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MONOLAYER PROPERTIES OF CHLOROPLAST LIPIDS

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Summary

The properties of seven monogalactosyldiacylglycerols and six digalactosyldiacylglycerols, isolated from photosynthetic membranes and possessing different levels of fatty acid unsaturation, have been studied by the monolayer technique and compared with those of the fully saturated compounds. In addition, the monolayer properties of sulphoquinovosyldiacylglycerols and phosphatidylglycerols from higher plant chloroplasts, and several hexadecenoic acids have been measured.

Monogalactosyldiacylglycerols containing saturated fatty acids form a condensed monolayer similar to that of saturated phosphatidylcholines. The naturally occurring monogalactosyldiacylglycerols, of which the double bond index ranged from 0.6 to 3.9, possessed comparable force-area curves suggesting that headgroup interactions play a more important role in packing behaviour than in phosphatidylcholines. Although digalactosyldiacylglycerols containing fully saturated fatty acids form a more expanded monolayer than the corresponding monogalactosyldiacylglycerols, the degree of expansion of the monolayer due to the presence of unsaturated fatty acids in the naturally occurring digalactosyldiacylglycerols is much less than in monogalactosyldiacylglycerols. Monogalactosyldiacylglycerols and digalactosyldiacylglycerols from a single species have very similar monolayer properties, and the presence of sulphoquinovosyldiacylglycerols and phosphatidylglycerols in the propor-

Abbreviations: DGG, digalactosyldiacylglycerols; MGG, monogalactosyldiacylglycerols; SL, sulphoquinovosyldiacylglycerols.

tions in which they occur in higher plant chloroplasts does not have any condensing effect on a monolayer of galactolipids

Introduction

Physical studies on model membrane systems have contributed much to our understanding of the structure and function of biological membranes. Such studies, however, have concentrated on the physical properties of phospholipids, their interaction with neutral lipids such as sterols, and the effect of temperature on the fluidity of the sample. The thylakoid membrane system of chloroplasts which is the site of the light-harvesting reactions of photosynthesis contains large proportions of glycolipids, and phospholipids are relatively minor components [1]. The major lipid components of the thylakoid membrane system are the uncharged lipids monogalactosyldiacylglycerols (MGG) and digalactosyldiacylglycerols (DGG) and the acidic lipids sulphoquinovosyldiacylglycerols (SL) and phosphatidylglycerols [1]. In higher plant chloroplasts, MGG and DGG are characterized by high concentrations of α-linolenic acid (all-*cis*-9,12,15-octadecatrienoic acid) (18 : 3), while SL contain large amounts of 18 : 3 and palmitic acid (16 : 0) and phosphatidylglycerols are unique in containing about 30% *trans*-3-hexadecenoic acid (*trans*-3 16 : 1), which is only found in the 2-position of the glycerol molecule [1].

The distinctive nature of the chloroplast thylakoid polar lipids, and the fact that the uncharged MGG and DGG may comprise 80% of the total polar lipid, might be expected to result in a membrane bilayer whose properties differ from those containing only charged phospholipids. We have previously presented the chemical shift data obtained from ^{13}C-NMR measurements of the chloroplast glycolipids and compared the longitudinal relaxation times of the sugar and hydrocarbon segments of the molecules [2—4]. The present paper describes a monolayer study of the four chloroplast lipids isolated from a higher plant and compares the monolayer properties of MGG and DGG with different fatty acid compositions.

Materials and Methods

Beta vulgaris was purchased at a local market. *Ulva lactuca* was collected from a rock shelf in the intertidal zone at Putty Beach, New South Wales. *Gymnodinium microadriaticum* was isolated from the mantle of the clam, *Tridacna crocea*, at Lizard Island, North Queensland as previously described [5]. *Prochloron* sp., a prokaryotic green alga [6], was isolated from the ascidian, *Didemnum molle*, also at Lizard Island. *Synechococcus* sp. number RRIMP. N1 (kindly provided by Dr. L. Borowitzka, Roche Research Institute of Marine Products, Dee Why, New South Wales) was grown at 25°C in the medium F/2 of Guillard and Ryther [7]. *Anacystis nidulans* and *Anabaena variabilis* were grown at 25°C in Stanier's medium BG11 [8] and the medium of Kratz and Myers [9], respectively.

