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THE EFFECT OF PRESSURE AND THE VOLUME OF ACTIVATION ON THE MONOVALENT CATION AND GLUCOSE PERMEABILITIES OF LIPOSOMES OF VARYING COMPOSITION

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SUMMARY

Measurement of the cation permeability of phospholipid microvesicles as a function of pressure confirmed that a single rate-determining step occurred in each case.

The volume of activation was 20 ml · mole^{-1} for Na^+ and K^+, and about 40 ml · mole^{-1} for valinomycin-mediated K^+ permeability. It was virtually independent of membrane composition. The results were explained in terms of Träuble's theory of kink–substrate dissociation at the membrane interface involving possible $2g$ 1 and $2g$ 2 kink isomers.

The volume of activation for D-glucose was 37 ml · mole^{-1}, which was not significantly different from that for any of the valinomycin-mediated K^+ permeabilities. However, other data suggest that the rate-limiting steps for the sugar and cation permeabilities are not the same.

INTRODUCTION

The precise mechanisms of cation and sugar permeability through phospholipid membranes is not known. Thermodynamic quantities, such as the free energy of activation, can provide an indication of how such a process takes place.

The most frequently measured parameter is the effect of temperature on a process, which gives the enthalpy of activation ΔH^*, but the effect of pressure on a process can provide additional useful information, and is rather less difficult to interpret.

Papahadjopoulos and Watkins [1] measured values of ΔH^* for the exchange of K^+ and Cl^- across phospholipid vesicles. Johnson and Bangham [2] showed

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that although valinomycin greatly enhanced the rate of exchange of K^+ across liposome membranes the value of ΔH^* remained constant at 15 ± 1 kcal · mole^{-1} within the limits of experimental error. They interpreted their results in terms of the absolute rate theory [3] as applied to membrane permeability by Zwolinski et al. [4], and found that the most appropriate equation for cation permeability was that describing a rate-determining step at the aqueous/organic interface. Proton magnetic resonance studies by Lee et al. [5] have shown that the phospholipid bilayer is most closely packed at this interface in the glycerol region of the phospholipid molecules, and electron spin resonance studies by Hubbell and McConnell [6] gave similar results.

The equation used for the K^+ permeability with or without valinomycin was

$$P = \frac{\lambda_{sm}}{2} \frac{kT}{h} \exp\left(-\Delta G^*_{sm}/RT\right) \tag{1}$$

where P was the permeability coefficient, λ_{sm} the "jump distance" for an ion crossing the interface, k and h are Boltzmann's and Planck's constants, respectively, T the absolute temperature, R the universal gas constant and ΔG^*_{sm} the free energy of activation for crossing the aqueous membrane interface. More generally, ΔG^* is the free energy of activation for any rate-determining step. Since at constant temperature

$$\Delta V^* = \frac{\partial(\Delta G^*)}{\partial \pi} \tag{2}$$

where ΔV^* is the volume of activation and π the absolute pressure. Differentiation of Eqn 1 with respect to pressure and substitution of Eqn 2 yields

$$\frac{\partial(\ln P)}{\partial \pi} = -\frac{\Delta V^*}{RT} \tag{3}$$

The volume of activation may thus be obtained by studying permeability as a function of pressure.

EXPERIMENTAL

Sources of materials, the techniques employed and methods of calculation have been described in detail in a recent publication [7], except that a permeability correction for the retardation of the glucose by the dialysis bag was essential. The method of finding the dialysis bag permeability was given in ref. 8, and a computer was used to find the glucose liposome permeability from the equation given in the same paper. Briefly, liposomes were formed from solvent-free mixed lipids by adding 0.6 ml 0.155 M ^{42}KCl or ^{24}NaCl buffered at pH 7.6 (20 °C) with Tris–HCl, sometimes containing 0.01 M D-[^{3}H]glucose, and by sonicating the suspension to clearness in a sealed tube under nitrogen with a bath sonicator. Untrapped isotope was separated from the liposomes by passage over a 30-cm column of Sephadex G-50 prepared in a non-radioactive salt solution of similar chemical composition to that containing the liposomes. 1-ml portions of the liposome suspension in Visking dialysis bags were allowed to exchange isotope with 10 ml of the non-radioactive salt solution, and the rate of loss of isotope from the liposomes was measured. The valinomycin concentration used was 2–6 μmoles valinomycin per mole lipid. Samples were exposed to hydrostatic pressure in stainless-steel pressure vessels.

RESULTS

The results obtained are shown in Fig. 1.

