Biochimica et Biophysica Acta 940 (1988) 158–164
Elsevier

BBA 73906

Neutron diffraction studies of digalactosyldiacylglycerol

Robert V. McDaniel

Department of Physiology and Biophysics, California College of Medicine, University of California, Irvine, CA (U.S.A.)

(Received 16 September 1987)
(Revised manuscript received 4 December 1987)

Key words: Neutron diffraction; Digalactosyldiacylglycerol; Lipid bilayer; Glycolipid structure

The structure of the digalactosyldiacylglycerol bilayer is calculated using neutron diffraction data. The polar head group of this lipid is oriented parallel to the plane of the bilayer such that the galactose moieties are tightly packed at the bilayer surface into a 0.8 nm thick polar layer. The thickness of this layer is independent of water activity over a wide range (15–100% relative humidity). The constant thickness of both the galactose layer and the hydrocarbon layer constrain the structure factor amplitudes to lie on a single continuous transform for repeat periods between 4 and 5 nm.

Introduction

Lipid-bound sugar residues are involved in a wide variety of biological membrane functions such as receptor modulation [1], cell-cell recognition [2], viral infection [3], and toxin binding [4]. To understand the molecular basis of sugar-interactions with membrane surfaces, it is useful to study model membranes formed from purified glycolipids. In particular, physical studies of glycolipid bilayers have given insight into such complicated phenomena as myelin stability [5], erythrocyte mobility in an electric field [6,7], and membrane adhesion [8].

In glycolipid bilayers, the balance between hydrogen-bonding of sugars to water and to the bilayer surface has a profound influence on the structure of the membrane. Examples of 'dry' and 'wet' glycolipid head groups can be found among the glycosphingolipids. Cerebroside bilayers can be classified as 'dry' because they bind only 4 or 5 molecules of water per saccharide group [9]. In cerebroside bilayers, water can be excluded from the membrane surface by inter-saccharide hydrogen bonds between polar head groups. Hydrogen-bonding of the sugar in the head group to the amide moiety of the sphingosine backbone favors a parallel orientation of the head group with respect to the plane of the bilayer [10]. In contrast, bilayers containing charged gangliosides have the head groups of the glycolipid oriented perpendicular to the plane of the bilayer [11]. The extended head groups of gangliosides exert a hydrodynamic drag on water flow near the membrane surface [6,7,12]. When the charges are removed from gangliosides incorporated into bilayers, the glycolipids cluster in the plane of the bilayer [13], presumably by excluding some of their bound water and hydrogen bonding to each other.

In addition to the sphingoglycolipids (cerebrosides and gangliosides), the glyceroglycolipids can be used to form bilayer membranes. Glucosyldiacylglycerols are found in the membrane of *Acholeplasma laidlawii*. Nuclear magnetic resonance studies suggest that the head groups of diglucosyldiacylglycerol are oriented parallel to

Correspondence: R.V. McDaniel, Department of Physiology and Biophysics, California College of Medicine, University of California, Irvine, CA 92717, U.S.A.

the plane of the lipid bilayer [14] and have low levels of hydration (less than four water molecules bound per saccharide group). Galactosyldiacylglycerols are major components of chloroplast thylakoid membranes [15]. Digalactosyldiacylglycerol is thought to play a role in maintenance of the lamellar structure of thylakoid membranes because it forms liquid crystalline, stacked bilayer arrays over a wide range of hydration and temperature [16]. The hydration of digalactosyldiacylglycerol bilayers has been studied by force measurements [8] and by X-ray diffraction [16,17], although the structure factors were not calculated.

In this report, the structure factors for digalactosyldiacylglycerol bilayers are estimated from neutron diffraction data. Neutron density profiles and $H_2O/^2H_2O$ exchange are used to calculate the polar layer thickness and head group orientation.

Materials and Methods

Digalactosyldiacylglycerol was obtained from Lipid Products (Surrey, U.K.) and purified by chromatography on DEAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden) in re-distilled methanol (Aldrich HPLC grade, Milwaukee, WI, U.S.A.) to adsorb negatively charged impurities. After purification, digalactosyldiacylglycerol showed a single spot on thin-layer chromatograms (Silica gel G, benzene/acetone/water (91 : 30 : 8, by vol.) or chloroform/methanol/water (65 : 25 : 4, by vol.) and had zero electrophoretic mobility as multilamellar vesicles in 0.1 M NaCl buffered to pH 7.5 with 1 mM Mops at 25 °C.

