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Interaction of soluble and membrane proteins with monolayers of glycosphingolipids

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1. The interactions of four proteins (albumin, myelin basic protein, melittin and glycophorin) with eight neutral or acidic glycosphingolipids, including sulphatides and gangliosides, five zwitterionic or anionic phospholipids and some of their mixtures, were studied in lipid monolayers at the air/145 mM-NaCl interface. 2. In lipid-free interfaces, the surface pressure and surface potential reached by either soluble or integral membrane proteins did not reveal marked differences. 3. All the proteins studied showed interactions with each of the lipids but the maximal interactions were found for basic proteins with acidic glycosphingolipids. 4. Surface-potential measurements indicated that different dipolar organizations at the interface can be adopted by lipid–protein interactions showing the same value for surface free energy. 5. The individual surface properties of either the lipid or protein component are modified as a consequence of the lipid–protein interaction. 6. In mixed-lipid monolayers, the composition of the interface may affect the lipid–protein interactions in a non-proportional manner with respect to the relative amount of the individual lipid components.

Glycosphingolipids exhibit different interfacial properties both in natural and model-membrane systems, depending on their oligosaccharide chain composition (for review, see Maggio *et al.*, 1981). Some of these lipids induce membrane fusion in chicken erythrocytes (Maggio *et al.*, 1978*c*; Monferrán *et al.*, 1979), permeability changes in liposomes in the presence of biogenic amines (Maggio *et al.*, 1977*b*) and neurotransmitter movements in nerve endings (Cumar *et al.*, 1980). Several of these effects have been correlated with the interactions of glycosphingolipids with natural and synthetic phospholipids (Maggio *et al.*, 1978*b*) and, in some cases, it was possible to obtain information on the intermolecular organization at the interface (Maggio *et al.*, 1980). It was previously shown that some of the effects of certain glycosphingolipids on natural membranes could be abolished in the presence of exogenously added proteins (Monferrán *et al.*, 1979; Cumar *et al.*, 1980).

Very little information exists on the interactions of glycosphingolipids with membrane or soluble proteins. Employing monolayers at the air/145 mM-NaCl interface, we have studied the interaction of several glycosphingolipids, differing in the complexity of their polar head groups, with four different types of proteins. Some preliminary results obtained with a small number of glycosphingolipids and two basic proteins have been reported previously (Fidelio *et al.*, 1981).

Abbreviations used: Cer, ceramide (*N*-acylsphingoid); NeuAc, *N*-acetylneuraminate; GalCer, Galβ1→1Cer; LacCer, Galβ1→4Glcβ1→1Cer; Gg_4Cer, Galβ1→3GalNAcβ1→4Galβ1→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←2αNeuAc8←2αNeuAc)β1→4Glcβ1→1Cer; $(Pam)_2$PtdCho, dipalmitoylglycerophosphocholine; $(Pam)_2$PtdEtn, dipalmitoylglycerophosphoethanolamine; PtdA, phosphatidic acid; PtdIns, phosphatidylinositol; $(Hxd)_2P$, dihexadecylphosphate. Abbreviations are those recommended by IUPAC–IUB (cf. Maggio *et al.*, 1978*a*) for neutral glycosphingolipids and by Svennerholm (1963) for gangliosides.

Materials and methods

Melittin was from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and further purified as described by Mollay *et al.* (1976). Myelin basic protein was purified as described previously (Maggio & Cumar, 1974) and glycophorin was obtained by the method of Segrest *et al.* (1979). These proteins showed a