Total lipids were extracted with chloroform/methanol, 2 : 1 (containing 0.1% butylated hydroxytoluene as antioxidant), and individual components

were isolated by chromatography on silica gel [5] and CM-cellulose [10]. Purity was established by thin-layer chromatography on silica gel G plates using as solvents, acetone/benzene/water (91 : 30 : 8) [11], and chloroform/methanol/acetic acid/water (85 : 15 : 10 : 3) [12]. Fatty acid composition was determined by gas chromatography of methyl esters as previously described [5]. All compounds used for monolayer studies were at least 99% pure. Phospholipids with defined fatty acid composition were purchased from Serdary Research Laboratories (London, Ontario, Canada) and further purified where necessary, using the above techniques.

Monolayer measurements were performed by conventional Wilhelmy plate techniques at 20 ± 1°C in an all-Teflon trough, 59 cm long and 12.9 cm wide. The subphase was highly purified water (pH 5.2). The sample (50 nmol) dissolved in chloroform was added to the surface with a microsyringe and the solvent was allowed to evaporate for 5 min before commencing compression. The compression rate was 1.3 cm/min and each measurement occupied about 20 min. Three to six measurements were made on each lipid, the standard deviations being 1—2 $Å^2$/mol in each case.

Results

Monolayer studies on phospholipids have, in general, been confined to synthetic phospholipids with a clearly defined fatty acid composition (e.g., Refs. 13—17), whereas naturally occurring phospholipids have a diverse fatty acid composition. A similar diversity occurs in chloroplast glycolipids although MGG and DGG from higher plants contain very high levels of trienoic acids, mainly 18 : 3. Table I shows the fatty acid composition of the galactolipids used in this study. MGG and DGG from *Beta* contain more than 80% 18 : 3, and MGG contain in addition 11% 16 : 3. In contrast, *Ulva* MGG contain 50% tetraenoic acids and 40% trienoic acids, while DGG from the same source contain less than 4% tetraenoic acids and 40% trienoic acids. This variation in the fatty acid composition of MGG and DGG from the same organism is quite remarkable when it is generally considered that DGG are synthesized by galactosylation or dismutation of MGG [18,19]. However, the variation was consistently observed in all samples of *Ulva* examined and similar analyses have been reported for galactolipids of *Ulva* collected in the northern hemisphere [20].

The galactolipids of *G. microadriaticum* are noteworthy for their content of octadecapentaenoic acid (18 : 5) [5], which has only been detected in lipids of dinoflagellates [21]. The galactolipids of *Synechococcus* and *Anabeana* contain smaller proportions of polyenoic acids and significant amounts of 16 : 0 while those of *Anacystis* and *Prochloron* lack polyunsaturated fatty acids and are largely comprised of fatty acids containing 16 carbon atoms. Two factors which influence the monolayer characteristics of a lipid are the length of the fatty acyl chain and its degree of unsaturation. Decreasing the chain length and/or increasing the degree of unsaturation reduces the interaction forces and increases the area per molecule. The double bond index of a fatty acid indicates an average value for the number of double bonds in each fatty acid although it does not allow for variations in chain length. The value of

TABLE I

FATTY ACID COMPOSITION OF GALACTOLIPIDS FROM THYLAKOID MEMBRANES

Double bond index (DBI) was calculated as Σ(fatty acid %) X (number of double bonds in fatty acid) X 10^{-2} for all unsaturated fatty acids in the molecule. Results are expressed as percentages.

