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Geometric and thermodynamic restrictions for the self-assembly of glycosphingolipid-phospholipid systems

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The thermodynamic and geometrical features of possible self-assembled structures of a series of chemically related glycosphingolipids differing in the complexity of their polar headgroup, and of their mixture with phospholipids, have been predicted according to the theory of self-assembly of hydrocarbon amphiphiles of Israelachvili et al. ((1980) Q. Rev. Biophys. 13, 340–357). The type and number of carbohydrate residues in the oligosaccharide chain of the polar headgroup are of paramount importance to determine the characteristics and thermodynamic stability of the possible self-assembled structure. In single component systems, the general prediction of the theory is that smaller aggregates may form as the polar headgroup of the glycosphingolipid is more complex and as the lateral surface pressure is smaller. In noninteracting two-component glycosphingolipid-phospholipid systems, the thermodynamic stability and the overall geometry of the possible aggregate appear to be determined by the proportion and type of glycosphingolipid present. Large and abrupt changes of the possible free energy per molecule, radius of curvature, and predicted asymmetry ratio for a particular glycosphingolipid may be triggered by relatively small changes of the molecular parameters, lipid composition, lateral surface pressure or vice-versa. If intermolecular interactions are taken into account with respect to the predictions for an ideal, noninteracting system, the theory indicates that two-component bilayer vesicles of polysialoganglioside-phosphatidylcholine may be thermodynamically and geometrically more stable. On the other hand, for systems constituted by phosphatidylcholine and neutral glycosphingolipids or monosialogangliosides, the possible bilayer vesicle is predicted to be less stable than in the ideal, noninteracting case. The results emphasize the general validity of the theory as applied to glycosphingolipid-containing systems.

Abbreviations: Cer, ceramide (*N*-acylsphingoid); GalCer, Galβ1 → 1Cer; Sulphatide, Gal(3-SO_3^-)β1 → 1Cer; GlcCer, Glcβ1 → 1Cer; LacCer, Galβ1 → 4Glcβ1 → 1Cer; Gg_3Cer, GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer; Gg_4Cer, Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer; G_{M_3}, NeuGcα2 → 3Galβ1 → 4Glcβ1 → 1Cer; G_{D_3}, NeuAcα2 → 8NeuAcα2 → 3Galβ1 → 4Glcβ1 → 1Cer; G_{M_2}, GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; G_{M_1}, Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; $G_{D_{1a}}$, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; G_{T_1}, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; G_{T_1}, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 3Gal(3 ← 2αNeuAc8 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer. Abbreviations are those recommended by IUPAC-IUB [4] for neutral glycosphingolipids and by Svennerholm [27] for gangliosides.

Introduction

A theory on the possibility of self-assembly of lipids into micelles or bilayer vesicles according to geometric and thermodynamic constraints has been developed during the past years [1–3]. This theory has been succesfully applied to the description of three-dimensional aggregates formed by one type of phospholipid or by two-component systems including lysophosphatidylcholine and cholesterol [2]. It has been shown that the type of self-assembled structure allowed for a particular type of lipid molecule or mixture of lipids results from finely tuned competing effects of interaction free energies, entropy and geometric intermolecular packing constraints.

In spite of its importance, this theory has not yet been widely applied to different lipid systems. This is probably because a possible limitation of its general applicability relies in the necessary requirement for a precise knowledge of several molecular parameters such as hydrocarbon volume, average length of the hydrocarbon portion and polar headgroup, and molecular area of a component at a specified value of interfacial free energy. Until recently, these data have only been available for some phospholipids and cholesterol.

Several molecular parameters have now been described in a systematic manner for a series of closely related glycosphingolipids in which the hydrocarbon portion is, on average, very similar; these lipids differ in the complexity of the oligosaccharide chain present in their polar headgroup in terms of number and type of carbohydrate residues present [4,5]. Glycosphingolipids are important constituents of nerve membranes and some of them are thought to participate in membrane instability processes leading to alterations of the normal metabolism and structure of myelin [5,6] and to neurotransmitter movements in nerve endings [7,8]. In view of the possible importance of their intermolecular organization to determine the stability of a biological membrane [5,9,10], an understanding and possible theoretical prediction of the molecular properties and constraints influencing the self-assembly in aqueous media of lipid structures containing glycosphingolipids is highly desirable.

In this work, I will make use of the molecular data previously obtained for several glycosphingolipids [4,11,13] and apply the criteria of the theory of self-assembly of hydrocarbon amphiphiles [3]. A specific aim is to investigate how the complexity of the polar headgroup of glycosphingolipids can influence energetic and geometric constraints that may affect the possibility of existence of a particular aggregate. Similarly, assuming no intermolecular interactions in mixed systems with phospholipids, it will be theoretically explored how the proportion of glycosphingolipid can influence the geometry and occurrence of a mixed aggregate. Several molecular parameters for a limited number of interacting glycosphingolipid/phospholipid mixtures have become available during the past years [11]. For these systems, the results obtained assuming no intermolecular interactions will be compared to the cases where interactions between molecules are taken into account. The results of this analysis can provide a useful framework within which several theoretical possibilities and new insights may be experimentally imagined and tested.

I. Calculation of parameters for self-assembly

The equations and terminology used for the several parameters necessary to describe the self-assembled structures have been developed and described in detail by Israelachvili and co-authors [1–3]. The relationships used in this work are briefly summarized below.

(a) Single-component systems

The minimum free energy per molecule in the self-aggregated structure is $\mu_N^0 = 2\gamma a_0$ where γ is the interfacial free energy and a_0 is the optimal area per molecule. If an amphipathic molecule can pack into a variety of structures for which the molecular surface area can remain close to a_0, entropy will favour the structure with the smallest aggregation number N. Packing constraints due to molecular shape will determine, in turn, which structure is possible so that the molecular area leads to the lowest possible μ_N^0 while keeping the lowest N at the same time [1]. The critical packing condition (Pc) for micelles of different shapes (spherical, elipsoidal of different eccentricities, or cylindrical), bilayer vesicles, or planar bilayers

formed by a molecule of area a_0, volume v and maximum hydrocarbon length l_c is $Pc = v/a_0 l_c$. This parameter may take the following values:

$0.00 < Pc \leqslant 0.33$ for spherical micelles

$0.33 \leqslant Pc \leqslant 0.44$ for elipsoidal micelles of different eccentricities

$0.44 \leqslant Pc \leqslant 0.50$ for cylindrical micelles

$0.50 \leqslant Pc \leqslant 1.00$ for bilayer vesicles

$Pc \leqslant 1.00$ for planar bilayers

The theory predicts that inverted micelles or hexagonal II phases may be possible for values of $Pc > 1.00$ but it has not yet been developed to allow calculations of the geometrical features of these structures [3].

