Ganglioside GD3 Lactones: Polar Head Group Mediated Control of the Intermolecular Organization[†]

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ABSTRACT: The individual properties and intermolecular organization of ganglioside GD3 and of two of its lactone forms (GD3Lactone I and GD3Lactone II) were studied in lipid monolayers. The formation of the first lactone ring in GD3Lactone I eliminates one negative charge and leads to a decrease of the molecular area at all surface pressures. The intermolecular dispersion energy and collapse pressure are higher in GD3Lactone I compared to those in the parent GD3. The surface potential per unit of molecular surface density and the resultant molecular dipole moment are increased in GD3Lactone I with respect to those in GD3 at comparable values of molecular area. In GD3Lactone I the molecular parameters suggest an oligosaccharide chain oriented similarly to that of GD3. On the average, this is perpendicular to the surface, and the resultant polar head-group dipole moment points away from the interface. In GD3Lactone II the negative charges are eliminated, resulting in considerably larger molecular areas than for GD3 and GD3Lactone I at all pressures. The intermolecular dispersion energy of GD3Lactone II is also greatly diminished and the collapse pressure is further increased compared to those of GD3Lactone I. However, the surface potential per unit molecular surface density and the resultant molecular dipole moment of GD3Lactone II are higher than in GD3 Lactone I at similar values of molecular areas. This is probably due to a positive polar head-group dipole moment contribution induced by the additional lactone ring in GD3Lactone II. These changes result from a distorted conformation of the oligosaccharide chain owing to the presence of fused carbohydrate rings which require a greater intermolecular spacing compared to GD3 and GD3Lactone I.

A variety of modulatory effects of gangliosides on the function of cell membranes have been reported. These lipids influence cellular plasticity during development, intercellular recognition, and receptor function (Svennerholm et al., 1980; Hakomori, 1981; Makita et al., 1982; Ledeen et al., 1988). Gangliosides modulate neurotransmitter release (Cumar et al., 1980, 1983), membrane protein phosphorylation (Goldenring et al., 1985; Yu, 1988), and phospholipase activity against organized interfaces (Bianco et al., 1989, 1990). At the molecular level, gangliosides and related glycosphingolipids induce dramatic modifications of the membrane organization and topology (Maggio et al., 1988a,b). Gangliosides modify the intermolecular packing, phase state, curvature, asymmetry, interfacial micropolarity, electrostatics, and free energy of the membrane. This depends on the type, number, and conformation or carbohydrate residues in the polar head group (Maggio et al., 1981, 1985a,b, 1986; MacLaughlin, 1989; Beitinger et al., 1989; Montich et al., 1985, 1988). Gangliosides also modulate the access to the lipid interface of toxins (Cumar et al., 1982), soluble and membrane proteins (Fidelio et al., 1982), and proteolytic and lipolytic enzymes (Bianco et al., 1989, 1990; Williams et al., 1986). Most of these effects and those induced in cells and subcellular fractions depend on the proportion of gangliosides in the system.

Ganglioside lactones occur naturally in the brain (Nores et al., 1987: Riboni et al., 1986). It has been suggested that interconversion of gangliosides between their acidic and lactonized forms may be important for the regulation of membrane function (Yu et al., 1985; Ando et al., 1983). This mechanism would not require major modifications of the composition of gangliosides but only their in situ transformation to different structures. The primary and secondary structures of the lactone form of GM3[1] and two lactones of GD3 have been precisely characterized by high-resolution proton NMR (Yu et al., 1985; Ando et al., 1989). The negative charges in the ganglioside oligosaccharide chain are eliminated or reduced in number in these lactones. Besides, the structure and conformation of the whole polar head group are markedly different, becoming highly rigid in the lactone structures (Yu et al., 1985; Ando et al., 1989). The recognition of the oligosaccharide moiety by monoclonal antibodies is modified after lactonization (Ando et al., 1989). The biological significance of these chemical alterations and their effects on the intermolecular organization of the membrane are not known. In the present investigation we studied comparatively the intermolecular packing and surface potential of GD3 and its lactone derivatives in monolayers.