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single band corresponding to the reported molecular weight (see the Results and discussion section) on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis; in the case of glycophorin, two main periodate/Schiff-positive bands and minor higher-molecular-weight aggregates were observed (cf. Schulte & Marchesi, 1978). Albumin (fatty acid-free), $(Pam)_2PtdCho$, $(Pam)_2PtdEtn$, PtdIns, PtdA, $(Hxd)_2P$ and hexadecyltrimethylammonium were from Sigma Chemical Co. and used without further treatment. The source and purity of glycosphingolipids, the equipment used and the preparation of single or mixed monolayers were given previously (Maggio *et al.*, 1978*a*; Fidelio *et al.*, 1981). Special care should be taken when preparing gangliosides for monolayer work; we have observed that if individual ganglioside species are obtained by a purification through a column of silica gel G (Svennerholm, 1972) it may lead to gangliosides that behave anomalously in monolayers and as if their sialosyl residues were wholly or partially protonated. This results in surface-pressure–area isotherms on 145mM-NaCl, pH5.6, showing higher values of collapse pressure (above $50 mN \cdot m^{-1}$), reduced limiting molecular areas (within 0.6–$0.9 nm^2$) and lower values of surface potential (below 40mV at the limiting molecular area) than those previously found and which are unusually similar for mono- or poly-sialogangliosides and insensitive to protonation of their sialosyl residues by changes of the pH of the subphase (cf. Maggio *et al.*, 1978*a*, 1981). These samples of gangliosides, even if they were over 95% pure according to the single band shown on t.l.c. plates and on the basis of the ratio of NeuAc content to dry weight, contained unexpected amounts (up to 30% of the NeuAc in certain batches) of esters as determined by the hydroxamic acid reaction with a standard of glucose pentaacetate (Weissman & Meyer, 1954). These are probably due to some internal ester formation (cf. Mestrallet *et al.*, 1976; Gross *et al.*, 1977; Iwamori *et al.*, 1978; Svennerholm, 1980) involving the carboxylate group of NeuAc. Treatment of these samples in alkaline conditions (0.01M-NaOH, at room temperature, for at least 24h), purification through a column of DEAE-Sephadex by elution with ammonium formate (Ledeen *et al.*, 1973; G. Nores & R. Caputto, unpublished work) and conservation in chloroform/methanol/0.01M-NaOH (40:20:3 by vol.) led to gangliosides that revealed no formation of internal esters and exhibited surface-pressure– and surface-potential–area curves that were in agreement with the values previously reported for mono-, di- and tri-sialogangliosides and that responded in the expected way according to the protonation of sialosyl residues on subphases at different pH (cf. Maggio *et al.*, 1978*a*, 1981).

Interactions between lipids and proteins were studied as described previously (Fidelio *et al.*, 1981) by automatically measuring as a function of time the changes in surface pressure and surface potential after injecting the protein into a subphase (145mM-NaCl, pH5.6) under a clean interface or a lipid monolayer at a specified surface pressure. In some experiments, after equilibrium in surface pressure was reached, the lipid films penetrated by proteins were compressed at a constant rate until collapse. The attainment of equilibrium is a slow process, especially at low protein concentrations, and the term equilibrium pressure is herein used when the rate of change of surface pressure is below $0.05 mN \cdot m^{-1} \cdot min^{-1}$. For spreading proteins we used either Trurnit's method (cf. Gaines, 1966) or deposition of a drop of protein solution on the aqueous surface; no difference was observed for the interfacial behaviour of proteins spread by the two methods. Also, melittin and albumin could be spread from chloroform/methanol/water (40:20:3, by vol.) solutions and gave similar values for surface-pressure– and surface-potential–area isotherms to those obtained by spreading from an aqueous solution. Experiments were done at least in duplicate and reproducibility was within $\pm 1 mN \cdot m^{-1}$ for surface pressure and $\pm 10 mV$ for surface potential.

Results and discussion

Interfacial behaviour of single proteins

Myelin basic protein (mol.wt. 18400) has an expanded conformation (Eylar, 1972) with segments of non-polar amino acid residues among more polar sequences. The non-polar regions can penetrate lipid interfaces, whereas the more polar segments remain in the aqueous phase (Demel *et al.*, 1973; London *et al.*, 1973) and, on this basis, this molecule may be considered as a partly embedded membrane protein. It was not possible to obtain a stable insoluble monolayer by spreading (Fidelio *et al.*, 1981), but it showed surface activity when injected into the subphase. The values of surface pressure obtained were stable and increased with the amount of protein injected, reaching maximum values of about 9–$10 mN \cdot m^{-1}$ (Fig. 1) and a surface potential of 315mV.

Glycophorin, the MN-blood-group-determinant-bearing glycoprotein of human erythrocyte membrane, is a single-chain polypeptide of 131 amino acid residues with an average of 16 oligosaccharide chains containing sialosyl residues (Tomita & Marchesi, 1975); it ran on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as two main periodate/Schiff-positive bands with mol.wts. 50000 and 97700. Compared with other intrinsic proteins substantially embedded in the membrane, glycophorin spans the lipid bilayer by a sequence of only about 23 non-polar amino acid residues that

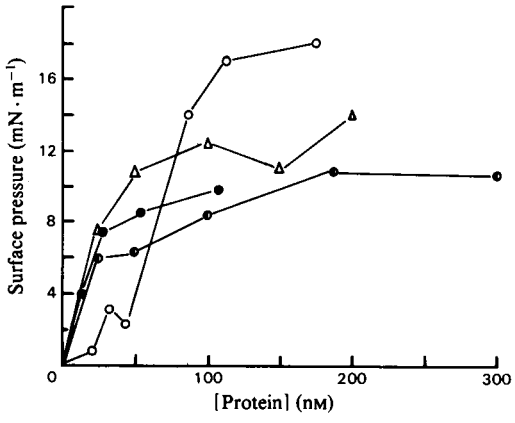

Fig. 1. *Surface activity of proteins*
The surface activity at different concentrations of protein in the subphase 145mM-NaCl, pH5.6 is shown for melittin (○), albumin (◐), myelin basic protein (●) and glycophorin (△).

