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Effect of calcium and temperature on mixed lipid-valinomycin monolayers

A comparison of glycosphingolipids (ganglioside GT1b, sulphatides) and phosphatidylcholine

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The influence of calcium and temperature on pure lipid (bovine brain PC, sulphatides, ganglioside GT1b), valinomycin and mixed lipid-valinomycin monolayers at the air/water interface was studied. In mixed films, evidence was found that the two components were miscible. On the other hand, at higher surface pressures, phase separation occurs in the cases of PC and sulphatides. Measuring the area requirement and the collapse pressure the stability of both lipid and the peptide was increased in particular due to ganglioside-valinomycin interaction. The addition of 10^{-5} M calcium into the subphase at 20 and 37°C and surface pressures of 10 and 20 mN/m led to a condensing effect in ganglioside mixtures, with formation of aggregates as indicated also by the nearly ideal behaviour of two component monolayers.

Lipid-valinomycin monolayer; Glycosphingolipid; Phosphatidylcholine; Ca^{2+}; Temperature; Surface pressure

1. INTRODUCTION

In biological membranes the lipids are assumed to be laterally distributed and changes in their composition and distribution could be responsible for effects on peptides. The nature of the interaction between lipids and membrane peptides, however, is still an open question. Investigations on monolayers as artificial and simple semimembrane systems might be a possible means of analyzing the physicochemical behaviour of different cell membrane molecules and their mutual interactions. This method reflects the lipid asymmetry between the outer and inner leaflet of the membrane bilayer and suggests that each of these layers is to some extent independent of the other in its physicochemical properties [1,2]. Gangliosides are glycosphingolipids containing different numbers of negatively charged sialic acids. These molecules are highly enriched in nerve cell membranes of vertebrates and might be intimately involved in neuronal functions. The effect of gangliosides on functional membrane proteins has been intensively investigated [3], but their direct relation to membrane properties still remains open. With regard to synaptic transmission, calcium-ganglioside complexes may act as neuromodulators, in particular, under different environmental conditions [4–6].

Sulphatides, negatively charged glycosphingolipids without sialic acid, are important constituents of myelin membranes and phosphatidylcholine is one of the most important lipid components of cell membranes.

In experimental membrane research the cyclododecadepsipeptide, valinomycin, can be regarded as an important example of a diffusible ion carrier consisting of a hydrophobic exterior and a polar interior ring.

In this study, the interactions of glycosphin-

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golipids (ganglioside GT1b, sulphatides) and phosphatidylcholine with valinomycin in mixed monolayer films were investigated, by recording the surface pressure/molecular area isotherms. We expect that the average area/molecule is modified as a consequence of lipid-peptide interactions which may be modified in some cases by addition of calcium and by change of temperature.

2. MATERIALS AND METHODS

The trisialoganglioside, GT1b, was purchased from Biosynth AG (Staad, Switzerland), sulphatides and bovine brain lecithin were obtained from Sigma (München, FRG), and valinomycin was obtained from Boehringer-Mannheim (Mannheim, FRG). Monolayers were formed on 5 mM triethanolamine (TEA)/HCl buffer (pH 7.4) as a subphase containing 0.01 mM $CaCl_2$ in some cases. The surface pressure/area isotherms were measured at three temperatures (10, 20 and 37°C). A rectangular Teflon trough (area, 345 cm^2; depth, 0.8 cm) was used, with a constant compression rate of 20 cm^2/min. The surface pressure was measured with a Wilhelmy balance, using a filter paper. All individual samples and mixtures had a concentration of 1 mM and were spread as solutions in chloroform/methanol (2:1, v/v) by means of a Hamilton syringe. Further details have been described in a previous paper [7].