MATERIALS AND METHODS

Ganglioside GD3 was isolated from human brain (Ariga et al., 1982) and further purified as reported before (Fidelio et al., 1982). The additional purification of the acidic form of gangliosides by treatment with mild alkaline conditions,

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[1] Abbreviations: GD3, $II^3(NeuAc)_2$-LacCer; GD3Lactone I or GD3LacI, GD3 with one lactone ring involving the external NeuAc (C) and the side chain of the internal NeuAc (A) (see Figure 1); GD3-Lactone II or GD3LacII, GD3 with two lactone rings involving, besides the lactone of GD3LacI, the internal NeuAc (A) and the galactose residue (II) (see Figure 1); GM3, II^3NeuAc-LacCer; GM1, II^3NeuAc-$GgOse_4$Cer; GD1a, IV^3NeuAc,II^3NeuAc-$GgOse_4$Cer.

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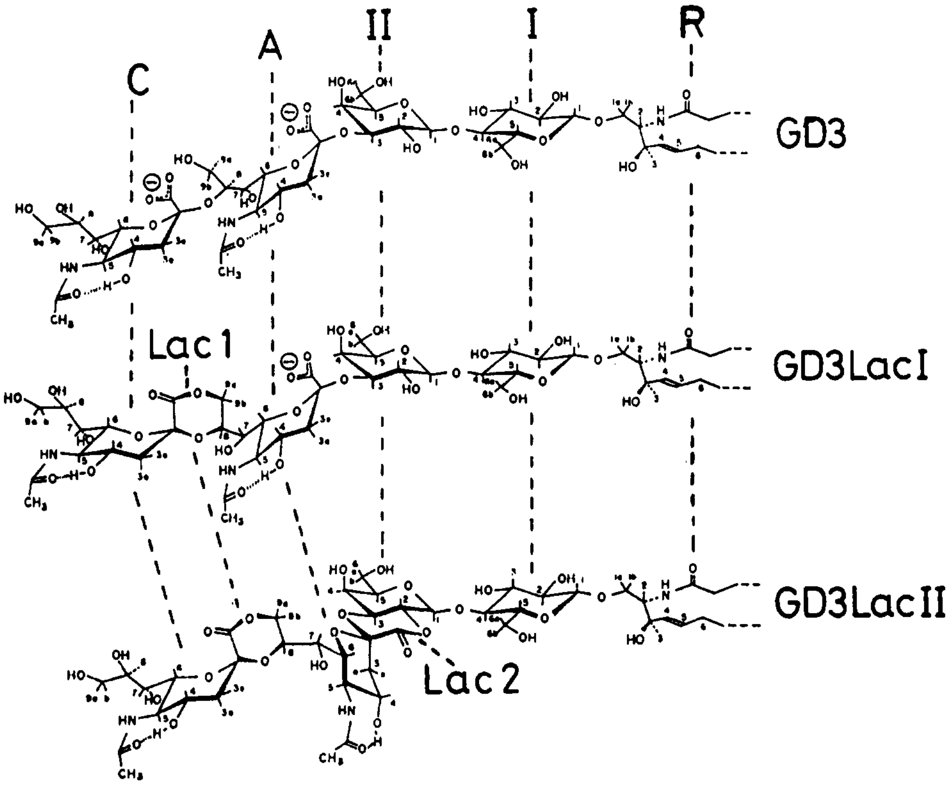

FIGURE 1: Chemcial structure of the oligosaccharide chain of GD3 and GD3 lactones as determined by high-resolution proton NMR. R, ceramide portion; I, glucosyl residue; II, galactosyl residue; A, inner sialosyl residue; C, external sialosyl residue.

dialysis, and rechromatography on DEAE-Sephadex are especially important in order to obtain the correct molecular parameters. This is due to the great sensitivity of the monolayer technique. Altered values for intermolecular packing and surface potential of gangliosides in monolayers can be obtained, depending on the method used for ganglioside purification, if the additional treatment is not performed (Fidelio et al., 1982). The preparation of GD3LacI and GD3LacII, their characterization, and the purity of the ganglioside preparations assessed by HPTLC and high-resolution proton NMR were described previously (Ando et al., 1989).