connect the oligosaccharide-bearing *N*-terminal and *C*-terminal more-hydrophilic segments. It formed an unstable film from spreading and a reproducible surface-pressure–area isotherm could not be obtained by compression. By injection into the subphase, stable values of surface pressure and surface potential at different concentrations with maxima of $14\,mN \cdot m^{-1}$ (Fig. 1) and 230mV respectively were observed. A similar result was obtained on a subphase of 150mM-Tris/HCl, pH7.4. The value of surface pressure reported by van Zoelen *et al.* (1977) for glycophorin injected at 40nM into a subphase of 1mM-Tris/HCl, pH7.4, was $6\,mN \cdot m^{-1}$.

Melittin is an amphipathic protein with 26 amino acid residues and mol.wt. 2840; residues 1–20 are predominantly hydrophobic, whereas residues 21–26 are hydrophilic and contain four basic amino acids (Habermann, 1972). This molecule can exist either as a monomer or a tetramer in aqueous solutions; the monomeric form has an extended flexible conformation, whereas in the self-aggregated tetramer or in the presence of detergent micelles or phospholipid bilayers the molecule adopts an α-helical structure that is independent of the type of hydrophobic environment (Lauterwein *et al.*, 1979). It spreads as a stable insoluble monolayer that can be compressed up to a collapse pressure of $19\,mN \cdot m^{-1}$ at the limiting molecular area of $1.78\,nm^2$ in agreement with Sessa *et al.* (1969) and a surface potential of 465mV. When injected into the subphase the values of surface pressure obtained varied with the concentration in a sigmoidal manner (Fig. 1), suggesting some co-operativity in the adsorption process. The maximum values of surface pressure and surface potential found in these conditions were equal to those obtained at the limiting molecular area of the surface-pressure–area isotherm of the spread film. If it is assumed that an α-helical structure with an inner diameter of 0.5nm (cf. Conn & Stumpf, 1976) and with lateral amino acid chains of average length of 0.5nm is oriented perpendicular to the interface, the molecular area is calculated as $1.80\,nm^2$, in agreement with the limiting area found in monolayers.

Albumin, a soluble protein (mol.wt. 67000; cf. Jones, 1975), gave reproducible surface-pressure–area curves by spreading, with a collapse pressure of $15\,mN \cdot m^{-1}$ at the limiting molecular area of $50\,nm^2$, in agreement with the work of Muramatsu & Sobotka (1962) and Mitchell *et al.* (1970), and a surface potential of 260mV. The maximum values of surface pressure and surface potential obtained when albumin was injected into the subphase were $10.8\,mN \cdot m^{-1}$ (Fig. 1) and 262mV respectively at a concentration of 200nM. The value for surface pressure is lower than that observed by spreading, suggesting either that less protein remains at the interface when the protein is injected into the subphase than when it is spread, possibly due to the progressive build-up of an interfacial energy barrier that opposes further adsorption (MacRitchie, 1978), or that a conformation with a different stability is reached in the two conditions. This latter possibility is less likely, since there are both theoretical and experimental reasons suggesting that the thermodynamically stable conformation adopted by proteins at interfaces by adsorption and spreading are similar (cf. Mitchell *et al.*, 1970; MacRitchie, 1978). In our experiments the surface-pressure–area isotherm of albumin and melittin showed the same behaviour when spread from aqueous- or organic-solvent solutions (see the Materials and methods section), solvents in which the original conformation of each protein is different (cf. Singer, 1971; Tanford, 1973). In addition, the values of surface potential obtained at each equilibrium pressure by adsorption did not differ by more than 10% of the values observed after spreading the protein at the interface and compressing it to the same surface pressure; also, if after a particular equilibrium pressure was reached by adsorption, the protein film was subsequently compressed, the film collapsed within $1\,mN \cdot m^{-1}$ of the pressure value obtained by spreading. These results also suggest that the surface characteristics of protein films are independent of the original protein conformation.

The overall results in Fig. 1 indicate that these different types of protein may lead to different values of surface free energy depending on the concentration of protein available and there seems not to be much difference in the surface pressure that can be reached at the interface by the proteins

considered 'soluble' or 'integral' of biological membranes studied.

