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THE ORIGIN OF A BREAK IN ARRHENIUS PLOTS OF MEMBRANE PROCESSES

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The break in Arrhenius plots of membrane permeation rates or protein activities is interpreted as a consequence of the inherent energy-entropy compensation at the lipid phase transition. An experimental result in support of this interpretation is presented.

Various membrane processes such as permeation or incorporation of small molecules [1] or activities of integral proteins [2] exhibit a marked temperature dependence around the lipid phase transition. If their rates are depicted in an Arrhenius plot, straight lines with different slopes above and below the phase transition are found which intersect at the transition temperature. Typical values for the corresponding activation energies are 10–20 kcal/mol above and 50–200 kcal/mol below the phase transition. This behavior immediately poses two questions: how can an activation energy as large as 100 kcal/mol be interpreted, and how can the rate vary continuously at the phase transition if the activation energy changes abruptly? Answers which have been proposed considered phase separation between lipid and protein or the coexistence of fluid and ordered lipid domains at the phase transition [3–6]. These answers, however, do not get to the heart of the problem since for permeation of a molecule through a pure lipid membrane the same temperature dependence is found (see below), although phase separation and coexistence of domains do not occur in this case (the latter at least not over a temperature range larger than 1 K around the phase transition). We present an alternative interpretation: because of the cooperative nature of the lipid phase a considerable number of lipid molecules is involved in the permeation or incorporation of one molecule. This number is comparable to the number of boundary lipids around a protein and is of the order of 20–50 lipid molecules. For permeation of a molecule through the ordered phase this number of lipid molecules has to be fluidized, causing the high activation energy. At the phase transition, however, this energy is exactly compensated by entropy, hence the permeation rate is continuous at the phase transition.

For an amphiphilic molecule a lipid bilayer represents an energy barrier, i.e. permeation is an activated process and the permeation rate is of the form

$$k \sim \exp[-\Delta\phi/kT] \quad (1)$$

where T is the temperature, k Boltzmann's constant, and $\Delta\phi$ the barrier height expressed in terms of the appropriate thermodynamic potential ϕ. If we assume the temperature and volume to be the externally fixed parameters, ϕ is the free energy F. Then $\Delta\phi = \Delta F = \Delta U - T\Delta S$ contains an energy (ΔU) and an entropy (ΔS) contribution which in the general case, however, both vary with temperature. The temperature dependence of ΔF for our

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problem is derived within the following model.

If a particle permeates through a lipid bilayer which is in the fluid state, the barrier is determined by the interaction between particle and bilayer arising from their amphiphilic properties, $\Delta F = \Delta F_a$. Permeation through the bilayer in the ordered state requires in addition a small lipid region to become fluid, $\Delta F = \Delta F_a + \Delta F_{melt}$. The amphiphilic properties have no marked temperature dependence around the phase transition, hence $\Delta F_a = \Delta U_a - T\Delta S_a$ with constant ΔU_a and ΔS_a. The free energy ΔF_{melt}, on the other hand, varies pronouncedly with temperature since fluidization of lipid molecules in the ordered phase is difficult far below the phase transition and facilitated on approaching the phase transition. To be specific, we assume that ν lipid molecules must adopt a fluid state, hence $F_{melt} = \nu\Delta f$ with $\Delta f = f_{fl} - f_{ord}$ denoting the difference in free energy per lipid molecule between the fluid and ordered state, at a given temperature $T < T_t$. This assumption represents a crude approximation since (i) the change in order at the ordered-fluid interface is considered to occur as a sharp boundary and (ii) the interfacial energy is neglected. Actually, however, an improved treatment based on a continuous region of boundary lipids leads to the same conclusions. With $\Delta f = 0$ for $T > T_t$ we obtain the expression

$$k(T) \sim \exp(-[\Delta U_a + \nu\Delta f(T)]/kT) \tag{2}$$

This result yields a qualitative answer to the questions raised initially. Above the phase transition where $\Delta f = 0$, the rate k is determined by an activation energy ΔU_a of ordinary magnitude. Below the phase transition, Δf has a finite value and after enhancement by the factor ν of melted lipid molecules leads to an extreme temperature dependence of k described by a high activation energy. Upon approaching the phase transition, however, Δf decreases and at the transition vanishes by definition of the transition temperature $T_t = \Delta u/\Delta s$ implying $\Delta f(T_t) = \Delta u - T_t\Delta s = 0$. Hence k remains continuous at the phase transition due to an energy-entropy compensation inherent in the cooperative lipid system.

For the quantitative treatment of Δf we refer to a description of the ordered-fluid transition within the framework of a general theory for phase transitions [7,8]. It is easy to show that in this description

$$\Delta f(T) = \Delta q \frac{T_t - T}{T_t} \text{ for } T < T_t \tag{3}$$

where Δq is the latent heat per lipid molecule at the ordered-fluid transition. The latent heat enters as the characteristic energy difference of the system; it is scaled by a temperature factor proportional to $T_t - T$ which is characteristic for pretransitional effects in the vicinity of a phase transition and assures that $\Delta f(T_t) = 0$. Insertion of Eqn. 3 into Eqn. 2 leads to

$$k(T) \sim \exp\left(-\left[\frac{\Delta U_a}{kT} + \frac{\nu\Delta q}{k}\left(\frac{1}{T} - \frac{1}{T_t}\right)\right]\right) \text{ for } T < T_t \tag{4}$$

This result states that below the phase transition the activation energy is $\Delta U = \Delta U_a + \nu\Delta q$, and that at the phase transition the dominant contribution $\nu\Delta q$ due to melting is compensated by the melting entropy. Using as typical values 200 kcal/mol for $\nu\Delta q$ and 10 kcal/mol for Δq yields $\nu = 20$ for the number of melted lipid molecules. This value is comparable to the number of boundary lipids around a membrane protein and characteristic for the number of lipid molecules perturbed by an incorporated particle [8]. Thus quantitatively the high activation energy below the phase transition is a consequence of the large number of lipid molecules which must become fluid for permeation of one particle.