The critical packing condition Pc determines which structure is possible on the basis of the molecular parameters. According to its value, the radius of curvature, aggregation number and minimum free energy per molecule in the aggregate are subsequently calculated as indicated by the theory [1–3] for each allowed shape.

(b) Two-component vesicles

The basic equation for attaining the condition of minimum free energy in a two-component vesicle [2] is:

$$X_0 = F + \left[\frac{\gamma(1-F)F}{R_0 kT}\right]\left\{[Fa_A + (1-F)a_B](D_A - D_B) + \left[\frac{R_i}{R_0}\right]^2 [FD_A + (1-F)D_B]\left(\frac{a_A}{R_A} - \frac{a_B}{R_B}\right)t\right\} \quad (1)$$

with

$$X_i = F - \left[\frac{R_0}{R_i}\right]^2 (X_0 - F) \quad (2)$$

subject to the geometric packing constraints described by:

$$4\pi R_0^2 = Nf[X_0 a_A + (1-X_0)a_B] = Nf\bar{a} \quad (3)$$

$$4\pi R_i^2 = N(1-f)[X_i a_A + (1-X_i)a_B] \quad (4)$$

$$\tfrac{4}{3}\pi(R_0^3 - R_i^3) = N[Fv_A + (1-F)v_B] \quad (5)$$

$$\tfrac{4}{3}\pi\left[R_0^3 - (R_0 - l_c)^3\right] = Nf[X_0 v_A + (1-X_0)v_B] = Nf\bar{v} \quad (6)$$

X_0 and X_i are the molar fraction of lipid A in the outer and inner monolayer, respectively; F is the molar fraction of lipid A in the system; N is the total number of lipids per vesicle or aggregation number; f is the fraction of total lipid in the outer monolayer; R_0 and R_i are the outer and inner radii of curvature in the mixed vesicle; $t = R_0 - R_i$ is the hydrocarbon bilayer thickness; a_A, a_B, v_A, v_B, D_A, D_B are the molecular area, hydrocarbon portion volume and polar head-group length of lipid A and B, respectively; l_c is the average maximum hydrocarbon length; R_A and R_B are the outer radius of curvature of vesicles constituted by the pure component A or B; $\bar{a}$ and $\bar{v}$ are defined by $\bar{a} = X_0 a_A + (1 - X_0)a_B$ and $\bar{v} = X_0 v_A + (1 - X_0)v_B$ and constitute the mean molecular area and volume of a two-component system following an ideal non-interacting behaviour in which the individual molecular parameters are not modified [2]. The above relations constitute a set of simultaneous non-linear equations for X_0, R_0, R_i, N, f and X_i and are solved by iteration from an initial guess $X_0 = F$ [2]. The iterative procedure has been carried out to a convergence of less than $1/10^5$ with a MS-51 microcomputer (Microsistemas S.A., Córdoba, Argentina). Once the six above variables are known, these are introduced into the following system of four linear equations for the number of molecules of lipid A and B in the outer (A_0, B_0) or inner (A_i, B_i) monolayer of the two-component vesicle:

$$4\pi R_0^2 = (A_0 + B_0)[X_0 a_A + (1-X_0)a_B] \quad (7)$$

$$\frac{A_0}{X_0} = \frac{B_0}{(1-X_0)} = A_0 + B_0 \quad (8)$$

$$4\pi R_i^2 = (A_i + B_i)[X_i a_A + (1-X_i)a_B] \quad (9)$$

$$N = A_0 + B_0 + A_i + B_i \quad (10)$$

from which A_0, B_0, A_i, B_i and their ratio A_0/B_0, B_0/B_i that provide information on the asymmetric distribution of compounds in the two-component vesicle are readily obtained.

II. Description of molecular parameters

The average optimal volume and hydrocarbon length are calculated [1,12] as:

$$v \cong (27.4 + 26.9n)n_{ch}$$

and

$$l_c \cong 0.8(1.5 + 1.265\ n) - (0.9\ n_{db})$$

The polar headgroup length was calculated on the basis of the orientation adopted by the oligosaccharide chain in the aqueous phase [4,5] and molecular model building [5,13]. Considering that, on a planar projection, the diameter of a pyranose ring measures about 4 Å, the length of the oligosaccharide chain was found to approximately follow the empirical relation $D = (4\ n_h) \cdot 0.75$ for neutral glycosphingolipids and $D = (4\ n_h) \cdot 0.68$ for gangliosides; the factor 0.75 and 0.68 allow for a shortening of the polar headgroup due to a greater conformational freedom as it becomes longer [5].

In the above relations n, n_{db}, n_{ch} and n_h are the number of methylene groups, number of double bonds in each hydrocarbon chain, the number of chains, and the number of carbohydrate residues in the molecule, respectively.

The molecular areas vary with the interfacial free energy and have been determined previously for the different glycosphingolipids from surface pressure-area isotherms obtained with monolayers at the air-NaCl interface [4,5]. When intramolecular interactions are considered, the values assigned to $\bar{a}$ and $\bar{v}$ in the above equations correspond to the actual mean molecular area and volume at a specified value of surface pressure and system's composition as directly determined in mixed glycosphingolipid-phosphatidylcholine monolayers [11].

Results and Discussion

(a) Single-component systems

The type and geometrical features of the self-aggregated structure depend strongly on the type of oligosaccharide chain present in the polar headgroup of the glycosphingolipid. Table I summarizes the values obtained for Pc and the possibility for adopting a particular structure according to the values of the interfacial free energy.

Neutral glycosphingolipids. Ceramide, with its very small polar headgroup constituted only by the -OH group of the sphingosine moiety leads to values of Pc > 1.00 (Table I) below an interfacial free energy of 52 $mN \cdot m^{-1}$ (i.e., above a surface lateral pressure of 20 $mN \cdot m^{-1}$); the probable preferred self-aggregated state would be that of inverted micelles [3]. Only with fully extended highly crystalline hydrocarbon chains and at values of surface free energy above 42 $mN \cdot m^{-1}$ the critical packing parameter Pc is near unity. This would allow packing into planar bilayers or large vesicles with high radius of curvature and entropically unfavourable aggregation numbers exceeding 400 000 molecules. The theory, therefore, predicts that it is unlikely that ceramide (like cholesterol) will disperse by its own and form stable organized structures in aqueous media.

