Interaction of glycosphingolipids with melittin and myelin basic protein in monolayers

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The penetration of melittin and myelin basic protein into glycosphingolipid monolayers depends on the lipid polar head group, the protein concentration available and the initial surface pressure. The lipid–protein interaction leads to modification of the surface properties of both the glycosphingolipid and the proteins.

The interfacial properties of glycosphingolipids depend on the number and type of carbohydrate residues present in their oligosaccharide chain (Maggio *et al.*, 1978a). The type of polar head group in these lipids also influences the interaction between themselves, with phospholipids and with Ca^{2+} (Maggio *et al.*, 1978b, 1980). The present work shows that in glycosphingolipid monolayers the presence of different carbohydrate residues in the polar head group affects their interaction with myelin basic protein and melittin, two proteins able to modify membrane properties (Sessa *et al.*, 1969; Monferrán *et al.*, 1979; Cumar *et al.*, 1980).

Materials and methods

The equipment used, the preparation of lipid monolayers and the source and purity of the glycosphingolipids were given previously (Maggio *et al.*, 1978a). The myelin basic protein was purified as described previously (Maggio & Cumar, 1974), and melittin was from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and further purified as described by Mollay *et al.* (1976). The molecular weights of melittin and myelin basic protein were taken as 2840 (Habermann, 1972) and 18400 (Eylar, 1972) respectively. All the experiments were carried out on a subphase of unbuffered 145 mM-NaCl, pH 5.6, at $20 \pm 1^\circ\text{C}$. Interactions between glycosphingolipids and proteins were studied by measuring the increase in surface pressure or change in surface potential after injecting the protein into the subphase under a glycolipid monolayer brought to a specified surface

pressure. The Teflon trough had an area of 23 cm^2 and was 0.8 cm deep. The proteins were injected into the subphase in a small volume (maximum $30\text{ }\mu\text{l}$) from aqueous solutions and the subphase was stirred for 0.5 min with a magnetic bar. Subsequent changes in surface pressure and surface potential at constant area, which can be taken as a measure of penetration and interaction (cf. Quinn & Dawson, 1969; Colacicco, 1970; Maggio *et al.*, 1977), were automatically recorded as a function of time. In some experiments, after reaching equilibrium in surface pressure, the penetrated films were compressed at a constant rate ($7.1\text{ cm}^2/\text{min}$) until collapse. In some conditions the attainment of a final equilibrium pressure is a slow process, especially at low protein concentration; the term equilibrium pressure will be used to indicate a situation in which the rate of change of surface pressure is below $0.05\text{ mN}\cdot\text{m}^{-1}\cdot\text{min}^{-1}$. Experiments were done in duplicate or triplicate and reproducibility was within $\pm 1\text{ mN}\cdot\text{m}^{-1}$ for surface pressure and $\pm 5\text{ mV}$ for surface potential.

Results and discussion

Melittin is an amphipathic protein having α -helical structure, with 20-non-polar amino acids and six polar residues (Habermann, 1972). It showed high surface activity and spread as an insoluble stable film with a limiting molecular area of 17.8 nm^2 at a collapse pressure of $19\text{ mN}\cdot\text{m}^{-1}$, similar to the data reported by Sessa *et al.* (1969). When injected into the subphase in concentrations of 22–114 nM the equilibrium surface pressures and surface potentials obtained varied from 1 to $18\text{ mN}\cdot\text{m}^{-1}$ and from 300 to 450 mV respectively. Assuming that all the protein injected is at the surface, the molecular areas calculated and the surface potentials measured

Abbreviations used: Cer, ceramide (*N*-acylsphingoid); GalCer, Gal β →1Cer; LacCer, Gal β 1→4Glc β 1→1Cer; GgCer, Gal β 1→3GalNAc β 1→4Gal β 1→4Glc β 1→1Cer; sulphatide, Gal(3←sulphate) β 1→1Cer. Abbreviations are those recommended by IUPAC–IUB.

differed only by 4–10% from those obtained from the force–area curve of the spread protein at the same surface pressures. In agreement with the value for the collapse pressure of the melittin film ($19\text{ mN}\cdot\text{m}^{-1}$), higher values of equilibrium pressure could not be obtained by injecting more protein (up to 176 nm) into the subphase. This suggests that below 114 nm the melittin injected may roughly be taken as being at the surface, although this was not directly measured in this work. The myelin basic protein has an expanded conformation (Eylar, 1972), with dispersed segments of non-polar residues along the molecule, which can penetrate lipid interfaces (London *et al.*, 1973). It did not form an insoluble monolayer and it was not possible to obtain a force–area curve, but it showed some surface activity, giving a maximum equilibrium surface pressure and surface potential of $10\text{ mN}\cdot\text{m}^{-1}$ and 315 mV when injected into the subphase at a concentration of 108 nm .