The result of Eqn. 4 can be verified further by studying the permeation of a particle through bilayers of lipids which differ in chain length. The latent heat Δq increases with chain length, whereas ν should remain constant. For particles shorter than the chain length, ΔU_a also should remain constant. Hence, above the phase transition the activation energy is expected to be independent of chain length, whereas below the phase transition it should increase proportional to Δq. These predictions were investigated experimentally. The permeation rate of the amphiphilic dye dansylgalactoside through lipid bilayers of different lipid chain lengths was measured (Fig. 1). Above the respective phase transitions of the different lipids the activation energy ΔU_a is independent of chain

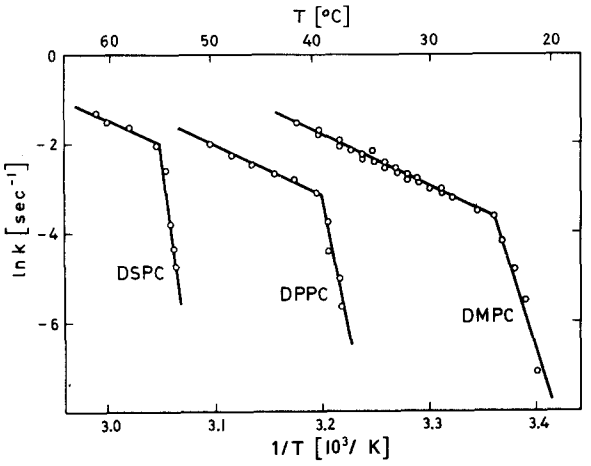

Fig. 1. Permeation rate k of dansylgalactoside through bilayers of vesicles made of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and distearoylphosphatidylcholine (DSPC). In a stopped-flow apparatus a dispersion of small unilamellar vesicles consisting of phosphatidylcholine with a particular chain length was rapidly mixed with a solution of dansylgalactoside, an amphiphilic fluorescent dye. The fluorescence intensity is higher if the dye is associated with the membrane than with water. By a fast diffusion-controlled process the dye molecules attain a partition equilibrium between water and the outer surface of the vesicles, signalized by a rapid increase of fluorescence intensity. In a second step the inner surface of the vesicles is occupied. This process involves permeation through the bilayer and is slow. The accompanying increase of fluorescence intensity is detected and evaluated for k. The molar lipid/dye ratio is 100:1.

length and equal to 22 kcal/mol. At the phase transition a break occurs in each case (the existence of a sharp break does not ncessarily collide with the fact that for the small vesicles utilized the phase transition is broad as observed by calorimetry; the broadening may arise from fluctuations of lipid order which do not affect the activation step for permeation). The activation energy ΔU below the phase transitions increases with increasing chain length, the values being given in Table I. They can be evaluated for ν using Eqn. 4, the above value for ΔU_a, and Δq values from the literature [9]. The result is included in Table I. Although Δq varies by more than a factor of 2 for the different lipids, the ν values remain approximately constant at a value of about 25. Thus the experimental results agree with the predictions of the theory.

It should be mentioned that another type of

TABLE I

ACTIVATION ENERGIES ΔU FOR PERMEATION OF DANSYLGALACTOSIDE THROUGH BILAYERS IN THE ORDERED PHASE AND NUMBER ν OF MELTED LIPID MOLECULES

The values for the latent heat Δq are taken from calorimetric data for liposomes [9] and therefore represent an overestimate for our case of vesicles.

	DMPC	DPPC	DSPC
ΔU(kcal/mol)	153	235	330
Δq(kcal/mol)	5.4	8.7	10.6
ν	24	24	29

temperature dependence of the permeation rate, namely a maximum at the phase transition, has been observed with smaller molecules such as sugars or metal ions [10]. In this case, a different mechanism for permeation has been proposed [8]. Permeation is postulated to require only local compression of a small lipid region surrounding the permeating particle. Since the lateral compressibility is maximal at the phase transition as a consequence of the cooperative behavior of the lipids, the permeability also becomes maximal there.

The above interpretation of a break in an Arrhenius plot of the permeation rate may be generalized to the case of membrane protein activity showing a break at the lipid phase transition. The basic assumption is still that according to Eqn. 2 the protein activation free energy is the sum of an ordinary term and a lipid melting term which vanishes at the phase transition because of energy-entropy compensation in the melting free energy. Such a possibility was already discussed by Wynn-Williams [4] but rejected, because he estimated from one experimental example the number ν of lipid molecules which melt to be rarely larger than 1 and regarded this value as too small for melting to take place. However, observed activation energies in many cases lead to higher ν values [3,5]. More importantly, complete melting does not seem necessary in the case of a permanently incorporated membrane protein since the boundary lipids around a protein are already relatively fluid below the phase transition. A slight further fluidization of them may be sufficient for the activation

of a protein. Hence, small differences in protein activation energies below and above the lipid phase transition may result as a consequence of small effective Δq values with ν still of the order of 20 or larger depending on the protein size. In the case of a broad phase transition as in biological membranes the existence of domains probably has a superimposed effect [4,6]. For example, they may lead to a second break at lower temperatures [5].

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