3. RESULTS AND DISCUSSION

The surface pressure/area isotherms of lipids and valinomycin and the influence of temperature (10, 20 and 37°C) and 0.01 mM $CaCl_2$ on the surface behaviour of pure monolayers are shown in fig.1. During compression of films, the glycosphingolipid isotherms exhibited a shoulder, which can be interpreted as a transition between a liquid-expanded and a liquid-condensed phase [8–10]. At 37°C, no phase transition is observed, and the collapse pressure is reduced for all lipid as compared to 10 and 20°C. In the case of sulphatides, the curves are shifted to lower values of area/molecule at a given surface pressure and the collapse pressures increased as compared to the ganglioside, due to the decreased number of carbohydrate residues. This is qualitatively in agreement with Maggio et al. [11], insofar as the introduction of negatively charged sialyl residues causes a decrease of the collapse pressure. Generally, in the presence of Ca^{2+}, a condensing effect on glycosphingolipid films could be detected, the phase transitions occurred at lower surface pressures and the collapse pressures increased

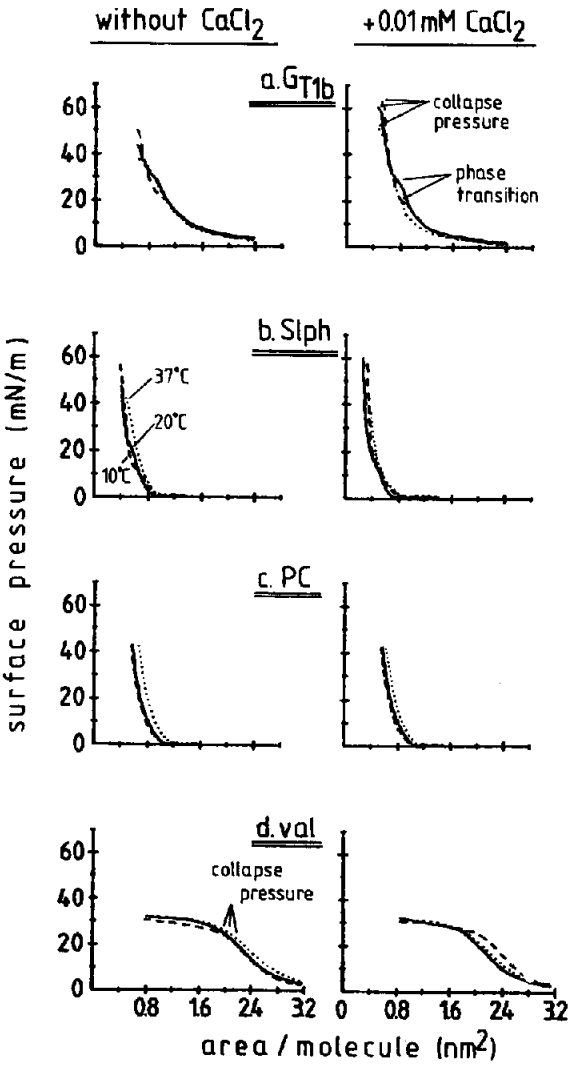

Fig.1. Surface pressure/area isotherms of G_{T1b} (a), Slph (b), PC (c) and valinomycin (d) at different temperatures: 10°C (– – –); 20°C (——); 37°C (···). Subphase: 5 mM triethanolamine/HCl buffer (pH 7.4) with and without $CaCl_2$.

significantly. The surface pressure/area isotherms for PC showed rather condensed films under all conditions tested and nearly no temperature- and calcium-dependent variations were detected. Valinomycin monolayers were only slightly affected by changes in temperature and calcium. This may simplify the analysis for mixtures of the peptide and lipids, since only the molecular areas of the lipids are influenced by both parameters [12].

Mixed monolayers were formed with lipid/valinomycin molar ratios between 40:1 and 1:2. Surface pressure/area isotherms are presented in fig.2. The recorded surface pressure follows the same curve upon expansion as on compression, showing that there are no relaxation processes involved in the measurement of the surface pressure/area isotherms under our conditions.