The method for measuring the surface potential and molecular areas at different surface pressures has been described in detail before (Maggio et al., 1978a). The surface compressibility and intermolecular interaction energy were calculated as previously reported (Maggio et al., 1981, 1985a). Solvents to prepare the lipid solutions were freshly distilled before use. Water was bidistilled over potassium permanganate [made alkaline with 0.05% KOH (w/v)] in an all-glass apparatus. The NaCl employed in the subphase solution (145 mM, pH 5.6) was previously roasted at 500 °C for 4 h. The absence of surface active compounds in the subphase solution or in the solvents was controlled daily. Sufficient time was allowed for adsorption of possible impurities that might be present in trace amounts in the subphase (Bianco & Maggio, 1989). Solvents were examined for purity by spreading 100 μL over the interface. The available surface area was then reduced to less than 10% of its original value. Only subphases or solvents for which no changes of surface pressure or surface potential occurred were used. Lipid monolayers were spread (10–20 μL) from solutions in chloroform–methanol–water (2:1:0.15) for GD3 or in chloroform–methanol (2:1) for the lactones. After 5 min the monolayer was compressed at a constant rate (routine speed was 3.6 $\text{cm}^2 \cdot \text{m}^{-1}$; total time for one run was 8–10 min). The surface tension and surface potential were automatically recorded as a function of the area available, at 22 ± 1 °C. Duplicate or triplicate isotherms were averaged. At least three cycles of compression and decompression of the same film, each lasting 10 min, were performed as routine controls. The surface pressure–area and surface potential–area isotherms were reproducible to within ± 0.3 nm^2 and ± 10 mV. This demonstrates that no hydrolysis of the ganglioside lactones and no loss of film molecules occurred during the experiment. The markedly different molecular areas and surface potentials of GD3 and the lactones (see below) make possible detection of less than 3% conversion, even if the behavior of the mixture was ideal [cf. Gaines (1966) and Maggio et al. (1978b)]. Different rates of film compression were checked to select the routine rate; a reproducible behavior of the isotherms, independent on the velocity of compression, is necessary to ensure the absence of transient or short-lived metastable organization in the monolayer that could lead to artifactual molecular parameters.

RESULTS AND DISCUSSION

Ganglioside GD3. Figure 1 shows the structure of ganglioside GD3 and the two lactones whose structures were determined by proton NMR (Ando et al., 1989). The surface pressure–area and surface potential–area isotherms of ganglioside GD3 (Figure 2a) agree with those reported previously (Maggio et al., 1978a). The molecular area is consistent with an average orientation of the oligosaccharide chain approximately perpendicular to the surface (Maggio et al., 1978a, 1981). The minimum length of an oligosaccharide chain with four carbohydrate residues in a straight conformation is at least 1.2 nm (Maggio, 1985). If the chain was oriented parallel to the surface, the molecular area could not be less than 4.52 nm^2. On the other hand, if two horizontally extended chains of two neighboring molecules overlapped completely, the required limiting molecular area would be at least 2.26 nm^2. Even below a surface pressure of 1 $\text{mN} \cdot \text{m}^{-1}$, the measured molecular