Acid	Hydrogenated *Beta vulgaris*		*Beta vulgaris*		*Ulva lactuca*		*Gymnodinium microadriaticum*		*Anabaena variabilis*		*Synechococcus* sp.		*Anacystis nidulans*		*Prochloron* sp.
	MGG	DGG	MGG	DGG	MGG	DGG	MGG	DGG	MGG	DGG	MGG	DGG	MGG	DGG	MGG
14:0	—	—	—	—	—	—	1.0	4.8	+	+	+	+	0.6	1.2	1.1
14:1	—	—	—	—	—	—	—	1.7	+	+	+	+	—	—	12.3
15:0	—	—	—	—	—	—	—	—	—	—	—	—	2.7	2.8	—
16:0	10.5	16.4	2.7	10.0	2.2	24.6	3.6	7.4	34.2	28.3	38.0	30.3	39.1	41.6	31.1
16:1	—	—	0.3	—	1.4	1.8	1.4	1.3	6.1	5.4	13.7	21.1	47.6	48.6	54.7
16:2	—	—	0.7	—	1.2	4.9	0.3	0.2	4.9	4.8	0.5	1.5	—	—	—
16:3	—	—	11.0	—	5.5	11.3	—	—	—	—	—	—	—	—	—
16:4	—	—	—	—	36.3	1.9	—	—	—	—	—	—	—	—	—
17:1	—	—	—	—	—	—	—	—	—	—	—	—	1.2	0.6	—
18:0	89.5	83.6	—	0.9	0.3	0.4	0.2	+	0.4	—	—	—	0.7	0.3	0.4
18:1	—	—	—	3.5	3.3	7.8	0.9	0.5	7.7	8.2	24.9	12.8	8.0	4.9	0.4
18:2	—	—	2.1	3.0	3.7	17.0	1.3	0.8	12.1	10.9	8.4	7.2	—	—	—
18:3	—	—	83.1	82.6	32.4	28.4	10.0	8.3	34.4	42.4	14.4	27.1	—	—	—
18:4	—	—	—	—	13.7	1.9	49.9	51.4	—	—	—	—	—	—	—
18:5	—	—	—	—	—	—	29.3	12.5	—	—	—	—	—	—	—
20:5	—	—	—	—	—	—	1.2	4.8	—	—	—	—	—	—	—
22:6	—	—	—	—	—	—	0.8	5.4	—	—	—	—	—	—	—
DBI	0	0	2.9	2.6	3.3	1.9	3.9	3.6	1.5	1.7	1.0	1.3	0.6	0.5	0.7
% C_{16} acids	10.5	16.4	14.7	10.0	46.6	44.5	5.3	8.9	45.2	38.5	52.2	52.9	89.4	93.0	85.8

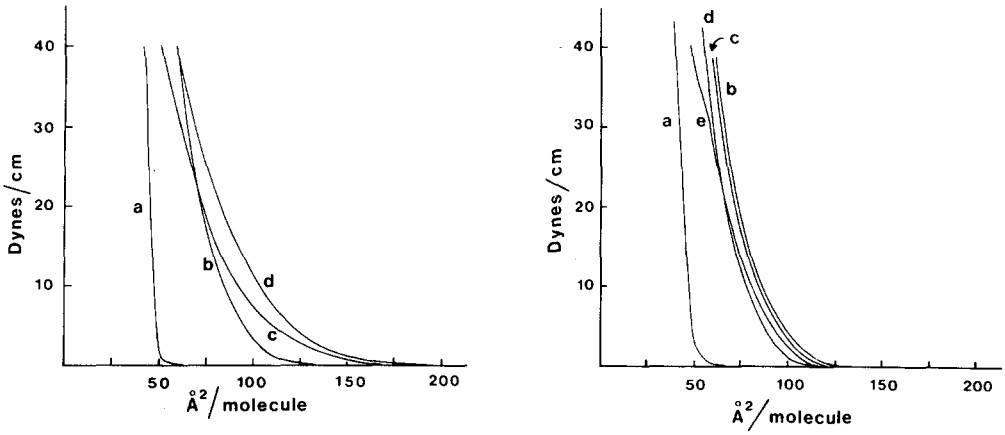

Fig. 1. Force-area curves of synthetic phosphatidylcholines. (a) di-18 : 0 phosphatidylcholine; (b) di-18 : 1 phosphatidylcholine; (c) di-18 : 2 phosphatidylcholine; (d) di-18 : 3 phosphatidylcholine.