Digalactosyldiacylglycerol was also prepared from the seeds of *Briza humilis* (gift of C.R. Smith, ARS, USDA, Peoria, IL), using the procedure of Smith and Wolff [18]. Digalactosyldiacylglycerol from *Briza* was purified by preparative thin-layer chromatography (Silica gel GF, 2 mm thick, in benzene/acetone/water (91 : 30 : 8, by vol.)) followed by DEAE Sephadex A-25 chromatography as described above.

Neutron diffraction experiments were performed at the High Flux Beam Reactor H-3 crystal station [19] of Brookhaven National Laboratories (Upton, NY). 10–20 mg of lipid, dissolved in chloroform or benzene/acetone (10 : 1, v/v), was dried under a stream of nitrogen onto acid cleaned 1 mm thick quartz microscope slides. A razor blade was used to trim the edges of the dried lipid film to final dimensions of 40 × 15 mm. After 12 h equilibration with the atmosphere above a saturated aqueous KCl solution at 11 °C, the quartz slide was mounted in a sealed aluminum chamber containing a vial of saturated salt solution at 21.3 °C.

The incident neutron beam was 4 mm in diameter and intersected the 40 mm long lipid film as an ellipse. As the quartz slide was rotated over a 10 ° angle (increments of 0.1 °) from the beam axis, the major axis of this ellipse varied as the sin of the rotation angle, leading to a geometrical correction of $s = (h/d)$ for the diffracted intensities. Another factor of s arose from the Lorentz correction [20] so that the measured intensities were corrected by multiplying them by $s^2 = (h/d)^2$. Entire elliptical reflections were collected on a two-dimensional position-sensitive gas flow detector. The integration and background subtraction procedures have been described previously in detail [19].

Results

4 or 5 orders ($1 < h < 5$) of lamellar diffraction were observed at 21 °C when the relative humidity (rh) was varied from 15 to 98% by the use of saturated salt solutions. The repeat period (d), varied from 4.1 to 5.1 nm. To construct neutron density profiles of the digalactosyldiacylglycerol bilayer, it was necessary to collect phased higher order structure factors. The observed structure factor amplitudes varied smoothly with the reciprocal spacing $s = h/d$, as illustrated by the points in Fig. 1. Phases were assigned to the three regions of the smooth curve by using swelling theory [21]. In regions I, II, and III of Fig. 1, the assigned phases are −, +, −, respectively. This phase assignment is consistent with results from $^2H_2O/H_2O$ exchange [22] at $d = 4.13$, 4.45, and 4.95 nm. The agreement between the phases from swelling theory and the phases from ^2H_2O exchange can be seen in Fig. 2, where the phased structure factors were used to generate neutron density profiles at various ^2H_2O contents. In these profiles, the peaks represent the high density polar

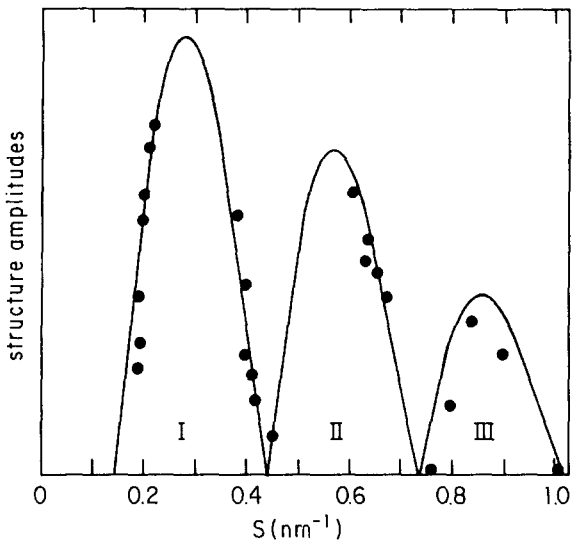

Fig. 1. Structure amplitudes for a series of swelling experiments on oriented digalactosyldiacylglycerol multilayers in 100% H_2O. The abscissa is the reciprocal space coordinate $s = h/d$, where h is the order of reflection and d is the repeat period. The repeat period was varied by changing the activity of water in the gas phase over oriented multilayers. The activity of water (relative humidity, rh) was buffered with saturated aqueous solutions of K_2SO_4 (98% rh), KCl (86% rh), NaCl (76% rh), $NaNO_2$ (66% rh), and LiCl (15% rh) at 21.3° C.

