Journal of Bioenergetics and Biomembranes, Vol. 19, No. 6, 1987

MINI-REVIEW

Structural and Functional Consequences of Galactolipids on Thylakoid Membrane Organization[1]

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Received July 27, 1987

Abstract

Photosynthetic membranes of higher plant chloroplasts are composed primarily of polar, but uncharged, galactolipids unlike most mammalian membranes which contain large amounts of phosphatidylcholine. It is unclear what role(s) the galactolipids play in maintaining the differentiated thylakoid membranes, or in stabilizing the photosynthetically active enzyme complexes. Some of the membrane complexes show no lipid selectivity for maintaining structural or functional integrity. Others are poisoned or dissociated in the presence of high concentrations of a trace lipid class. The efficiency of energy transfer and the reconstitution of protein complexes into liposomes are dependent on the lipid class employed. The lipids are asymmetrically arranged along and across the thylakoid membranes but not as distinctly as the proteins.

Key Words: Galactolipids; photosynthesis; chloroplast; membranes; structure; function; reconstitution; fusion; liposomes; lipases.

Introduction

In the past decade we have made remarkable progress in describing the spatial and functional organization of the photosynthetic thylakoid membranes of higher plant chloroplasts. Most of the advances have been in our understanding of protein organization rather than of lipid organization. We now know,

[1] Abbreviations; DGDG, digalactosyldiglyceride; MGDG, monogalactosyldiglyceride; SQDG, sulfoquinovosyldiglyceride; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PSI, photosystem I; PSII, photosystem II; LHC, chlorophyll *a*/*b* light-harvesting complex; cyt b_6f, cytochrome b_6f complex; CF_0/CF_1; coupling factor ATPase; DCIP, 2,6-dichlorophenolindophenol; LRa, galactolipase from *Rhizopus arrhis*.

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0145-479X/87/1200-0691$05.00/0

for example, which protein components are segregated to appressed and nonappressed regions, which polypeptide is involved in membrane adhesion, and that phosphorylation of this polypeptide is involved in the regulation of energy distribution between the two photosystems (see reviews by Staehelin and Arntzen, 1983; Staehelin, 1986). Only more recently has the investigation of the other ~40% of the thylakoid membranes received as much attention as the protein components.

The unusual lipid composition of the thylakoid membranes is well documented (Douce and Joyard, 1979). Recent works suggests that lipid asymmetries exist along and across the thylakoids, but we have yet to understand what role galactolipids play in maintaining the unique organization of the thylakoid membranes (Gounaris *et al.*, 1983d; Murphy and Woodrow, 1983a, b; Rawyler and Siegenthaler, 1981, 1985). There have been speculations that boundary lipids may be required for activity of the various membrane components. Unsaturated fatty acyl chains may act to increase cold hardiness, while specific lipids may be required to maintain thylakoid structure. Overwhelming experimental evidence does not exist in support of these, or any other, hypotheses that suggest an *absolute* requirement of the lipids actually found in the native membrane. Our current understanding of the role of the lipids in structure and function of the photosynthetic components is much more subtle: The native lipids tend to produce results (structural organization or functional activity) that more closely resemble the values seen *in vivo* than do other lipids. This suggests that we have not as yet considered the proper parameter in thylakoid organization that will require the lipid composition seen in the native thylakoid membrane.

In this review I will consider possible roles for galactolipids in maintaining structure and function in thylakoid membrane organization. From this compilation I hope to demonstrate that in fact we have gained greater insights into the role of lipids in membrane organization, but that in this case we seem not to have found the crux of the issue.

Thylakoid Membrane Organization

Thylakoid membranes have several unique characteristics which distinguish them from other membrane systems. Not only do they house all the components necessary for the light reactions of photosynthesis, but they do so in a highly organized manner. The membranes are differentiated into appressed (granal or stacked) and nonappressed (stromal or unstacked) regions with marginal membranes bordering the ends of the granal stacks. There are two important characteristics that should be considered: the membranes are very closely associated in the appressed regions, and the

margins are areas of extremely high curvature. We shall return to these features after a discussion of the membrane lipids.