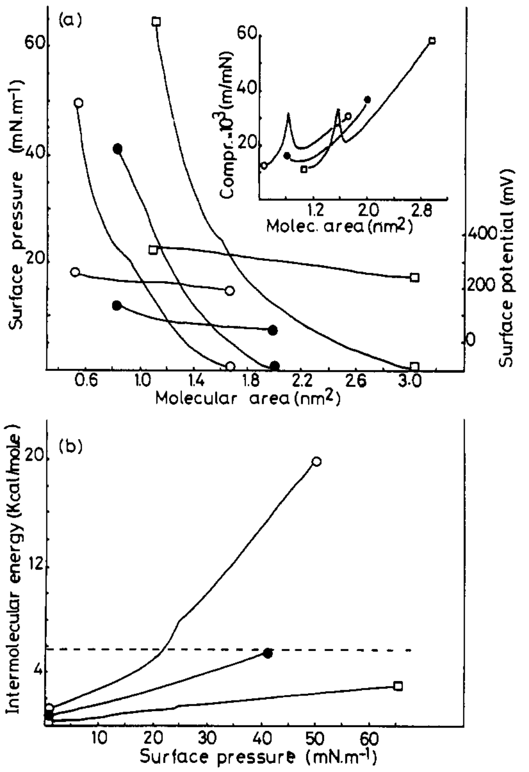

FIGURE 2: Molecular areas, surface potential, and interaction energy for ganglioside GD3 and GD3 lactones. (a) Surface pressure- and surface potential-area isotherms of GD3 (●), GD3LacI (○), and GD3LacII (□). The inset shows the surface compressibility as a function of the molecular areas. (b) Intermolecular dispersion energy as a function of the surface pressure. The symbols are the same as in panel a. The horizontal dashed line indicates the value of 10*RT*.

area for GD3 is less than this figure (Figure 2a). The oligosaccharide chain must therefore be dipping into the water subphase. The intermolecular spacing of GD3 at the limiting molecular area exceeds by 50% that required by two closely packed hydrocarbon chains. The extra space can be accounted for by electrostatic repulsion among the negatively charged sialic acids and a considerable hydration shell associated with the ganglioside polar head groups (Maggio et al., 1981; Bach et al., 1982; Fidelio et al., 1986; Bianco et al., 1988).

The values for the compressibility (Figure 2a, inset) indicate that the isotherm is of the fully liquid-expanded type. The apparent free energy of compression represents the thermodynamic work required to pack the molecules from 0.1 mN·m^{-1} (the lower sensibility limit of our equipment) to the limiting molecular area before collapse. The value of 2.8 kcal/mol obtained for GD3 is similar to that found for GD1a (Fidelio et al., 1986). This is lower than that for asialo-GM1 or globoside having the same number of carbohydrate residues in the polar head group as GD3 but no negative charges (Maggio et al., 1985a). Similar to those of other gangliosides (Maggio et al., 1978b, 1981), the cohesive intermolecular interaction energies are not above 10*RT* even at the highest surface pressures (Figure 2b). This indicates that the interface formed by GD3 is fully delocalized along the lateral plane [cf. Aveyard and Haydon (1973)]. Considerable intermolecular expansion exists in gangliosides compared to neutral glycosphingolipids (Maggio et al., 1978a, 1981, 1985a). This is due to the presence of negative charges, mutually repelling carbohydrate dipole moment vectors, and a large hydration shell (Maggio et al., 1981; Bach et al., 1982; Fidelio et al., 1986) in the ganglioside molecules.

The electrostatic field gradient at the interface is measured by the surface potential. When the surface potential is compared at different molecular areas, it has to be normalized to per unit of molecular surface density ($\Delta V/n$) (Gaines, 1966; Maggio et al., 1978a). In general, the dipolar components of the polar head group in gangliosides and neutral glycosphingolipids point with their positive termini toward the subphase (Maggio et al., 1978a, 1981; Beitinger et al., 1989). This is opposite to the orientation of the hydrocarbon chain dipoles (+0.35 D/chain, positive toward the air; Vogel & Mobius, 1988). This contributes a considerable negative electrical field at the interface (Maggio et al., 1978a, 1981; Beitinger et al., 1989). In addition, the negative charges on the ganglioside generate a negative double-layer electrostatic potential (McLaughlin, 1989). This is included in the measured surface potential (Gaines, 1966; Maggio et al., 1978a). Though partially screened in electrolyte solutions, this potential is also felt laterally. It further contributes to the area expansion of gangliosides compared to neutral glycosphingolipids (Maggio et al., 1978a, 1981). Similar to other gangliosides (Maggio et al., 1978a, 1981; Beitinger et al., 1989), the resultant dipole moment of the polar head group of GD3 is negative. For proper calculation of the resultant dipole moments, the double-layer potential should always be subtracted from the measured surface potential. This is to avoid obtaining apparent values that would operationally depend on the concentration and type of electrolyte used. However, in the case of gangliosides, the resultant polar head-group dipole moments obtained remain negative regardless of whether the double-

