ELECTROSTATIC INTERACTIONS AT CHARGED LIPID MEMBRANES. CAN THE LIPID PHASE TRANSFORMATION AFFECT ENZYME ACTIVITY THROUGH THE MEDIATION OF PROTONS?

Paul WOOLLEY
Magdalene College, Cambridge, England

and

Hansjörg EIBL
Max-Planck-Institut für biophysikalische Chemie, 34 Göttingen, FRG

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1. Introduction

In a previous paper [1] an expression for the electrostatic contribution to the free energy of a charged lipid membrane was derived and applied to the variation in the crystal–liquid crystal transition temperature (T_t) with degree of dissociation (α) of the lipid's ionisable head-group. It was shown that although the change in molecular area at the transition results in a change in the apparent dissociation constant of the polar group, this change does not affect T_t. However the change is in itself interesting, for the transition superposes a 'step' onto the otherwise smoothly-changing dissociation constant.

The 'α-step' $\Delta\alpha$ and 'pH-step' ΔpH which result from this may be shown using the thermodynamical method of M. Teubner [2] to be related by equation [1]:

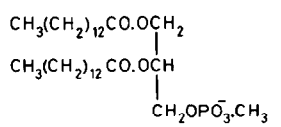
$$\frac{\partial \Delta G}{\partial \alpha} = -\Delta\alpha \frac{\partial^2 G}{\partial \alpha^2} + 2.303RT\,\Delta\mathrm{pH} = \Delta S^* \frac{\partial T_t}{\partial \alpha} \quad (1)$$

where G is the membrane's total Gibbs' free energy, S^* is the entropy of the neutral membrane, ∂ refers to partial differentiation and Δ refers to changes at the phase transition. Equation (1) is always satisfied at the transition. In concentrated, unbuffered dispersions the membrane itself controls the pH, so that $\Delta\alpha \approx 0$ and equation (2) is obtained. This has been investigated in detail

$$\Delta\mathrm{pH} = \frac{\Delta S^*}{2.303RT} \frac{\partial T_t}{\partial \alpha} \quad (2)$$

for the case of dimyristoyl methyl phosphatidic acid (MPA) (fig.1) by M. Teubner and the late H. Träuble [3] using the pH-electrode and a complete theory of this effect, incorporating competition from buffers, has been given by H. Träuble and F. Jähnig [4]. In this communication two further aspects of equation (1) are described: the utilisation of pH-steps to regulate the activity of an enzyme and the (biologically more relevant) observation of α-steps.

MPA:



PmPC:

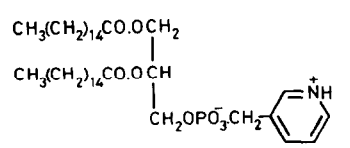


Fig.1. MPA (dimyristoyl methyl phosphatidic acid) and PmPC (dipalmitoyl phosphatidyl-*m*-pyridylcarbinol). MPA was synthesized by the method of ref. [1]. PmPC was synthesized by phosphorylation of 1,2-dipalmitoyl glycerine with phosphoryl chloride and treatment of the product with *m*-pyridyl carbinol.

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These are observed in buffered systems ($\Delta pH = 0$) and their magnitude, derived using relationships in ref. [1] with or without the use of equation, is given by equation (3), where f is the molecular area of

$$\Delta\alpha = \frac{2\alpha(1-\alpha)}{2 - 2\alpha + \coth\frac{e\psi_0}{kT}} \frac{\Delta f}{f} \approx \frac{2\alpha(1-\alpha)}{3-2\alpha} \frac{\Delta f}{f} \quad (3)$$

the lipid in the bilayer. The approximate expression is accurate to better than 10% at surface potential (ψ_0) values above 75 mV.

2. Experimental and results

2.1. *Coupling of enzymic activity to the membrane's phase transition*

The lipid used was dimyristoyl methyl phosphatidic acid (MPA). Bilayers were produced by sonication of a 5 mM dispersion of the sodium salt. The pH at the required Na_2SO_4 concentration was adjusted under rigorously CO_2-free conditions and an unbuffered solution of the enzyme added thereto. The enzyme used was carbonic anhydrase from bovine erythrocytes, kindly donated by Dr E. Grell. Carbonic anhydrase activity is pH-dependent in the required pH-range, so that pH-changes due to the membrane are expressed in changes in enzyme activity. The enzyme is negatively charged at this pH, so that interference between membrane and enzyme will not occur.

To assay the enzyme, the hydrolysis of *p*-nitrophenyl acetate was observed at a concentration well below K_m. The first-order rate constant is a measure of the enzyme's activity and its pH-dependence, other things being equal, reflects the pH-dependence of k_2/K_m. Thus the activity of the enzyme is coupled by the protons in solution to the state of the membrane, and the pH-step is superposed upon the usual Arrhenius activation plot, which is itself a composite of the enthalpic components of k_2, K_m, $pK_{a(enzyme)}$, $pK_{a(membrane)}$ etc. The experimental outcome is shown in fig.2 in the form of an Arrhenius plot; the step in the activity curve accompanying rising temperature is negative, as predicted by the theory and shown by the electrode measurements [2,3] i.e., the enzyme's activity is reduced at the transition. The