Thylakoid membranes are composed of 60–65% protein and 35–40% lipid, depending on growth conditions. The protein complexes involved in photosynthetic energy production are well described biochemically, and localized topographically to specific regions of the thylakoids (see review by Staehelin, 1986). Only the cytochrome (cyt)[1] b_6f complex appears to be uniformly distributed over the entire thylakoid membranes (Allred and Staehelin, 1985). The coupling factor CF_0/CF_1 is found only in nonappressed regions, presumably due to the bulky nature of the CF_1 portion of the complex (Miller and Staehelin, 1976; Wellburn, 1977). Photosystem I (PSI) appears to be housed primarily (> 85%) in the nonappressed membranes whereas the converse is true for PSII, ~ 85% in the appressed regions (Andersson and Anderson, 1980). The chlorophyll *a*/*b* light-harvesting complex (LHC) is found in both stacked and unstacked areas. Most (70–90%) of the LHC is in the stacked membranes, the actual amount varying with phosphorylation levels (Kyle *et al.*, 1983).

The lipid composition of the thylakoids is unique among eukaryotic cellular membranes because it includes ~ 77% neutral galactosyldiglycerides (Douce and Joyard, 1979). Of the total nonpigmented membrane lipids ~ 51% are monogalactosyldiglycerides (MGDG) and ~ 26% are digalactosyldiglycerides (DGDG). These lipids are uncharged, but slightly polar (Gounaris and Barber, 1983). This is in sharp contrast to most eukaryotic membranes which contain high proportions of the zwitterionic phosphatidylcholine (PC). Thylakoid membranes contain only ~ 3% PC. Small amounts of two acidic lipids are also present: sulfoquinovosyldiglyceride (SQDG, ~ 7%) and phosphatidylglycerol (PG, ~ 5%). Thus, the first significant characteristic of the thylakoid lipids may be their lack of charge.

The second important characteristic that may be relevant to overall membrane structure is the high degree of unsaturation in thylakoid lipids (see reviews by Quinn and Williams, 1983; Murphy 1986a, b). Both thylakoid galactolipids contain primarily *cis*-unsaturated 16 : 3 and 18 : 3 acyl chains, which occupy a large cross-sectional area when rotated through three dimensions around all the acyl C–C bonds. At the opposite end of the molecule there is either a single or a pair of galactosyl residues in MGDG and DGDG, respectively. The space occupied by the pair of sugars is close to that of the unsaturated tails, indicating that the DGDG molecule approximates a cylinder in overall shape. The single sugar moiety comprising the head group of MGDG occupies a considerably smaller cross-sectional area than the unsaturated acyl chains (Bishop *et al.*, 1980), leading Murphy and Woodrow (1983a, b) to suggest that MGDG resembles a cone in shape. The consequence of this difference in shape is that DGDG readily packs into planar

bilayer membranes while MGDG adopts hexagonal II configurations (inverted micelles or tubes, "lipid particles") after reconstitution in aqueous solutions (Shipley *et al.*, 1973; Bishop *et al.*, 1980; Sprague and Staehelin, 1983, 1984; Brental *et al.*, 1985). Mixtures of MGDG and DGDG in proportions that approach that in native thylakoids are similarly resistant to bilayer formation (Sen *et al.*, 1981a, b, 1982). Reconstitution of mixtures containing all the nonpigmented thylakoid lipids produces vesicles primarily bilayer in nature, but still exhibiting hexagonal II structures within the bilayer (Ryrie and Fuad, 1982; Gounaris *et al.*, 1983b, c; Sprague and Staehelin, 1984). This is in direct contrast to the situation observed in the native thylakoids where one sees a bilayer lipid membrane with many embedded proteins and no evidence for hexagonal II structures unless the membranes are stressed (Gounaris *et al.*, 1983a, 1984).

Theoretical Considerations

Attempts to integrate the experimental observations that the predominant thylakoid lipid (MGDG) does not form lamellar phases in aqueous solutions with those that demonstrate the bilayer structure of native thylakoids have focussed on the highly curved marginal regions and the hydrophobic portions of the integral proteins within the membrane. The highly curved margins would provide an ideal site on the inner leaflet of the membrane for cone-shaped molecules such as MGDG (Murphy, 1982, 1986a, b). Unfortunately the estimated membrane area represented in the margins could house only ~one-third of the MGDG found in the thylakoids (Murphy, 1982). The remaining MGDG must be localized in the planar regions of the thylakoids: even if the margins are saturated with MGDG, 34% of the lipid in the planar membrane regions is a nonbilayer-forming species. Murphy suggests that the remainder of the MGDG is packaged around the hydrophobic portions of the partially or totally embedded membrane proteins in the form of "lipidic particles." This location would be consistent with the experiments of Williams and Dominy (1987) that show parallel loss of MGDG and stromal membranes after photobleaching of PSI. The organization of the MGDG into "lipidic particles" in *native* thylakoids currently has no experimental support as inverted micelles have never been observed in freeze-fractured thylakoids. In addition, Murphy and Woodrow (1983a, b) calculate that the amount of lipid available in the appressed regions is not sufficient to provide more than a monolayer around the protein complexes. It is difficult to reconcile this calculation with the proposal that some of the lipid is sequestered within the bilayer as "lipidic particles." Sakai *et al.* (1983) similarly propose that MGDG is contained in inverted micelles within the bilayer or in the inner,