The time course for the changes in surface pressure and surface potential of a monolayer of GalCer at an initial surface pressure of $10\text{ mN}\cdot\text{m}^{-1}$ after injection of melittin (88 nm) or myelin basic protein (108 nm) into the subphase at zero time is shown in Fig. 1(a). The penetration was rapid, and at 5 min (or before for other lipids) the change was about 70–80% of the final value. For interactions with melittin the value of surface potential increased

with the penetration, tending to a constant value with time; by contrast, after giving an initial increase of the surface potential at a time that corresponded to a rapid increase of the surface pressure, the myelin basic protein led to surface potentials that decreased with time. This biphasic effect was not present in conditions in which penetration was poor (i.e. at high initial surface pressures of the lipid film or low protein concentrations), and only a decrease in the surface potential was observed. This effect may be due to an initial positive contribution to the surface potential by protein rapidly penetrating the lipid film, followed by a secondary interaction that appears more slowly with time and does not contribute to the surface pressure; in conditions where little penetration occurs, only the second effect is observed. For the other glycolipids studied the time course for the interactions followed a pattern qualitatively similar to that described above for either protein (results not shown).

Fig. 1(b) shows that the interactions depend on the polar head groups of the lipids and the concentration of protein. At low protein concentration, one or more neutral carbohydrate residues in the polar head group of the glycolipid inhibited penetration with respect to that observed for ceramide. For the negatively charged sulphatide the effect was the opposite and showed a greater effect with myelin basic protein than with melittin. At high

Fig. 1. Penetration of melittin and myelin basic protein into glycosphingolipid monolayers

(a) Time course obtained for the changes in surface pressure (○) and surface potential (●) after penetration of melittin (---, 88 nm) or myelin basic protein (—, 108 nm) into a monolayer of GalCer at an initial surface pressure of $10\text{ mN}\cdot\text{m}^{-1}$ and initial surface potential of 315 mV . (b) Maximum increases in surface pressure obtained after penetration of melittin (---) or myelin basic protein (—) into the glycosphingolipid monolayers indicated, at an initial surface pressure of $10\text{ mN}\cdot\text{m}^{-1}$ at protein concentrations of 108 nm (▼), 14 nm (●), 88 nm (▲) or 33 nm (■). Vertical bars represent s.e.m. for duplicate or triplicate experiments.

protein concentration the complexity of the neutral oligosaccharide chain had no influence on the penetration and only the effect with sulphatide was different.

The behaviour of the surface potential also depends on the type of polar head group on the lipid and the initial surface pressure of the film. At low concentration of protein, the changes in the surface potential were small and a clear pattern could not be obtained. At high protein concentration (Fig. 2a), the surface potential obtained after penetration usually increased with the complexity of the polar head group, and the influence of a negative charge in sulphatide with respect to GalCer appeared to be similar to that obtained by adding more neutral carbohydrates to the polar head group. Therefore, in spite of the change in surface pressure being independent of the type of neutral polar head group of the glycosphingolipid at high protein concentration (cf. Fig. 1b), the change of the surface potential depended on this. Conversely, even if similar changes in surface potential are found for sulphatide and Gg₄Cer at high protein concentration, different increases in surface pressure were found. These results indicate that both the packing and dipolar properties of the lipid-protein organi-

zation at the surface are strongly dependent on the polar head group of the lipid but the effect of both proteins is similar.

