Effect of glycerol on the molecular properties of cerebrosides, sulphatides and gangliosides in monolayers

Ismael D. BIANCO, Gerrado D. FIDELIO and Bruno MAGGIO*

Departamento de Quimica Biologica-CIQUIBIC, Facultad de Ciencias Quimicas-CONICET, Casilla de Correo 61, 5016 Cordoba, Argentina

The presence of glycerol, free from surface-active impurities, modifies the molecular area, surface potential/molecule and thermodynamic parameters of compression of monolayers of galactosylceramide, sulphatide and gangliosides G_{M1} , G_{D1a} and G_{T1b} . This may be due to changes of the composition and structural properties of the glycosphingolipid solvation shell with an influence on the intermolecular organization.

INTRODUCTION

The polar head group of glycosphingolipids (GSLs) has a remarkable influence on their intermolecular organization, interactions with phospholipids, Ca^{2+} and proteins, the phase state and the three-dimensional organization of the lipid aggregate (Maggio *et al.*, 1981, 1986; Maggio, 1985; Cantu *et al.*, 1986; Curatolo & Neuringer, 1986).

A considerable amount of non-freezable water is associated with the polar head group of the more complex GSLs (Bach *et al.*, 1982), and the possible modification of the structure of interfacial water by polysialogangliosides has been ascribed an evolutionary importance for maintaining the integrity of membranes (Hilbig & Rahmann, 1986). On the other hand, the formation of metastable phases of the more simple GSLs such as cerebrosides depends on the degree of hydration and is affected by agents that disrupt water structure (Curatolo, 1985). The thermodynamics of compression of GSLs and the excess thermodynamic functions for mixed monolayers of anionic GSLs with proteins indicated that hydration–dehydration processes can be important for intermolecular organization (Fidelio *et al.*, 1984, 1986a,b). Studies using merocyanine-540 (Montich *et al.*, 1985) and anilino-naphthalenesulphonate (G. G. Montich & G. Maggio, unpublished work) revealed a more polar dielectric environment for interfaces containing anionic GSLs than for phosphatidylcholine or neutral GSLs, and this can be modified by the presence of Ca^{2+} or basic proteins (Montich *et al.*, 1985). These effects may be due either to changes of the interfacial potential (McDaniel *et al.*, 1986; Maggio *et al.*, 1981; Fidelio *et al.*, 1984) or to a different hydration of the interface.

The effect of the water environment on the organization of lipids has been studied with monolayers spread on subphases containing solutes that may affect water structure (Cadenhead & Bean, 1972; Maggio & Lucy, 1978; Crowe *et al.*, 1984; Johnston *et al.*, 1984). Some of

these results were related to the protective effects of certain carbohydrates on membranes (Cadenhead & Bean, 1972; Crowe & Crowe, 1984) or to membrane changes occurring during fusion (Maggio *et al.*, 1976; Boni *et al.*, 1981). Recently Arnett *et al.* (1986) suggested an explanation for some of the inconsistencies among results previously obtained. We have confirmed that these are due to the presence of surfactant impurities in trace quantities in reputedly highly pure solutes. In the present work we report the effect of glycerol, carefully controlled to ensure absence of surfactants, on the interfacial properties of some neutral and anionic GSLs.

MATERIALS AND METHODS

We employed GalCer, Sulf, G_{M1} , G_{D1a} and G_{T1b} as GSLs with oligosaccharide chains of different complexity. The purification and sources of GSLs and the technique, equipment, calculations and reproducibility for measurement of monolayer properties were described in detail previously (Maggio *et al.*, 1978).

Glycerol was from E. Merck (Darmstadt, Germany). Special care was taken to control the presence of surface-active impurities (Arnett *et al.*, 1986). Solutions of glycerol in 145 mM-NaCl, pH 5.6, were previously boiled with activated charcoal. After this treatment, no increase in the surface pressure or changes in surface potential are detected when the interfacial area is reduced to a minimum value even after 1 h was allowed for possible adsorption of impurities. The isotherms are fully reproducible at different compression rates and under successive expansion–recompression cycles. The thermodynamic parameters for compression were calculated from the temperature dependence of the free energy of compression (ΔF_c) (Gershfeld, 1974). The system defined for these calculations consists of the lipid monolayer at the initial molecular area taken at $2 \text{ mN} \cdot \text{m}^{-1}$. Some