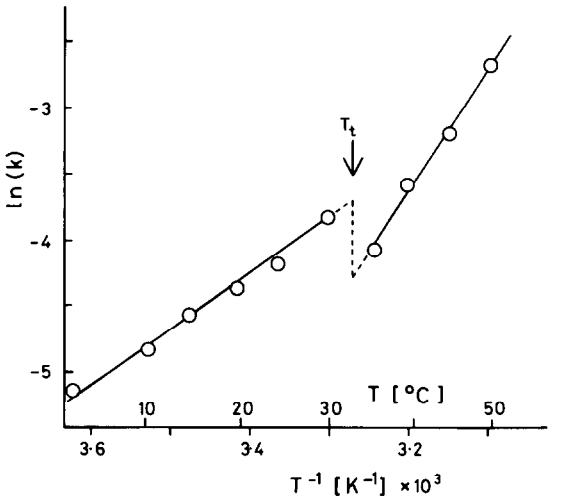

Fig.2. Arrhenius plot of enzyme activity, showing a pH-step at the crystal–liquid crystal phase transition, caused by the release of protons from the membrane into the solution. The first-order rate constant k is in s^{-1}. Dispersed MPA 5 mM, Na_2SO_4 10 mM, pH 6.3 at 18°C. 4 μl *p*-nitrophenyl acetate (0.01 M in acetonitrile) added to 0.4 ml dispersion in a 2 mm cell; observation at 347.5 nm. Controls using buffered lecithin or MPA, outside the range of pH and salt concentration where pH-steps are significant, showed no such steps in enzyme activity.

factor of approximately 40% by which the rate is reduced is consistent with the known magnitude of the pH-steps (here, 0.21 units).

A change by a factor of ten, which might correspond to the sometimes-postulated 'switching' function of the membrane's phase transition, would therefore require an enzyme or multi-enzyme complex with n pH-sensitive groups with $n \geqslant 3$ (for $\Delta pH = 0.5$) or $n \geqslant 5$ (for $\Delta pH = 0.2$). pH-Steps above 0.5 units for MPA [2,3] and 0.3 units for phosphatidic acid [5] have never been observed.

2.2. *Changes in dissociation at the membrane surface*

Although lipids can easily reach a concentration in cells above 5 mM, on account of the very small volumes between biological membranes, the systems in question are usually well buffered, so that equation (1) is more usually satisfied by $\Delta\alpha \neq 0$ and $\Delta pH = 0$ than by the converse.

To observe $\Delta\alpha$, the special lipid dipalmitoyl-phosphatidyl-*m*-pyridylcarbinol (PmPC) was synthe-

sised. This is a structural analogue of lecithin (see fig.1). However the proton on the nitrogen atom of PmPC may be removed, giving a net negative charge, so that the surface density of charge is variable as in the case of MPA. In addition the ultraviolet absorbance of the pyridine group enables α to be measured spectrophotometrically.

The ultraviolet extinction coefficient of the pyridine ring at the maximum of 260 nm varies from approximately 1000 in alkaline solution to 2000 in acidic solution. Thus, making a correction for light-scattering, the change in absorbance at this wavelength is 1000 [PmPC] $\Delta\alpha$, whence $\Delta\alpha$ can be calculated. Using dispersions buffered with phosphate between pH values 6 and 8, $\Delta\alpha$ values were measured and are plotted in fig.3 against $2\alpha(1-\alpha)/(3-2\alpha)$. The resulting straight line has an expected gradient of $\Delta f/f$ and the value measured, 0.23 ± 0.04, is in good agreement with the values 0.22 known for lecithin [6,7].

This change in degree of dissociation of the lipid is related to the change in degree of dissociation which an ionising non-lipid molecule at the surface would experience, as long as it were not so large as to reduce significantly the electrostatic field at the ionising group. If the degree of dissociation of the guest molecule is α', then $\Delta\alpha'$ is given by equation (4), and attains a maximum at membrane potentials above ca. 75 mV and $\alpha \approx 1$, given in (4). Thus the greatest

$$\Delta\alpha' = \frac{2\alpha'(1-\alpha')}{2-2\alpha+\coth\frac{e\psi_0}{kT}}\,\frac{\Delta f}{f} \leqslant 2\alpha'(1-\alpha')\frac{\Delta f}{f} \quad (4)$$

possible increase in the activity of an enzyme controlled by an ionising group in the membrane surface is given by $\frac{\Delta\alpha'}{\alpha'} = 2\,\frac{\Delta f}{f}$, equal for most membranes to not more than 0.5, requiring $n \geqslant 6$ for the activity of a multi-enzyme complex to change by a factor of ten or more.

We thus conclude that both theory and experiment show that α-steps and pH-steps, while measurable, are insufficient to produce significant changes in enzymic activity unless the effect is reinforced by a high degree of co-operativity.

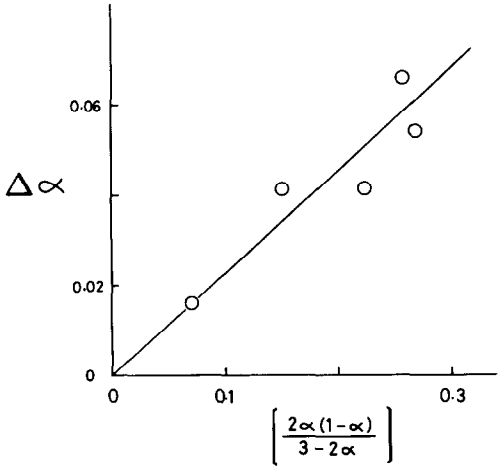

Fig.3. Plot showing $\Delta\alpha$ (see text). Dispersed PmPC 0.5 mM, sodium phosphate buffer 20 mM, pH values 6–8, ultraviolet path length 10 mm; wavelengths 247.3 nm, 260.5 nm and 280.5 nm, light-scattering corrected for using the empirical formula based on lecithin. Absorption due to pyridine = $[A_{260.5} - \frac{1}{2}(A_{280.5} + A_{247.3})]$.

Acknowledgements

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