Surface and phase behavior of cholesterol/ganglioside mixtures

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Summary. Gangliosides occupy 1.54 nm^2 per molecule at zero surface pressure at an air/water interface. Gangliosides and cholesterol interact to produce 2 distinct complexes: one composed of 1 molecule ganglioside: 3 molecules cholesterol; the other composed of 2 molecules ganglioside: 1 molecule cholesterol.

Gangliosides are sialic acid-containing glyco-sphingolipids which have been shown to be involved in a variety of membrane-mediated phenomena; they may serve as receptors for bacterial toxins[1,2], they have been implicated in forming part of the receptor for the peptide hormones thyrotropin[3], human chorionic gonadotropin[4], luteinising hormone[5] and follicle stimulating hormone[5] and appear to be intimately associated with the 5-hydroxytryptamine receptor[6].

A knowledge of the interactions of gangliosides with other membrane components is essential before an understanding of the role of these molecules within the membrane can be realized; however, such interactions have received little attention. Due to the difficulty in obtaining large amounts of pure single ganglioside and to allow comparison of results with the studies of other groups[7-10], where mixtures of gangliosides have been consistently used, it was decided to examine the interaction of a ganglioside mixture with the membrane component cholesterol.

Materials and methods. The interactions of gangliosides with cholesterol at an air/water interface were studied on a Langmuir surface trough made of chromium plated brass. Surface pressures were measured by a phospho-bronze torsion wire which was sensitive to 0.02 dynes cm^{-1}. The mixed solutions of cholesterol and ganglioside were spread on the clean water surface and the film compressed in order to obtain pressure-area curves. Phase equilibria behavior of cholesterol/ganglioside mixtures was investigated by noting the melting point of the mixtures; it was hoped to use a thermal analysis procedure but heating the gangliosides to temperatures greater than 210 °C led to irreversible decomposition.

The cholesterol used in this work was obtained from Sigma Co. and was chromatographically pure; the gangliosides were also obtained from Sigma Co. (type III, extracted from bovine brain) and were used without further purification. Curatolo et al.[7] have shown that this fraction is reasonably pure (approximately 99%) and contains primarily G_{D1a}, G_{D1b}, G_{M1} and small amounts of G_{M2} with an average mol.wt of approximately 1700; the average mol.wt of the mixture used in this study, estimated from surface data, was 1750.

Results and discussion. Figure 1 shows the pressure-area curves for ganglioside-cholesterol mixtures on a Langmuir surface trough and indicates that the surface area per molecule of ganglioside at zero pressure is 1.54 nm^2 (= 154 $Å^2$ $molecule^{-1}$). This value is in good agreement with the maximum figure obtained by X-ray diffraction of hydrated bovine brain gangliosides for the area of the polar head group (158 $Å^2$ $molecule^{-1}$)[7].

Figure 2 shows the area occupied per molecule, at zero pressure, for varying compositions of membrane. Much lower areas for the monolayer than expected for ideal mixtures are observed in that the presence of cholesterol condenses the ganglioside film in a manner similar to

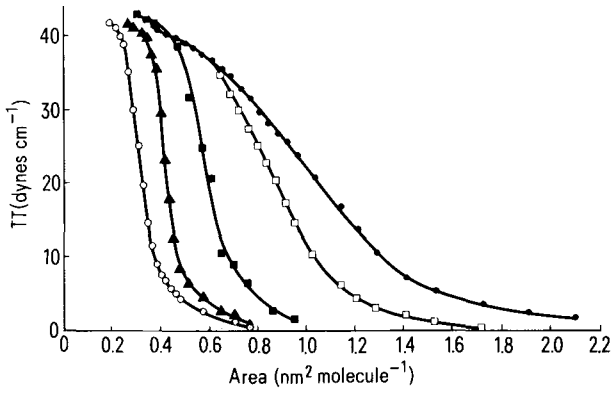

Figure 1. Surface pressure-area curves for mixed monolayers of gangliosides with cholesterol. Using an average mol.wt of 1750 for the gangliosides the figure shows force-area curves for the following mole fractions of cholesterol: (—○—) pure cholesterol i.e. mole fraction 1.0; (—▲—) mole fraction 0.7; (—■—) mole fraction 0.5; (—□—) mole fraction 0.2; (—●—) mole fraction 0 i.e. pure ganglioside.

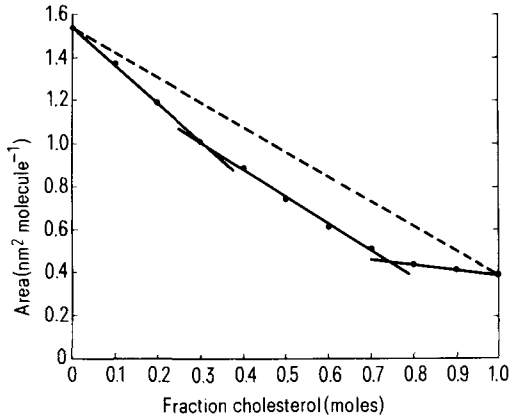

Figure 2. The mean area per molecule in mixed monolayers of cholesterol and gangliosides to the proportions of the 2 components. Pure cholesterol on the right; pure ganglioside on the left. The dotted line is the expected plot for ideal mixtures.