Fig. 2. Force-area curves of monogalactosyldiacylglycerols. (a) Hydrogenated *Beta* MGG, double bond index 0; (b) *Beta* MGG, double bond index 2.9; (c) *Ulva* MGG, double bond index 3.3; (d) *Synechococcus* MGG, double bond index 1.0; (e) *Anabaena* MGG, double bond index 1.5.

the double bond index in the MGG samples ranges from 0 to 3.9, and from 0 to 3.6 in the DGG samples. The double bond indexes of *Beta*, *Ulva* and *Gymnodinium* MGG were higher than that of the corresponding DGG, while the reverse was true in *Synechococcus* and *Anabaena*. The double bond indexes of *Anacystis* MGG and DGG were about the same but *Prochloron* contained very little DGG, which precluded its isolation in sufficient quantity. A sample of diglucosyldiacylglycerol from *Bacillus subtilis* was also examined. This compound has a double bond index of 0, but contains predominantly branched-chain fatty acids (br-15 : 0, 55.3%; br-16 : 0, 3.3%, *n*-16 : 0, 5.2%; br-17 : 0, 34.9%).

The force-area curves of the MGG are shown in Figs. 2 and 3 and compared with those for phosphatidylcholines containing 18-carbon fatty acids with different degrees of unsaturation (Fig. 1). The curves for the phosphatidylcholines molecules are in agreement with those of other workers [13—15] and show that the di-18 : 0 compound forms a very condensed monolayer. More expanded monolayers are formed as the degree of unsaturation of the fatty acid chain is increased, although the major change occurs with the introduction of one double bond per fatty acid molecule [14]. The force-area curve of hydrogenated MGG (Fig. 2) is similar to that of di-18 : 0 phosphatidylcholine in being very condensed and approaching the limiting area occupied by two long-chain saturated fatty acid molecules. The MGG from *Beta*, which contain 94% trienoic acids and have a double bond index of 2.9 do not form a monolayer as expanded as di-18 : 3 phosphatidylcholine but rather one which is similar to di-18 : 1 phosphatidylcholines. It should be noted, however, that 1-palmityl-2-docosahexenoyl phosphatidylcholine, which also has a double bond index of 3, forms a much more condensed monolayer than di-18 : 3 phosphatidylcholine [14] and this indicates the limitations of attempting direct correlations between the degree of expansion of a monolayer and the

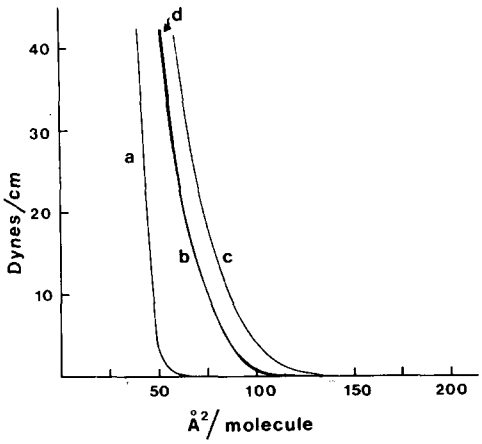

Fig. 3. Force-area curves of monogalactosyldiacylglycerols. (a) Hydrogenated *Beta* MGG, double bond index 0; (b) *Anacystis* MGG, double bond index 0.6; (c) *Gymnodinium* MGG, double bond index 3.9; (d) *Prochloron* MGG, double bond index 0.7.

double bond index of the lipid. Nonetheless, the very similar fatty acid compositions of *Beta* MGG and di-18 : 3 phosphatidylcholine indicate strongly that the more condensed curve of the MGG is due to stronger interactions between the glycolipid headgroups than those occurring in the phosphatidylcholine headgroup.