Pressures of up to 400 atm were used in the determination and no deviations from linearity were observed using Eqn 3. ΔV^*_{sm} is thus independent of pressure within our experimental error in the range studied and characterises the rate of decrease of permeability with pressure. The volumes of activation obtained for each

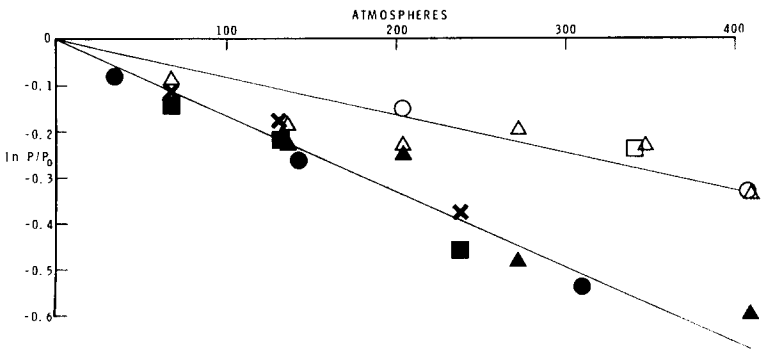

Fig. 1. The effect of pressure on the K^+, valinomycin-mediated K^+ and glucose permeabiliteis of phospholipid membranes. Membrane composed of phosphatidylcholine with the following phospholipids or sterol present in the molar proportions indicated. Unless otherwise stated, points refer to K^+ permeabilities. ○, 4 % phosphatidic acid at 37 °C; □, 4 % phosphatidic acid at 23 °C; △, 6 % phosphatidylserine at 37 °C; ●, 4 % phosphatidic acid at 21 °C with valinomycin; ■, 2 % phosphatidic acid + 50 % cholesterol at 21 °C with valinomycin; ▲, 6 % phosphatidylserine at 23 °C with valinomycin; ×, 4 % phosphatidic acid at 23 °C, D-glucose permeability. The two lines are the regression lines for the phosphatidic acid and phosphatidylserine points at 37 °C, gradient and standard error, 0.00082 ± 0.00006, and all the valinomycin points at 21 and 23 °C, gradient 0.00166 ± 0.00007. From the regression lines the two values of ΔV^* are 20.8 ± 1.5 ml · mole^{-1} and 40.2 ± 1.7 ml · mole^{-1}, respectively.

TABLE I

THE EFFECT OF PRESSURE ON THE PERMEABILITY OF IONS THROUGH PHOSPHOLIPID MEMBRANES

Ion	Temperature (°C)	Membrane composition*	$\Delta V^* \pm$S.E. ml · mole^{-1}	No. of points at pressure**
K^+	23	6 % Phosphatidylserine	19 ± 3.5	5
K^+	37	6 % Phosphatidylserine	21 ± 2.2	6
K^+	37	4 % Phosphatidic acid	21 ± 0.7	2
Na^+	37	4 % Phosphatidic acid	20 ± 1.9	6
Valinomycin–K^+	23	6 % Phosphatidylserine	38 ± 2.4	4
Valinomycin–K^+	21	4 % Phosphatidic acid	43 ± 1.2	3
Valinomycin–K^+	21	2 % Phosphatidic acid 50 % Cholesterol	45 ± 2.4	3
D-glucose	22	4 % Phosphatidic acid	37 ± 2.0	3

* Balance molar % phosphatidylcholine.

** Each point is the mean of three determinations.

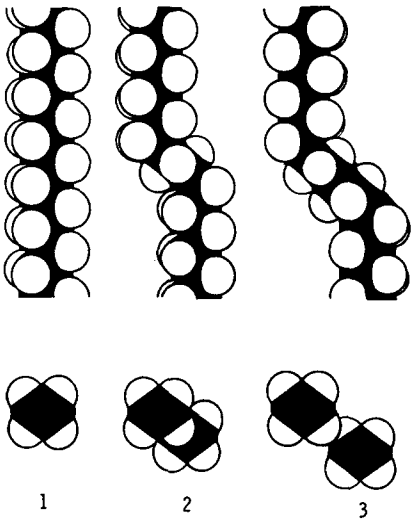

Fig. 2. Diagram showing the formation of kink isomers in a hydrocarbon chain, Pechold [11]. Side and surface views of: 1, straight hydrocarbon chain; 2, 2 *g* 1 kink; 3, 2 *g* 2 kink.

case are summarised in Table I. The results fall into two distinct groups, and none of the ΔV^* values within each group differs significantly at the 95 % confidence level. The volumes of activation for permeability without valinomycin are grouped about 20 ml · mole^{-1} independent of whether Na^+ or K^+ is the permeant and of change of composition from phosphatidylserine–phosphatidylcholine to phosphatidic acid–phosphatidylcholine. Valinomycin-mediated K^+ permeability has a volume of activation roughly twice this, again showing no gross dependence on membrane composition. The volume of activation for D-glucose was 37 ml · mole^{-1}, which did not differ significantly from any of the valinomycin-mediated K^+ permeabilities. No allowance was made for the effect of pressure on the dialysis-bag permeability; this may be marginally significant in the case of the sugar.