The mixtures lecithin (PC)/valinomycin and sulphatides (Slph)/valinomycin show a similar behaviour, whereas mixtures of the ganglioside GT1b/valinomycin behave differently. The collapse of the peptide clearly occurs at the same surface pressure (around 30 mN/m) in mixtures with PC and Slph, independent of the molar fraction of the peptide. The collapse pressure of the lipid is only slightly influenced by the presence of the peptide.

In contrast, for mixed monolayers of GT1b and valinomycin this collapse moves gradually to higher surface pressures with an increasing fraction of the ganglioside. The collapse pressure of the lipid is also influenced by moving up from 50 mN/m (GT1b/val = 1:2) to 65 mN/m (GT1b) in the presence of Ca^{2+}.

These results indicate a stronger interaction between the ganglioside and valinomycin than between the phospholipid or sulphatides and the peptides, respectively. In fact, the peptide and the phospholipid or sulphatide may not form a mixed monolayer but may be organized in separate phases, whereas GT1b and valinomycin may form a mixed monolayer. Two criteria can be applied to characterize the monolayers with respect to miscibility: additivity rule for the mean area and the dependence of the collapse pressure on the

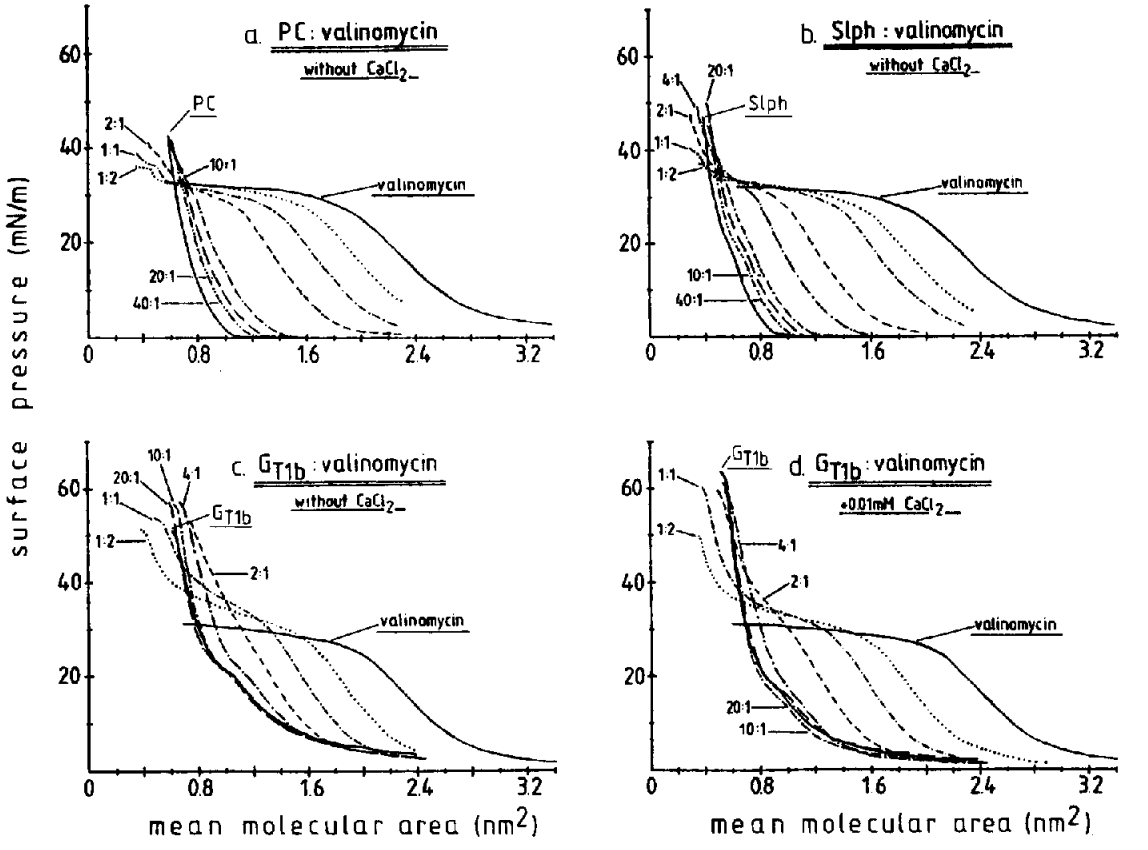

Fig.2. Isotherms of mixed monolayers lipid-valinomycin with different molar ratios: (a–c) without; (d) with 0.01 mM $CaCl_2$. The pure glycosphingolipids exhibited a lower collapse pressure without calcium and a higher collapse pressure with $CaCl_2$ than that of the mixtures.

mole fraction of one component. The additivity rule is expressed by [13],

$$A_{mix} = f_{lip} \cdot A_{lip} + f_{val} \cdot A_{val}$$

where A_{mix} is the mean molecular area of the mixture, f values are the mole fractions of the two components, and A values are the molecular areas of the pure component films. A deviation from the additivity indicates mixing of the components. When the results are in agreement with the additivity rule, the system could be ideal, i.e. ideal mixing