region (galactose-galactose-glycerol) of the digalactosyldiacylglycerol molecule and the central minimum between the polar head group peaks is the water layer. The low density region in the center of the bilayer (at the edge of each profile) is the hydrocarbon chain region. Water and ^2H_2O cannot maintain a high concentration in the hydrocarbon region of the membrane, so the superimposition of the profiles in Fig. 2 over a 2.6 nm wide region defines an estimate for the hydrocarbon thickness. Since strip model calculations are consistent with this estimate over the entire range of d-spacings of Figs. 1–3, 2.6 nm was assumed to be the constant hydrocarbon thickness. A more direct method for hydrocarbon thickness measurement would involve specific deuteration of the interfacial carbons on each acyl chain, but this might perturb the structure of the interface.

Figs. 3 and 4 show that the thickness of the digalactosyldiacylglycerol bilayer is independent of water content. In Fig. 3, the separation of the two polar head groups across the hydrocarbon region remains constant at 3.3 nm for $5.1 < d < 4.5$ nm while the change in water spacing accounts for the entire change in repeat period. In Fig. 4,

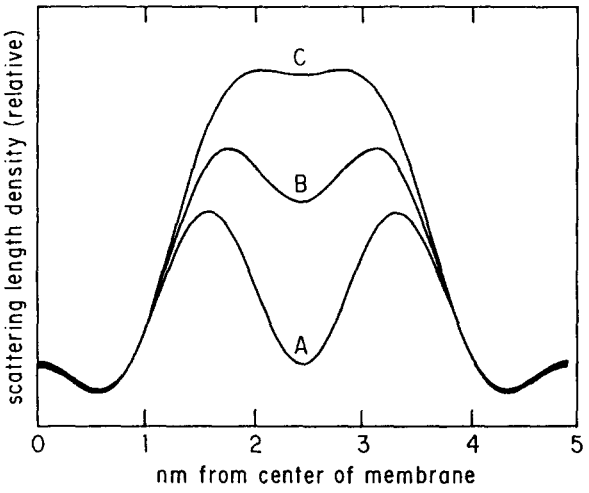

Fig. 2. Neutron density profiles for digalactosyldiacylglycerol commercially extracted from chloroplasts (see Methods). Resolution = 1 nm, $d = 4.9$ nm. The ^2H_2O concentration was varied from 0% (A) to 15% (B) to 33% (C) by gas phase equilibration with saturated K_2SO_4 (98% rh) in the given volume percentage of ^2H_2O. The profiles superimpose over a hydrocarbon region that is 2.6 nm wide.

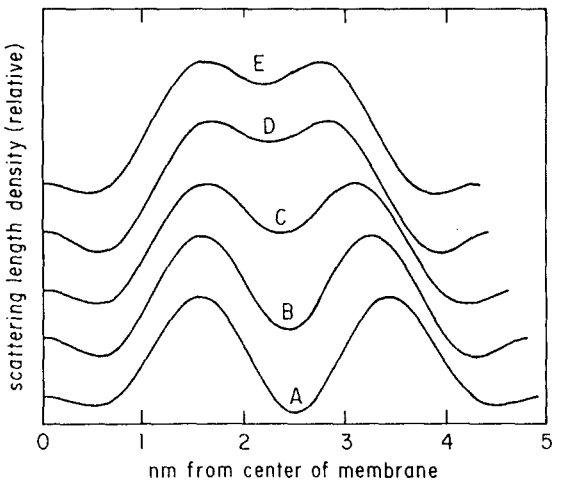

Fig. 3. Neutron density profiles for digalactosyldiacylglycerol derived from chloroplasts, at 1 nm resolution. The distance from the center of the hydrocarbon region (at 0 nm) to the peak density is 1.65 nm. Thus, the peak to peak bilayer width is 3.3 nm. The activity of water was buffered with saturated aqueous solutions of K_2SO_4 (A), KCl (B), NaCl (C), $NaNO_2$ (D), and LiCl (E), at 21.3° C.

