The electrophoretic properties and aggregation behaviour of dipalmitoylphosphatidylcholine vesicles incorporating glycophrin A at the gel to liquid-crystalline phase transition temperature

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The incorporation of glycophrin into the bilayers of diacetylphosphatidylcholine vesicles results in changes in their physical properties in the region of the gel to liquid-crystalline phase transition (Goodwin et al., 1982a). The enthalpy of the transition decreases linearly with the glycophrin to lipid molar ratio (Goodwin & Jones, 1980) and the rates of agglutination with wheatgerm agglutinin becomes approximately independent of temperature (Goodwin et al., 1982b). Below the gel to liquid-crystalline phase transition temperature (Tc), like many intrinsic membrane proteins (glycoproteins), glycophrin is present in patches or clusters (Chapman et al., 1979); these clusters dissociate at Tc resulting in a more uniform distribution at higher temperatures.

The N-terminal domain of glycophrin A (residues 1-74) is glycosylated and carries 16 oligosaccharide chains terminated by sialic acid residues (Furthmayr, 1977). Sialic acids are largely responsible for the surface charge of the human erythrocyte (Eylar et al., 1962) and glycophrin A accounts for 40% of the sialic acid on the cell surface (Levine et al., 1983). It is of some interest to investigate the effect of glycophrin incorporation on the electrophoretic properties of phospholipid vesicles in the region of the lipid phase transition where the bilayer distribution of glycophrin changes.

Human glycophrin A (Sigma Chemical Company Ltd., product number G9511) was incorporated into dipalmitoylphosphatidylcholine (DPPC) sonicated vesicles as previously described (Goodwin et al., 1982a) to give a final lipid concentration of 0.0125% (w/v) and glycophrin to lipid molar ratios in the range 0.9 - 10×. Two buffers were used in the preparations: buffer A (10 mm-Tris/HC1/100 mm-NaCl, pH 7.4, I 0.1077 M) and buffer B (1 mm-Tris/HC1/10 mm-NaCl, pH 7.4, I 0.0108 M). The electrophoretic mobilities of the vesicles were measured using a Rank Bros. Mark II microelectrophoresis apparatus. For aggregation studies sonicated vesicles were prepared in water with an initial lipid concentration of 0.025% (w/v) and a glycophrin to lipid molar ratio of 2 × 10⁻³. The absorbance changes on addition of Ca²⁺, Mg²⁺ and La³⁺ ions were measured using a Pye Unicam SP 1700 spectrophotometer thermostatted at 42.43°C against a reference containing vesicles at the same concentration as the sample.

Fig. 1(a) shows the zeta potentials (ζ) at 42°C (T, for DPPC), as a function of glycophrin to lipid molar ratio calculated from the measured electrophoretic mobilities by using the Smoluchowski equation (Hunter, 1981). The data shows a marked initial increase in ζ with increase in glycophrin level. Increasing the ionic strength lowers ζ consistent with compression of the electrical double layer. The values of ζ are comparable with those of the human erythrocyte. At 25°C and ionic strength 0.1, ζ = −19 mV (Hunter, 1960) for erythrocytes compared with −15.5 mV for DPPC vesicles (glycophrin to DPPC molar ratio 8 × 10⁻⁴) at 42°C ionic strength 0.10. Fig. 1(b) shows ζ as a function of temperature in the range 30-35°C. The gel to liquid-crystalline phase transition is approximately 42°C (Chen et al., 1980). However, there is no evidence that the lipid phase transition has any effect on electrophoretic mobility or ζ. The electrophoretic mobilities increase between 30 and 50°C but this is entirely accounted for by the decrease in the viscosity of water. It follows that since ζ is the potential at the plane of shear, any changes in the distribution of glycophrin in the lipid bilayer must be ‘smoothed-out’ at the plane of shear. If the human erythrocyte is modelled by a polyelectrolyte-coated particle the best agreement with the measured mobility is obtained with a polyelectrolyte layer of thickness 7.5 nm (Levine et al., 1983) containing a uniform distribution of sialic acid residues. The ionic diffuse layer containing the shear plane extends from the outer boundary of the polyelectrolyte layer. Should glycophrin-bearing vesicles behave similarly then it follows that the mean charge density at the outer boundary of the glycophrin oligosaccharides is independent of glycophrin distribution.

At Tc, the vesicles (glycophrin to DPPC molar ratio 2 × 10⁻³) are aggregated by La³⁺ ions with a maximum rate at 12.5 μM. Ca²⁺ (up to 100 mM) and Mg²⁺ (up to 20 mM) induce a transient increase in absorbance in

Abbreviation used: DPPC, dipalmitoylphosphatidylcholine.

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time intervals 0–300 s. It follows that the vesicles bind multivalent ions and for La$^{3+}$ ions the aggregation behaviour is indicative of charge reversal. The origin of the transient changes in absorbance when the vesicles are exposed to Ca$^{2+}$ and Mg$^{2+}$ ions may relate to the rate of change in the lateral distribution of glycophorin on exposure to these ions.

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The dynamic relationships between the cytoskeleton, Ca$^{2+}$, the plasma membrane, and the cell wall: a viscoelastic model of morphogenesis

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The giant unicellular green alga, Acetabularia mediterranea, regenerates after removal of its apical cap by undergoing a well-defined sequence of morphogenetic transformations. The first few steps of this process are shown diagrammatically in Fig. 1, and indicate the transitions involved in making first a growing tip, which then flattens before the appearance of a ring of hairs called a whorl.

This sequence of events involves the spatio-temporal organization of a set of processes which include electrophysiological, biochemical and mechanical aspects (Bentrup, 1977; Goodwin & Pateromicheleakis, 1979; Schweiger & Berger, 1981). Ca$^{2+}$ is particularly important in regulating regeneration, the sequence of morphogenetic transformations being subject to control by the concentration of this divalent cation in the sea water (Goodwin et al., 1983). In an attempt to understand the dynamic organization of the regenerative process, we have modelled the cytoskeleton in interaction with Ca$^{2+}$, the plasmalemma and the cell wall, as a mechnochemical system with viscoelastic properties. The main focus of attention is the reciprocal influences between Ca$^{2+}$ and the cytoskeleton, and the possibility of spontaneous transitions to spatial patterns of mechanical strain and Ca$^{2+}$ concentration which are similar to the morphogenetic patterns observed in regenerating plants.

From the literature on the effects of Ca$^{2+}$ on the mechanical state of the cytoskeleton (gel–sol transitions, contraction of actomyosin filaments, viscosity, etc.), estimates were made for changes in elasticity and viscosity parameters as a function of Ca$^{2+}$ concentration over the range $10^{-7}$–$10^{-4}$ M. An equation was derived for the regulation of Ca$^{2+}$ concen-