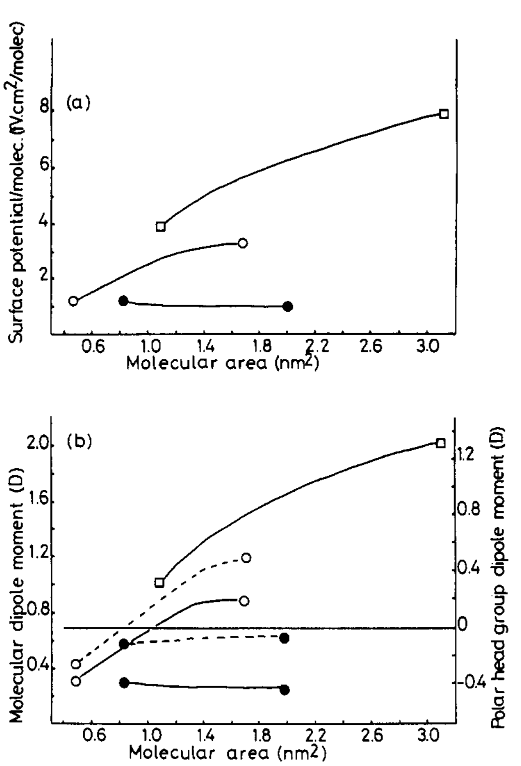

FIGURE 3: Surface potential and molecular dipole moments for ganglioside GD3 and GD3 lactones. (a) Normalized surface potential per unit of molecular surface density as a function of the molecular area for GD3 (●), GD3LacI (○), and GD3LacII (□). (b) Resultant molecular dipole moment (left ordinate) and polar head-group dipole moment (right ordinate) as a function of the molecular area. The polar head-group dipole moments were calculated by subtracting the dipole moment of two closely packed hydrocarbon chains [cf. Vogel and Mobius (1988)] from the molecular dipole moment. Similar results are obtained by subtracting the dipole moment contribution of the ceramide portion [cf. Maggio et al. (1978a)] from the molecular dipole moment. The symbols are the same as in panel a. The dashed lines for GD3 and GD3LacI represent the dipole moments calculated from surface potential values after the electrostatic double-layer potential was subtracted (see text).

layer potential is subtracted or not prior to their calculation (Maggio et al., 1978a, 1981) (Figure 3b). This is due to the large negative contribution of the oligosaccharide chain (Maggio et al., 1978a, 1981; Beitinger et al., 1989). The values calculated with or without prior subtraction of the double-layer potential are shown in Figure 3b. The variation of $\Delta V/n$ and of the molecular dipole moment with the intermolecular packing is negligible (Figure 3). This indicates that little modifications of the dipoles associated to the oligosaccharide chain of GD3 occur when the molecular area is decreased.

Lactone GD3LacI. The carboxyl group of the external sialic acid of the oligosaccharide chain in this lactone (Figure 1) is part of a new six-membered ring involving an internal ester with the C_9–OH of the internal sialic acid residue. There is only one negative charge left in the polar head group located at the internal sialic acid (Ando et al., 1989). These structural changes in the oligosaccharide chain induce marked modifications of the surface behavior. The mean molecular area is shifted to smaller values at all pressures (Figure 2a). A kink is present in the isotherm at a surface pressure of 22 $mN\cdot m^{-1}$ (molecular area of about 0.84 nm^2). At this point, a maximum of the monolayer compressibility is observed (Figure 2a, inset). The kink probably represents a two-dimensional phase transition to a more closely packed state due to modifications of the polar head-group interactions (Maggio et al., 1978a; Fidelio et al., 1986; Beitinger et al., 1989). The phase behavior of glycosphingolipids is highly dependent on the type and conformation of the oligosaccharide while the influence of the hydrocarbon portion is less (Maggio et al., 1985a,b, 1986, 1988a,b).