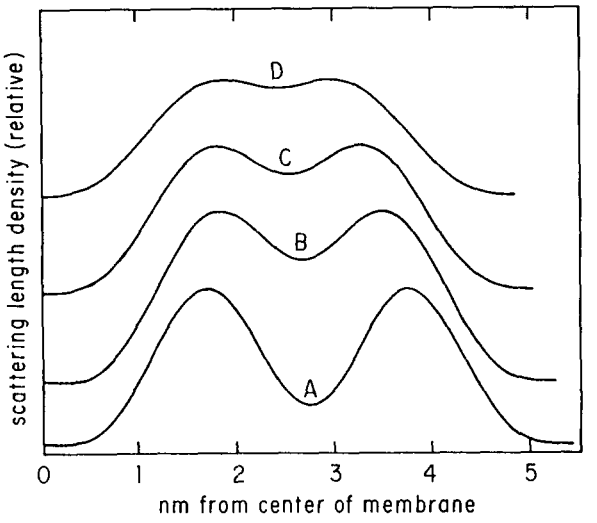

Fig. 4. Neutron density profiles for digalactosyldiacylglycerol derived from seeds (see Methods). 100% H_2O; resolution = 1.3 nm. The peak to peak bilayer width is 3.6 nm. The activity of water was buffered with saturated aqueous solutions of K_2SO_4 (A), KCl (B), NaCl (C), and $NaNO_2$ (D), at 21.3° C.

similar results are shown for the digalactosyldiacylglycerol of *Briza humilis* seeds. The bilayer is slightly thicker (3.6 nm, peak to peak separation) in Fig. 4 than in Fig. 3, presumably because the degree of saturation of the fatty acid chains is higher in the seed lipid than in the chloroplast lipid [18,23].

The thickness of the digalactosyldiacylglycerol bilayer (hydrocarbon region plus polar head group) can be defined in several ways. First, the repeat period at low water content approximates the thickness of a single bilayer. At 15% rh, where the repeat period is 4.1 nm, $^2H_2O/H_2O$ exchange does not significantly alter the amplitudes of the observed structure factors. This indicates that little water is between the bilayers. If we assume that the measurement of hydrocarbon thickness at 98 and 66% rh is valid at 15% rh, then the hydrocarbon region thickness (2.6 nm) plus twice the polar region thickness equals 4.1 nm, giving 0.8 nm as the head group thickness. X-ray diffraction has also been used to measure the repeat period of digalactosyldiacylglycerol at low water content. Shipley et al. [16] measured 4.48 nm as the repeat period for *Pelargonium* chloroplast digalactosyldiacylglycerol with no added water, but obtained no independent measure of the hydrocarbon thickness. Assuming a 2.6 nm thick hydrocarbon region in their preparation gives 0.94 nm as the head group thickness. From molecular models, the maximal extension of the terminal galactose from the hydrocarbon/water interface (carbonyl group) is about 1.8 nm, showing that the head groups of digalactosyldiacylglycerol are not fully extended.

The method of Luzzati [24] examines the dependence of repeat period (d) on the weight fraction of water in the lipid/water mixture. Shipley et al. [16] applied this method to digalactosyldiacylglycerol at weight fractions of water between 10% and 40%. The calculated lipid thickness of 4.2 nm was constant (within 0.1 nm) when d varied from 4.7 to 5.4 nm. For a 2.6 nm thick hydrocarbon region, this again leads to a 0.8 nm thick head group.

Swelling theory and strip models [25] provide a more direct approach to the measurement of bilayer thickness. In general, when the fluid space between bilayers changes and the bilayer thickness remains fixed, the structure factor amplitudes lie on a single smooth curve when plotted vs. $s =$