Interaction of proteins with single-component lipid monolayers

Effect of the lipid–protein interaction on the interfacial free energy and electrical potential. As described before (Fidelio *et al.*, 1981) the changes in surface pressure and surface potential brought about by the injection of proteins into the subphase under the lipid monolayers studied were rapid and reached about 80% of the final value in 5–10 min after injection, as shown in Fig. 2 (inset). The values of surface pressure obtained depended on the type of lipid and protein in the system and are shown in Fig. 2(*a*). These values represent maximum surface pressures and increasing the concentration of protein by 100% in the subphase did not change the surface pressure by more than 10%.

The interactions of glycophorin and albumin with neutral or negatively charged glycosphingolipids were similar, with maximum values of surface pressure that did not differ by more than $4\,mN \cdot m^{-1}$. This was also found for the interactions of melittin and myelin basic protein with neutral glycosphingolipids. By contrast, the interaction of these basic proteins with negatively charged glycosphingolipids, such as sulphatide and gangliosides, occurred with higher values of surface-pressure increments. Since higher values of surface pressure indicate lower values of surface free energy, the latter interfaces are more stable than those obtained with the basic proteins and neutral glycosphingolipids or with all the lipids studied with albumin or glycophorin. These interactions can lead to considerable decreases in surface free energy. If it is assumed that the spontaneous incorporation of protein into the lipid interface is occurring at the cost of an increase in packing along the pressure–area isotherm of the pure lipid, it can amount to a gain in the system's free energy of more than 4.9 kJ/mol. These values are higher than the gain in excess free energy of mixing obtained through the spontaneous interaction of gangliosides with phosphatidylcholine (Maggio *et al.*, 1978*b*) in monolayers. Therefore, in the absence of other constraints it is likely that the negatively charged glycosphingolipids would be preferentially associated with basic proteins in a more-complex lipid–protein system, such as a biological interface, and this has been found to be so in some cases (Brunngraber & Ziboh, 1974). As described previously (Fidelio *et al.*, 1981), the complexity of the neutral oligosaccharide chain does not affect the lipid–protein interactions at high protein concentration and a similar effect of a negative charge in the polar head group of the glycolipid was obtained for sulphatide compared with GalCer or for the more complex ganglioside

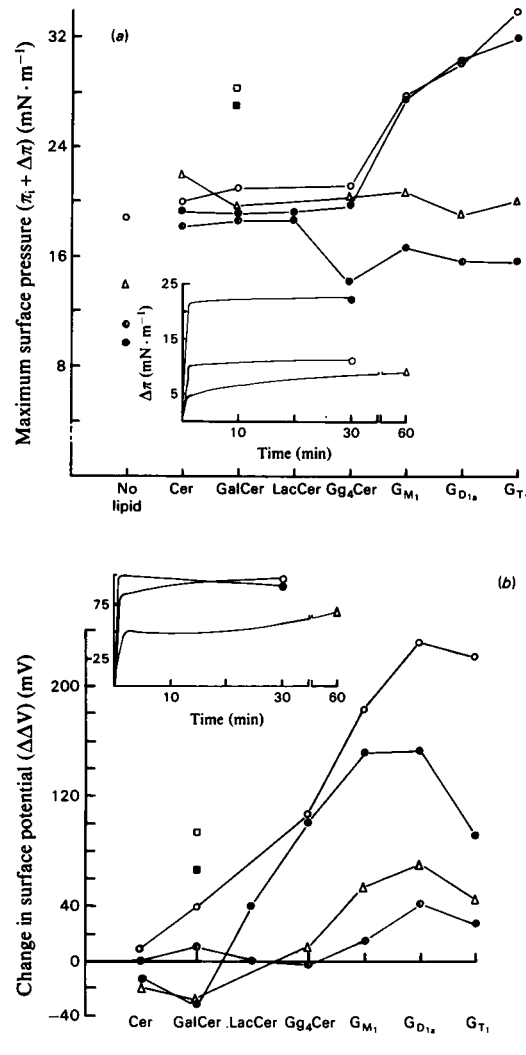

Fig. 2. *Effect of glycosphingolipid–protein interactions on the surface pressure and surface potential*

The Figure shows maximum values of surface pressure ($\pi_i + \Delta\pi$) (*a*) and changes in surface potential (*b*) induced in the glycosphingolipid monolayers indicated at an initial surface pressure of $10\,mN \cdot m^{-1}$ by: ○, melittin (88 nM for Cer to G_{M_1} and 176 nM for $G_{D_{1a}}$ and G_{T_1}); ◐, albumin (200 nM for Cer to Gg_4Cer and 300 nM for G_{M_1} to G_{T_1}); ●, myelin basic protein (108 nM for Cer to G_{T_1}); △, glycophorin (200 nM for Cer to G_{T_1}). Interaction of sulphatide with melittin (88 nM; □) and myelin basic protein (108 nM; ■) is also shown; the interaction of sulphatide with albumin and glycophorin was similar to that of GalCer. The insets show the time-course for the increases in surface pressure (*a*) and changes in surface potential (*b*) for the interaction of: G_{T_1} with 108 nM-myelin basic protein (●); Gg_4Cer with 88 nM-melittin (○); $G_{D_{1a}}$ with 200 nM-glycophorin (△).