without interaction terms, or a phase separated system. Then, the criteria of the dependence of the collapse pressure have to be applied. A constant collapse pressure with variation of the composition indicates phase separation, whereas a continuous change of the collapse pressure with an increasing fraction of one component is taken as evidence for homogeneous mixing.

The mean areas at 10 mN/m and 20 mN/m are plotted in fig.3 vs the fraction of valinomycin for mixtures of the peptide with the three lipids in the absence and the presence of Ca^{2+}. In the case of the phospholipid PC deviations from additivity to larger mean areas are observed both in the absence and presence of Ca^{2+}. This effect is more pronounced with the sulphatides. This is compatible with the formation of mixed films with a positive free energy of mixing at the surface pressures of 10 and 20 mN/m, respectively. However, the collapse pressure attributed to the valinomycin is independent of the fraction of the peptide in monolayers of both lipids (see fig.2). This leads to the conclusion that phase separation occurs in mixed monolayers of valinomycin with PC and sulphatides, respectively, at high surface pressures.

With monolayers of the ganglioside GT1b, the deviation of the mean area from additivity is negative in the absence of Ca^{2+}. In the presence of Ca^{2+}, the behaviour is nearly ideal. From the isotherms (see fig.2) it is seen that the collapse pressure of valinomycin increases steadily with an increasing fraction of GT1b in the mixture. Therefore, as already shown for the gangliosides GM1 and GD1a [14], GT1b and valinomycin form mixed monolayers with a homogeneous distribution of the components.

In the presence of Ca^{2+}, however, the condensing effect on the GT1b should lead to formation of ganglioside associates like dimers and higher aggregates that do not build up a separate phase. Such a situation is in agreement with the strongly reduced deviation from ideal behaviour in the presence of Ca^{2+}, with respect to monolayers in the absence of Ca^{2+}.

