

Poly(Ethylene Glycol), Surface Potential and Cell Fusion

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(Received 28 May 1976)

Poly(ethylene glycol), glycerol and dimethyl sulphoxide markedly decrease the surface potentials of monolayers of phosphatidylcholine and phosphatidylethanolamine. This finding is discussed in relation to the properties of hen erythrocytes undergoing fusion induced by poly(ethylene glycol).

Poly(ethylene glycol)-6000 induces the fusion of plant protoplasts (Kao & Michayluk, 1974), hen erythrocytes (Ahkong *et al.*, 1975a), hen erythrocytes with yeast protoplasts (Ahkong *et al.*, 1975b), and mammalian cells (Pontecorvo, 1975). We have studied the actions on monolayers of phospholipids of poly(ethylene glycol), glycerol, sorbitol, sucrose and dimethyl sulphoxide, all of which fuse hen erythrocytes (Ahkong *et al.*, 1975a), to obtain information relevant to the molecular mechanisms by which these molecules may induce cell fusion.

Materials and Methods

AnalaR NaCl, sucrose, sorbitol, glycerol, poly(ethylene glycol) mol.wt. 1500 and poly(ethylene oxide) mol.wts. 3×10^5 and 5×10^6 were from BDH Chemicals, Poole, Dorset, U.K. Poly(ethylene glycol) and poly(ethylene oxide) describe the same polymer, $-(CH_2-CH_2-O)_n-$, prepared from different monomers. The term poly(ethylene glycol) is used in this present paper, individual polymers being identified by their molecular weight. Dimethyl sulphoxide was from Sigma (London) Chemical Co., London S.W.6, U.K. Dipalmitoylglycerolphosphorylcholine, dipalmitoylglycerolphosphorylethanolamine and poly(ethylene glycol) mol.wt. 6000 were from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Phospholipid monolayers were prepared from solutions in chloroform, and the surface pressures, molecular areas and surface potentials of the monolayers were recorded automatically, as described previously (Maggio & Lucy, 1975, 1976). All subphases contained NaCl (145mM); their pH was adjusted to 5.6 with HCl (5M) or NaOH (5M). The surface potential and surface pressure of each subphase were adjusted to give a zero reading before spreading of the phospholipid monolayer. Isotherms were obtained in duplicate; they were usually reproducible within

$\pm 0.02 \text{ nm}^2$, $\pm 1 \text{ mN} \cdot \text{m}^{-1}$ ($\pm 1 \text{ dyn} \cdot \text{cm}^{-1}$) and $\pm 10 \text{ mV}$. For some subphases containing high concentrations of organic solute, reproducibility was not better than 0.04 nm^2 in molecular areas, $\pm 2 \text{ mN} \cdot \text{m}^{-1}$ in surface pressure and $\pm 20 \text{ mV}$ in surface potential; the isotherm was then obtained in triplicate, and the curves were averaged.

Hen erythrocytes were prepared for experiments on cell fusion as described previously (Ahkong *et al.*, 1973).

Results

Phospholipid monolayers

Fig. 1 shows the surface potential-area and surface pressure-area curves for monolayers of phosphatidylcholine and phosphatidylethanolamine on subphases containing two small organic solutes and two polymers. Comparable observations (not shown) were made with subphases containing the other small organic solutes and polymers studied (see the Materials and Methods section). With subphases containing lower concentrations of organic solutes than those used to obtain the results shown in Fig. 1, the behaviour of the phospholipid monolayers progressively approached that observed on a pure NaCl subphase, both for surface potential (Fig. 2) and for surface pressure (not shown).

