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An Explanation of Apparent Sudden Change in the Activation Energy of Membrane Enzymes

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If an enzyme–lipid mixture forms phases of pure lipid and enzyme–lipid solution, and enzyme activity depends on the composition of the enzyme–lipid solution, the temperature-dependence of lipid solubility in the enzyme–lipid solution leads to apparent sudden changes in enzyme activation energy without activity discontinuities at lipid phase transition temperatures.

Discontinuities in enzyme activity at a particular temperature are known (e.g. Linden *et al.*, 1973), but it is more common to find, in membrane enzymes and transport proteins, that there is apparently a sudden change in enzyme activation energy, without a change in reaction rate, at a particular temperature, which may be the crystalline-to-liquid-crystalline transition temperature of the membrane (see, e.g., Overath & Träuble, 1973; Grisham & Barnett, 1973; Warren *et al.*, 1975*a*); there is a discontinuity in dV/dT but not in V.

It is surprising that a change in the enzyme so dramatic as to affect the activation energy of the rate-limiting step has no effect on its rate. The rate of an enzyme reaction is $A \cdot e^{-\frac{\Delta H^*}{RT}}$, where ΔH^* is the activation energy of the rate-limiting step and A is a velocity constant. If the reaction rate is continuous it must be the same in the two phases [X and Y (low- and high-temperature equilibrium states respectively)] at the membrane transition temperature T_0. If ΔH^* is different in the two phases, A must be different too, so that $\ln(A_X/A_Y) = (\Delta H_X^* - \Delta H_Y^*)/RT$. The change in A must take into account any relevant changes in K_m or the pK of important groups at the active centre etc.

Instead of assuming such a close and convenient connexion between ΔH^* and A, suppose the active centre of the enzyme is quite unaffected by the lipid phase change. The apparent change in activation energy may then be explained in two ways.

A Possible Explanation

Consider first a membrane which is a single phase, namely a solution of enzyme in lipid. Suppose the formation of the activated state required the transfer of n lipid molecules from their equilibrium state to state Z. Lipid molecules have the same free energy G in their low-temperature (X) and high-temperature (Y) equilibrium states at T_0. The enzyme reaction rate depends on the difference (ΔG^*) between G in the equilibrium and activated states, from which the dependence on ΔH^* may be derived. If the free energy of the active centre is increased by $\Delta G'$ on formation of the activated state, $\Delta G^* = \Delta G' + n(G_Z - G_{X,Y})$. The reaction rate will be:

$$B \cdot e^{-\frac{\Delta G^*}{RT}} = B \cdot e^{-\frac{\Delta G' + n(G_Z - G_{X,Y})}{RT}}$$

$$= \left(B \cdot e^{\frac{\Delta S' + n(S_Z - S_{X,Y})}{R}}\right) \cdot e^{-\frac{\Delta H' + n(H_Z - H_{X,Y})}{RT}}$$

(where B is a velocity constant) so the activation energy will be $\Delta H' + n(H_Z - H_{X,Y})$, and since $G_X = G_Y$ at T_0 the reaction rate will be the same in the two phases at T_0.

For dipalmitoyl phosphatidylcholine at 41.8°C, $H_Y - H_X = 40.5$ kJ/mol (9.7 kcal/mol), the latent heat of the crystalline-to-liquid-crystalline phase transition (Hinz & Sturtevant, 1972). The apparent activation energy of sarcoplasmic-reticulum ATPase* in dipalmitoyl phosphatidylcholine decreases by about this amount above 41°C (Warren *et al.*, 1975*a*), so n would be about 1. Most enzymes having sudden changes in their activation energies change them by about this order of magnitude, so n would rarely be much more than 1.

The Z state cannot be the same as the Y state; the requirement cannot be that at least one lipid molecule is in the high-temperature equilibrium state. This would mean that state Z would be a phase containing one, or only a very few, molecules, which is certainly not the same as normal phase Y. Further, G_Z is independent of the state of the solvent lipid, and state Z is formed from a solvent lipid molecule. This suggests that formation of the activated state would involve absorption well within the enzyme and away from the solvent of a lipid molecule previously free in solution.

* Abbreviation: ATPase, adenosine triphosphatase.

Although this explanation leads to a curve for V against T of the correct form, the model is rather unsatisfactory; it is difficult to see why the enzyme should have evolved this mechanism. By using a slightly more complex model for the membrane but a much simpler model for the enzyme, a curve of the correct form may be derived in a straightforward way by quite a different approach.

The Preferred Explanation

Suppose the enzyme and lipid are only partially miscible. The membrane will contain two phases at all relevant temperatures, except T_0 at which it will have three. One phase will be a solution of enzyme and lipid, and one, or two at T_0, will be pure lipid. Such partial miscibility of enzyme and lipid would not be surprising. Partial miscibility of lipids is known even for two lipids in the liquid-crystalline state (Wu & McConnell, 1975). Proteins affect lipid phase transitions (Papahadjopoulos *et al.*, 1975), and, below membrane transition temperatures, phase separation into pure lipid and protein–lipid solutions is evident (Grant & McConnell, 1974; Kleemann & McConnell, 1974; Shechter *et al.*, 1974). Simple two-component (protein and lipid) phase diagrams have been used in interpreting these experiments (Kleemann & McConnell, 1974). Above the transition temperatures results are inconclusive, but it is a reasonable extrapolation to suppose such separations could exist here too under certain circumstances. The behaviour of sarcoplasmic-reticulum ATPase in lipid solution has been interpreted as showing that the lipid is divided into two classes: some is free in solution and some associated with the enzyme as an annulus around it (Warren *et al.*, 1975*a*,*b*). My model differs only in that there is little contact between the two lipid classes. The free lipid forms one phase and the enzyme and associated lipid another.