The smaller intermolecular spacing of GD3LacI than of GD3 results in higher intermolecular cohesive energies. In the range of the two-dimensional discontinuity these values are in the order of $10RT$. The intermolecular energies increase steadily to more than $40RT$ at the closest packing (Figure 2b). Thus, the interface formed by GD3LacI transforms from a laterally nonlocalized to a localized state above 25 $mN\cdot m^{-1}$. The collapse pressure point depends on a balance of forces among the film-forming molecules and the affinity for the aqueous subphase (Gaines, 1966). The marked increase of the collapse pressure of GD3LacI compared to that of GD3 indicates a greater stability of the former lipid to remain organized in the monolayer arrangement. The collapse pressure of glycosphingolipid monolayers is markedly dependent on the hydrophilic–hydrophobic balance. This is affected by the length and charge of the oligosaccharide chain in relation to the hydrocarbon portion (Maggio et al., 1978a; Fidelio et al., 1986). The elimination of one negative charge in gangliosides is known to cause between 7 and 15 $mN\cdot m^{-1}$ of increase in the collapse pressure (Maggio et al., 1978a). The collapse pressure also depends on the hydration and phase state of the interface (Fidelio et al., 1986; Bianco et al., 1988). On the other hand, it is rather insensitive to changes of the oligosaccharide chain conformation (Maggio et al., 1985a). The greater intermolecular cohesion of GD3LacI causes the free energy of compression (measured up to 41 $mN\cdot m^{-1}$) to decrease to 2.1 kcal/mol compared to that of GD3. Due to the smaller areas, an additional 0.8 kcal/mol has to be added to pack GD3LacI from the area at 41 $mN\cdot m^{-1}$ to its limiting area.

The elimination of one negative charge and the formation of a new six-membered lactone ring induce a marked increase of the surface potential compared to that of GD3 (Figure 3a). The normalized potential per unit of molecular surface density also remains higher than for GD3 (Figure 3a). This indicates that the increase in potential is not a false effect due to variations in the packing areas. The hydrocarbon chains are the same in both molecules. Thus, the increased potential must arise, directly or indirectly, from a new balance of dipoles associated with the lactonized polar head group. More positive molecular and polar head-group dipole moments are present in GD3LacI compared to GD3 (Figure 3b).

Due to the loss of one negative charge, the lateral electrostatic repulsion among GD3LacI molecules decreases. The electrostatic double-layer potential (ψ_0) can be estimated to a good approximation according to the Gouy–Chapman theory. This theory has provided reasonable agreement between the experimental and theoretical values even when the charges are separated by about 1 nm from the interface as occurs in gangliosides (McLaughlin, 1989). The values of ψ_0 calculated by the Gouy–Chapman equations (Gaines, 1966; Aveyard & Haydon, 1973; McLaughlin, 1989) include implicitly the normalization due to the surface charge density. Compared to GD3, the increase of the molecular surface density of GD3LacI compensates the effect on the potential brought about by the elimination of one negative charge. As a consequence, the values for ψ_0 and their variation from the area at 0.1 $mN\cdot m^{-1}$ to the limiting molecular area are similar for both lipids (from −90 to −140 mV for GD3 and from −75 to −135 mV for GD3LacI). Since ψ_0 is not significantly modified, the higher surface potential for GD3LacI cannot be explained by variations of the double-layer potential. Contrary to GD3, the variation of $\Delta V/n$ for GD3LacI with the intermolecular packing is considerable. This reflects the variation of the fundamental dipoles that becomes especially marked above 10–15 $mN\cdot m^{-1}$ (below molecular area of 1.2–1.0 nm^2). This brings about an important difference in the surface properties of GD3LacI compared to GD3: the electrostatic field gradient associated with the polar head group of GD3LacI can be either negative or positive (Figure 3b) depending on the lateral packing.

Modifications of the oligosaccharide chain orientation could be the cause of the increased surface potential. However, this is not likely. Let us assume that all the fundamental dipole moment vectors in the polar head group of GD3LacI are the same as in GD3. It can be calculated (Gaines, 1966; Maggio et al., 1981, 1985a) that in order to justify the increase of $\Delta V/n$ (i.e., above 20 $mN\cdot m^{-1}$) the oligosaccharide chain of GD3LacI would have to be displaced by an angle of at least 60° from the perpendicular direction to the interface. However, in this case the projection of the extended oligosaccharide chain onto the interface plane would require an area of no less than 2.80 nm^2 at the same pressures. The experimental value for GD3LacI is less than 0.95 nm^2 above that at 20 $mN\cdot m^{-1}$. Not even a very close overlapping of horizontally extended oligosaccharide chains from two neighboring molecules could account for the measured areas. The molecular areas of GD3LacI are only compatible with an average orientation of the oligosaccharide chain perpendicular to the interface (not more than 30° displacement). Therefore, the more plausible explanation is that the lactonized polar head group contains a considerable resultant dipole moment pointing toward the interface. This does not necessarily mean that the new lactone ring as such is the sole factor responsible for the new dipoles. Its presence may alter intra- and intermolecular dipole–dipole or dipole–ion interactions. Also, it is likely that the hydration shell of the ganglioside polar head group becomes modified after lactonization. These possibilities cannot be ascertained with the experiments done so far.