According to the molecular parameters [4,9], the theory predicts that GalCer, GlcCer and Sulphatide may form bilayer vesicles if the lateral surface pressure is between 5 and 20 $mN \cdot m^{-1}$ (i.e., for interfacial free energies between 67 and 52 $mN \cdot m^{-1}$). The outer vesicular radius of curvature for these vesicles range between 400 and 3000 Å, the aggregation numbers between $1 \cdot 10^4$ and $5 \cdot 10^6$, bilayer thickness between 38 and 57 Å and average free energy per molecule in the aggregate between 6 and 10 kcal/mol. Even if the structures predicted at lateral surface pressures below 20 $mN \cdot m^{-1}$ are theoretically possible, the very high aggregation numbers obtained suggest that these become unlikely on entropic terms. At surface pressures above 20 $mM \cdot m^{-1}$ (i.e., interfacial free energies below about 52 $mN \cdot m^{-1}$) the critical packing parameter Pc for GalCer, GlcCer and Sulphatide takes values of 1.00, allowing for entropically unfavourable planar bilayers or very large vesicles with extremely high aggregation numbers. At higher lateral surface pressure, values greater than unity can occur (see Table I) indicating the possibility for other structures such as inverted micelles whose geometry will depend on the water content of the system [3,14]. Lamellar, cubic, hexagonal and micellar phases have been described for this type of lipids which exhibit a complex polymorphic behaviour in aqueous media, with presence of metastable phases that depend on the degree of hydration [14–16,31].

As the number of neutral carbohydrate residues in the polar headgroup increases, the critical packing parameter Pc gradually shifts to values between 0.50 and 1.00 allowing for vesicular struc-

TABLE I

CRITICAL PACKING PARAMETER (Pc) FOR GLYCOSPHINGOLIPIDS AT DIFFERENT SURFACE PRESSURES

Values in brackets at the top of each column correspond to interfacial free energies taking the value of 72 $mN \cdot m^{-1}$ for a lipid-free air/NaCl 145 mM interface. b: bilayer vesicle; cylindrical micelle; e: elipsoidal micelle; i: inverted micelle or other not well defined structure; s: spherical micelle. *: theoretically, the value for Pc allows for a bilayer vesicle but the structure is not possible since the length of the polar headgroup exceeds the value for the inner vesicular radius. The next favoured structure corresponds to a cylindrical micelle and it was labelled as such.

Lipid	Lateral surface pressure ($mN \cdot m^{-1}$)			
	5 (67)	10 (62)	20 (52)	30 (42)
Dipalmitoylphosphatidylcholine	0.63 b	0.86 b	0.96 b	0.96 b
Cer	0.97 b	0.98 b	0.98 b	> 1.00 i
GalCer	0.94 b	0.98 b	0.99 b	⩾ 1.00 i
GlcCer	0.88 b	0.96 b	0.99 b	⩾ 1.00 i
LacCer	0.76 b	0.83 b	0.91 b	0.96 b
Gg_3Cer	0.61 b	0.71 b	0.87 b	0.97 b
Gg_4Cer	0.63 b	0.74 b	0.85 b	0.95 b
Sulphatide, pH 5.6	0.82 b	0.95 b	0.98 b	⩾ 1.00 i
G_{M_3}, pH 5.6	0.42 e	0.48 c	0.54 c*	0.63 c*
G_{M_2}, pH 5.6	0.45 c	0.49 c	0.55 c*	0.62 c*
G_{M_1}, pH 5.6	0.42 e	0.46 c	0.49 c	0.61 c*
$G_{D_{1a}}$, pH 5.6	0.29 s	0.34 e	0.40 e	0.46 e
G_{D_3}, pH 5.6	0.28 s	0.32 s	0.39 e	0.49 e
G_{T_1}, pH 5.6	0.20 s	0.24 s	0.29 s	0.33 e
Sulphatide, pH 1.2	0.81 b	0.99 b	0.99 b	⩾ 1.00 i
G_{M_3}, pH 1.2	0.62 b	0.72 b	0.92 b	0.98 b
G_{M_2}, pH 1.2	0.54 c*	0.61 b	0.76 b	0.87 b
G_{M_1}, pH 1.2	0.54 c*	0.63 b	0.70 b	0.79 b
$G_{D_{1a}}$, pH 1.2	0.43 e	0.48 c	0.57 b	0.66 b
G_{D_3}, pH 1.2	0.61 c*	0.69 b	0.84 b	0.97 b
G_{T_1}, pH 1.2	0.43 e	0.49 c	0.61 b	0.76 b

tures. Their geometrical parameters are determined by the molecular area adopted which, in turn, depends on the lateral surface pressure [4,5,13].

Fig. 1 shows the dependence on the lateral pressure of the predicted external vesicular radius, bilayer thickness, free energy per molecule, aggregation number and outside/inside ratio for possible vesicles of LacCer and Gg_4Cer. It is clear that the predicted radius, bilayer thickness and aggregation number increase with the lateral pressure (i.e., as the interfacial free energy becomes lower). Conversely, the free energy per molecule in the aggregate and the vesicular asymmetry decrease, this being consistent with the adoption of a less strained, larger and thermodynamically more favoured vesicle at higher lateral surface pressures; the opposing factor to this is the entropically unfavourable abrupt increase of the aggregation number taking place between 20 and 30 $mN \cdot m^{-1}$. It can be seen in Fig. 1b that the molecules located at the inner monolayer appear as the more strained in smaller vesicles. This is due to the unfavourable increase imposed upon their molecular area with respect to the optimum average (cf. Ref. 1). The thermodynamic and geometric constraints, therefore, influence more the molecules located at the inner side of the bilayer vesicle than those in the outer monolayer.

It is likely that the two mutually opposing factors of energy and entropy may found a favourable balance at average pressures above