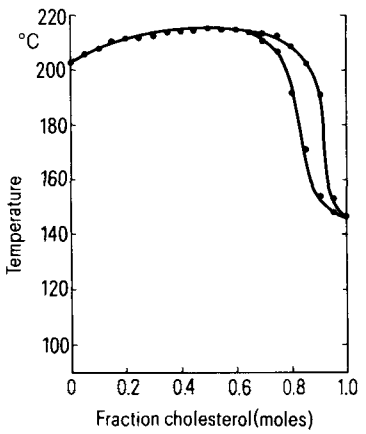

Figure 3. Phase equilibrium diagram for ganglioside-cholesterol mixtures obtained from melting point determinations.

lecithin-cholesterol monolayers[11]. Willmer[11], De Bernard[12] and Snart[13] all concluded that this type of behavior results from the formation of distinct surface structures. In this case of ganglioside/cholesterol monolayers there are 2 different surface structures: one due to excess cholesterol with the surface structure being composed of 1 molecule ganglioside:3 molecules cholesterol and the other surface structure due to excess ganglioside being composed of 1 molecule cholesterol:2 molecules ganglioside. The intermediate compositions being mixtures of these 2 basic structures.

In an attempt to study in more detail the miscibility of gangliosides in cholesterol mixed melting points were determined from which the phase equilibrium diagram shown in figure 3 has been obtained. In mixtures containing an excess of cholesterol 2 temperature dependent phase changes were noted: as the temperature was increased softening at the edges of the mixtures was noted. Rapid melting followed by irreversible decomposition of the mixture occurred a few degrees higher. At mole fractions of cholesterol less than 0.7 the softening at the edges of the mixture was not observed; final melting of the mixtures became less distinct with the mixture 'charring' and decomposing rather than melting. The phase behavior exhibited by mixtures containing an excess of cholesterol (mole fraction cholesterol greater than 0.7) is of the type associated with constituents that are completely miscible in both the solid and liquid states; although such behavior is not normally associated with complex formation the diagram (fig. 3) is interpreted in terms of the formation of a cholesterol/ganglioside complex in the molecular ratio of 3:1 with this complex and cholesterol being completely miscible. The phase equilibrium diagram however, provides no evidence for the formation/behavior of a 1:2 cholesterol: ganglioside complex.

The property of gangliosides to form complexes with other membrane constituents (cholesterol) raises the possibility of such complexes having a role in the structure of hormone receptors where gangliosides are known to form at least part of the receptor – a problem which is under investigation in this laboratory at the present time.

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Effects of N-ethyl maleimide on urea facilitated transport across toad gall bladder

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Summary. An SH reactive agent, N-ethyl maleimide (10^{-3} M for 2 min in the luminal fluid) selectively inhibits the urea transepithelial flux across the toad gall bladder. Thiourea and antipyrine flux is not inhibited. The inhibitory effect on urea flux seems to be exerted on the carrier mechanism of urea transport.

It is known that the gall bladder is the site of an active transport mechanism for urea and presumably other amides, and that such transport exhibits saturation kinetics and is selectively inhibited by phloretin[1,2].

Little can be said about the chemical nature of the carrier molecules involved; however, the selective inhibition by cycloheximide[3] seems to indicate that they are of a protein nature. The activity of several carrier molecules possessing sulphydryl groups is strongly inhibited by SH-reactive agents such as N-ethyl maleimide (NEM) and mersalyl[4].

In order to define the urea carrier molecule more precisely, we have studied the effects of NEM on urea transport across toad gall bladder.

Methods. Gall bladders were isolated from female toads *(Bufo bufo)* and then opened, washed free of bile with Ringer solution and mounted between 2 lucite chambers containing 7 ml of incubation fluid, gassed with air at 22 ± 2 °C. The exposed area was 0.2 cm^2 and both sides were bathed with the same fluid containing (in mmole/l): NaCl 112, KCl 5, $CaCl_2$ 1, $NaHCO_3$ 2.5, test molecule 1, pH = 8.1.

Transepithelial fluxes from the serosa to the mucosa were measured using the following labeled molecules: ^{14}C-urea, ^{14}C-thiourea and N-methyl ^{14}C-antipyrine (obtained from N.E.N., Frankfurt, FRG).

The labeled molecule was added to the serosal compartment (final activity 1 μCi/ml) and after 2 h of equilibration, samples were withdrawn every hour from the opposite compartment. After a control period, NEM (10^{-3} M) was added to the mucosal side for 2 min; samples were withdrawn every hour thereafter for 2 h.

For counterflow experiments, the isolated tissue, perfused as previously reported, was loaded for 70 min with Ringer solution containing ^{14}C-urea (10^{-6} M); final activity on both sides was 5 μCi/ml. During this period the perfusion fluids were stirred continuously with magnetic bars. At the end of the loading period the perfusion fluids were recovered and the chambers were thoroughly washed twice