Abbreviations used: dmPC, dimyristoyl phosphatidylcholine; dpPC, dipalmitoyl phosphatidylcholine; GSLs, glycosphingolipids; Cer, ceramide (*N*-acylsphingoid); NeuAc, *N*-acetylneuraminic acid; GalCer, Gal β 1-1Cer; Sulf, Gal(3-sulphate) β 1-1Cer; G_{M1} , Gal β 1-3GalNAc β 1-4Gal(3-2 α NeuAc) β 1-4Glc β 1-1Cer; G_{D1a} , NeuAc α 2-3Gal β 1-3GalNAc β 1-4Gal(3-2 α NeuAc) β 1-4Glc β 1-1Cer; G_{T1b} , NeuAc α 2-3Gal β 1-3GalNAc β 1-4Gal(3-2 α NeuAc α 8-2 α NeuAc) β 1-4Glc β 1-1Cer.

* To whom correspondence and reprint requests should be sent.

limitations and assumptions inherent to these calculations and the use of the thermodynamic values in a comparative manner for these systems were discussed elsewhere (Fidelio *et al.*, 1986a).

RESULTS

Glycerol has no effect on the surface behaviour of dmPC or dpPC, in agreement with Johnston *et al.* (1984). A decrease of the collapse pressure is observed for G_{D1a} ,

Fig. 1. Interfacial behaviour of GSLs on subphases containing glycerol

The surface-pressure–area (a) and surface-potential/molecular-area (b) isotherms at 20 °C are shown for GalCer (filled symbols) and G_{D1a} (open symbols) on subphases containing 145 mM-NaCl (▲, △); 145 mM-NaCl + glycerol (50%, v/v) (●, ○).

whereas no change is present for the other GSLs in the presence of up to 50% (v/v) glycerol. The influence on the intermolecular packing can be grouped into two different behaviours (Fig. 1a). GalCer, Sulf and G_{M1} show expansions of the surface pressure–area isotherm below 20 mN·m⁻¹ that increase with the concentration of glycerol. G_{D1a} and G_{T1b} show marked condensation at all pressures, as shown in Fig. 1(a) for a subphase with 50% glycerol.

The effect of glycerol on the surface potential per unit of molecular density ($\Delta V/n$; cf. Maggio *et al.*, 1978) is complex and depends on the concentration of glycerol and the polar head group of the GSLs (Fig. 1b). A decrease of $\Delta V/n$ at molecular areas below 0.45 nm² (45 Å²)/molecule is found for GalCer on subphases containing 10% (v/v) glycerol, and an increase of concentration does not induce further effects. Sulf and G_{M1} show an increase of $\Delta V/n$ at all molecular areas on subphases with 10 or 20% (v/v) glycerol, and on increasing the concentration to 50%, either a decrease (Sulf) or values similar to those for NaCl (G_{M1}) are obtained. A marked reduction of $\Delta V/n$ for G_{D1a} and G_{T1b} is observed with 50% glycerol (Fig. 1b), and no change is present at lower concentrations.

Table 1 shows that the increase of free energy of compression (ΔF_c) with temperature for GalCer is less in the presence of 50% glycerol than on NaCl. Thus the entropy of compression (ΔS_c) of GalCer is less negative on subphases containing glycerol. On the other hand, ΔF_c of G_{D1a} on NaCl decreases with temperature and S_c is positive (Fidelio *et al.*, 1986a); glycerol has a marked effect on this, and it changes the slope of the ΔF_c –temperature curve, leading to a negative ΔS_c (Table 1).

DISCUSSION

The effect of the water environment and hydrophilic solutes on the lipid organization were interpreted as increased solute penetration into the interface (Cadenhead & Bean, 1972), variations in the long-range order of the bulk-phase water affecting the interactions in the lipid polar-head-group region (Maggio *et al.*, 1976; Maggio & Lucy, 1978), and direct solute–lipid–polar-head-group interactions (Crowe *et al.*, 1984; Johnston *et al.*, 1984; Rudolph *et al.*, 1986). Several of these conclusions may be questionable, owing to the presence

Table 1. Thermodynamic parameters of compression of GalCer and G_{D1a}

The apparent free energy of compression (ΔF_c) at different temperatures was calculated by graphical integration of the surface-pressure–area isotherms between the value of initial molecular area taken at 2 mN/m and the limiting molecular area. The entropy of compression was obtained from the slope of the ΔF_c –temperature curve (Fidelio *et al.*, 1986b). Abbreviation: e.u., entropy units (cal·K⁻¹·mol⁻¹); note that 1 cal = 4.184 J (S.I. units).