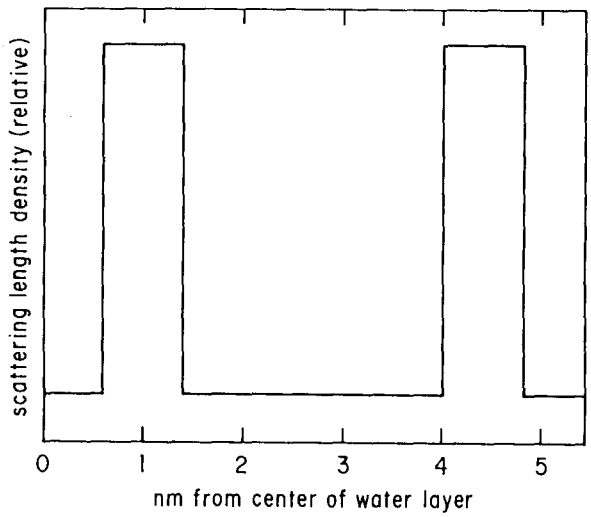

Fig. 5. Strip density model of the digalactosyldiacylglycerol bilayer shown in Figs. 2 and 3. The Fourier transform of this model is shown as the curve in Fig. 1. The middle of the hydrocarbon region is at the center of the strip model. Since the narrowest strip in this model represents the finest resolvable detail (1 nm), only the sum of structure factor amplitudes upto a resolution of 1 nm^{-1} was used as a scaling factor. Higher frequency components of the calculated transform were discarded and assumed to be zero.

(h/d) [21]. The shape of this curve is quite sensitive to the bilayer thickness, even for low resolution diffraction data [25]. The Fourier transform of the smooth curve gives a real space density distribution for the membrane. The smooth curve in Fig. 1 is the Fourier transform of the real space density distribution shown in Fig. 5. In this model distribution, the digalactosylglycerol polar regions are represented by two strips of width 0.8 nm. The central hydrocarbon region (2.6 nm wide) and the water layers (outside the polar head group strips) have the same neutron density. The Fourier transform of this strip model approximates the continuous transform of the digalactosyldiacylglycerol membrane because the measured structure amplitudes (points in Fig. 1) lie on the curve. Therefore, a 0.8 nm thick polar region is consistent with the neutron diffraction data over a wide range of hydrations. Fig. 6 shows that the shape of transforms from simple strip models is extremely sensitive to the headgroup orientation. When the headgroup strip density is distributed over a 1.8 nm thick layer, (fully extended uniform headgroup), Fig. 6B is obtained as the predicted continuous transform. This model of extended orientation is inappropriate for digalactosyldiacylglycerol because it does not match the data of Fig. 1. For instance, it has a prominent node near 0.6 nm^{-1}, just where a peak is seen in the measured transform.

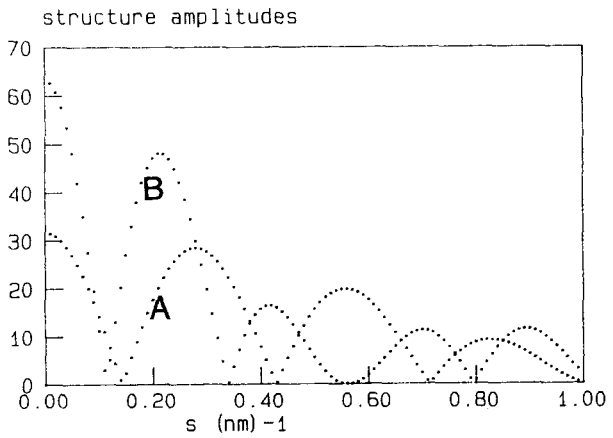

Fig. 6. Sensitivity of the continuous transform to head group orientation. (A) The experimentally observed transform of Fig. 1, with 0.8 nm thick headgroups, as in Fig. 5. (B) Transform for the case of fully extended headgroups: 1.8 nm thick polar layer. All other features of the strip-model are as in (A). Note that this transform must be rejected because it predicts nodes where experimental peaks are observed and vice versa (compare Fig. 1). Other models for intermediate headgroup orientations are bounded by transforms A and B.

The neutron density of the 0.8 nm wide head group region can be estimated from $^2H_2O/H_2O$ exchange, as shown in Fig. 2. The head group peaks are contrast matched with the water layer when the ^2H_2O concentration is 33%, corresponding to a neutron scattering length density of 1.7 (10^{-11} cm/nm^3) [26] in both the polar and fluid layers. The atomic composition of the digalactosyldiacylglycerol polar region (excluding water and acyl chains) is $C_{17}H_{25}O_{15}$ [16], corresponding to a neutron scattering length of 1.07 (10^{-11} cm). Taking the molecular area of digalactosyldiacylglycerol as 0.75 nm^2 [16], the volume available to each head group in the 0.8 nm thick polar layer is $0.75 \times 0.8 = 0.6$ nm^3, giving a calculated neutron density of $1.07/0.6 = 1.78$ (10^{-11} cm/nm^3), equivalent in density to 35% ^2H_2O, in good agreement with Fig. 2C. Exchange of 33% of the 7 non-carbon-bonded H atoms with deuterium in 33% ^2H_2O would increase the calculated neutron density to 2.2 (10^{-11} cm/nm^3), suggesting that little $^2H/H$ exchange occurred at the COH groups of digalactosyldiacylglycerol. Based on contrast matching, the scattering length densities of the head groups of digalactosyldiacylglycerol and phosphatidylcholine are similar [26]. A caution is required here, especially where hydrogen-bonding may be involved in the stabilisation of saccharide orientation. That is, deuterium substitution for hydrogen may have an unobserved effect on contrast matching experiments, because the orientation of the contrast-matched sugar groups is not directly determined.