All of the compounds tested produced large decreases in surface potential with phospholipid monolayers at close molecular packings (Figs. 1a and 1b). With sorbitol (Figs. 2a and 2b), and with sucrose, there was an optimum concentration of organic solute (approx. 1M) for the maximum decrease in surface potential with monolayers of each of the phospholipids studied. Conceivably this may be related to the optimum concentration of sorbitol (2.5-3.0M) found previously for cell fusion (Ahkong *et al.*, 1975a). Interestingly, with the preparations of poly(ethylene glycol), lower concentrations of polymer were required to decrease

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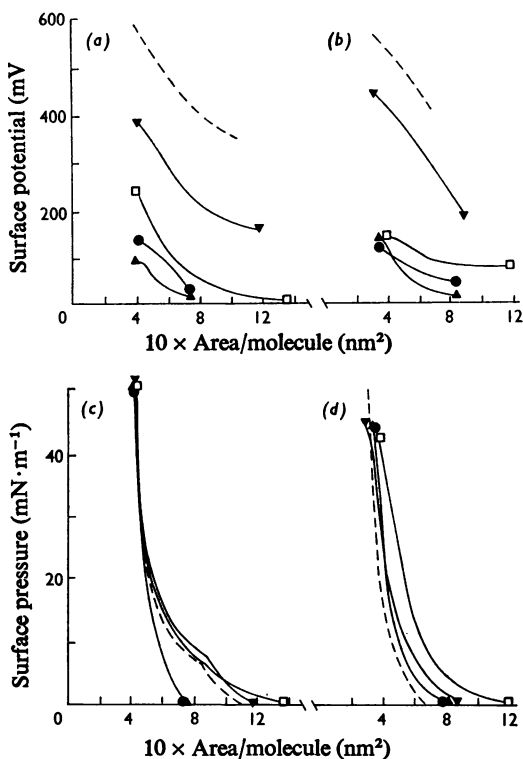


Fig. 1. Surface pressure–area and surface potential–area curves for monolayers of phospholipids on different subphases

The Figure shows surface potential–area curves for dipalmitoylglycerolphosphorylcholine (a) and dipalmitoylglycerolphosphorylethanolamine (b), and surface pressure–area curves for dipalmitoylglycerolphosphorylcholine (c) and dipalmitoylglycerolphosphorylethanolamine (d). The subphase was 145 mM-NaCl, pH 5.6, containing 5.4 M-glycerol (\square), 2 M-sorbitol (\blacktriangledown), 4.2 mM-poly(ethylene glycol)-6000 (\blacktriangle) or 0.5 μ M-poly(ethylene glycol)- 3×10^5 (\bullet). In each case the broken line represents the isotherm obtained on a subphase of 145 mM-NaCl.

the surface potential with increasing molecular weight (Figs. 2a and 2b).

For dipalmitoylglycerolphosphorylcholine below a surface pressure of about $30 \text{ mN} \cdot \text{m}^{-1}$, slightly greater areas per molecule were obtained with subphases containing glycerol ($\geq 3.8 \text{ M}$), dimethyl sulphoxide ($\geq 3 \text{ M}$), sucrose ($\geq 1.5 \text{ M}$) and sorbitol ($\geq 2 \text{ M}$), as compared with a subphase containing only NaCl (Fig. 1c). Expansion effects of partially expanded films and decreases of surface potential of lipid monolayers on subphases containing glycerol have been reported previously (Cadenhead & Bean, 1972). Smaller areas per molecule were obtained

below $30 \text{ mN} \cdot \text{m}^{-1}$ for subphases containing poly(ethylene glycol) of mol.wt. 6000 ($\geq 4.2 \text{ mM}$), of mol.wt. 1500 ($\geq 15 \text{ mM}$), of mol.wt. 3×10^5 ($\geq 0.25 \mu\text{M}$) and of mol.wt. 5×10^6 ($\geq 1 \text{ nM}$). At higher surface pressures the isotherms were like those on NaCl alone, indicating that alterations in molecular packing induced by the organic solutes occurred only when intermolecular interactions between the acyl chains were weak.