The well-known effect of a decrease in penetration with an increase of the initial surface pressure of the lipid monolayer (cf. Phillips *et al.*, 1975) was found for both proteins above a concentration of 88 nM for melittin and 54 nM for myelin basic protein (Fig. 2b). However, for lower amounts of protein injected the penetration is not always inversely proportional to the initial surface pressure and, depending on the type of lipid, the protein concentration and the initial surface pressure, some optima for penetration are found, as shown for the interaction of melittin with Gg₄Cer (Fig. 2b, inset). Fig. 2(b) shows that the increase of surface pressure are considerable even when the initial surface pressure of the lipid monolayer was equal to or higher than the equilibrium pressure corresponding to the injection of the same amount of protein under a clean interface. For the myelin basic protein, the maximum equilibrium pressure obtained without a lipid film is 10 mN·m⁻¹, and the increase obtained by penetration of lipid monolayers at this initial pressure is 9 mN·m⁻¹ for neutral glycosphingolipids and 17 mN·m⁻¹ for sulphatide. With melittin

Fig. 2. Changes in surface potential and surface pressure after penetration of melittin and myelin basic protein into glycosphingolipid monolayers at different initial surface pressures

(a) Maximum changes in surface potential of the glycosphingolipid monolayers indicated that an initial surface pressure of 5 mN·m⁻¹ (○) or 10 mN·m⁻¹ (●), for myelin basic protein (—, 108 nM) or melittin (---, 88 nM). (b) Maximum increases in surface pressure after penetration of myelin basic protein (—, 108 nM) or melittin (---, 88 nM) into monolayers of Cer (Δ), Gg₄Cer (●) and sulphatide (○) at different initial surface pressures (π_i) with respect to the equilibrium surface pressure of the proteins injected below a lipid-free interface (π_{eq} is 10 mN·m⁻¹ for myelin basic protein and 14 mN·m⁻¹ for melittin). The inset shows the increase in surface pressure after penetration by melittin at a concentration of 88 nM (●), 44 nM (▲) or 33 nM (○) into films of Gg₄Cer at different initial surface pressures. Vertical bars represent s.e.m. for duplicate or triplicate experiments.

a similar effect was found, but unlike the myelin basic protein it must be noticed that, independently of the equilibrium pressure, the maximum surface pressure that can support a pure melittin film is $19 \text{ mN} \cdot \text{m}^{-1}$ (the collapse pressure). Nevertheless, the maximum surface pressure ($\Delta\pi + \pi_i$) found for the melittin-lipid interactions was about $4 \text{ mN} \cdot \text{m}^{-1}$ above the collapse pressure of melittin alone for neutral glycosphingolipids and, for sulphatide, about $12 \text{ mN} \cdot \text{m}^{-1}$ above the collapse pressure of melittin. The above results indicate that the glycolipid-protein interactions modify the surface properties of the proteins which can support a higher lateral pressure or packing in the presence of lipids.

Increased penetration of myelin basic protein into sulphatide with respect to cerebroside monolayers was reported by London *et al.* (1973). The effects that we found on the protein surface properties are greater for sulphatide than for the other lipids and the changes are more marked for myelin basic protein than for melittin, since the $\Delta\pi + \pi_i$ values obtained for both proteins are similar with any of the lipids (Fig. 2b), but the higher surface pressure obtained for myelin basic protein without lipid is $10 \text{ mN} \cdot \text{m}^{-1}$, whereas for melittin it is $19 \text{ mN} \cdot \text{m}^{-1}$.

The surface properties of the lipids, in turn, are modified by the proteins, depending on the lipid polar head group. This is indicated by experiments in which the collapse pressure obtained by compression of the penetrated film after reaching equilibrium was compared with the collapse pressure of a monolayer of pure lipid. Films of Cer and GalCer that exhibited considerable increases of surface pressure after penetration by myelin basic protein or melittin showed a collapse pressure significantly higher (Student's *t* test, $P < 0.01$) by about $4 \text{ mN} \cdot \text{m}^{-1}$ than that of the lipid alone. Conversely, for Gg₄Cer and sulphatide, which by themselves have high values of collapse pressure (above $58 \text{ mN} \cdot \text{m}^{-1}$; cf. Maggio *et al.*, 1978a; Monferrán *et al.*, 1979), the penetrated lipid-protein films showed significantly lower collapse pressures by about $3 \text{ mN} \cdot \text{m}^{-1}$ ($P < 0.01$).

The present work shows that the interaction of glycosphingolipids with proteins depends on the type

of lipid polar head group, the protein concentration available and the initial surface packing; when interaction occurs the surface properties of the ensemble are different from those of the individual components. Therefore a single parameter does not uniquely describe these lipid-protein interactions, but several combined factors are likely to control in a dynamic way the surface organization of glycosphingolipid-protein associations in biological membranes.

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