concave leaflet of bilayer cusps and bulges as suggested for phosphatidylethanolamine (De Kruijff *et al.*, 1979; Miller, 1980). These are areas where "cone-shaped" molecules could readily be accommodated. Cusps and bulges would not deplete the available lipids as extensively as would intrabilayer inverted micelles.

Correlation of the experimentally determined lipid behavior, the theoretical calculations, and the real thylakoid bilayer remains a challenge. The close appression of the granal membranes is mediated *in vivo* by charge interactions involving phosphorylated and dephosphorylated LHC and Mg^{2+}. These interactions are primarily associated with the protein components of the membrane. The presence of uncharged galactolipids in the membrane may facilitate the process (Barber, 1980).

More difficult to rationalize is the presence of a nonbilayer-forming lipid as the major bilayer lipid in thylakoids. Research with other glycolipids has shown that the molecular shape of the molecule, rather than the overall membrane fluidity, may be important in enzyme activity and membrane stability (Carruthers and Melchior, 1986; Weislander *et al.*, 1980). In addition, Iwamoto *et al.* (1982) suggest that the head-group interactions between glycolipids may be stronger than those between PC head groups, possibly allowing cone-shaped glycolipids to adopt spacings within the membrane that differ from those followed by PC. More work will be required to answer these questions.

Reconstitution and Lipid Supplementation of Isolated Protein Complexes

Investigations of the role(s) of galactolipids in structure and function have focussed primarily on (1) comparing the structural integrity of LHC, PSI, PSII, CF_0/CF_1 and/or cyt b_6f in liposomes formed from PC or native thylakoid lipids, and (2) maximizing the efficiency of energy transfer between the LHC and the reaction centers of each photosystem. Very few studies have addressed both structural and functional parameters simultaneously.

All of the photosynthetically active membrane complexes have been reconstituted in liposomes formed from PC and/or various mixtures of thylakoid lipids. Freeze-fracture electron microscopy has been used to compare the size of the protein particles in the artificial bilayers as well as the overall membrane organization.

PSI complexes prepared by different methods have been reconstituted into PC liposomes and give rise to particles (a) of ~ 10.6 nm diameter, and (b) in two size classes of 10–11 nm and 12–13 nm (Mullet *et al.*, 1980; Dunahay and Staehelin, 1985, respectively). These sizes are consistent with values suggested for PSI complexes in fracture faces from the inner leaflet of

unstacked thylakoids (PFu). The appearance of the larger size class in the latter study may indicate the existence of populations of PSI with varying amounts of bound antenna complexes.

The cyt b_6f complex produced ~8.3 nm particles when reconstituted in either PC or DGDG liposomes (Mörschel and Staehelin, 1983). Particles in this size range are seen in fracture faces from the inner lipid leaflet of both stacked (PFs) and unstacked (PFu) thylakoid membranes (see Staehelin, 1986). This is not unexpected in light of the recent immunolocalization work that showed a random and roughly uniform distribution of the cyt b_6f complex over the granal and stromal membranes (Allred and Staehelin, 1985).

Reconstitution of the chloroplast coupling factor into liposomes of PC and DGDG produced particles with a diameter of ~9.5 nm (Mörschel and Staehelin, 1983). This complex has been localized, using antibodies to CF_1 in thin-sectioned chloroplasts, to stroma facing membranes which exhibit a large number of particles in the 9.5 nm size class (Miller and Staehelin, 1976; see Staehelin, 1986).

All of the above complexes have produced bilayer liposomes with dispersed protein particles in all of the lipids used for reconstitution. The differences between the zwitterionic PC and the polar DGDG appear not to influence the organization of the membranes, or affect the distribution of the individual complexes in the bilayers. This is not true for the major pigment–protein complex of the thylakoids (LHC), or for a PSII core complex under some reconstitution conditions.