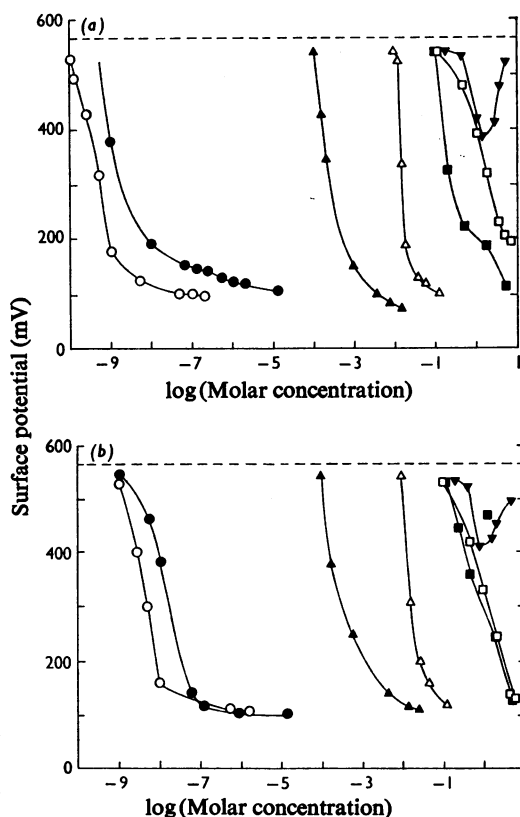
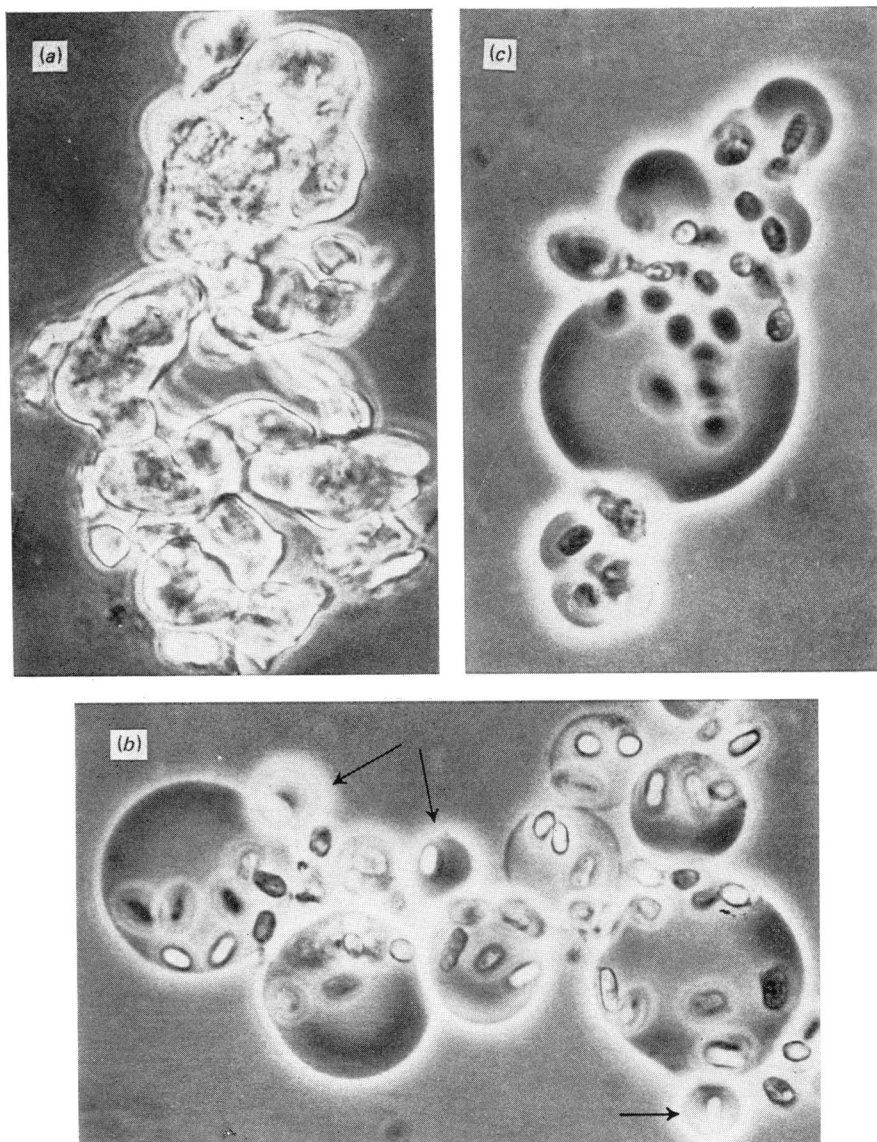


Fig. 2. Surface potentials of monolayers of phospholipids on subphases containing differing concentrations of organic solutes