Lactone GD3LacII. In GD3LacII all negative charges are eliminated. The NMR data on the structure of the oligosaccharide chain indicate an additional internal ester linkage involving the inner sialic acid residue (Figure 1). The additional lactone ring generates a rigid six-membered ring that is fused with the galactopyranosyl residue (Ando et al., 1989).

These structural changes result in additional modifications of the intermolecular organization of GD3LacII compared to either GD3LacI or GD3. The polar head group of GD3LacII is neutral, and no intermolecular electrostatic repulsion should occur. Nevertheless, a marked increase of the molecular area at all pressures is observed compared to that of the negatively charged parent compound (Figure 2a). A slight but reproducible kink is observed at 25 mN·m^{-1} (molecular area of 1.58 nm^2). This is clearly revealed by a peak in the compressibility curve (Figure 2a, inset). The compressibility values along the whole isotherm are similar to those of GD3. Since in GD3LacII the intermolecular spacings are relatively large, the cohesive intermolecular interaction energies are well below $10RT$ over the entire isotherm (Figure 2b). This is similar to GD3, but GD3LacII is even more delocalized. The free energy of compression up to 41 mN·m^{-1} is slightly greater (3.2 kcal/mol) than that for GD3. An additional 1.4 kcal/mol is necessary to pack GD3LacII to its limiting molecular area at the collapse pressure. The interface formed by GD3LacII has the highest collapse point (64 mN·m^{-1}) compared to those of GD3LacI (50 mN·m^{-1}) and GD3 (41 mN·m^{-1}). This suggests that the hydrophobic–hydrophilic balance in the molecule has been shifted in favor of a higher hydrophobicity (Maggio et al., 1978a).

GD3LacII contains the same ceramide portion present in GD3LacI and GD3. The ceramide moiety forms a highly condensed monolayer. Depending on the lateral surface pressure ceramide exhibits cross-sectional molecular areas varying between 0.60 nm^2 at 0.1 mN·m^{-1} and 0.38 nm^2 at the collapse point occurring at 35 mN·m^{-1} (Maggio et al., 1978a, 1981). Above 30 mN·m^{-1} the presence of four neutral carbohydrates in a straight-chain conformation in the polar head group causes less than 10–20% variation of the molecular area compared to that in ceramide (Maggio et al., 1978a, 1981). However, the limiting molecular area of GD3LacII is 1.11 nm^2. This is 0.27, 0.62, and 0.72 nm^2 larger than the limiting molecular area of GD3, GD3LacI, and ceramide, respectively. The huge increase of intermolecular spacing in GD3LacII is not caused by a more expanded state of the monolayer. Apart from the peak due to the kink at 23 mN·m^{-1}, the compressibilities are similar to those of GD3 (Figure 2a, inset). These values are well within the range corresponding to monolayers in the liquid state (Sears & Stark, 1973). The dramatic change of the mean molecular area of GD3LacII must result, directly or indirectly, from conformational changes in the polar head group induced by the two lactone rings. Marked resonance shifts of carbohydrate protons and carbons of GD3 lactones were observed by high-resolution proton NMR. These shifts suggested a significant conformational change in the oligosaccharide chain (Ando et al., 1989). In addition, our present results show directly that this affects remarkably the long-range intermolecular organization and surface potential.