The Gibb's free energy G of a molecule is the same in its stable pure phase as in a solution saturated with it. G is a continuous function of both temperature and solute concentration for a solute molecule, and a continuous function of temperature for the pure solute, even when the solute undergoes a phase transition. The solubility of a substance must therefore be a continuous function of temperature through the phase-transition temperature of the pure substance.

The variation of solubility s with temperature is given by $\mathrm{d}(\ln s)/\mathrm{d}T = \Delta H''/RT^2$, where $\Delta H''$ is the heat of solution. For temperature ranges within which $\Delta H''$ may be taken as constant, $\ln(s/s_0) = \frac{\Delta H''}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)$. $\Delta H''$ will change when the solute undergoes a phase transition, being less above ($\Delta H_Y''$) than below ($\Delta H_X''$) the transition temperature. $\Delta H_X'' - \Delta H_Y'' =$ the latent heat of the transition. The solubility will be a continuous function of temperature, the slope $\mathrm{d}(\ln s)/\mathrm{d}(1/T)$ changing suddenly at the transition temperature of the solute. An example of this behaviour is the aqueous solubility of NH_4NO_3 near 32.1°C (Stephen & Stephen, 1963), one of its phase-transition temperatures.

Suppose the maximal activity V of the enzyme is proportional to the nth power of the lipid content c of the enzyme–lipid solution. At constant lipid concentration c_0, let V vary as $V = V_0 \cdot e^{-\frac{\Delta H^*}{RT}}$. When c varies, $V = V_0 \cdot e^{-\frac{\Delta H^*}{RT}}(c/c_0)^n$. If pure lipid phase is present, so that the enzyme–lipid phase is saturated:

$$\begin{aligned} V &= V_0 \cdot e^{-\frac{\Delta H^*}{RT}} \cdot (s/c_0)^n \\ &= V_0 \cdot e^{-\frac{\Delta H^*}{R}} \cdot (s_0/c_0)^n \cdot e^{-\frac{n\Delta H''}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)} \\ &= V_0 \cdot (s_0/c_0)^n \cdot e^{\frac{\Delta H''}{RT_0}} \cdot e^{-\frac{\Delta H^* + n\Delta H''}{RT}} \end{aligned}$$

The apparent activation energy of the enzyme reaction, $\Delta H^* + n\Delta H''$, will change at T_0, being n times the latent heat of the transition greater below than above T_0. As shown above, for sarcoplasmic-reticulum ATPase, and probably most other enzymes, n is about 1, so the enzyme activity would seem to be proportional to the lipid content of the enzyme–lipid solution.

If the shape of the enzyme molecule changes slightly on formation of the activated state the enzyme activity would depend on the compressibility of the enzyme–lipid solution. Near their transition temperature, lipids are highly compressible (Overath & Traüble, 1973; Hui *et al.*, 1975), so the compressibility of the enzyme–lipid solution might be approximately proportional to its lipid content. The activity of an enzyme in an enzyme–lipid solution might then be approximately proportional to the lipid content of the solution, providing a simple explanation of the apparent change in activation energy. Although this relationship is only approximately valid it may apply reasonably well, allowing for experimental error, over the small temperature ranges usually examined.

Discussion

The use of an enzyme–lipid phase diagram to explain the behaviour of membrane enzymes is a highly adaptable approach. It is quite reasonable to use a fairly complicated diagram, especially for concentrated enzyme solutions where protein–protein interactions could be complex. Small alterations in the diagram can be made to accommodate new results without affecting existing explanations. A phase diagram of this type can provide plausible explanations for the behaviour of sarcoplasmic-reticulum ATPase (A. T. Wynn-Williams, unpublished work)

as described in the literature (Warren *et al.*, 1974*a*,*b*, 1975*a*,*b*; Hardwicke, 1976).

Two important consequences of this theory follow. First, the activation energy of the rate-limiting step of an enzyme reaction cannot be deduced from the slope of $\ln V$ against $1/T$. This is true for any membrane enzyme unless it is shown that the compositions of all phases containing the enzyme are temperature-independent. Secondly, the phase change causing the apparent change in activation energy may not be taking place in the phase containing the enzyme; the enzyme–lipid solution will be affected by a change in the pure lipid phase.

Cell membranes may contain several phases (Grant & McConnell, 1974; Kleemann & McConnell, 1974; Shechter *et al.*, 1974) either of pure lipid of different types, which may not be completely miscible even in the liquid-crystalline state (Wu & McConnell, 1975), or of protein–lipid solutions. Changes in the pH (Traüble & Eibl, 1974; Jacobson & Papahadjopoulos, 1975; Ito *et al.*, 1975) or the concentrations of proteins (Papahadjopoulos *et al.*, 1975) or metal ions such as Ca^{2+} (Galla & Sackmann, 1975; Jacobson & Papahadjopoulos, 1975; Ito *et al.*, 1975) or Mg^{2+} (Traüble & Eibl, 1974) could affect the compositions and states of these phases. By these simple mechanisms the cell may affect the compositions of certain of the membrane phases and so the activities of a selection of its enzymes and transport proteins. These enzymes and proteins need not all be affected to the same extent or in the same way. This provides a simple mechanism for a complex control system of cellular activity.

Note Added in Proof (Received 12 April 1976)

There will also be an apparent sudden change in enzyme activation energy, without any discontinuity in activity, at a temperature at which pure lipid begins to, or ceases to separate out from, an enzyme–lipid solution. On cooling, pure lipid might begin to separate out from a solution containing comparable weights of enzyme and lipid at less than 1°C below the lipid phase-transition temperature (Grant & McConnell, 1974).

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