Comparison of the force-area curves of MGG from other species indicate that they form expanded monolayers similar to that of *Beta* MGG even though the double bond index varies from 0.6 to 3.9. The most unsaturated MGG, that from *Gymnodinium* (double bond index 3.9) have a lift-off area only approx. 20 Å^2/molecule greater than the least unsaturated MGG, in contrast to a group of naturally occurring phosphatidylcholine molecules where a similar increase in the dimensions of the lift-off area is obtained when the double bond index increases from only 1.2 to 1.6 [22]. In general, those MGG with lower double bond indexes have a higher content of 16-carbon acids than those with higher indexes, although *Ulva* MGG are an exception. Nonetheless, the similarity of the force-area curves for all seven naturally occurring MGG indicate that once some unsaturation is introduced, which disrupts the hydrophobic association found when only *n*-saturated acids are present, the degree of unsaturation has relatively little effect on the monolayer properties of these lipids, suggesting that headgroup interactions dominate the monolayer properties. The force-area curve of a monoglucosyldiacylglycerol isolated from *Acholeplasma laidlawii* grown on elaidic acid, and which contained 64% of that acid [16], is intermediate between those of the saturated and unsaturated MGG. However, *trans* double bonds always induce more highly condensed monolayers than do the corresponding *cis* compounds [23,24].

The force-area curves of DGG, with double bond indexes between 0 and 3.6 are shown in Figs. 4 and 5. The fully saturated compounds form a monolayer which is more expanded at low pressures than the saturated MGG (lift off at approx. 110 Å^2/mol as compared to approx. 68 Å^2/mol) and which

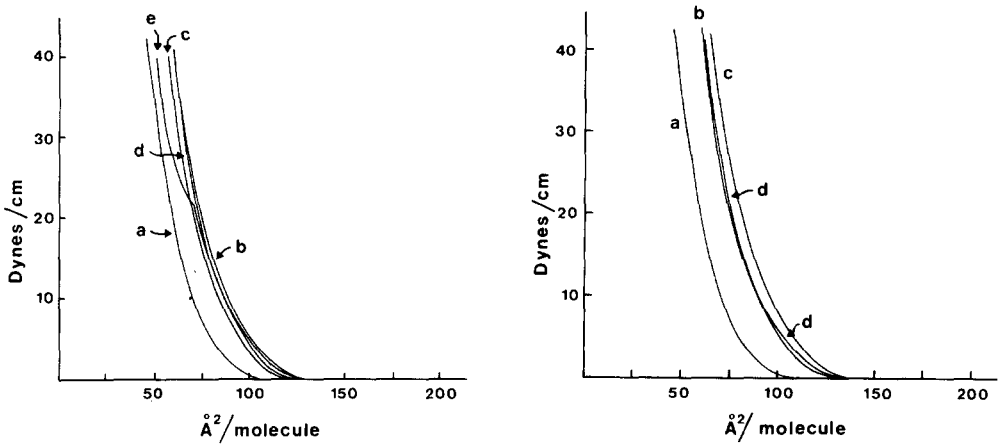

Fig. 4. Force-area curves of digalactosyldiacylglycerols. (a) Hydrogenated *Beta* DGG, double bond index 0; (b) *Beta* DGG, double bond index 2.6; (c) *Ulva* DGG, double bond index 1.9; (d) *Synechococcus* DGG, double bond index 1.3; (e) *Anabaena* DGG, double bond index 1.7.