G_{M_1} compared with Gg_4Cer (Fig. 2*a*). For melittin and myelin basic protein at a lower concentration of protein injected, however, the neutral oligosaccharide chain inhibits the penetration according to its complexity (Fidelio *et al.*, 1981).

The increase in surface pressure obtained for the penetration of melittin or myelin basic protein into gangliosides, as these contain more sialosyl residues, suggests that the interaction is dependent on the number of negative charges in the glycolipid. However, as shown in Fig. 3, a greater stability does not seem to be only a consequence of simple electrostatic interactions between an anionic lipid and a basic protein, since the penetration of melittin, myelin basic protein, glycophorin or albumin with neutral or acidic phospholipids was similar to that obtained with neutral glycosphingolipids.

The interactions do not reveal a simple preference of a protein for a particular physical state of the lipid at the interface, since melittin showed a different penetration into the similarly liquid-expanded sulphatide and Gg_4Cer (cf. Maggio *et al.*, 1978*a*; Monferrán *et al.*, 1979), but a similar penetration into the liquid-expanded Gg_4Cer or the more liquid-condensed $(Pam)_2PtdCho$ (cf. Phillips, 1972). In addition, glycophorin or albumin did not show much difference in their ability to penetrate into different kinds of lipids, regardless of their interfacial physical state. The similar interactions of glycophorin with any of the lipids studied found herein is in agreement with the work of Lee & Grant (1980), who found that the oligosaccharide chain behaviour in glycophorin was independent of the composition of the lipid with which the protein was interacting. On the other hand, van Zoelen *et al.* (1977) found a preferential interaction ascribed to 'glycophorin' with negatively charged phospholipids. However, it must be pointed out that a desialylated glycophorin, rather than the native protein, was employed in their experiments.

It is noteworthy that maximum values of surface pressure in the range 18–24 $mN \cdot m^{-1}$ were obtained for melittin, albumin, glycophorin and myelin basic protein penetrating into spread melittin films (Fig. 3, inset). This indicates that arrangements of a similar stability at the interface can be obtained either by lipid–protein or protein–protein interactions. These effects may be of importance in biomembranes where the protein/lipid ratio is high and protein–protein interactions should represent an important contribution to the interfacial stability.

The changes of surface potential showed, in general, a tendency to increase towards more positive values as the oligosaccharide chain of the glycosphingolipid becomes more complex up to ganglioside $G_{D_{1a}}$ (Fig. 2*b*). The interactions of all four proteins with ganglioside G_{T_1} tended to shift the positive interfacial potential to lower values. These

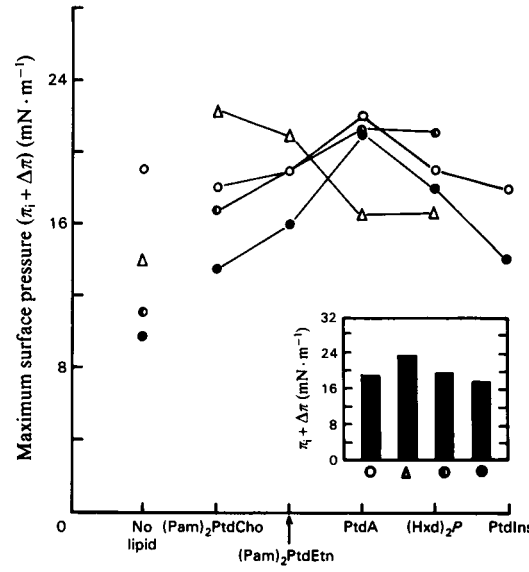

Fig. 3. *Effect of phospholipid–protein and protein–protein interaction on the surface pressure*

Maximum values of surface pressure ($\pi_i + \Delta\pi$) for the interaction of the phospholipid indicated at an initial surface pressure of 10 $mN \cdot m^{-1}$ with: 88 nM-melittin (○); 200 nM-albumin (◐); 108 nM-myelin basic protein (●); 100 nM-glycophorin (△). The inset shows the interaction of melittin at an initial surface pressure of 10 $mN \cdot m^{-1}$ with: 88 nM-melittin (○); 200 nM-albumin (◐); 108 nM-myelin basic protein (●); 100 nM-glycophorin (△).

results indicate that for similar values of final surface free energy the dipolar organization at the interface can be quite different (compare the system myelin basic protein–G_{T_1} and melittin–G_{T_1}, with maximum surface pressures of 32 and 34 $mN \cdot m^{-1}$ respectively, showing interfacial potentials differing by more than 120 mV).