The surface potentials of monolayers of dipalmitoylglycerolphosphorylcholine (a) and dipalmitoylglycerolphosphorylethanolamine (b), each at surface pressures of $35 \text{ mN} \cdot \text{m}^{-1}$, were derived from isotherms obtained on subphases of 145 mM-NaCl, pH 5.6, containing poly(ethylene glycol)- 5×10^5 (\circ), poly(ethylene glycol)- 3×10^5 (\bullet), poly(ethylene glycol)-6000 (\blacktriangle), poly(ethylene glycol)-1500 (Δ), dimethyl sulphoxide (\blacksquare), glycerol (\square) or sorbitol (\blacktriangledown). The horizontal broken line at 570 mV represents the values of the surface potentials for the phospholipid monolayers, spread at a surface pressure of $35 \text{ mN} \cdot \text{m}^{-1}$, on a subphase of 145 mM-NaCl.



EXPLANATION OF PLATE I

Morphological changes during cell fusion

(a) Hen erythrocytes (3×10^8 cells/ml), after incubation for 15 min at 37°C in 1 ml of modified Eagle's basal salt solution (1.8 mM-Ca^{2+}) at pH 7.4 (Ahkong *et al.*, 1973) containing poly(ethylene glycol)-6000 (400 mg/ml), showing extensive cellular aggregation and shrinking. In (b), the aggregated cells were diluted with the (warmed) salt solution (5 ml) free from poly(ethylene glycol), centrifuged (800g for 5 min), resuspended in the salt solution (1 ml), and incubated at 37°C for 30 min. Multinucleated and single swollen cells (arrowed) are present. The cells in (c) were treated as in (b), except that the salt solution used for dilution and for the second incubation contained EDTA (5 mM). Phase-contrast microscopy: magnification $\times 1000$.

Monolayers of dipalmitoylglycerolphosphoryl-ethanolamine exhibited greater areas per molecule at all surface pressures, for subphases containing poly(ethylene glycol)-6000 or poly(ethylene glycol)- 3×10^5 as well as with glycerol and sorbitol (Fig. 1*d*). The minimum concentrations of the components in the subphase at which the changes occurred were lower than with phosphatidylcholine: $\geq 0.5M$ for glycerol, dimethyl sulphoxide, sucrose and sorbitol, $\geq 0.2M$ for poly(ethylene glycol)-6000, $\geq 10mM$ for poly(ethylene glycol)-1500, $\geq 60\mu M$ for poly(ethylene glycol)- 3×10^5 and $\geq 0.25mM$ for poly(ethylene glycol)- 5×10^6 .

Cell fusion

Preparations of poly(ethylene glycol) having mol.wts. of 1500, 6000, 3×10^5 and 5×10^6 gave maximum agglutination of hen erythrocytes (see Plate 1*a*) during a 30min incubation at 37°C (pH 7.4) in progressively decreasing concentrations, namely 266mM, 66mM, 0.17mM and 5 μM respectively. Cellular aggregation might therefore involve polymer bridging between cells (Vincent, 1974). However, a comparison of the polymer concentrations for maximum cellular agglutination with those giving maximum decrease in the surface potential of phospholipid monolayers (Figs. 2*a* and 2*b*) indicates that changes in surface potential may also be involved in cellular agglutination.

The fusion of hen erythrocytes by poly(ethylene glycol) is apparently not related to molecular weight in a simple manner, since polymers with mol.wts. of 1500 and 3×10^5 were less effective, and 5×10^6 much less effective, than poly(ethylene glycol)-6000 in causing cell fusion. Cells that were agglutinated and made to shrink by treatment with poly(ethylene glycol)-6000 (66mM) did not fuse *in situ*, possibly because polymer molecules between the cells act as a barrier. Extensive fusion occurred, after removal of most of the poly(ethylene glycol), between swollen less-aggregated cells (Plate 1*b*). Excess of EDTA (approx. 4mM) inhibited fusion when present throughout, but it was unable to inhibit fusion when added after the cells had been incubated with the polymer in the presence of Ca^{2+} (Plate 1*c*). Once events leading to cell fusion have been initiated by the treatment with poly(ethylene glycol)-6000, exogenous Ca^{2+} is thus not required for fusion to be completed in this system.