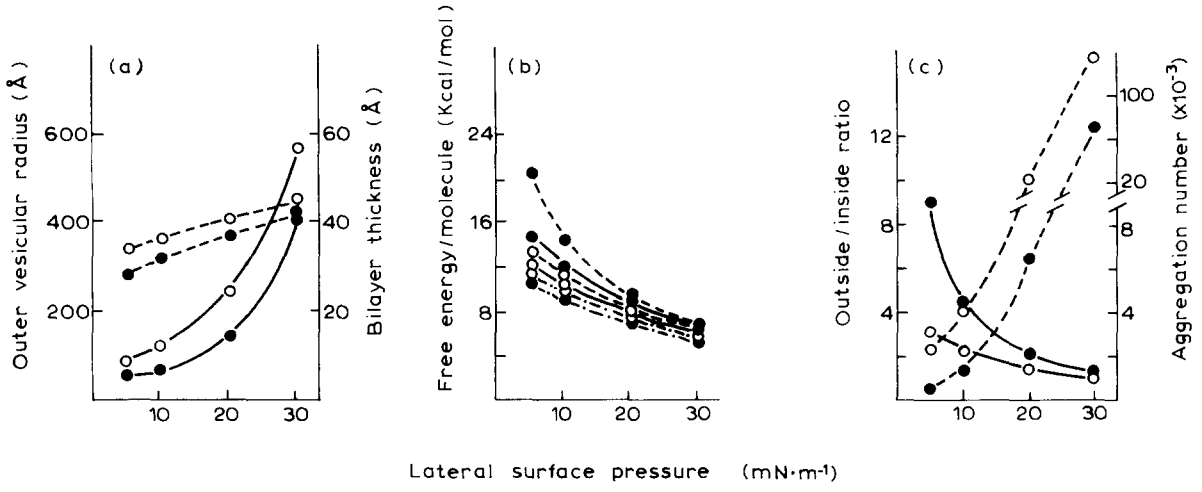

Fig. 1. Possible geometrical features of single-component vesicles of LacCer and Gg_4Cer according to the lateral surface pressure. (a) Outer vesiscular radius (———) and bilayer thickness (— — —) for LacCer (○) and Gg_4Cer (●). (b) Average free energy per molecule (———) and free energy per molecule in inner (— — —) and outer (· - · - ·) monolayer for LacCer (○) and Gg_4Cer (●). (c) Outside/inside distribution ratio (———) and aggregation number (— — —) for LacCer (○) and Gg_4Cer (●).

20–30 $mN \cdot m^{-1}$, a range of values for the lateral pressure that is of probable relevance for natural membranes [3,17,18]. However, it should not be disregarded that the lateral surface pressure actually exhibits large local fluctuations around the above mean values that can amount to more than ± 10 $mN \cdot m^{-1}$ (cf. Ref. 19). Throughout the present analysis, the values of lateral surface pressure at which the molecular areas are taken for calculating the geometrical and thermodynamic parameters have been arbitrarily chosen to represent expanded or closely packed arrangements in oriented interfaces. Therefore, the predictions on the thermodynamics and geometry of the possible structure at each value of surface pressure only apply to a theoretical case where the lateral surface pressure experienced by the molecules at the interface of the tri-dimensional aggregate remains constant at the value chosen. Since this is not the case for real systems, the lateral surface pressure can not be considered an independent variable. The radius of curvature, asymmetry ratio and free energy per molecule in a vesicle or micelle will influence the lateral surface pressure of the molecule at the interface and this, in turn, may bring about modifications of the initial values of molecular area. Therefore, while the results predicted represent possible shapes and thermodynamic features of self-aggregated structures locked at some fixed value of lateral surface pressure, the overall pattern of variation should be interpreted on the basis of dynamic variations of the surface pressure within the range of values analysed. Important biological consequences may derive from the fact that even small fluctuations of the lateral surface pressure brought about by dynamic phase changes [18] and intermolecular interactions involving reductions or expansions of the molecular area [5,11] may have large and amplified consequences on the local radius of curvature, bilayer thickness, free energy per molecule and lipid asymmetry (see also Fig. 7).

Fig. 2 summarizes the results obtained for the different glycosphingolipids. At surface pressures below 20 $mN \cdot m^{-1}$, as the polar headgroup becomes more complex, the predicted external radius of curvature and aggregation number (see also Fig. 1c) decrease; this leads to smaller vesicles with high asymmetry ratios (Fig. 2a) and free energy per molecule in the possible aggregate (Fig. 2b). At lateral surface pressures above 20 $mN \cdot m^{-1}$ the outer radius of allowed vesicles formed by glycosphingolipids with more than two carbohydrate residues in the oligosaccharide chain reaches the lowest values. However, the theory indicates that the vesicles are still large enough at these pressures so as not to develop a great geometrical strain and

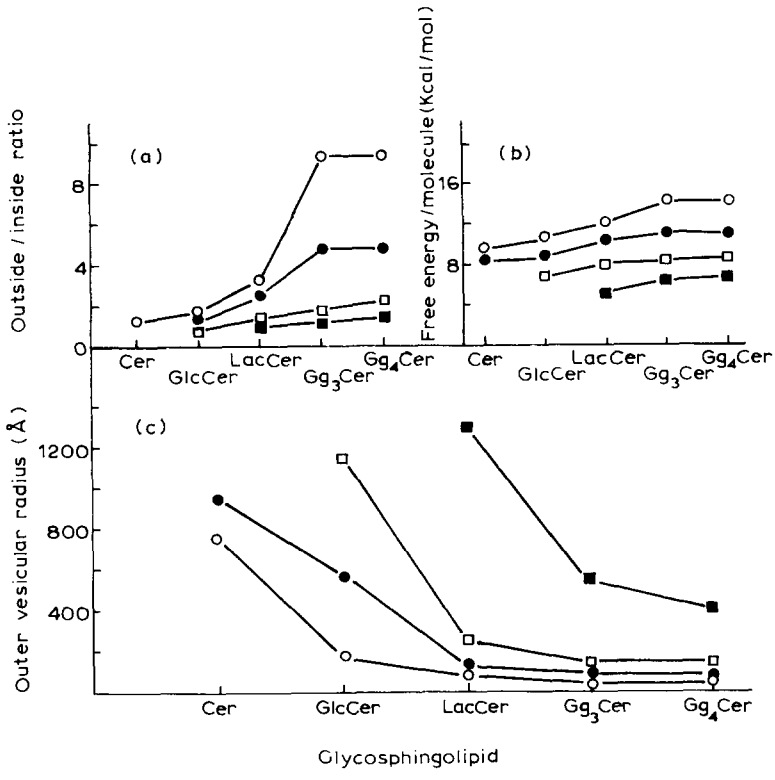

Fig. 2. Possible geometrical features of single-component vesicles of different neutral glycosphingolipids. (a) Outside/inside distribution ratio; (b) free energy per molecule and (c) outer vesicular radius are shown for the glycosphingolipids indicated at a lateral surface pressure of 5 (○), 10 (●), 20 (□) and 30 (■) $mN \cdot m^{-1}$.

the predicted asymmetry ratio, free energy per molecule and aggregation number remain low enough to allow for a presumably comfortable molecular arrangement.

Gangliosides. Except for gangliosides G_{M_3} and G_{M_2} for which the data predict that highly tensioned bilayer vesicles might eventually be formed only at lateral pressures well above 40 $mN \cdot m^{-1}$ and with fully stretched hydrocarbon chains, the theory indicates that the allowed structure for self-aggregated gangliosides at pH 5.6, correspond to the micellar type in its different variations (spherical, globular or elipsoidal, cylindrical, see Table I). The maximal radius of curvature are between 18 and 26 Å and typical aggregation numbers between 50 and 500, depending on the surface pressure. The values predicted are in excellent aggreement with the few experimental data available for gangliosides that indicate the presence of spherical and elipsoidal micelles with aggregation numbers between 120 and 225 for G_{M_1}, $G_{D_{1a}}$ and G_{T_1} [20]. At pH 1.2, the sialosyl residues are protonated and the electrostatic repulsion and unfavorable hydration layer effects between the polar headgroups are probably reduced; this leads to smaller areas [4] and increased intermolecular interaction energies for these lipids [5]. It is noteworthy that, in these conditions, and at lateral surface pressures of 20 $mN \cdot m^{-1}$ or above depending on the particular ganglioside, the theory predicts that these lipids may adopt a structural arrangement corresponding to bilayer vesicles (Table I). For these possible aggregates the geometrical parameters follow the general trend already found for neutral glycosphingolipids (Fig. 3).