DISCUSSION

The diffusion of an ion across a lipid bilayer is a complex process whose mechanism is not well understood. However the absence of a gross dependence of the magnitude of ΔV^* on pressure indicates that a single rate-determining process is dominant over this range in our experiments. The observed activation volume is the same for K^+ and Na^+ and is independent of the acidic lipid in the bilayer. This invariance of the activation volume is paralleled in the observed activation enthalpies which are 14–16 kcal/mole for Na^+ and K^+ in phosphatidic acid–phosphatidylcholine and phosphatidylserine–cholesterol sonicated vesicle bilayers [2, 9] and in bilayers of other non-cation-selective phospolipids [1]. Both ΔH^* and ΔV^* are larger than normally observed for diffusion in liquids, e.g. in CS_2 ΔV^* is 4 ml/mole [10]. Although changes in membrane thickness with temperature and pressure might contribute somewhat to such large values they cannot be explained by this mechanism alone, and it seems more reasonable to suppose that the values do not represent

changes occurring during translocation through the bilayer interior but rather represent a rate-determining step at the aqueous–lipid interface. The nature of such a step is not clear, but since it is independent of both the ion and the acidic head group of the lipid it may involve a structural rearrangement in the glycerol region of the bilayer as previously suggested [2].

In the case of valinomycin–K^+ ΔV^* represents the volume change as the complex pushes through the rate-limiting region, because the energy requirement for formation of a void of adequate size for valinomycin to jump into is prohibitive. The cooperative changes of the stiff acyl chains in the glycerol region together with the simultaneous relaxation of the dipole–ion interactions at the interface would produce a ΔV^* of the correct sign and a structural disturbance of this nature is in keeping with the ΔS^* previously reported [2]. The value for the enthalpy for the valinomycin-mediated K^+ diffusion is 15 kcal/mole, the same as for K^+ in the absence of valinomycin [2], but the activation volume is twice as large as for K^+ alone.

Although the volume of activation for the diffusion of glucose through these liposomes is not experimentally distinguishable from that of valinomycin–K^+, previous evidence [7] suggests that the rate-limiting step occurs at a different place from that of the ions or ionophore.

It is interesting to consider these results in the light of the theory of kink formation and diffusion in the acyl chains as described by Pechold [11] and Träuble [12, 13], although the kinetic theory of Träuble is only appropriate to unchanged permeants, since the rate is limited by kink diffusion in the membrane interior. The accommodation of a solute into the ordered acyl region is accomplished by the formation of kinks formed when two adjacent C–C bonds rotate from a gauche to a trans position (a $2g$ 1 kink in their terminology); two such kinks separated by one trans bond is designed $2g$ 2. The enthalpy of activation for both these kinks should be similar, although their volumes and entropies of activation differ. Kinks can extend over many units of the hydrocarbon chain. Hence the number of kinks required by a permeant species depends, within limits, on its two shorter dimensions rather than its total volume. Valinomycin in its "bracelet" conformation has a cross section about 8 Å × 4 Å [14] and could fit diagonally into a $2g$ 2 kink. From the crystal studies of McDonald and Beevers [15, 16], glucose is estimated to occupy a space some 5.4 Å × 3.8 Å, and so should fit a $2g$ 2 kink. The hydrated ions of Na^+ (3.6 Å diameter) and K^+ (2.4 Å) would fit in a $2g$ 1 kink.

The introduced volume of a $2g$ 1 kink has been estimated as 50–25 ml/mole [12] but this applies to a quasi-solid system and in the fluid bilayers considered here the true value must be much less. Nonetheless the estimate has the correct sign and provides an upper limit of magnitude which is not inconsistent with our ΔV^* values. The apparent quantisation of our values, though possible arising by chance, suggests that measurements of ΔV^* for a range of non-polar solutes would provide an appropriate test of the Träuble kink diffusion theory. The use of pressure also offers the possibility of studying the temperature coefficient of permeation or diffusion at constant bilayer density thus avoiding problems of interpretation due to dimension changes.

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