.4 ± 1.5

[a]The 100% values correspond to 209 ± 35 nmol PG/mg Chl for TM, 208 ± 37 nmol PG/mg Chl for T3, and 185 ± 31 nmol PG/mg Chl for B3-420S; n = number of experiments. The 18:0/16:1(3t) and 18:0/16:0 PG molecular species were found only in trace amounts. For abbreviations see Tables 1 and 2.

concentrated at different locations in the membrane where they are associated with their interacting protein(s). But, owing to their scarcity, they do not change the global composition and the distribution of the four classes of lipids between the membrane domains considered in this study (Tables 1–4).

In conclusion, we propose that the current model of thylakoid membrane lipid composition is one that contains simultaneously bulk and specific lipids. Current experimental evidence (as described in this investigation) indicates that only the latter ones display lateral heterogeneity in the membrane.

ACKNOWLEDGMENTS

This research was supported in part by the Swiss National Science Foundation (grants number 31.336 93.92 and 31.432 97.95). The authors thank very much Per-Åke Albertsson and his collaborators for help and advice in preparing thylakoid vesicles. This work is part of a doctoral program that has been carried out by Sylvie Duchêne in the Laboratoire de Physiologie végétale, Université de Neuchâtel, Switzerland.

REFERENCES

1. Siegenthaler, P.A. (1998) Molecular Organization of Acyl Lipids in Photosynthetic Membranes of Higher Plants, in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.A., and Murata, N., eds.), Vol. 6, pp. 119–144, Kluwer Academic Publishers, Dordrecht.
2. Dubacq, J.P., and Trémolières, A. (1983) Occurrence and Function of Phosphatidylglycerol Containing Δ^3-*trans*-Hexadecenoic Acid in Photosynthetic Lamellae, *Physiol. Vég. 21*, 293–312.
3. Nishihara, M., Yokota, K., and Kito, M. (1980) Lipid Molecular Species Composition of Thylakoid Membranes, *Biochim. Biophys. Acta 617,* 12–19.
4. Xu, Y.N., and Siegenthaler, P.-A. (1996) Phosphatidylglycerol Molecular Species of Photosynthetic Membranes Analyzed by High-Performance Liquid Chromatography: Theoretical Considerations, *Lipids 31*, 223–229.
5. Costes, C., Bazier, R., and Lechevallier, D. (1972) Rôle Structural des lipides dans les membranes des chloroplastes de Blé, *Physiol. Vég. 10*, 291–317.
6. Ouijja, A., Farineau, N., Cautrel, C., and Guillot-Salomon, T. (1988) Biochemical Analysis and Photosynthetic Activity of Chloroplasts and Photosystem II Particles from a Barley Mutant Lacking Chlorophyll *b*, *Biochim. Biophys. Acta 932*, 97–106.
7. Gounaris, K., Sundby, C., Andersson, B., and Barber, J. (1983) Lateral Heterogeneity of Polar Lipids in the Thylakoid Membranes of Spinach Chloroplasts, *FEBS Lett. 156*, 170–174.
8. Henry, L.E.A., Mikkelsen, J.D., and Møller, B.L. (1983) Pigment and Acyl Lipid Composition of Photosystem I and II Vesicles and of Photosynthetic Mutants in Barley, *Carlsberg Res. Commun. 48*, 131–148.
9. Murphy, D.J., and Woodrow, I.E. (1983) Lateral Heterogeneity in the Distribution of Thylakoid Membrane Lipid and Protein Components and Its Implications for the Molecular Organization of Photosynthetic Membranes, *Biochim. Biophys. Acta 725*, 104–112.
10. Bednarz, J., Radunz, A., and Schmid, G.H. (1988) Lipid Composition of Photosystem I and II in the Tobacco Mutant *Nicotiana tabacum* NC 95, *Z. Naturforsch. 43c*, 423–430.
11. Heinz, E., and Siefermann-Harms, D. (1981) Are Galactolipids Integral Components of the Chlorophyll–Protein Complexes in Spinach Thylakoids? *FEBS Lett. 124*, 105–111.