The LHC is the complex responsible for membrane stacking in the thylakoids. This characteristic has been exploited in most isolation procedures by including cation aggregation and centrifugation to collect the large crystalline sheets of LHC (see Staehelin, 1986). These sheets cannot be dissociated after precipitation and give rise to extensive crystalline arrays of particles in liposomes formed from PC or mixed thylakoid lipids (McDonnel and Staehelin, 1980; Mullet and Arntzen, 1980; Ryrie *et al.*, 1980; Ryrie and Fuad, 1982; Ryrie, 1983, 1986). The extent of the crystalline arrays in PC liposomes was reduced by using an alternative isolation procedure that circumvents the cation aggregation step (Sprague *et al.*, 1985). A further reduction in LHC aggregation in liposomes was obtained by reconstituting the complex in DGDG. Aggregation of these LHC-liposomes occurred in the presence of Mg^{+2} at concentrations that cause stacking in native thylakoids (Sprague *et al.*, 1985). The LHC gives rise to ~8-nm particles in reconstituted membranes of all lipids tested to date.

PSII complexes are generally isolated as oxygen-evolving preparations or as PSII cores (see Staehelin, 1986). The former appear to be purified, paired, and appressed membrane fragments, and the latter correspond to a

freeze-fracture particle of the outer lipid leaflet of native thylakoids found in varying distributions between stacked (EFs) and unstacked (EFu) membranes (Andersson and Åkerlund, 1978; Dunahay *et al.*, 1984). Reconstitution of a PSII core complex with DGDG liposomes produced bilayer vesicles with ~7.5 nm-diameter particles. The parallel reconstitution experiment with PC failed to produce particle-containing liposomes. Freeze-fracture replicas of the samples showed protein complexes associated with the surface of, rather than incorporated into, the bilayers. In addition, the bilayers appeared highly convoluted, forming a tubular network rather than individual vesicles (Sprague *et al.*, 1985). Although these results do not indicate an absolute requirement for DGDG for the successful reconstitution of PSII core complexes and the LHC, they clearly indicate that high concentrations of the minor thylakoid lipid PC are not optimal. Despite the dramatic structural differences observed after reconstitution of PSII cores with PC and DGDG liposomes, light-dependent reduction of 2,6-dichlorophenolindophenol (DCIP) by diphenylcarbazide was independent of the lipid involved in reconstitution (Sprague *et al.*, 1985).

Functional studies involving reconstitution of isolated membrane complexes have focussed on the efficiency of energy transfer from the LHC to either or both photosystems. PC and mixed thylakoid lipids have been tested for their abilities to improve energy transfer. Larkum and Anderson (1982) used low-temperature fluorescence to show that PSII, PSI, and light-harvesting complexes reconstituted individually into mixed thylakoid lipids behaved as did the complexes in native thylakoids. Pairwise reconstitution of the complexes showed that energy transfer occurred from LHC to PSI and LHC to PSII, but not from PSI to PSII. Combination of all three complexes in the same liposomes showed energy transfer from LHC to both photosystems, but not between the photosystems. A similar study by Murphy *et al.* (1984) using PC for reconstitution showed that PSII activity (as DCIP reduction) increased 70% after addition of the lipid. Addition of LHC to the PSII complexes during reconstitution further increased the rate of DCIP reduction at low light intensities.

The relative effectiveness of all the thylakoid lipid species to increase energy transfer between LHC and PSII or PSI reaction centers was tested by Siefermann-Harms *et al.* (1982) using Triton X-100-solubilized complexes. Native (unsaturated) MGDG was the only lipid that restored the 77 K fluorescence to the level observed in intact thylakoids. Saturated MGDG, DGDG, PC, PG, SQDG, and PE did not affect the fluorescence. DGDG promoted aggregation of the protein complexes, but did not alter the fluorescence. The authors suggest that the ability of Triton X-100 to form bilayers with "cone-shaped" lipids may be important in the restoration of activity by MGDG (see Madden and Cullis, 1982).

Gounaris *et al.* (1983e) investigated the role of lipids in stimulating activity in a Triton X-100-prepared oxygen-evolving PSII sample. The preparation was selectively depleted in DGDG. MGDG had no effect on activity. Unsaturated lipids were stimulatory, but acidic lipids (SQDG and PG) were completely inhibitory. A mixture of the total thylakoid lipids and isolated DGDG stimulated oxygen evolution. The authors postulate that the unsaturated acyl chains are required to optimally associate physically with the complex, that the inability of MGDG to stimulate activity may be associated with its inability to form bilayers, and that the increased amounts of negative charge associated with the acidic lipids destabilizes the complex.