In agreement with some previous results (Fidelio *et al.*, 1981) another parameter influencing the establishment of a particular value for the interfacial free energy is the concentration of protein in the subphase. Fig. 4 shows that at low concentrations of melittin the penetration into films of $G_{D_{1a}}$ and Gg_4Cer is similar, but becomes higher for the negatively charged lipid as the protein concentration is increased. On the other hand, at low protein concentration, the penetration of myelin basic protein into GalCer was more than three times that into sulphatide, whereas at higher protein concentration the penetration into this lipid was about twice that into GalCer. Similarly, Fig. 4 (inset) shows that the penetration of albumin into $G_{D_{1a}}$ is

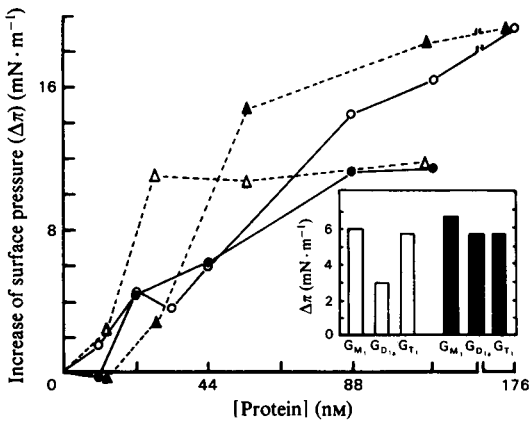

Fig. 4. *Effect of the protein concentration on the surface pressure*

The increases in surface pressure are shown for the interaction of melittin (——) with $G_{D_{1a}}$ (○) and Gg_4Cer (●) at an initial surface pressure of $10\,mN \cdot m^{-1}$, and for the interaction of myelin basic protein (– – –) with GalCer (△) and sulphatide (▲) at an initial surface pressure of $5\,mN \cdot m^{-1}$. The inset shows the interaction of the glycosphingolipids indicated with 200nM-albumin (□) and 300nM-albumin (■).

less than that into G_{M_1} or G_{T_1} at a protein concentration of 200nM, but becomes similar at 300nM. A similar dependence of penetration on the protein concentration has been reported by Hanssens & van Cauwelaert (1978) for α-lactalbumin interacting with some phospholipid monolayers.

With respect to the dependence of penetration on the initial surface pressure, it was reported previously (Fidelio *et al.*, 1981) that at protein concentrations below those required to reach a maximum value of surface-pressure increase the penetration is not always inversely proportional to the initial surface pressure and some optima for the interactions were found (results not shown). However, at high protein concentrations (leading to maximum values of surface-pressure change) the usual decrease in penetration was observed with an increase in the surface pressure of the lipid film (cf. Quinn & Dawson, 1969; Phillips *et al.*, 1975; Fidelio *et al.*, 1981).

Effect of the lipid–protein interaction on the interfacial properties of individual components. For some lipid–protein or protein–protein interactions the increases in surface pressure are considerable even when the initial surface pressure of the lipid or protein interface is equal to, or higher than, the equilibrium pressure corresponding to the injection of the same amount of protein under a clean interface. This may lead to surface-pressure values that can be well above the collapse or equilibrium pressure of the protein itself. The results in Figs. 2(*a*) and 3 show that the values of surface pressure obtained are clearly above the equilibrium or collapse pressure of the protein for the interactions of melittin with sulphatide and gangliosides, albumin with melittin, zwitterionic and acidic phospholipids or Cer, GalCer, LacCer and sulphatide, glycophorin with melittin, all the glycosphingolipids and zwitterionic phospholipids and myelin basic protein with melittin and all the lipids studied. These results indicate that the surface properties of proteins can be modified, become more stable at the interface and can support a higher lateral pressure or packing as a consequence of the interactions occurring with certain lipids or proteins. This increase in stability of proteins as a consequence of its association with lipids occurred with both the 'soluble' and 'membrane' proteins studied.