Fig. 5. Force-area curves of diglycosyldiacylglycerols. (a) Hydrogenated *Beta* DGG, double bond index 0; (b) *Anacystis* DGG, double bond index 0.5; (c) *Gymnodinium* DGG, double bond index 3.6; (d) *Bacillus subtilis* diglucosyldiacylglycerol, double bond index 0.

does not compress to the same extent. This is no doubt due to the much larger size of the DGG headgroup, but it is also apparent that the presence of double bonds in the naturally occurring DGG has much less effect on the properties of the monolayer than was the case with MGG. Thus, headgroup interactions are also dominant in determining the monolayer properties of the DGG. As was the case with MGG, a low double bond index in the DGG is accompanied by a high content of 16-carbon acids. The diglucosyldiacylglycerol from *B. subtilis*, which has a double bond index of 0 but contains predominantly branched-chain acids, also has a force-area curve similar to that of the unsaturated DGG.

The fatty acid compositions of *Beta* SL and phosphatidylglycerols are shown in Table II. Both lipids have a characteristic fatty acid composition, SL con-

TABLE II

FATTY ACID COMPOSITION OF *BETA VULGARIS* SULPHOLIPIDS (SL) AND PHOSPHATIDYLGLYCEROLS (PG)

Results are expressed as percentages.

Fatty acid	SL	Hydrogenated SL	PG
16:0	47.4	46.4	34.4
trans-3 16:1	—	—	29.1
18:0	1.3	53.6	0.6
18:1	1.8	—	2.0
18:2	4.5	—	6.2
18:3	44.8	—	27.6
Double bond index	1.5	0	1.3
% C_{16} acids	47.4	46.4	63.5

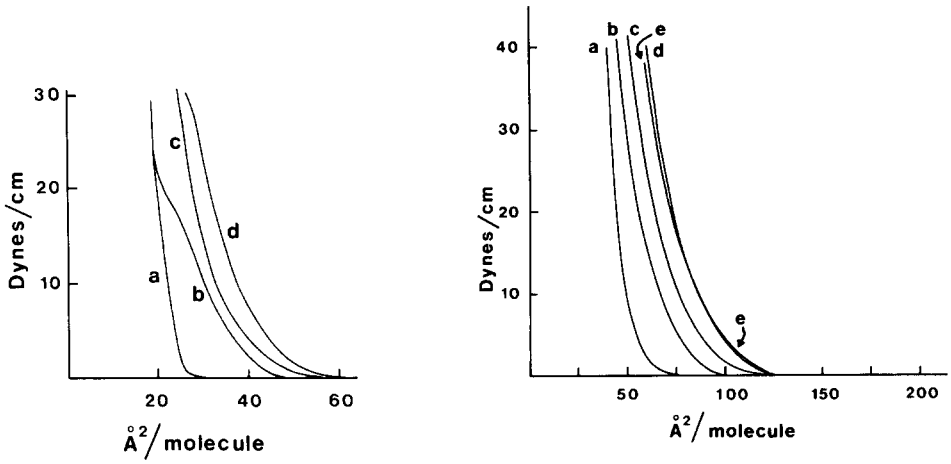

Fig. 6. Force-area curves of 16-carbon atom fatty acids. (a) 16 : 0; (b) *trans*-3 16 : 1; (c) *trans*-9 16 : 1; (d) *cis*-9 16 : 1.

Fig. 7. Force-area curves of *Beta* chloroplast lipids. (a) Hydrogenated SL, double bond index 0; (b) phosphatidylglycerols, double bond index 1.3; (c) SL, double bond index 1.5; (d) MGG, double bond index 2.9; (e) DGG, double bond index 2.6.

taining almost equal amounts of 16 : 0 and 18 : 3 acids, with the unusual property that most of the 16 : 0 is located at the 2-position of the glycerol molecule [25] which is the reverse of the general situation in most naturally occurring phospholipids, where saturated acids occur at the 1-position. Phosphatidylglycerols contain almost equal amounts of 16 : 0, 18 : 3 and *trans*-3 16 : 1, the latter being esterified only at the 2-position of the glycerol molecule.