12. Trémolières, A., Dubacq, J.P., Ambard-Bretteville, F., and Rémy, R. (1981) Lipid Composition of Chlorophyll–Protein Complexes, *FEBS Lett. 130*, 27–31.
13. Rémy, R., Trémolières, A., Duval, J.C., Ambard-Bretteville, F., and Dubacq, J.P. (1982) Study of the Supramolecular Organisation of Light Harvesting Chlorophyll Protein (LHCP), *FEBS Lett. 137*, 271–275.
14. Doyle, M.F., and Yu, C.A. (1985) Preparation and Reconstitution of a Phospholipid Deficient Cytochrome b_6-f Complex from Spinach Chloroplasts, *Biochem. Biophys. Res. Commun. 131*, 700–706.
15. Gounaris, K., and Barber, J. (1985) Isolation and Characterisation of a Photosystem II Reaction Center Lipoprotein Complex, *FEBS Lett. 188*, 68–72.
16. Pick, U., Gounaris, K., Weiss, M., and Barber, J. (1985) Tightly Bound Sulpholipids in Chloroplast CF_0–CF_1, *Biochim. Biophys. Acta 808*, 415–420.
17. Sigrist, M., Zwillenberg, C., Giroud, C., Eichenberger, W., and Boschetti, A. (1988) Sulfolipid Associated with the Light-Harvesting Complex Associated with Photosystem II Apoproteins of *Chlamydomonas reinhardtii, Plant Sci. 58*, 15–23.
18. Murata, N., Higashi, S.-I., and Fujimura, K. (1990) Glycerolipids in Various Preparations of Photosystem II from Spinach Chloroplasts, *Biochim. Biophys. Acta 1019*, 261–268.
19. Trémolières, A., Dainese, P., and Bassi, R. (1994) Heterogeneous Lipid Distribution Among Chlorophyll-Binding Proteins of Photosystem II in Maize Mesophyll Chloroplasts, *Eur. J. Biochem. 221*, 721–730.
20. Radunz, A. (1981) Application of Antibodies in the Analysis of Structural Configuration of Thylakoid Membranes, *Ber. Dtsch. Bot. Ges. 94*, 477–489.
21. Voss, R., Radunz, A., and Schmid, G.H. (1992) Binding of Lipids onto Polypeptides of the Thylakoid Membrane. I. Galactolipids and Sulpholipid as Prosthetic Groups of Core Peptides of the Photosystem II Complex, *Z. Naturforsch. 47c*, 406–415.
22. Kruse, O., and Schmid, G.H. (1995) The Role of Phosphatidylglycerol as a Functional Effector and Membrane Anchor of the D_1-Core Peptide from Photosystem II Particles of the Cyanobacterium *Oscillatoria chalybea, Z. Naturforsch. 50c*, 380–390.
23. Trémolières, A., and Siegenthaler, P.A. (1998) Reconstitution of Photosynthetic Structures and Activities with Lipids, in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.A., and Murata, N., eds.), Vol. 6, pp. 175–189, Kluwer Academic Publishers, Dordrecht.
24. Nussberger, S., Dörr, K., Wang, D.N., and Kühlbrandt, W. (1993) Lipid-Protein Interactions in Crystals of Plant Light-Harvesting Complex, *J. Mol. Biol. 234*, 347–356.
25. Krupa, Z., Williams, J.P., Khan, M.U., and Huner, N.P.A. (1992) The Role of Acyl Lipids in Reconstitution of Lipid-Depleted Light-Harvesting Complex II from Cold-Hardened and Non-hardened Rye, *Plant Physiol. 100*, 931–938.
26. Albertsson, P.A., Andreasson, E., Stefansson, H., and Wollenberger, L. (1994) Fractionation of Thylakoid Membrane, in *Aqueous Two-Phase Systems* (Walter, H., and Johansson, G., eds.) *Methods in Enzymology,* Vol. 228, pp. 469–482, Academic Press, New York.
27. Andreasson, E., Svensson, P., Weibull, C., and Albertsson, P.A. (1988) Separation and Characterization of Stroma and Grana Membranes—Evidence for Heterogeneity in Antenna Size of Both Photosystem I and Photosystem II, *Biochim. Biophys. Acta 936*, 339–350.
28. Wollenberger, L., Stefansson, H., Yu, S.-H., and Albertsson, P.-A. (1994) Isolation and Characterization of Vesicles Originating from the Chloroplast Grana Margins, *Biochim. Biophys. Acta 1184*, 93–102.
29. Xu, Y.N., and Siegenthaler, P.-A. (1997) Low Temperature Treatments Induce an Increase in the Relative Content of Both