Compound	$\Delta F_{c,10^\circ\text{C}}$ (kcal/mol)	$\Delta F_{c,20^\circ\text{C}}$ (kcal/mol)	$\Delta F_{c,35^\circ\text{C}}$ (kcal/mol)	ΔS_c (e.u.)
GalCer				
NaCl (145 mM)	0.30	0.33	0.64	–14.2
+ Glycerol (50%, v/v)	0.74	0.76	0.87	–5.2
G_{D1a}				
NaCl (145 mM)	2.09	2.04	1.87	+9.0
+ Glycerol (50%, v/v)	1.35	1.77	1.95	–20.0

of surface-active impurities in some of the solutes (Arnett *et al.*, 1986). However, the present work shows that glycerol solutions (free of surfactants) modify the interfacial properties of GSLs.

If solute molecules only alter the possibilities for orientation of the oligosaccharide chain with respect to the interface according to pressure (Maggio *et al.*, 1985), both the magnitude and the variation of $\Delta V/n$ with the molecular area should change. In addition, the values of $\Delta V/n$ for GalCer, Sulf and G_{M1} with the highest concentration of glycerol should converge to those obtained from glycerol-free subphases at the closest packing, because their limiting molecular areas are not affected by glycerol. This is not observed. For GalCer, Sulf, and G_{M1} , glycerol (5–50 %, v/v) mostly affects the magnitude of molecular dipoles, but its variation with the intermolecular packing is similar to that on NaCl. This is also the case for G_{D1a} and G_{T1b} over 10 or 20 % (v/v) glycerol, and at 50 % glycerol the magnitude, the dependence of $\Delta V/n$ with the molecular area and the limiting molecular area itself are modified. These changes of intermolecular organization may result from a modification of the composition and structural properties of the solvation shell of the GSLs.

About 10 water molecules per lipid molecule are strongly perturbed by the polar head group of GalCer; for gangliosides this reaches about 40–60 water molecules per lipid (Curatolo *et al.*, 1977; Bach *et al.*, 1982). The hydration shell is considerably greater than this if water in secondary layers is taken into account (Crowe & Crowe, 1984). Glycerol exhibits a large difference among its partial molar volume in aqueous solutions and its van der Waals volume; this is an indication of a marked perturbation of the long-range order of the water phase (Back *et al.*, 1979). Glycerol and other polyols can compete with, and for, water in the polar-head-group region. This affects the hydration of phospholipids and cerebroside with a consequence on their phase behaviour (McDaniel *et al.*, 1983; Curatolo, 1985).

Solutes capable of replacing water hydrogen-bonded to lipid polar head groups should lead to entropically favourable changes (Johnston *et al.*, 1985). It was previously shown that the two-dimensional ordering of monolayers of the more complex GSLs on 145 mM-NaCl occurs with a favourable entropy change. This suggests that water molecules are released by coalescence of the large hydration shell of the oligosaccharide chain when these lipids become closely packed (Fidelio *et al.*, 1986a). When the GSLs contain a small and relatively less hydrated polar head group, such as in GalCer, the entropy of compression on NaCl is negative (Table 1). This may be due to the unfavourable increase of interfacial order of the lipid not compensated for by release of enough subphase molecules from the polar-head-group region. On subphases containing glycerol, expansion occurs at low pressures, and the entropy of compression is less negative. This suggests that a greater amount of subphase molecules may associate with the polar head group in the liquid-expanded state and be released from the solvation shell at higher pressures. On the other hand, with a large and strongly hydrated oligosaccharide chain, as in G_{D1a} , the entropy of compression on NaCl is positive, suggesting that, as the lipid becomes more ordered, a considerable amount of solvent may be released into the bulk phase and this more than compensates for the unfavourable variation

of the lipid entropy. In the presence of glycerol the solvation shell is probably reduced in size, as indicated by the smaller areas per molecule at all pressures. A release of fewer subphase molecules from the interface when the intermolecular packing is increased may explain why the entropy of compression becomes negative. All these results suggest that the existence of a considerable amount of water ordered around the oligosaccharide chains is not thermodynamically convenient compared with maintaining water in a relatively more disordered bulk phase when glycerol is present. In summary, a subtle thermodynamic and structural balance in the water milieu influences the intermolecular organization and surface potential of interfaces containing GSLs; this depends on the type of lipid oligosaccharide chain extending into the aqueous phase and may therefore be important for modulating cell-surface phenomena (Maggio *et al.*, 1981, 1986).