The force-area curve of *trans*-3 16 : 1, and those of some related 16-carbon acids are shown in Fig. 6. The presence of the *trans*-3 double bond produces a more highly condensed monolayer than the *trans*-9 double bond, which itself produces a slightly more condensed monolayer than the *cis*-9 isomer. These results are in agreement with similar measurments performed on 18-carbon fatty acids [26] in which the order of film expansion is saturated < *trans*-ethylenic < *cis*-ethylenic, and in which the presence of a double bond near either end of the chain leads to a further condensation of the monolayer. The condensed nature of the monolayer of *trans*-3 16 : 1 acid suggests that the presence of the acid in phosphatidylglycerols will contribute to the condensed nature of the phosphatidylglycerols monolayer. The presence of *trans* double bonds in molecules of phosphatidylethanolamines, phosphatidylcholines and ceramides produces monolayers which are more condensed than those containing the corresponding *cis* acids [23,24].

The force-area curves for the four chloroplast lipids of *Beta* are shown in Fig. 7, together with that of hydrogenated SL. The difference between the curve for SL and that previously reported for algal SL [27] is probably due to differences in the ionic strength of the subphases, since the curves for the uncharged higher plant MGG and DGG are in good agreement with the present results. The SL from *Beta* have a higher double bond index than the algal SL [28] and might be expected to yield a more expanded monolayer. It can be

clearly seen that the curves for MGG and DGG are almost the same, despite the disparity in the size of their headgroups, and despite the only minor differences in their fatty acid composition. Comparison of the curves of MGG and DGG from each of the other species (Figs. 2—5) confirms the similarity in the monolayers of the two galactolipids from any one source. Although both SL and phosphatidylglycerols form more highly condensed monolayers than the galactolipids, they do not exert any condensing effect on the force-area curve of the mixture of lipids which is identical with that of the two galactolipids which constitute the major proportion of the mixture (tracing not shown for clarity).

Discussion

Measurement of force-area curves can provide information concerning the interaction between molecules in a monolayer. The use of synthetic phospholipid species has demonstrated that molecular packing at the air/water interface is largely determined by the nature of the hydrocarbon chains, except when only long-chain saturated hydrocarbon residues are present, in which case the nature of the headgroup exerts an effect [13,29,30]. There has been little extension of these studies into the monolayer properties of naturally occurring phosphoglycerides, which exhibit a great diversity in fatty acid composition. It has been shown [22], however, that rats fed on diets differing widely with respect to lipid composition accumulated liver phosphatidylcholines with marked differences in their fatty acid compositions. The force-area curves of these phosphatidylcholines became more expanded with increase in unsaturation [22], although the range in the double bond index (1.2 to 1.6) was much smaller than for the galactolipids used in the present study.

The results presented in this paper demonstrate that the monolayer properties of chloroplast glycolipids differ markedly from those of the phosphoglycerides. This difference is most clearly seen in the monolayers formed by MGG and DGG from *Beta*, which are more condensed than that of di-18 : 3 phosphatidylcholine, although the fatty acid compositions are very similar. The present data are at variance with those of Trosper and Sauer [31] who found that MGG and SL could be compressed to an area as little as 20 $Å^2$/molecule. Such values are hard to explain when it is generally accepted that the limiting area for a molecule containing two fatty acyl chains is approx. 40 $Å^2$/molecule.

We attribute the present results to both the size and orientation of the glycolipids headgroups in the monolayer and also to their capacity for strong intermolecular attraction probably due to hydrogen bonding. In the case of MGG, the hydrogenated compound forms a very condensed monolayer, more condensed than that of di-18 : 0 phosphatidylcholine and similar to that of di-18 : 0 phosphatidylethanolamine [32]. In the case of phosphatidylethanolamines and MGG, the small size of the headgroup allows strong interaction between saturated hydrocarbon chains. Naturally occurring unsaturated MGG and phosphatidylethanolamine show similar swelling behaviour [28] and both are difficult to hydrate [28,33], the reason advanced for phosphatidylethanolamines being that a net neutralization of charge between the polar

groups leads to tight packing and bound water is packed only near the glycerol backbone. Monoglycosyldiacylglycerols, like phosphatidylethanolamines, do not readily form bilayers in aqueous dispersions but exist predominantly in a hexagonal II phase [28,34]. ^{13}C-NMR measurements of MGG have indicated a strong intermolecular headgroup association [3]. Although the presence of unsaturated fatty acids in MGG can exert an expanding effect on the monolayer, that effect is counteracted by the tendency of the headgroup to associate and thus leads to the observation that monolayers of *Beta* MGG are more condensed than those of di-18 : 3 phosphatidylcholine even though the two compounds have very similar fatty acid compositions.