Linolenic and Δ^3-*trans*-Hexadecenoic Acids in Thylakoid Membrane Phosphatidylglycerol of Squash Cotyledons, *Plant Cell Physiol. 38*, 611–618.
30. Kito, M., Takamura, H., Narita, H., and Urade, R. (1985) A Sensitive Method for Quantitative Analysis of Phospholipid Molecular Species by High-Performance Liquid Chromatography, *J. Biochem. 98*, 327–331.
31. Bruinsma, J. (1961) A Comment on the Spectrophotometric Determination of Chlorophyll, *Biochim. Biophys. Acta 52*, 576–578.
32. Lichtenthaler, H.K. (1987) Chlorophylls and Carotenoids: Pigments in Photosynthetic Biomembranes, in *Plant Cell Membranes* (Packer, L., and Douce, R., eds.), *Methods in Enzymology,* Vol. 148, pp. 350–382, Academic Press, New York.
33. Siegenthaler, P.A., Sutter, J., and Rawyler, A. (1988) The Transmembrane Distribution of Galactolipids in Spinach Thylakoid Inside-out Vesicles Is Opposite to That Found in Intact Thylakoids, *FEBS Lett. 228*, 94–98.
34. Albertsson, P.A. (1995) The Structure and Function of the Chloroplast Photosynthetic Membrane—A Model for the Domain Organization, *Photosynth. Res. 46*, 141–149.
35. Rawyler, A., and Siegenthaler, P.A. (1980) Role of Lipids in Function of Photosynthetic Membranes Revealed by Treatment with a Lipolytic Acyl Hydrolase, *Eur. J. Biochem. 110*, 179–187.
36. Siegenthaler, P.A., and Rawyler, A. (1986) Acyl Lipids in Thylakoid Membranes: Distribution and Involvement in Photosynthetic Functions, in *Encyclopedia of Plant Physiology* (Staehelin, L.A., and Arntzen, C., eds.), Vol. 19, New Series, pp. 693–705, Springer-Verlag, Berlin.
37. Siegenthaler, P.A., and Trémolières, A. (1998) Role of Acyl Lipids in the Function of Higher Plants' Photosynthetic Membranes, in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.A., and Murata, N., eds.), Vol. 6, pp. 145–173, Kluwer Academic Publishers, Dordrecht.
38. Murphy, D.J. (1986) The Molecular Organisation of the Photosynthetic Membranes of Higher Plants, *Biochim. Biophys. Acta 864*, 33–94.
39. Siegenthaler, P.A., Rawyler, A., and Smutny, J. (1989) The Phospholipid Population Which Sustains the Uncoupled Non-Cyclic Electron Flow Activity is Localized in the Inner Monolayer of the Thylakoid Membrane, *Biochim. Biophys. Acta 975*, 104–111.
40. Duchêne, S., Smutny, J., and Siegenthaler, P.A. (2000) The Topology of Phosphatidylglycerol Populations Is Essential for Sustaining Photosynthetic Electron Flow Activities in Thylakoid Membranes, *Biochim. Biophys. Acta 1463*, 115–120.

[Received December 30, 1999, and in final revised form and accepted May 12, 2000]