Lateral heterogeneity of polar lipids in the thylakoid membranes of spinach chloroplasts

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Isolated appressed and non-appressed membrane fractions of spinach thylakoids have been subjected to lipid class and fatty acid composition analyses. The ratio of monogalactosyldiacylglycerol to digalactosyldiacylglycerol was much higher in the fractions obtained from the appressed membranes compared with those derived from non-appressed membranes. Moreover, appressed thylakoids contained a larger amount of anionic lipids in comparison with non-appressed thylakoids. No major differences were observed in the fatty acid composition between the thylakoid fractions. The results are discussed in terms of the possible significance of lipid heterogeneity in the chloroplast thylakoid membrane.

Chloroplast lipid   Lipid asymmetry   Membrane lateral heterogeneity   Phase partition
Thylakoid organization   Thylakoid subfractionation

1. INTRODUCTION

The preparation of thylakoid membrane fragments using phase partition procedures [1] has supported, and extended, the concept that the various protein complexes involved in photosynthetic electron transport and photophosphorylation are laterally separated in the plane of the membrane [2–6]. The appressed lamellae of the grana contain photosystem 2 (PS 2) and the associated light-harvesting chlorophyll *a/b* complexes. On the other hand, those lamellae which have their outer surface exposed to the stromal phase and are not involved in close membrane–membrane interactions, contain photosystem one (PS 1), the coupling factor complex (CF$_0$–CF$_1$)

and perhaps some PS 2 and light-harvesting chlorophyll *a/b* complex. The location of the cytochrome b_6–f complex is unclear and could be either distributed throughout both the appressed and the non-appressed membrane regions [7,8] or localized only at the interphase of these two regions [9,10].

The functional and structural asymmetry of the protein complexes in the thylakoid membrane raised the question of whether a similar heterogeneity exists for lipid distribution and if so, what would be its significance in terms of the overall function and structure of the thylakoids. At present the information on this distribution and function of the thylakoid membrane lipids is not clear. Various functions have been attributed to both the galactolipids and the anionic lipids of the thylakoids [11–13] but the experimental data is rather sparse and the exact functions not proven. In addition, studies on the lateral and transverse distribution of the thylakoid membrane lipids [14–16] have provided little evidence supporting the concept of lipid asymmetry.

Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine; OPL, other phospholipids; DMSO, dimethylsulfoxide; PS, photosystem

To improve our knowledge of lipid distribution within the thylakoid membrane we have studied the lipid class and fatty acid content of isolated appressed and non-appressed membranes of spinach chloroplasts.

2. EXPERIMENTAL

2.1. Isolation of thylakoid fractions

Chloroplasts were isolated from spinach leaves as in [17], giving a high proportion of intact chloroplasts, thereby minimizing extra chloroplast membrane contamination. Stroma lamellae vesicles and inside-out vesicles, representative of non-appressed and appressed thylakoid regions, respectively, were isolated after Yeda press fragmentation of washed and stacked thylakoids [17]. Stroma lamellae vesicles were separated from fast sedimenting grana by centrifugation at 40 000 × g for 30 min and thereafter pelleted at 100 000 × g for 60 min (Y-100). The 40 000 × g pellet was suspended and passed twice more through the press and the inside-out vesicles were obtained by phase partition. Some modifications were made to the original phase partition procedure [17] in order to reduce contamination of right-sided PS 1-enriched material. The polymer concentrations were lowered to 5.55% (w/w) for both dextran and polyethylene glycol. The inside-out vesicles were obtained after 4 repartition steps of the lower phase yielding fraction B5 while the right-sided vesicles were obtained after one repartition of the upper phase (T2). The B5 and the T2 material were collected at 100 000 × g for 45 min. All thylakoid fractions were suspended in 100 mM sorbitol/1 mM KOH/1 mM Hepes (pH 7.6) (HCl)/5% DMSO and stored in liquid nitrogen. The fractions were tested for purity by analysis of chlorophyll–protein complexes by mild SDS–PAGE [3].

2.2. Lipid extraction

Total lipid extracts of spinach thylakoid membranes and membrane fractions were prepared as in [18]. Chlorophyll was determined as in [19] and the lipids were stored in chloroform under nitrogen at −20°C for further analysis.

2.3. Lipid class separation and fatty acid analysis

Lipid classes were separated by thin-layer chromatography of the lipid extract on ammonium sulphate impregnated silica gel G with a solvent system of acetone–benzene–water (91:30:8, by vol.) as in [20]. The lipids were visualised by spraying with 0.01% 2′,7′-dichlorofluorescein in methanol and viewed under UV light. Individual lipid classes were removed from the plates into screw-capped culture tubes. Analysis of the fatty acid methyl esters was carried out as in [21].

The data given below represents the average of three independent analyses. Standard errors varied between 0.2–0.8% in the fatty acid analysis and between 0.5–2% in the total lipid class content.

3. RESULTS

Prior to lipid analyses the thylakoid fractions were tested for their PS 1 content as a purity control. The Y-100 fraction showed a very high PS 1-enrichment since some 70% of its chlorophyll belonged to the P700–chlorophyll a proteins (CPI+ CPI$_a$) compared to 28% for the original thylakoids. In marked contrast, the B5 fraction contained only 7% of PS 1 chlorophyll, which is an improvement to the original preparation [3], thereby ensuring a low contamination of non-appressed thylakoids. The T2 fraction had 35% of its chlorophyll associated with CPI + CPI$_a$ demonstrating a slight PS 1 enrichment.