(b) Two-component systems

In a two-component system, the competing factors of energy, entropy and curvature effects are operating as above. However, in this case, the molecules may distribute asymmetrically in the structure to allow for their different packing constraints and, at the same time, result in a structure with the lowest possible aggregation number. In consequence, two-component vesicles will, in general, be asymmetric if the geometrical packing of

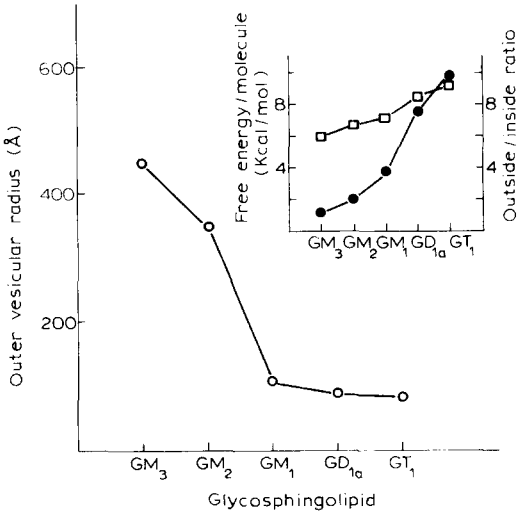

Fig. 3. Possible geometrical features of single-component vesicles of protonated gangliosides. The outer vesicular radius (○), free energy per molecule (inset, □) and outside/inside ratio (inset, ●) are shown for the different gangliosides at pH 1.2 at a lateral surface pressure of 30 $mN \cdot m^{-1}$.

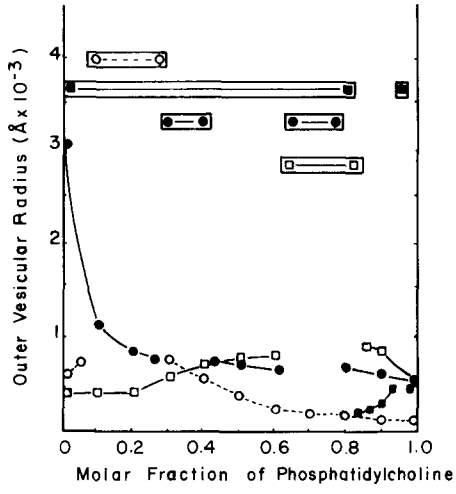

Fig. 4. Compositional dependence of the outer vesicular radius of two-component vesicles. The outer vesicular radius of two-component vesicles of dipalmitoylphosphatidylcholine and GlcCer at 10 $mN \cdot m^{-1}$ (○) or 20 $mN \cdot m^{-1}$ (●); Gg_4Cer (□) and G_{M_1} (■ at 30 $mN \cdot m^{-1}$ are shown for different molar fractions of the phospholipid. The horizontal bars indicate the regions of composition where the critical packing condition $Pc > 1.00$ and a vesicular structure is not possible for the systems containing the glycolipid defined by the symbol enclosed within the bar.

the individual molecules is different. The ultimate asymmetry is restricted by the unfavourable term of entropy demixing between the inner and outer monolayers of the resulting bilayer vesicle (cf. Ref. 3).

Non-interacting phosphatidylcholine-glycosphingolipid systems. This section describes the predicted geometrical parameters for self-assembled structures constituted by natural or synthetic phosphatidylcholines and glycosphingolipids in different molar fractions. First, an ideal non-interacting behaviour will be considered.

In these conditions, the individual molecular parameters are not modified by the presence of the other component. The calculation of the parameters for self-assembly for two component systems requires a previous knowledge of the parameters for the self-assembled single-component system. Therefore, the calculations are obviously not possible for cases in which the critical packing condition Pc for an individual component exceeds 1.00 (see Table I and section I).

Fig. 4 shows the predicted variation of the outer radius of a vesicle formed by dipalmitoylphosphatidylcholine (lipid A) and GlcCer, Gg_4Cer or G_{M_1} (lipid B) as the molar fraction of the lipids is theoretically varied and at two different values of surface pressure for GlcCer. The discontinuities of the lines in Fig. 4 represent particular regions of composition (highlighted by the corresponding horizontal bars in the upper part) where the combination of molecular parameters for the mixed system leads to values for $Pc > 1.00$ which is not compatible with the adoption of a vesicular structure. The general behaviour of systems containing egg phosphatidylcholine or other glycosphingolipids is similar, differences being the range of composition where a mixed structure is theoretically possible.

Some molecular parameters such as l_c and v are not experimentally measured in this work but calculated according to the approximate formulae given in Section II. These values are assumed to be fixed constants by the theory of Israelachvili and co-authors [1–3]. Therefore, the values in Fig. 4 only represent the variation of the radius of curvature with composition over regions in which a vesicular structure is predicted as possible ($Pc \leqslant 1.00$) for values of l_c and v assumed as invariant.

However, it is known that in real lipid systems the configuration and length of the hydrocarbon chains can change dynamically as a consequence of local interactions and lipid phase transitions that involve the formation of different types of rotational isomers [3]. Within the confines of the present theory, l_c can not be allowed to vary; however, it might be presumed that if a different value for l_c than that predicted by the formula in Section II was possible in a real vesicle, then a different dependence of the radius of curvature with composition than that shown in Fig. 4 would be obtained. Qualitatively, if in real vesicles l_c could vary either discontinuously or continuously (i.e. due to defined interconversions between *trans*, *gauche* and *cis* carbon-carbon bonds along the chain) and in a way that a vesicular structure could be maintained, then curves with different branches of maximum or minimum values for R_0 for particular lipid compositions might be generated. However, although conceivable, this would require a more elaborate theory and is not formally allowed in its present state so that no analysis of this possibility can be made.