Energy transfer into PSI was investigated by Ikegami (1983) using chlorophyll-depleted and P-700-enriched preparations obtained by ether extraction of lyophilized PSI particles. The efficiency of energy transfer from antenna pigments to the P-700 reaction center was increased if the samples were supplemented with chlorophyll *a* and MGDG or PC. In contrast PE suppressed the association of chlorophyll *a* with the PSI material.

Lipase Treatment

Thylakoids have been treated with a variety of lipases to determine the transverse distribution of the lipid classes within the membrane as well as to attempt a correlation to lipid class with some aspect of structure or function of the membranes as a whole. Both phospholipases and galactolipases have been used. The data obtained with the phospholipases have been simpler to interpret because total hydrolysis can be obtained with removal of only 5–15% of the membrane lipids.

Early work with phospholipase A_2 indicated that 80% of the phospholipids could be hydrolyzed (Hirayama and Nomotobori, 1978), and that PG was the predominant lipid affected (Duval *et al.*, 1979). Jordan *et al.* (1983) reported reduction of PG and PC in damaged thylakoids by 75 and 60%, respectively. Treatment of intact thylakoids with the same enzyme released primarily PG, further suggesting that this lipid is predominantly localized in the outer lipid leaflet (Rawyler and Siegenthaler, 1981a). These authors also reported that PC was released from the membranes only if disrupted thylakoids were used, suggesting that PC was primarily housed in the inner leaflet. Subsequent work using potato lipolytic acid hydrolase and phospholipase C allowed Rawyler and Siegenthaler (1981b) to estimate the proportion of PG and PC in the outer membrane leaflet at 70 and 35%, respectively.

Loss of photochemical activity followed loss of lipids. PSII activity was lost with phospholipase A_2 treatment, but PSI activity was stimulated or unaffected (Jordan *et al.*, 1983; Rawyler and Siegenthaler, 1981a, b; Duval

et al., 1979; Hirayama and Matsui, 1976). Potato lipolytic acid hydrolase inhibited activity around both photosystems, but not electron flow directly through either photosystem (Rawyler and Siegenthaler, 1980). Incomplete hydrolysis of phospholipids inhibited cyclic electron flow somewhat, but not linear flow, prompting the suggestion that pools of phospholipids exist that are more resistant to lipase attack because they are more tightly bound or sequestered, and that only such tightly bound lipids are important in maintaining electron transport activity (Hirayama and Nomotobori, 1978; Rawyler and Siegenthaler, 1981a). At this time it appears that an intact pool of PC is required in the inner leaflet for PSII electron transport activity, and that outer and inner leaflet PG pools are required for PSI activity (see Siegenthaler and Rawyler, 1986).

Galactolipase treatments of thylakoid membranes have utilized enzymes from potato tubers (Hirayama and Matsui, 1976), tomato and bean leaves (Michalski and Kaniuga, 1980), *Rhizopus arrhis* (Rawyler and Siegenthaler, 1985; Siegenthaler and Giroud, 1987), and runner bean chloroplasts (Jacob and Miller, 1986). Rawyler and Siegenthaler (1985) demonstrated that 65% of MGDG is accessible to *R. arrhis* lipase (LRa), and therefore resides in the outer leaflet, while only 15% of DGDG is within this half of the bilayer. Two or three "kinetic" pools exist, depending on the environmental conditions during the enzymatic treatment, suggesting that SQDG was almost entirely ($>95\%$) contained in the inner leaflet. In mole % of lipids, MGDG would account for approximately one-third of the inner leaflet lipid, enough to occupy the sharply concave regions of the granal membrane margins (see Murphy, 1982).

Siegenthaler and Giroud (1987) reported that treatment of thylakoids with LRa inhibited PSII electron flow, but not PSI activity. After 30 min of incubation with LRa 95% of MGDG was hydrolyzed, producing free fatty acids and lyso MGDG. Addition of BSA to the reaction mixture removed all the free fatty acids, and approximately one-half of the lyso MGDG. However, only 50% of the electron flow activity was protected in the presence of BSA, suggesting that an external leaflet pool of MGDG is required for activity. From their data Siegenthaler and Giroud (1987) suggest that the lack of MGDG affects PSII electron transfer between the primary acceptor and the Q_A moeity, and not at the level of the water-splitting system. PSI electron flow and energy distribution between the two photosystems were also disrupted by LRa treatment. Siegenthaler and Giroud suggest that inhibition due to MGDG degradation is in the region of cyt b_6f and PSI. In addition, energy distribution appears to be shifted to favor PSI over PSII.