The lipid class content of the various thylakoid fractions were markedly different (table 1). The most striking difference was seen for the ratio between the two galactolipids MGDG and DGDG. The ratio in the B5 fraction (2.77) was more than double to the ratio in the Y-100 fraction (1.15). These values differed from the value of around 2 of intact thylakoids, demonstrating an uneven distribution of the two galactolipids along the thylakoid membrane. Moreover, the level of anionic lipids, PG and SQDG, was higher in the appressed thylakoid fraction than in the intact thylakoids, while lower in the non-appressed thylakoid fraction. It is also of interest that the fraction originating from the appressed thylakoid region contained less lipid per chlorophyll when compared with the intact thylakoids, while the opposite situation applied for the non-appressed thylakoid fraction.

Table 2 summarises the fatty acid composition of the thylakoid fractions confirming the high

Table 1

Lipid class composition of the different thylakoid membrane fractions compared with that of the intact spinach thylakoids

Sample	mol Lipid/ mol total chl	Lipid class composition (mol%)						MGDG/ DGDG
		MGDG	DGDG	PG	SQDG	PC	OPL	
Cl II	2.38	46.9	23.9	14.1	7.6	3.0	4.5	1.96
T2	2.23	40.9	30.0	13.5	7.6	1.4	6.6	1.36
Y-100	2.70	36.0	31.4	12.5	6.2	3.7	10.2	1.15
B5	1.80	46.8	16.9	17.5	10.9	3.3	4.6	2.77

Table 2

Fatty acid composition of spinach thylakoids and membrane fractions

Sample	Fatty acid composition (mol%)							% Saturated fatty acids	Av. no. double bonds/ lipid mol.
	16:0	16:1	16:3	18:0	18:1	18:2	18:3		
Cl II	9.1	2.1	11.3	0.5	1.9	3.5	71.6	20.5	5.19
T2	8.4	2.2	11.3	0.4	1.6	3.7	72.4	19.8	5.25
Y-100	11.0	2.2	11.2	0.5	1.9	3.9	69.3	22.2	5.06
B5	8.3	2.9	10.7	0.5	1.8	3.3	72.5	19.1	5.21

degree of unsaturation of the thylakoid lipids, particularly for the galactolipids. In contrast to the lipid classes there were no obvious differences in the relative proportions of the individual fatty acids for the different membrane fractions and the intact thylakoids. An examination of the fatty acid composition of each lipid class and a comparison between the different membrane fractions was also made (not shown). For the dominant polar lipids (MGDG, DGDG, PG) there was no marked deviation from the composition in intact thylakoids. However, for the polar lipids present in minor amounts some differences could be seen. For SQDG and PC there was a higher degree of unsaturation in the Y-100 fraction compared with the B5 fraction. This was mainly the result of an increased proportion of linolenic acid (18:3) at the expense of palmitic acid (16:0).

4. DISCUSSION

These data show, that apart from the well-known asymmetry of lipids across biological membranes [22,23] there can also exist an asymmetry of lipids along the plane of a membrane. The lateral heterogeneity of the thylakoid lipids is only partial, since each lipid class was present in both thylakoid regions. In this respect it differs from the lateral heterogeneity in the distribution of most thylakoid–protein complexes, which is quite extreme [6,24]. Thus lateral membrane asymmetry seems analogous to transmembrane asymmetry, where the lipids only show a partial asymmetric distribution between the bilayer leaflets, in contrast to the absolute protein asymmetry.

Since each lipid class was found in both the appressed and the non-appressed thylakoid regions, it is hard to pin-point a specific functional role of a particular lipid class. Nevertheless it is very likely that specific lipids are needed for functional activity of the various membrane-bound thylakoid proteins, but such requirements may only involve a small amount of a particular molecular species [25–27]. Some possibilities for lipid function can be considered in the light of these data. The occurrence of relatively high levels of anionic lipids in the appressed membranes of the grana has been implicated from other work. In particular it has

been claimed, that PG, containing the *trans*-hexadecenoic acid, is preferentially associated with the light-harvesting chlorophyll *a/b* protein [13] which is now believed to be mainly located in the appressed regions of the grana [3]. Coupled with this is the suggestion that the formation of grana involves anionic lipids [28]. However, there has been no precise functional role attributed to the sulpholipid (SQDG) in the thylakoid, although in other membrane systems these types of lipids have been implicated with cation transport mechanisms [29]. Of interest in this respect is the recent proposal that the anionic lipid headgroups could act as proton-conducting pathways along the membrane surface [30]. It is conceivable that such a functional role could operate in the granal membranes in order to facilitate proton translocation to the coupling factor complex located in the non-appressed regions [6].

The role of MGDG in the organisation of the thylakoid membrane has been dealt with [31] in light of its geometry [32] and ability to form non-bilayer structures [33]. In this respect the high MGDG to DGDG ratio of the appressed thylakoids is interesting. The non-bilayer forming properties of monogalactosyldiacylglycerol could be important in allowing grana formation to occur or for packing hydrophobic proteins such as the light-harvesting chlorophyll *a/b* complex. Since the B5 vesicles probably do not contain the grana margins [34] our data cannot substantiate any role of MGDG in stabilising the highly curved regions of the grana margins [31].

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