INTERACTIONS OF WATER-SOLUBLE FUSOGENS WITH PHOSPHOLIPIDS IN MONOLAYERS

Bruno MAGGIO* and Jack A. LUCY+

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, 8 Hunter Street, London WC1N 1BP, England

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1. Introduction

In earlier work it was proposed that unsaturated fatty acids and their derivatives may induce erythrocytes to fuse by increasing the proportion of hydrocarbon chains in the membrane that are in a relatively liquid state [1,2]. From a spectrofluorimetric study, it has been suggested that the action on erythrocyte ghosts of the fusogenic lipid, glycerol mono-oleate, is also at least partially a fluidizing one [3], and from investigations using the 'membrane mobility agent A_2C' it has been concluded that it seems reasonable to suppose that some increase in local fluidity in membranes favours cell fusion [4]. Similarly, the fusion of phospholipid vesicles with one another in model systems requires the lipids of the interacting membranes to be in a fluid state [5].

Bij contrast dimethyl sulphoxide, which enhances the rate of fusion of acidic phospholipid vesicles [6], as well as inducing cell fusion [7], has been found to produce an increase in the transition temperature of acidic dimyristoylphosphatidylglycerol membranes [8]. It was therefore proposed that membrane fusion induced by dimethyl sulphoxide may, like the action of Ca^{2+}, involve isothermal phase transitions in the lipids of membranes [9].

We have previously reported that water-soluble fusogens interact with and markedly decrease the surface potential of monolayers of phosphatidylcholine and phosphatidylethanolamine at the air–water interface [10]. Changes in the surface pressure–area curves were also noted earlier, and these are now reported in more detail.

* Present address: Departmento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Cordoba, Cordoba, Argentina

+ To whom reprint requests should be addressed

2. Materials and methods

The materials, methods and equipment used were as in [10].

3. Results

With dipalmitoylglycerylphosphorylcholine at low surface pressures, the small organic solutes studied expanded the phospholipid film. Figure 1 shows the force–area curves of dipalmitoylglycerylphosphorylcholine on subphases containing dimethyl superoxide or sucrose at different concentrations. At pressures below about 20–30 $mN.m^{-1}$ this phospholipid occupied increased areas per molecule on subphases containing dimethyl sulphoxide ($\geq$ 3 M) and sucrose ($\geq$ 1.5 M). Apart from the curve becoming more liquid-expanded and shifting to greater molecular areas at low pressures, the point of onset of the two-dimensional phase transition was also raised from about 6 $mN.m^{-1}$ in the absence of an organic solute to about 9 $mN.m^{-1}$ in its presence, in accordance with a more liquid character of the film. Similar changes (not shown) were found for sorbitol ($\geq$ 2 M) and, less markedly, for glycerol ($\geq$ 3.8 M). Contrary to the effects induced by the small organic solutes, poly(ethylene glycol)-mol. wt

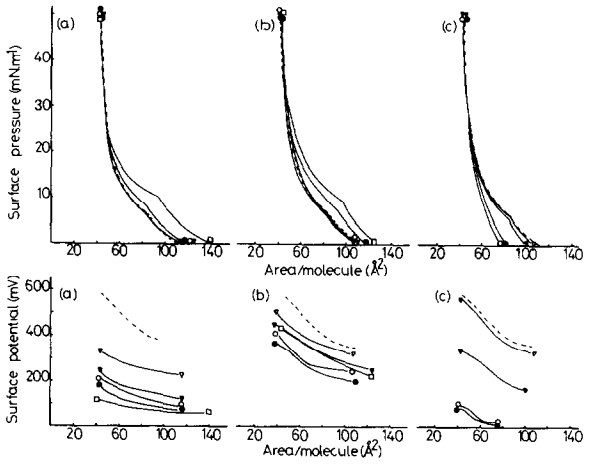

Fig.1. Surface pressure–area and surface potential–area curves for monolayers of dipalmitoylglycerylphosphorylcholine on different subphases. The figure shows the surface pressure–area (upper part) and surface potential–area (lower part) curves for monolayers of dipalmitoylglyceryl phosphorylcholine on different subphases. The subphase was 145 mM NaCl (pH 5.6) containing: dimethyl sulphoxide (a) at 0.2 M (▽), 0.5 M (▼), 2 M (○), 3 M (●) and 4.5 M (□); sucrose (b) at 0.2 M (▽), 0.5 M (▼), 1 M (○), 1.5 M (●) and 3 M (□); PEG-6000 (c) at 0.17 mM (▽), 2 mM (▼), 4.2 mM (○) and 17 mM (●). In each case the broken line represents the isotherm obtained on a subphase of 145 mM NaCl, at pH 5.6.

6000 (PEG-6000) condensed the film with elimination of the liquid-expanded–liquid-condensed phase transition (fig.1). Similar results were obtained with PEG-1500, PEG-3 × 10^5, and PEG-5 × 10^6, the concentration of polymer required to produce the change decreasing as its molecular weight increased. Dextran (mol. wt 82 000) did not produce changes in packing or surface potential at 0.9 mM, but a decrease of ~100 mV of the surface potential of the phospholipid monolayer, with no changes in packing, was observed at 2.8 mM.

In accordance with [10], both the small organic solvents and poly(ethylene glycol) caused decreases in surface potential, and fig.1 shows the changes observed with differing concentrations of dimethyl sulphoxide, sucrose, and PEG-6000.