In contrast to the selectivity of LRa for MGDG, Jacob and Miller (1986) report that runner bean chloroplast lipase hydrolyzes nearly 100% of MGDG and DGDG in 30 min. The products of the hydrolysis are free fatty

acids and soluble galactosylglycerides, suggesting that a significant portion (77%) of the membrane lipids are altered by the lipase. Again BSA will remove all the free fatty acids from the membrane, thus producing a structure with only a quarter of the normal lipid content. In spite of this dramatic change in lipid composition, the electrophoretic polypeptide pattern of the membranes shows no similar loss of polypeptides. The lipase-treated membranes were examined by freeze-fracture electron microscopy and shown to have lost the large outer lipid leaflet (EF) particles and tetrameric complexes of the inner surfaces (ES) of stacked membranes, both thought to represent the PSII-associated structure (see review by Staehelin, 1986). Since no polypeptides were lost from the membrane, the authors suggest that the particles have dissociated into smaller subunits in response to the removal of MGDG and DGDG from the membrane.

From these studies it is apparent that all the lipids play some role in the successful function of the photosynthetic electron transport chain. At this time the data indicate specific activities that are harmed by lipid depletion, but we are still far from understanding the mechanism(s) by which such depletions exert inhibition.

Lipid Enrichment of Thylakoid Membranes

This technique involves the addition of exogenous lipid to intact membranes by the stimulated fusion of liposomes to the thylakoids. Siegel *et al.* (1981) used repeated freeze-thaw cycles to incorporate either PG or PC into spinach thylakoids, and Millner *et al.* (1983) used an acid pulse to incorporate PC. Unlike the situation previously reported for inner mitochondrial membranes (Schneider *et al.*, 1980a, b), thylakoid membrane structure appeared to be easily disrupted by lipid addition. Siegel *et al.* (1981) reported fragmentation of thylakoid membranes, aggregation of the LHC into crystalline particle arrays within the membrane, and loss of a population of large intramembrane particles ($\sim$ 16 nm). Freeze-fracture electron microscopy showed a correlation between the amount of lipid incorporated and the distance between the intramembrane particles. However, the increase in interparticle distance was not sufficient to account for all the lipid that was incorporated, suggesting that the loss of large membrane particles resulted from the dissociation of a large membrane complex into smaller ones (presumably PSII-LHC particles into individual PSII and LHC units). Low-temperature fluorescence spectra of the lipid-enriched membrane fractions indicated a loss in energy transfer between LHC and PSII, which is consistent with the suggestion of Siegel *et al.* (1981) that a larger PSII-LHC particle be dissociated into individual units.

Similar structural results were obtained by Millner *et al.* (1983). These authors also reported rates of steady-state electron transport after lipid enrichment and showed a decrease in these activities with increased amount of lipid incorported into the membranes. In addition, they were able to supplement the membranes with liposomes containing exogenous plastoquinone and demonstrate that electron transfer activity through this region of the chain (PSII → cyt b_6f) was increased over that measured in membranes enriched with liposomes alone. Both groups suggest that at least part of the membrane destruction observed in these experiments could result from the addition of large amounts of charged lipids to a membrane that normally contains only 10–15% charged lipids. This is consistent with some of the reconstitution work discussed earlier: aggregation of the LHC into crystalline arrays occurs more frequently in PC liposomes than in DGDG liposomes (Sprague *et al.*, 1985). We have used liposomes formed from DGDG or from a total nonpigmented thylakoid membrane extract to enrich pea thylakoids (Sprague and Staehelin, in preparation) using a variety of fusion-inducing techniques. The use of endogenous lipids appears to promote greater incorporation of lipid with less structural perturbation of the thylakoids than was reported using phospholipids. However, all of the abnormalities previously reported by Siegel *et al.* (1981) and Millner *et al.* (1983) have been observed in thylakoids enriched by the acid pulse method of Schneider *et al.* (1980a, b) despite the use of liposomes composed of a mixture of native thylakoid lipids. More work is required to find a nondestructive method for adding lipid via fusion to thylakoids before the role(s) of lipids in maintaining structure and function can be thoroughly investigated.

Future Directions

It is clear that we are beginning to understand the importance of specific lipids in maintaining structure and function in thylakoid membranes. Unfortunately there are not yet enough data to allow unambiguous assignments to each of the specific lipid classes in the thylakoids. It is probable that the interactions of several lipid classes will be involved in maintaining a given characteristic of the photosynthetic membranes. The complexity of structure and function in higher plant thylakoid membranes will continue to provide a challenging system in which one can investigate these interactions.

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