Discussion

The large decreases of surface potential induced by individual organic solutes were of the same magnitude for both phospholipids (Fig. 2), and, since the synthetic phospholipids used have the same fatty acyl chains, the contributions made by the

hydrocarbon chains to the vertical component of the surface dipole and to the surface potential are presumably similar for both molecules (Davies & Rideal, 1963). Nevertheless no precise molecular interpretation of the observed changes in surface potential can be given, because the relative contributions of the phospholipid head groups and solute molecules to the measured surface potential cannot be separated. Interactions of the polar head groups of the phospholipids with the organic solutes probably occur. The solutes may also introduce various structural changes in the bulk-phase water (Tanford, 1961), which induce alterations in the orientation and hydration of the phospholipid head groups.

Fusogenic lipids interact with monolayers of phosphatidylcholine to allow closer packing in the monolayer and a rather small decrease in the surface potential (Maggio & Lucy, 1975, 1976). Since poly(ethylene glycol)-6000 is relatively non-toxic (as compared with fusogenic lipids), gives an exceptionally high incidence of chemically induced cell fusion (Pontecorvo, 1975) and decreases the surface potential of lipid monolayers by several hundred millivolts, a decrease in the surface potential of biological membranes may be of major importance in membrane fusion. Conversely, the toxic effects of lipid fusogens might result from an undue perturbation of the lipid bilayer of membranes.

Maroudas (1975) has suggested that glycerol and dimethyl sulphoxide decrease the exclusion volumes of membrane glycoproteins, thus facilitating cell adhesion by allowing naked lipid bilayers in the membranes of adjacent cells to interact; cf. also the aggregation of membrane proteins discussed by Ahkong *et al.* (1975*a*). Similar considerations may apply to poly(ethylene glycol). If the electrostatic field perpendicular to the surface of the lipid molecules of cell membranes is greatly diminished by these fusogens, as has been found here with lipid monolayers, extensive cellular aggregation would then be expected.

The bivalent-cation ionophore A23187 promotes the fusion of hen erythrocytes in the presence of exogenous Ca^{2+} , indicating that the entry of Ca^{2+} into the cellular cytoplasm mediates cell fusion (Ahkong *et al.*, 1975*c*). Entry of Ca^{2+} into hen erythrocytes, during incubation with poly(ethylene glycol)-6000, may conceivably therefore be responsible for cell fusion being independent of exogenous Ca^{2+} after treatment with the polymer, in view of the possibilities for the control of ionic permeability by surface potential that have been discussed by Gingell (1967).

This work was supported by the Medical Research Council, and by the award of a British Council Fellowship to B. M.

References

- Ahkong, Q. F., Fisher, D., Tampion, W. & Lucy, J. A. (1973) *Biochem. J.* **136**, 147-155
- Ahkong, Q. F., Fisher, D., Tampion, W. & Lucy, J. A. (1975a) *Nature (London)* **253**, 194-195
- Ahkong, Q. F., Howell, J. I., Lucy, J. A., Safwat, F., Davey, M. R. & Cocking, E. C. (1975b) *Nature (London)* **255**, 66-67
- Ahkong, Q. F., Tampion, W. & Lucy, J. A. (1975c) *Nature (London)* **256**, 208-209
- Cadenhead, D. A. & Bean, K. E. (1972) *Biochim. Biophys. Acta* **290**, 43-50
- Davies, J. T. & Rideal, E. K. (1963) *Interfacial Phenomena*, pp. 75-92, Academic Press, New York
- Gingell, D. (1967) *J. Theor. Biol.* **17**, 451-482
- Kao, K. N. & Michayluk, M. R. (1974) *Planta* **115**, 355-367
- Maggio, B. & Lucy, J. A. (1975) *Biochem. J.* **149**, 597-608
- Maggio, B. & Lucy, J. A. (1976) *Biochem. J.* **155**, 353-364
- Maroudas, N. G. (1975) *Nature (London)* **254**, 695-696
- Pontecorvo, G. (1975) *Somatic Cell Genet.* **1**, 397-400
- Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp. 114-200, John Wiley and Sons, New York
- Vincent, B. (1974) *Adv. Colloid Interface Sci.* **4**, 193-277