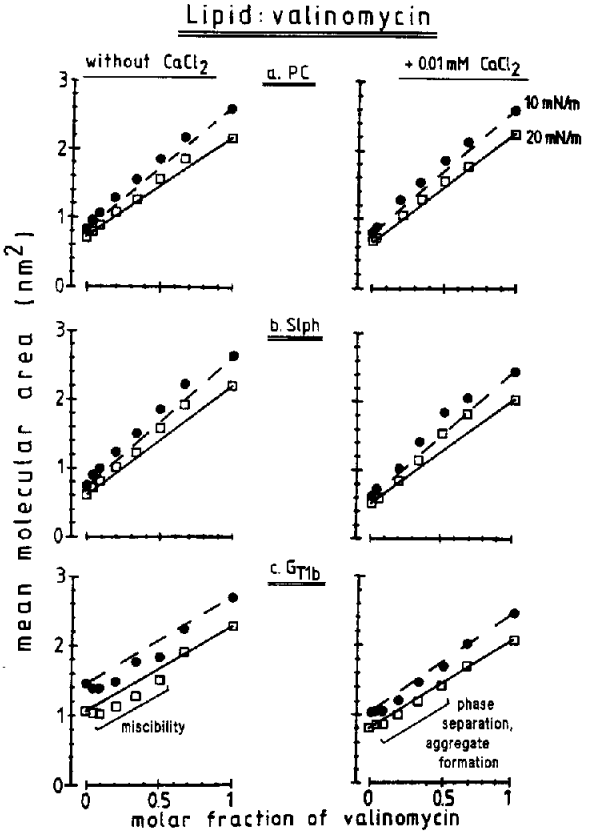

Fig.3. Interaction of lipid (PC, sulphatides, GT1b) and valinomycin. Mean molecular areas (y-axis) of mixed monolayers at surface pressures of 10 (●) and 20 (□) mN/m. 'Ideality lines' at 10 mN/m (– – –) and 20 mN/m (——) represent values calculated according to the additivity rule (Gaines), i.e., the components are immiscible (phase separation). Deviations (positive and negative) from ideality indicate miscibility of the components.

The possible involvement of gangliosides in the calcium-dependent short-term process of synaptic transmission and long-term thermal adaptation might be based on modulatory functions of gangliosides in nerve membranes [5]. In agreement with earlier results, our monolayer experiments demonstrate the stability, miscibility and phase separation of important membrane components, particularly gangliosides, in mixed monolayers.

Depending on the molar ratio, temperature and surface pressure, the availability or absence of free calcium could lead to local membrane variations like aggregate formations. Compared with phosphatidylcholine and sulphatides, there seems to be a more sensitive balance in ganglioside-peptide interactions, and this could have an effect on activation or inhibition of functional membrane proteins.

REFERENCES

[1] Schroeder, F. (1985) Subcell. Biochem. 11, 51–101.
[2] Smith, A.D. and Stubbs, C.D. (1986) in: Lipid Metabolism in the Normoxic and Ischaemic Heart (Stam, H. and Van der Vusse, G.J. eds) pp.93–97, Steinkopff, Darmstadt.
[3] Hakomori, S. (1987) in: Gangliosides and Modulation of Neuronal Functions (Rahmann, H. ed.) NATO ASI Series vol.7, pp.465–479, Springer, Berlin.
[4] Rahmann, H. (1983) Neurochem. Int. 5, 539–547.
[5] Rahmann, H. (1987) in: Gangliosides and Modulation of Neuronal Functions (Rahmann, H. ed.) NATO ASI Series vol.7, pp.501–521, Springer, Berlin.
[6] Probst, W. and Rahmann, H. (1987) in: Gangliosides and Modulation of Neuronal Functions (Rahmann, H. ed.) NATO ASI Series vol.7, pp.139–154, Springer, Berlin.
[7] Probst, W., Möbius, D. and Rahmann, H. (1984) Cell. Mol. Neurobiol. 4, 157–176.
[8] Curatolo, W., Small, D.M. and Shipley, G.G. (1977) Biochim. Biophys. Acta 468, 11–20.
[9] Baret, J.F., Bois, A.G., Dupin, J.J. and Firpo, J.L. (1982) J. Colloid Interface Sci. 86, 370–376.
[10] Gershfeld, N.L. (1982) J. Colloid Interface Sci. 85, 28–40.
[11] Maggio, B., Cumar, F.A. and Caputto, R. (1978) FEBS Lett. 90, 149–152.
[12] Fidelio, G.D., Maggio, B. and Cumar, F.A. (1986) Biochim. Biophys. Acta 854, 231–239.
[13] Gaines, G.L. (1966) Insoluble Monolayers at Liquid-Gas Interfaces, Wiley Interscience, New York.
[14] Beitinger, H., Probst, W., Möbius, D. and Rahmann, H. (1987) J. Biochem. 102, 963–966.