The values of $\Delta V/n$ are further increased at all pressures in GD3LacII compared with GD3 and GD3LacI (Figure 3a). This indicates additional increases of the polar head-group dipole moment vector in GD3LacII. The resultant polar head-group dipole moment of GD3 undergoes little variation under compression. For GD3LacI the variation becomes important only at molecular areas smaller than 1.20 nm^2 (Figure 3b). For GD3LacII, the variation of the polar head-group dipole moment occurs continuously from the beginning of the isotherm. In contrast to that in GD3 and GD3LacI, the contribution of the polar head group in GD3LacII to the electrostatic field gradient at the interface remains positive throughout (Figure 3b). The greater molecular areas found for GD3LacII are consistent with the changes in the polar head-group region. The rigid fused lactone rings appear to require a different orientation or a conformational bending in the oligosaccharide chain. This inevitably affects the intermolecular packing.

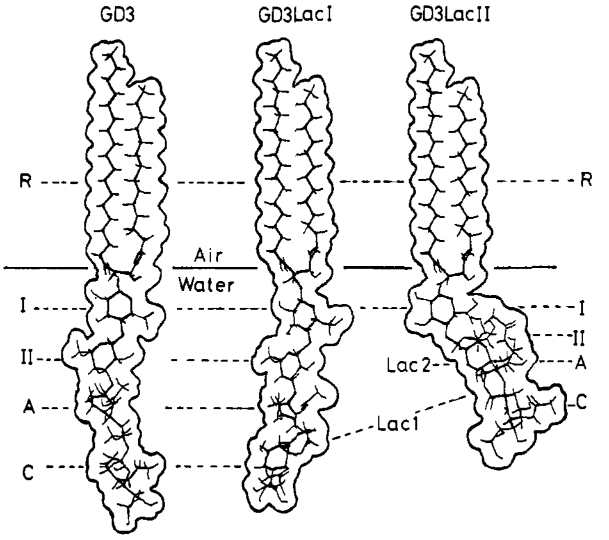

FIGURE 4: Molecular models of ganglioside GD3 and its lactone derivatives as possibly oriented at the monolayer interface. Dreiden and space-filled contours for GD3, GD3LacI, and GD3LacII are illustrated in the optimal conformation after convergence of an energy minimization algorithm for torsion angles, interatomic bond stretching, angle and out-of-plane bending, and van der Waals interactions (Tripos Assoc. Inc., St. Louis, MO). Letters denominating the carbohydrate residues are as in Figure 1.

CONCLUSIONS

Figure 4 shows the space-filling models of GD3 and its lactone derivatives. Similar to that of other glycosphingolipids, the oligosaccharide chain of GD3 is oriented in a perpendicular direction to the interface (Maggio et al., 1978a, 1981, 1985a). The changes in conformation of the oligosaccharide chain induced by lactonization as determined by NMR (Ando et al., 1989) are compatible with the results obtained in monolayers. The spatial orientation of the lactone ring in GD3LacI is perpendicular to the plane of the terminal sialic acid (Ando et al., 1989). However, there is free rotation about the bonds linking the lactone ring to the inner sialic acid (Figure 1). This still allows the adoption of an overall straight conformation of the oligosaccharide chain (Figure 4). The monolayer data indicate that this orientation does not require additional space. The molecular area of GD3LacI is therefore similar to that of monosialogangliosides GM3 and GM2 (Maggio et al., 1978a, 1981). The difference compared to GD3 is most probably due to a decrease of the electrostatic repulsion and/or interfacial hydration.

In GD3LacII two additional fused rings are formed between the inner sialic acid and the galactose residue. This causes a forbidden rotation around the second carbon of the inner sialic acid (Figure 1). As a consequence of these rigid structures, the oligosaccharide chain of GD3LacII is distorted and displaced from the vertical position (Figure 4). This requires larger intermolecular spacings than for GD3 and GD3LacI.

A wide range of electrostatic field gradients ranging from negative to positive values is encompassed by GD3 and its lactone derivatives. This depends on packing and may represent an important modulation of the surface electrostatics. Dramatic changes of the molecular organization and surface potential of ganglioside interfaces are induced by lactonization. Conceivably, if these changes were to occur in natural membranes, this should affect intermolecular and long-range intermembrane interactions involved in the regulation of surface recognition and transduction of signals. The relative proportion of gangliosides with respect to other membrane lipids and proteins would not need to be metabolically altered to induce these changes. The membrane behavior could thus be rapidly modified according to the surface and environmental conditions (Yu et al., 1985; Ando et al., 1989).

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