The more expanded nature of the monolayer of hydrogenated DGG as compared to MGG could arise both from the increased size of the headgroup and its orientation at the monolayer surface. The tighter association of MGG molecules is also reflected in the thermal transition measured by differential scanning calorimetry. Hydrogenated MGG has a transition temperature (73°C) about 30°C higher than hydrogenated DGG (43°C) and the enthalpy of the MGG transition is about twice that of the DGG (unpublished observations). It is apparent that in DGG, fatty acid composition has relatively little overall effect on the monolayer properties of the molecule, because the headgroup size and interactions are dominant. The similarity of the force-area curve of diglucosyldiacylglycerol from *B. subtilis* with those of the DGG indicate that this conclusion is probably true for all natural diglycosyldiacylglycerols. Unlike naturally occurring MGG, DGG can form stable bilayers in water [28] and ^{13}C-NMR evidence also indicates association of the polar headgroups [3]. The discontinuous force-area curves of both galactolipids from *Anabaena* are indicative of phase transitions [23]. Such transitions would not be predicted from the fatty acid composition unless there exists a significant content of molecular species containing two saturated fatty acids, in the galactolipids of *Anabaena*. No such discontinuity is present in the force-area curves of galactolipids of the other procaryotic algae although they also have similar total contents of saturated acids.

^{13}C-NMR measurements have indicated that the molecular packing of SL and DGG are similar, with the greater polar headgroup size of DGG being compensated for in SL by a stronger association with solvent molecules [4]. SL from *Beta* form a more condensed monolayer than either MGG or DGG, whereas a monolayer of hydrogenated SL is more expanded than that of hydrogenated MGG. This may be accounted for by the fact that the very condensed monolayer formed by hydrogenated MGG is a combination both of headgroup interaction and attractive forces between the hydrocarbon chains, whereas in hydrogenated SL, an increase in the size of the headgroup together with some charge repulsion due to the sulphonic acid group and stronger association with water molecules limits the packing area of the headgroup. The packing characteristics do not, however, provide any information either about the unique fatty acid composition of SL or about the location of the 16 : 0 acid in the 2-position of the glycerol molecule [25]. Interchange of saturated and unsaturated fatty acids in the 1 and 2 positions of phosphatidylcholines [14] and phosphatidylethanolamines [35] does not markedly affect the force-area curves. However, we have previously shown by ^{13}C-NMR

[4] that the motion of the 18 : 3 acid in the SL molecule is affected by the adjacent 16 : 0 acid and that at all equivalent positions which could be resolved in the spectrum of the two acyl chains, the motion in the unsaturated chain is greater than in the saturated chain.

The role of phosphatidylglycerols in chloroplast membrane function is of great interest because of the influence of pH, ionic strength, divalent ions and proteins on its transition temperature and monolayer properties [36–40]. Although they comprise less than 20% of the membrane polar lipid, phosphatidylglycerols and SL must play an important role because they are the only charged lipids in the membrane. Any charge interaction between protein and lipid in the membrane must involve these two components. Finally, it can be seen (Fig. 7) that the overall molecular packing of the isolated chloroplast membrane lipids is determined by the two galactolipids, whose force-area curves are almost the same. SL and phosphatidylglycerols do not appear to exert any condensing effect on the monolayer, although a mixture of phosphatidylglycerols and positively charged lysyl phosphatidylglycerols, the major lipid components of the membrane of *Staphylococcus aureus* [36], form a more condensed layer than predicted.

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