Discussion

The head group of digalactosyldiacylglycerol is probably oriented parallel to the plane of the bilayer because the thickness of the polar region of digalactosyldiacylglycerol is only 0.8 nm. Based on Corey-Pauling-Koltun molecular models, the anhydrous polar layer would be 1.8 nm thick if the head group were fully extended. From space-filling molecular models, the volume of two galactose groups is about 0.56 nm^3 and only 0.6 nm^3 of

volume is available to each digalactosyldiacylglycerol head group. Thus, the digalactosyl moieties must be tightly packed at the surface, leaving little room for water of hydration. Thermal measurements [17] suggest that little water is bound by the galactose groups of digalactosyldiacylglycerol: similar to diglucosyldiacylglycerol, where less than four water molecules are bound by each saccharide group [14]. The diglycosyldiacylglycerols may be categorized as 'dry' glycolipids, similar to cerebrosides, and different from the charged gangliosides.

Although this study has not dealt with mixtures of digalactosyldiacylglycerol with other lipids, there is circumstantial evidence that the head groups of glycosyldiacylglycerols are not fully extended, even when mixed with the charged phospholipid phosphatidylglycerol. In 0.1 M NaCl solutions, the electrophoretic mobilities of vesicles formed from diglucosyldiacylglycerol/phosphatidylglycerol mixtures [27] are identical to the mobilities of vesicles formed from mixtures of phosphatidylglycerol with phosphatidylcholine (for phosphatidylglycerol, see Ref. 28; for PC/phosphatidylglycerol 5 : 1, see Ref. 6) and twice the mobilities of phosphatidylcholine/ganglioside vesicles [6,7,12] of equivalent surface charge. If the head groups of phosphatidylcholine [29] and of diglucosyldiacylglycerol are parallel to the plane of the bilayer, they would exert little hydrodynamic drag effect on the electrophoretic mobility, as is observed.

In contrast, the head groups of the gangliosides are extended from the bilayer plane [11,12] so that they exert considerable hydrodynamic drag, even for the smallest ganglioside head group (G_{M3}), which is a trisaccharide [12]. Some monosaccharide head groups are also suspected of extended orientation, based on electrophoretic mobility measurements (phosphatidylinositol [30]), or on ^{2}H-NMR studies (glucocerebroside [31]; glucosyldialkylglycerol [32]). In the case of cerebroside, there is disagreement between groups as to the preferred orientation of the head group (compare Ref. 10 with Ref. 31). If the orientation of sugars at the surface of bilayer membranes is modulated by balancing electrostatic repulsion (e.g. sialic acid), hydration-swelling, and thermal disorder with hydrogen-bonding [33] and adhesion [8], then it is tempting to speculate that regulation of charge density and saccharide-composition in cellular membranes controls the extension of the glycocalyx into the aqueous phase.

Acknowledgements

The research at Brookhaven National Laboratory was carried out under the auspices of the U.S. Department of Energy with the help of Dr. Benno Schoenborn, Dr. Anand Saxena, and Dr. Jurg Schaefer. The author thanks Professor Stuart McLaughlin and Stephen H. White for financial support, and the Agricultural Research Service of the U.S. Department of Agriculture for supplying the seeds of *Briza humilis* and Dr. Glen King for use of his sample chamber.