It can be seen in Fig. 4 that the regions of composition for which a value of Pc compatible with a vesicular structure is obtained are different for systems containing glycosphingolipids of different complexities. This indicates that the establishment and the geometrical variation of these structures arise from constraints occurring within a multicomponent hypersurface containing several interdependent thermodynamic and molecular variables that can concomitantly lead to critical

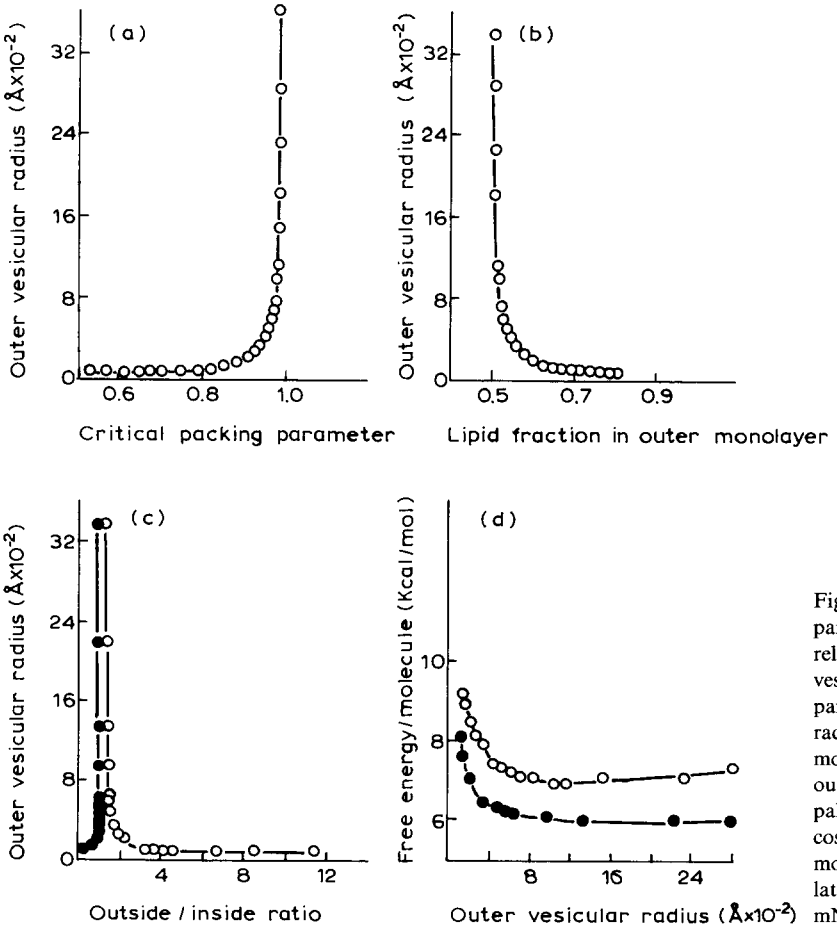

Fig. 5. Interdependence of geometrical parameters for two-component vesicles. Correlations between: (a) values for the outer vesicular radius and the critical packing parameter: (b) values of the outer vesicular radius and total lipid fraction in the outer monolayer; (c) outer vesicular radius and the outside/inside distribution ratio of dipalmitoylphosphatidylcholine (●) and the glycosphingolipid (○); (d) mean free energy per molecule and the outer vesicular radius at a lateral surface pressure of 20 (○) and 30 (●) $mN \cdot m^{-1}$.

values depending on the properties and proportions of each type of lipid in the system.

As can be seen in Fig. 5a, very small variations of Pc in the range 0.95–1.00 can bring about large and amplified variations of the vesicular radius of curvature. Also, the predicted asymmetry appears as fairly constant except for a narrow range of values at larger radius in which it rapidly tends to unity (Fig. 5c). At the same time, the total molar fraction of lipid in the outer monolayer (f) tends to 0.5 as expected for a more symmetrical vesicle (Fig. 5b). As expected, the free energy per molecule indicates that the smallest and more highly curved vesicles are predicted to be the more thermodynamically strained; a sharp variation of the molecular free energy is also predicted to occur at particular threshold values (below 400 Å) of the outer radius of vesicles formed by the different glycosphingolipid-phospholipid systems (Fig. 5d).

Similar to the case of single-component systems, as the polar headgroup of the glycosphingolipid becomes more complex the maximal possible outer radius of curvature of the two-component vesicle becomes smaller (Fig. 6). Also, the theory predicts that the geometric and thermodynamic constraints acting on the two-component vesicle will force the glycosphingolipid to be more favorably located at the outer monolayer, the more so the more complex or the more negatively charged oligosaccharide chain it contains in the polar headgroup (Fig. 6). This result is in excellent agreement with experimental data reporting on the location of these lipids in natural membranes [21,22].

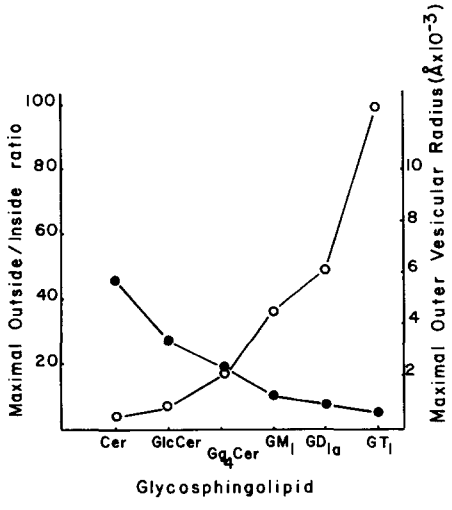

Fig. 6. Outer vesicular radius and possible distribution of different glycosphingolipids in two-component vesicles with dipalmitoylphosphatidylcholine. The outside/inside glycosphingolipid distribution (○) and outer vesicular radius (●) are maximal values obtained at a lateral surface pressure of: 20 mN·m^{-1} for Cer ($F = 0.2$) and GlcCer ($F = 0.4$); 30 mN·m^{-1} for Gg_4Cer ($F = 0.7$), G_{M_1} ($F = 0.93$), $G_{D_{1a}}$ ($F = 0.98$) and G_{T_1} ($F = 0.98$). F corresponds to the molar fraction of phospholipid (lipid A).

Interacting phosphatidylcholine-glycosphingolipid system. By systematic studies of the molecular requirements for interactions of some glycosphingolipids with phosphatidylcholine in mixed monolayers at the air/NaCl interface, we have previously shown that the intermolecular organization adopted depends on the type of oligosaccharide chain present in the polar head group of the glycosphingolipid [4,5,11]. On the basis of the intermolecular packing obtained after the interactions with phosphatidylcholine [11,13] two groups of glycosphingolipids could be distinguished: (1) neutral glycosphingolipids and monosialogangliosides which exhibit mean molecular areas larger than those theoretically expected for an ideal glycosphingolipid-phospholipid system and which occurred with thermodynamically unfavourable positive excess free energy of mixing; (2) polysialogangliosides that lead to systems showing thermodynamically favored interactions (negative excess free energy of mixing) and reductions of the mean molecular area with respect to the ideal system. Due to the changes of the mean molecular geometry brought about by these interactions with respect to the ideal state where no interactions are assumed, it was considered of interest to compare the results predicted by the theory in the two situations.