The stability of the lipids at the interface, in turn, can be modified by interaction with proteins (Fidelio *et al.*, 1981). This has been studied in experiments in which the collapse pressure obtained by compression of the penetrated film, after reaching equilibrium in conditions of maximum penetration, was compared with the collapse pressure of a monolayer of pure lipid. Some results obtained with melittin and myelin basic protein are shown in Table 1. In general, it was found that monolayers of lipids that, without protein, show collapse pressures of about $45\,mN \cdot m^{-1}$, or lower, exhibited values of collapse pressure of the protein-penetrated films that were significantly higher by about $4\,mN \cdot m^{-1}$. Conversely, lipids that in single-component monolayers showed collapse pressures of more than $55\,mN \cdot m^{-1}$ led to significant decreases of about $2–4\,mN \cdot m^{-1}$ after interaction with these proteins. These interactions therefore appear to have a 'buffering' effect on the overall maximum stability of the interface. In conditions of lower penetration, such as those obtained at high initial surface pressure of the lipid film or low protein concentration in the subphase, the collapse pressure of the penetrated lipid–protein film is more similar to the value exhibited by the monolayer of the pure lipid. This can be seen in Table 1 by comparing values of collapse pressure for penetrated films of melittin–GalCer at the same protein concentration (≥44nM) and different values of initial surface pressure, or the penetrated films of sulphatide–melittin at an initial surface pressure of $20\,mN \cdot m^{-1}$ and different concentration of melittin.

Interaction of proteins with mixed-lipid monolayers

These experiments were undertaken to investigate the influence that the composition of a lipid interface may have on the lipid–protein interaction. To this

Table 1. *Effect of the glycosphingolipid–protein interaction on their individual surface properties*

Results are means ± S.E.M. for the numbers of determinations indicated in parentheses. π_i indicates the initial surface pressure of the lipid monolayer; π_c represents the collapse pressure of the lipid or lipid–protein film. *P* was calculated by Student's *t* test for non-correlated samples. Abbreviations used: NS, non-significant; MBP, myelin basic protein.

Lipid monolayer	π_i ($mN \cdot m^{-1}$)	π_c of lipid ($mN \cdot m^{-1}$)	[Protein] (nM)	π_c of penetrated film ($mN \cdot m^{-1}$)	*P*
Cer	5	36.8 ± 0.6 (5)	Melittin ⩾ 44	40.0 ± 0.7 (7)	<0.01
Cer	5	36.8 ± 0.6 (5)	MBP ⩾ 54	40.3 ± 0.5 (8)	<0.01
GalCer	2	45.4 ± 0.3 (11)	Melittin ⩾ 88	49.0 ± 0.2 (8)	<0.001
GalCer	5	45.4 ± 0.3 (11)	Melittin ⩾ 44	47.6 ± 0.6 (5)	<0.01
GalCer	10	45.4 ± 0.3 (11)	Melittin ⩾ 44	45.2 ± 0.6 (5)	NS
GalCer	20	45.4 ± 0.3 (11)	Melittin ⩾ 44	44.6 ± 0.5 (5)	NS
Sulphatide	5	58.3 ± 0.2 (7)	Melittin ⩾ 44	55.5 ± 0.6 (4)	<0.001
Sulphatide	10	58.3 ± 0.2 (7)	Melittin ⩾ 44	54.8 ± 0.3 (5)	<0.001
Sulphatide	20	58.3 ± 0.2 (7)	Melittin ⩾ 44	55.5 ± 0.3 (4)	<0.001
Sulphatide	20	58.3 ± 0.2 (7)	Melittin < 44	58.0 ± 0.4 (3)	NS
Sulphatide	10	58.3 ± 0.2 (7)	MBP ⩾ 54	54.6 ± 0.3 (2)	<0.001
Cg_4Cer	5	62.3 ± 0.6 (6)	Melittin ⩾ 44	59.6 ± 0.5 (4)	<0.001
G_{M_1}	5	55.3 ± 0.6 (8)	Melittin ⩾ 88	50.0 ± 0.0 (3)	<0.001
G_{M_1}	10	55.3 ± 0.6 (8)	Melittin ⩾ 88	51.7 ± 0.9 (3)	<0.01

purpose, the sulphatide/melittin system was routinely used because of the ease of obtaining the components in purified form compared with the others and the relatively high values for surface-pressure increases obtained, which made comparisons possible.

Fig. 5(*a*) shows the penetration of melittin into films of sulphatide and $(Pam)_2PtdCho$ at different interfacial molar fractions. As the lipid interface is gradually enriched in sulphatide the increase in surface pressure brought about by the penetration of melittin increases rather rapidly and, at molar fractions of sulphatide greater than 0.3, values for surface-pressure increases within $1 mN \cdot m^{-1}$ of those obtained for a pure sulphatide film were obtained. The effect was similar at other initial surface pressures of the lipid films, for different concentrations of protein and for the myelin basic protein (results not shown). These results indicate that the ability of these proteins to penetrate a lipid interface is not a simple proportional function depending only on the additive behaviour of the individual interactions established and that there are thresholds in composition (i.e. between molar fractions of 0.1 and 0.3 for the melittin/sulphatide system), at which the behaviour can abruptly shift from that corresponding to one type of lipid–protein interaction to the other. These effects are not exclusive for the penetration of proteins into lipid interfaces; they were previously reported for tocopherols penetrating phospholipid films (Maggio *et al.*, 1977*a*).