References

1 Bremer, E.G., Hakomori, S., Bowen-Pope, D.F., Raines, E. and Ross, R. (1984) J. Biol. Chem. 259, 6818–6825.
2 Hakomori, S. (1981) Annu. Rev. Biochem. 50, 733–764.
3 Tsao, Y. and Huang, L. (1985) Biochemistry 24, 1092–1097.
4 Fishman, P.H. (1982) J. Membrane Biol. 69, 85–97.
5 Ruocco, M.J. and Shipley, G.G. (1984) Biophys. J. 46, 695–707.
6 McDaniel, R.V., McLaughlin, A., Winiski, A.P., Eisenberg, M. and McLaughlin, S. (1984) Biochemistry 23, 4618–4623.
7 McDaniel, R.V. and McLaughlin, S. (1985) Biochim. Biophys. Acta 819, 153–160.
8 Marra, J., Horn, R.G. and Israelachvili, J.N. (1985) in Abstracts of the 5th International Conference on Surface and Colloid Science, p. 411, Clarkson University, Potsdam, New York.
9 Ruocco, M.J. and G.G. Shipley, G.G. (1983) Biochim. Biophys. Acta 735, 305–308.
10 Pascher, I. and Sundell, S. (1977) Chem. Phys. Lipids 20, 175–191.
11 McDaniel, R.V. and McIntosh, T.J. (1986) Biophys. J. 49, 93–96.
12 McDaniel, R.V., Sharp, K., Brooks, D., McLaughlin, A.C., Winiski, A.P., Cafiso, D. and McLaughlin, S. (1986) Biophys. J. 49, 741–752.
13 Tillack, T.W., Wong, M., Allietta, M. and T.E. Thompson (1982) Biochim. Biophys. Acta 691, 261–273.
14 Wieslander, A., Ulmius, J., Lindblom, G. and Fontell, K. (1978) Biochim. Biophys. Acta 512, 241–253.
15 Gounaris, K. (1985) in Ion Interactions in Biological Energy Transport Systems. Proceedings of an International Workshop, Athens, Greece, April 8–12, 1985, pp. 133–140, UNESCO, Athens.
16 Shipley, G.G., Green, J.P. and Nichols, B.W. (1973) Biochim. Biophys. Acta 311, 531–544.

17 Sen, A., Mannock, D.A., Collins, D.J., Quinn, P.J. and Williams, W.P. (1983) Proc. R. Soc. London B. 218, 349–364.
18 Smith, C.R. and Wolff, I.A. (1965) Lipids 1, 123–127.
19 Schoenborn, B. (1984) in Neutrons in Biology (Schoenborn, B.P., ed.), pp. 211–226, Plenum Press, New York.
20 Saxena, A.M. and Schoenborn, B.P. (1977) Acta Cryst. A33, 813–818.
21 Worthington, C.R., King, G.I. and McIntosh, T.J. (1973) Biophys. J. 13, 480–494.
22 Blasie, J.K., Schoenborn, B.P. and Zaccai, G. (1975) Brookhaven Symp. Biol. 27, III-58–III-67.
23 Robertson, R.N. (1983) in The Lively Membranes, p. 22, Cambridge Press, Cambridge.
24 Luzzati, V. (1968) in Biological Membranes (Chapman, D., ed.), pp. 71–123, Academic Press, New York.
25 King, G.I., Jacobs, R.E. and White, S.H. (1984) Biochemistry 24, 4637–4645.
26 Atkinson, D. and Shipley, G.G. (1984) in Neutrons in Biology (Schoenborn, B.P., ed.), pp. 211–226, Plenum Press, New York.
27 Christiansson, A., Eriksson, L.E.G., Westman, J., Demel, R. and Wieslander, A. (1985) J. Biol. Chem. 260, 3984–3990.
28 Lau, A., McLaughlin, A. and McLaughlin, S. (1981) Biochim. Biophys. Acta 645, 279–292.
29 Buldt, G. (1984) in Neutrons in Biology (Schoenborn, B.P., ed.), pp. 189–200, Plenum Press, New York.
30 Hammond, K., Reborias, M.D., Lyle, I.G. and Jones, M.N. (1984) Biochim. Biophys. Acta 774, 19–25.
31 Skarjune, R. and Oldfield, E. (1982) Biochemistry 21, 3154–3160.
32 Jarrell, H.C., Giziewicz, J.B. and Smith, I.C.P. (1986) Biochemistry 25, 3950–3957.
33 Crowe, J.H., Spargo, B.J. and Crowe, L.M. (1987) Proc. Natl. Acad. Sci. USA 84, 1537–1540.