It can be seen in Table II that when intermolecular interactions are considered, the overall vesicular asymmetry predicted (N_0/N_i) is greater than for the case in which intermolecular interactions are ignored. Also, for neutral glycosphingolipids and monosialoganglioside G_{M_1}, the outer radius predicted for the mixed vesicle is smaller while the glycosphingolipid outside/inside ratio and average molecular free energy in the mixed aggregate are greater than in the ideal noninteracting case. This

TABLE II

TWO-COMPONENT SYSTEMS. COMPARISON OF GEOMETRICAL FEATURES FOR INTERACTING AND NON-INTERACTING SYSTEMS

The dipalmitoylphosphatidylcholine (DPPC) is taken as component A and the glycosphingolipid as component B. id.: ideal non-interacting mixture. Int.: interacting mixture. $^+$ values significantly greater ($P < 0.01$) for interacting systems compared to non-interacting systems. * values significantly lower ($P < 0.01$) for interacting systems compared to non-interacting sysems. The maximum S.E. for the different parameters were not above $\pm 3\%$ of the mean value. a: vesicles for ideal mixtures are not possible for phospholipid molar fractions below 0.85, the value 0.75 corresponds to the minimum possible value for the interacting system, the values for the ideal mixture correspond to a phospholipid mole fraction of 0.85. b: vesicles for ideal mixtures are not possible for phospholipid molar fractions below 0.92, the value 0.75 corresponds to the minimum possible value for the interacting system, the values for the ideal mixture correspond to a phospholipid mole fraction of 0.92. c: vesicles for ideal mixtures are not possible for phospholipid molar fraction below 0.98, the value 0.85 corresponds to the minimum possible value for the interacting system, the values for the ideal mixture correspond to a phospholipid mole fraction of 0.98.

System	Surface pressure ($mN \cdot m^{-1}$)	Phospholipid mole fraction (F)	Outer vesicular radius (Å)		Phospholipid asymmetry (A_0/A_i)		Glycosphingolipid asymmetry (B_0/B_i)		Outer/inner lipid ratio (N_0/N_i)		Molecular free energy (kcal/mol)	
			Id.	Int.	Id.	Int.	Id	Int.	Id.	Int.	Id	Int
GlcCer-DPPC	20	0.25	584	174 *	1.18	1.38 $^+$	1.17	1.36 $^+$	1.17	1.37 $^+$	6.61	6.81 $^+$
	20	0.50	817	269 *	1.11	1.24 $^+$	1.11	1.23 $^+$	1.11	1.23 $^+$	6.74	6.84 $^+$
	20	0.75	2527	322 *	1.03	1.22 $^+$	1.03	1.21 $^+$	1.00	1.22 $^+$	7.02	7.22 $^+$
Gg_4Cer-DPPC	30	0.25	560	282 *	1.04	1.24 $^+$	1.20	1.41 $^+$	1.16	1.30 $^+$	5.80	5.89 $^+$
	30	0.50	942	230 *	1.05	1.20 $^+$	1.14	1.62 $^+$	1.09	1.33 $^+$	5.62	5.79 $^+$
	30	0.75	3441	280 *	1.02	1.26 $^+$	1.04	1.56 $^+$	1.02	1.25 $^+$	5.60	5.69 $^+$
G_{M_1}-DPPC	30	0.75 [a]	114	92 *	1.21	1.29 $^+$	8.63	13.9 $^+$	1.71	1.87 $^+$	6.42	6.79 $^+$
$G_{D_{1a}}$-DPPC	30	0.75 [b]	189	1709 $^+$	1.24	1.31 $^+$	6.38	2.60 *	1.27	1.38 $^+$	7.45	7.22 *
G_{T_1}-DPPC	30	0.85 [c]	382	1086 $^+$	1.16	1.28 $^+$	2.82	1.84 *	1.18	1.37 $^+$	7.16	6.97 *

indicates that the actual arrangement predicted by the theory in the interacting case represents a more strained and less stable structure than that expected if there were no interactions. Conversely, for polysialoganglioside-phosphatidylcholine systems, larger vesicles with greater outer radius and smaller glycosphingolipid asymmetry and average free energy per molecule than in the non interacting case are predicted if interactions are taken into account. It is interesting that the differences in the average free energy per molecule predicted by the theory between both interacting and non-interacting situations are of a similar magnitude than the excess free energy of mixing experimentally obtained in lipid monolayers (i.e., 100–800 kcal/mol) [11].

In addition to the restrictions imposed by the equations described in Section Ib, the above results indicate that the actual experimental values for the mean molecular area, volume and the excess free energy of mixing should be taken into account for real glycosphingolipid-phospholipid systems. These values are related to the molecular parameters of the ideal non-interacting system as follows:

$$[a_m - \bar{a} = \Delta a]_\pi$$

$$[v_m - \bar{v} = \Delta v]_\pi$$

$$\Delta G_{xs} = \int_0^\pi [a_m - \bar{a}] d\pi$$

where $\bar{a}$ and $\bar{v}$ were defined in Section Ib and a_m, v_m and ΔG_{xs} are the experimentally determinable mean molecular area and volume at a particular value of the lateral surface pressure π, and the excess free energy of mixing (cf. Refs. 5, 11).

On the basis of the experimental molecular parameters resulting when intermolecular interactions are taken into account, the theory predicts that the system may accept a greater proportion of gangliosides while still existing in the form of vesicular aggregates than what appears to be theoretically possible if interactions are ignored (Table

II). This is also in general agreement with experiment since it has been reported that G_{M_1}, $G_{D_{1a}}$ and G_{T_1} can be incorporated to about 25%, 15% and 10%, respectively, into phospholipid vesicles [23]. By contrast, the maximal amount incorporated predicted by the theory for a non-interacting system is not above 15% for G_{M_1}, 8% for $G_{D_{1a}}$ and 2% for G_{T_1}.

The criteria of the theory of self-assembly of hydrocarbon amphiphiles of Israelachvili and coauthors [1–3] seems to provide plausible predictions for the self-assembly of phospholipid-glycosphingolipid systems in aqueous media. According to the data obtained, large and abrupt changes of the asymmetry ratio for a particular glycosphingolipid component, free energy per molecule and overall geometry in local areas of a complex lipid interface should be expected as a consequence of small changes of the radius of curvature or lateral surface pressure of the membrane. These could be triggered by transient fluctuations and local changes in composition established by variations of intermolecular forces and as a consequence of modifications of interactions and isothermal phase separations in local membrane domains [5,8,10,26]. Conversely, the theory indicates that a sligth modification of the molecular geometry, free energy per molecule or outside/inside glycosphingolipid ratio in a particular membrane region could have immediate and amplified consequence on the radius of curvature, local composition and overall geometry and stability of a glycosphingolipid-containing membrane. Fig. 7 illustrates in a schematic and oversimplified manner some of the dynamic shape changes that could be induced by a few of the above effects.