For dipalmitoylglycerylphosphorylethanolamine the differential effect between the small organic solutes and PEG was not present, and all of the compounds induced increases of the area occupied per molecule of phospholipid and a decrease in its surface potential. Unlike dipalmitoylglycerylphosphorylcholine, changes in the molecular packing of dipalmitoylglycerylphosphorylethanolamine were found at pressure > 20 $mN.m^{-1}$. Figure 2 shows observations made for monolayers of this phospholipid on subphases containing dimethyl sulphoxide, sucrose and PEG-3 × 10^5. For subphases containing glycerol (≥ 0.5 M), sorbitol (≥ 0.5 M), PEG-1500 (≥ 10 mM) and PEG-6000 (≥ 0.2 mM) the results were similar to those for sucrose. For subphases containing PEG-5 × 10^6 the isotherms were similar to those observed with PEG-3 × 10^5 or dimethyl sulphoxide in which the expansion was also accompanied by the appearance of a phase transition. With 0.9 mM dextran (mol. wt 82 000) no changes in packing or surface potential were noted with respect to the isotherm on 145 mM NaCl but when 2.8 mM dextran was present in the subphase similar changes to those seen for PEG-3 × 10^5 occurred.

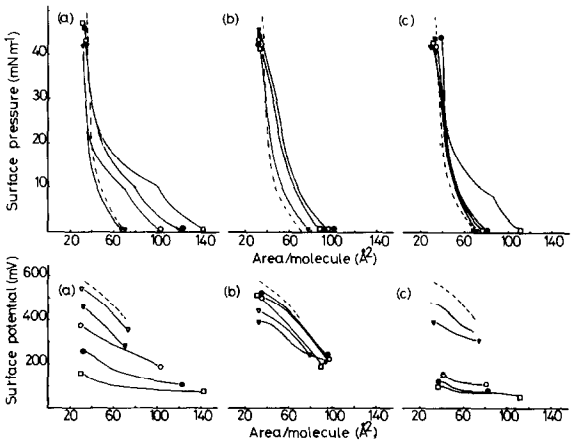

Fig.2. Surface pressure–area and surface potential–area curves for monolayers of dipalmitoylglycerylphosphorylethanolamine on different subphases. The figure shows the surface pressure–area (upper part) and surface potential–area (lower part) curves for monolayers of dipalmitoylglyceryl phosphorylethanolamine on different subphases. The subphase was 145 mM NaCl (pH 5.6) containing: dimethyl sulphoxide (a) at 0.1 M (▽), 0.2 M (▼), 0.5 M (○), 2 M (●) and 4.5 M (□); sucrose (b) at 0.5 M (▽), 0.8 M (▼), 1 M (○), 1.5 M (●) and 2 M (□); PEG-3 × 10^5 (c) at 5 nM (▽), 10 nM (▼), 60 nM (○), 0.125 μM (●) and 1 μM (□). In each case the broken line represents the isotherm obtained on a subphase of 145 mM NaCl, at pH 5.6.

4. Discussion

Although a precise molecular interpretation of our observations is not possible, some generalizations can nevertheless be made. All of the water-soluble compounds tested were able to cause noticeable decreases of the surface potential of the phospholipid monolayers at concentrations at which the changes in molecular area were not yet present. This and the fact that maximum changes were seen in the surface potential at high surface pressures, but much smaller or no modifications occurred in the molecular packing, suggest that the changes in the surface behaviour of these phospholipids (induced by the presence of the water-soluble fusogens in the subphase) primarily involve the properties of the phospholipid head groups. The water-soluble organic solutes can act as hydrogen bond donors and acceptors and are capable, as reported for glycerol [11] and alcohols [12], of changing the long-range structure of water in the bulk phase. This may affect the hydration, orientation and interactions taking place in and between the phospholipid polar head groups. Changes in the aqueous environment of the phospholipid polar head groups thus seem to be of great importance in determining the surface potential, molecular packing and fluidity of the phospholipids. Such changes are no doubt important in the increased permeability of erythrocytes to Ca^{2+} observed when the cells are treated with the water-soluble fusogens dimethyl sulphoxide and PEG [13].

The configurations of the hydrocarbon chains of phospholipids in liquid-condensed and liquid-expanded monolayers are normally expected to be similar to those of the chains in the gel and liquid crystalline states, respectively [14,15]. Thus it is usually assumed that there is an analogy between the two systems, and that changes of state occurring in phospholipid monolayers correspond to transitions occurring in the lyotropic mesomorphism of phospholipids. On this basis, DMSO apparently decreased the transition temperatures of both dipalmitoylglycerylphosphorylcholine and dipalmitoylglycerylphosphorylethanolamine, unlike the effect of DMSO on acidic phospholipids [8]. If it is possible to extrapolate from monolayers to membranes, it may be inferred that in the fusion of membranes containing phosphatidylcholine and phosphatidylethanolamine, rather than phosphatidylglycerol, small organic molecules act like fusogenic lipids to increase fluidity.

It is not at present clear why PEG apparently acts on phospholipid monolayers to increase the transition temperature of phosphatidylcholine (fig.1), and to decrease the transition temperature of phosphatidylethanolamine (fig.2). Caution is therefore necessary in attempting to extrapolate from these findings to the effects of the polymer on biological membranes in cell fusion. Such opposite effects of PEG on the two phospholipids in membranes would perhaps induce lateral phase separations that might lead to cell fusion [16]. It is important to note, however, that the concentrations of PEG used in our monolayer studies are much less than those normally needed to induce cells to fuse. Furthermore the relationship of interactions between solute molecules and phospholipids in monolayers to factors governing the behaviour of phospholipids in membranes may be complicated by special considerations applying to the compression of phospholipids at the air–water interface.

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