Particular interactions established in the mixed-lipid–protein interface seem to participate in these effects, since the proportion of glycosphingolipid to phospholipid is not the only requirement for a non-proportional behaviour. This is indicated from the comparison of the penetration of melittin into mixed films of sulphatide with $(Pam)_2PtdEtn$ (Fig. 5*b*) or into monolayers of GalCer mixed with $(Pam)_2PtdCho$ (Fig. 5*c*), in which the increases of surface pressure were linearly proportional to the composition of the lipid interface. This differential behaviour can probably be related to the different interactions between the lipids, since GalCer shows interactions with phosphatidylcholine of the same type as those exhibited by sulphatide with phosphatidylethanolamine, and both are characteristically different from those shown by sulphatide with phosphatidylcholine (Monferrán *et al.*, 1979). Preliminary results suggest that dipolar interactions between sulphatide and a phosphate group may be mediating the non-proportional penetration of the protein. This is supported by the finding that a similar behaviour to that obtained in the system sulphatide–$(Pam)_2PtdCho$ has been found for melittin interacting with mixed films of sulphatide–$(Hxd)_2P$ (Fig. 5*d*), and this was abolished if the mixed film contained hexadecyltrimethylammonium (Fig. 5*f*), which is known to establish strong dipolar interactions with the phosphate group of $(Hxd)_2P$ (cf. Shah, 1970; Maggio & Lucy, 1976). However, when $(Pam)_2PtdEtn$ is mixed with sulphatide the phosphate group of this phospholipid does not lead to the same behaviour towards the protein, and a penetration proportional to the mole fraction of each lipid was found. Obviously the rest of the polar

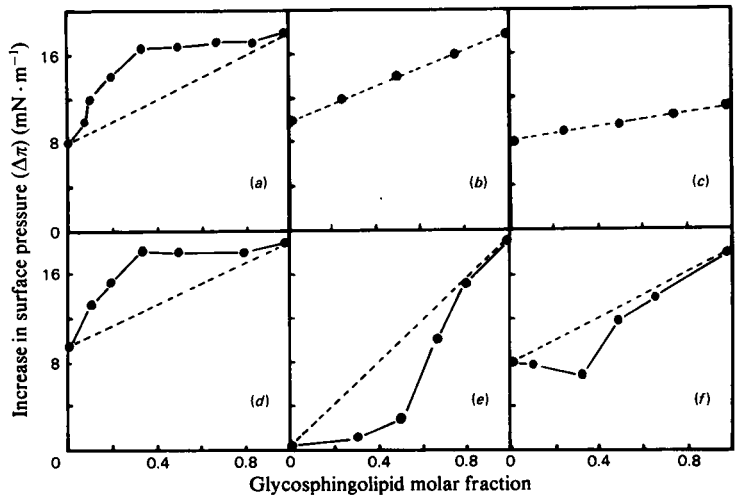

Fig. 5. *Effect of the interfacial lipid composition on the lipid–protein interactions*

The increases in surface pressure induced by melittin (88nM) on monolayers of GalCer or sulphatide mixed with other lipids in different proportions, at an initial surface pressure of 10nN · m^{-1}, are shown for: (*a*), sulphatide–(Pam)$_2$PtdCho; (*b*), sulphatide–(Pam)$_2$PtdEtn; (*c*), GalCer–(Pam)$_2$PtdCho; (*d*), sulphatide–(Hxd)$_2$*P*; (*e*), sulphatide–hexadecyltrimethylammonium; (*f*), sulphatide–((Hxd)$_2$*P*/hexadecyltrimethylammonium, 1:1).

moiety in the phospholipid is constraining the phosphate-group behaviour towards sulphatide and the protein in a different manner in (Pam)$_2$PtdCho and (Pam)$_2$PtdEtn. Some lipid–lipid interactions have been found previously to involve the particular properties of the polar group of phosphatidylcholine compared with phosphatidylethanolamine. In these cases it was suggested (Maggio & Lucy, 1976) that it could be related to the greater electrostatic constraints between the ammonium nitrogen and phosphate oxygen atoms in the latter phospholipid, which is known to lead to a greater stability with a more rigid and less hydrated lattice (cf. Hauser *et al.*, 1981) than in phosphatidylcholine.

In conclusion, it was shown that the interactions of the soluble and membrane proteins studied depend on the polar head group of the lipid, the concentration, interfacial and molecular features of the protein, the initial composition and packing of the surface and may lead to modifications of the individual properties of both the glycosphingolipid and the protein.

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