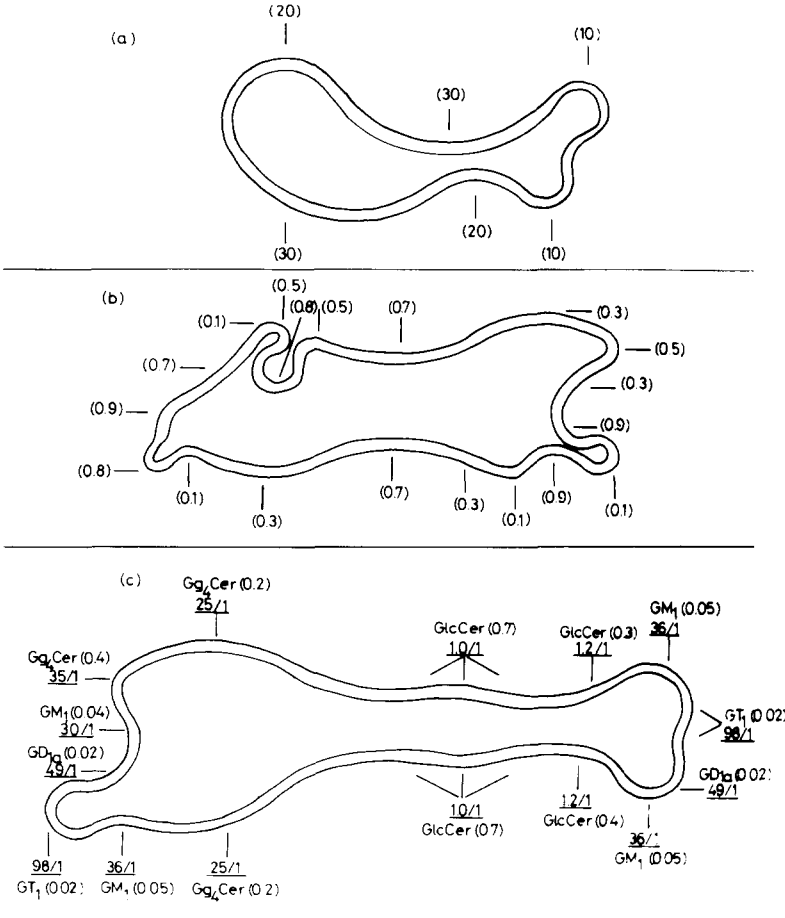

Fig. 7. Possible shape changes induced in bilayer vesicles by the combination of thermodynamic and geometric constraints. The modifications of the radius of curvature were induced by: (a) variations of the lateral surface pressure in allowed bilayer vesicles of LacCer; (b) variations of the molar fraction of GlcCer in allowed bilayer vesicles of a two-component system of GlcCer-dipalmitoylphosphatidylcholine at a constant lateral surface pressure of 20 $mN \cdot m^{-1}$; (c) variations of the molar fraction and type of glycosphingolipid in different domains of an allowed bilayer vesicle of a two-component system of the glycosphingolipid indicated with dipalmitoylphosphatidylcholine, at a constant lateral surface pressure of 20 $mN \cdot m^{-1}$. The numbers in brackets represent: (a) the local lateral surface pressure; (b) the local molar fraction of GlcCer; (c) the molar fraction of the glycolipid indicated in the local domain (underlined is the outside/inside distribution ratio of the glycosphingolipid indicated). The bilayer thickness was represented approximately to scale according to the data. The figure is highly schematic and intended to be only illustrative, by combination of the effects induced by the radius of curvature, lateral surface pressure and local composition multiple forms can be arbitrarily generated.

The theory of Israelachivili and co-authors [1–3] is not applicable to cases in which interactions between vesicles occur. However, if some of the different parameters that determine the stability of the membrane acquire critical values it may well occur that interactions or recombinations (i.e., fusion) of bilayers becomes possible or facilitated at certain regions of a deformable vesicle (as exemplified in the highly curved regions in Fig. 7b, c). In this, connection, some studies on the effect of bilayer cations in lipid monolayers in correlation with their effect on the surface free energy and possibilities for fusion of phospholipid vesicles of different radius of curvature have been made [28,29]. It has been proposed that an increase of the surface tension of 7.7 $mN \cdot m^{-1}$ and 9.5 $mN \cdot m^{-1}$ for small or large vesicles, respectively, can trigger membrane fusion processes in vesicles of phosphatidylserine [29]. This corresponds to a decrease of the lateral surface pressure from 27 $mN \cdot m^{-1}$ to 19.3 $mN \cdot m^{-1}$ or to 17.5 $mN \cdot m^{-1}$ for small (about 250 Å in diameter) or large (about 2000 Å or more in diameter) vesicles, respectively. The data in Fig. 1 for single-component vesicles indicate that if the lateral surface pressure decreases from about 30 $mN \cdot m^{-1}$ to about 15 $mN \cdot m^{-1}$ the radius of curvature is predicted to experience a considerable decrease and the expected free energy per molecule and asymmetry of the possible aggregate should increase. Similarly, for two-component vesicles, Fig. 5 predicts that when the expected radius of curvature shows values between 600–800 Å that would correspond to lateral surface pressures of about 15–20 $mM \cdot m^{-1}$, a critical situation may be reached. Further decreases of the surface pressure (i.e., increases of the surface tension) beyond these values lead to increasingly smaller vesicles with unfavourable increases of the free energy per molecule and asymmetry ratio of the two-component aggregate. The threshold for inducing highly tensioned vesicles is in the same range of surface pressures that correspond to the surface tension values previously proposed as critical limits for inducing spontaneous fusion and aggregation of bilayer vesicles [28,29]. Also, in this connection it has been shown that lipids capable of inducing natural membrane fusion such as polysialogangliosides [30] exhibit thermodynamically favored interactions with phosphatidylcholines characterized by increases of the surface tension of the mixed interface of 5–10 $mN \cdot m^{-1}$ in addition to that exptected if no interactions were present; the intermolecular arrangements established, in addition, occur with considerable decreases of the interfacial electrical potential [5,11]. Both effects would certainly facilitate or induce vesicle aggregation and fusion [5,28,29].

Apart from different types of interactions, additional complicating factors not taken into account by this analysis in its present state are the effects of intra- and intermolecular hydrogen bonding [14] and steric and hydration repulsive forces known to be important for these systems [24,25] that may influence the molecular parameters, mesomorphic state [31] and the type of self-aggregated glycosphingolipid-containing structure formed.

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