This work was supported in part by CONICOR and CONICET, Argentina. B.M. and G.D.F. are Career Investigators and I.D.B. is a Fellow of the latter institution.

REFERENCES

- Arnett, E. A., Harvey, N., Johnson, E. A., Johnston, D. S. & Chapman, D. (1986) *Biochemistry* **23**, 6912–6919
- Bach, D., Sela, B. & Miller, I. R. (1982) *Chem. Phys. Lipids* **31**, 381–384
- Back, J. F., Oakenfull, D. & Smith, M. B. (1979) *Biochemistry* **18**, 5191–5196
- Boni, L. T., Stewart, T. P., Alderfer, J. L. & Hui, S. W. (1981) *J. Membr. Biol.* **62**, 65–70
- Cadenhead, D. A. & Bean, K. E. (1972) *Biochim. Biophys. Acta* **290**, 43–50
- Cantu, L., Corti, M., Sonnino, S. & Tettamanti, G. (1986) *Chem. Phys. Lipids* **41**, 315–328
- Crowe, J. H. & Crowe, L. M. (1984) in *Biological Membranes* (Chapman, D., ed.), vol. 5, pp. 57–103, Academic Press, London
- Crowe, J. H., Crowe, L. M. & Chapman, D. (1984) *Arch. Biochem. Biophys.* **232**, 400–407
- Curatolo, W. (1985) *Biochim. Biophys. Acta* **817**, 134–138
- Curatolo, W., Small, D. M. & Shipley, G. G. (1977) *Biochim. Biophys. Acta* **468**, 11–20
- Curatolo, W. & Neuringer, L. J. (1986) *J. Biol. Chem.* **261**, 17177–17182
- Fidelio, G. D., Maggio, B. & Cumar, F. A. (1984) *Chem. Phys. Lipids* **35**, 231–245
- Fidelio, G. D., Maggio, B. & Cumar, F. A. (1986a) *Biochim. Biophys. Acta* **854**, 231–239
- Fidelio, G. D., Maggio, B. & Cumar, F. A. (1986b) *Biochim. Biophys. Acta* **862**, 49–56
- Gershfeld, N. L. (1974) in *Methods in Membrane Biology* (Korn, E. D., ed.), pp. 69–104, Plenum Press, New York
- Hilbig, R. & Rahmann, H. (1986) in *Gangliosides and Modulation of Neuronal Functions* (Rahmann, H., ed.), NATO ASI Series (H: Cell Biology), vol. 7, pp. 333–350, Springer-Verlag, Berlin
- Johnston, D. S., Coppard, E., Valencia, Parera, G. & Chapman, D. (1984) *Biochemistry* **23**, 6912–6919
- Johnston, D. S., Coppard, E. & Chapman, D. (1985) *Biochim. Biophys. Acta* **815**, 325–333

- Maggio, B. (1985) *Biochim. Biophys. Acta* **815**, 245–258
- Maggio, B. & Lucy, J. A. (1978) *FEBS Lett.* **94**, 301–304
- Maggio, B., Ahkong, Q. F. & Lucy, J. A. (1976) *Biochem. J.* **158**, 647–650
- Maggio, B., Cumar, F. A. & Caputto, R. (1978) *Biochem. J.* **171**, 559–565
- Maggio, B., Cumar, F. A. & Caputto, R. (1981) *Biochim. Biophys. Acta* **650**, 69–87
- Maggio, B., Ariga, T. & Yu, R. K. (1985) *Arch. Biochem. Biophys.* **241**, 14–21
- Maggio, B., Fidelio, G. D., Cumar, F. A. & Yu, R. K. (1986) *Chem. Phys. Lipids* **42**, 49–63
- McDaniel, R. V., McIntosh, T. J. & Simon, S. A. (1983) *Biochim. Biophys. Acta* **73**, 97–108
- McDaniel, R. V., Sharp, K., Brooks, D., McLaughlin, A. C., Winski, A. P., Cafiso, D. & McLaughlin, S. (1986) *Biophys. J.* **49**, 741–752
- Montich, G. G., Bustos, M. M., Maggio, B. & Cumar, F. A. (1985) *Chem. Phys. Lipids* **38**, 319–326
- Rudolph, A. S., Crowe, J. H. & Crowe, L. M. (1986) *Arch. Biochem. Biophys.* **245**, 134–143

Received 9 October 1987/8 January 1988; accepted 2 February 1988