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Permeability of alkylamines across phosphatidylcholine vesicles as studied by ^{1}H-NMR

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The exchange rate and enthalpy and entropy of activation of the diffusion of the first five *n*-alkylamines across egg phosphatidylcholine vesicles has been measured by ^{1}H-NMR spectroscopy employing the 1:2 Gd^{3+}-EDTA complex as a relaxation reagent. The permeability determined from the exchange rate of the ethyl through the pentyl derivatives increased sequentially with increasing chain length from $7 \cdot 10^{-7}$ to $4 \cdot 10^{-4}$ cm/s, respectively, at 25°C. The permeability of methylamine was similar to that of ethylamine ($1 \cdot 10^{-6}$ cm/s at 25°C) and exhibited a relatively smaller entropy increase. The enthalpy of activation for the transfer reaction was high for all amine derivatives (20 kcal/mol). The entropy of activation increased with increasing chain length. The results indicate that the rate of diffusion is dominated by the partition into the membrane. Methylamine, being the smallest molecule in this series, can probably diffuse also through vacancies formed by the internal motions of the lipid chains.

Introduction

Amines have been extensively used as probes for measuring either pH gradients across membranes [1–4] or surface charge of membranes [5,6]. In both cases, the diffusional permeability of the amine molecule dictates its employment. The function of biogenic amine derivatives such as the catecholamines and the polyamines may involve transfer through membranal barriers. There are no systematic data on amine diffusional permeability across artificial or natural membranes. In here, we present data on the kinetics of transfer of the first five homologs of the *n*-alkylamines across phosphatidylcholine vesicles. Studies of an ascending homologous series of permeants under the same conditions provide information on the various factors determining the transfer mechanism. Kinetic measurements were performed by employing ^{1}H-NMR spectroscopy, extending a previous developed methodology [7] to amine molecules.

Materials and Methods

Vesicles were prepared from pure egg phosphatidylcholine (Makor). All chemicals were of analytical grade and were used without further purification.

Vesicle preparation. 150 mg dried lipid was dispersed in 3 ml aqueous solution that comprised of 50 mM $GdCl_3$/100 mM disodium EDTA. This suspension was sonicated under nitrogen with a W-375 Heat Systems Sonicator in an ice-bath for 20 min. The crude vesicle suspension was centrifuged for 30 min at $40000 \times g$ at 4°C and the middle zone was removed for dialysis. The vesicles were then dialyzed for 4–12 h twice against 21 ml of 250 mM NaCl and twice against 20 ml of 250

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mM NaCl in ^2H_2O. The alkylamine was added to the dialyzed vesicle suspension at concentrations ranging between 0.3 to 0.5 M. A final adjustment of the acidity was performed prior to the NMR measurements using ^2HCl and NaO^2H. Phospholipid concentrations were determined by measuring the phosphate content of ashed aliquots.

Control solutions. Aqueous solutions containing the same concentrations of $GdCl_3$ and EDTA as in the initial lipid suspensions were dialyzed simultaneously with the vesicles. The addition of alkylamine and the adjustment of the pH were performed in the same manner as in the vesicle preparations. The final compositions of these solutions were approximately the same as those of the extravesicular media in the corresponding vesicle suspensions.

NMR measurements were performed on a Bruker WH-270 pulsed-FT spectrometer. Longitudinal relaxation rates were determined by the inversion recovery method.

Results

Relaxation reagent for alkylamines

The EDTA chelates of the trivalent lanthanides form ion pairs with alkylammonium ions, thereby inducing a shift and enhancing the relaxation rate of the amine's protons [8]. Titration studies with EDTA at a constant concentration of lanthanide ions (Dy^{3+} or Gd^{3+}) and at a constant and excess methylamine concentration indicated that a maximal shift or a maximal enhancement in linewidth ($\delta\Delta\nu_{1/2}$) is induced by the 1 : 2 lanthanide : EDTA chelate (Fig. 1). The T_1 and T_2 relaxation rates ($T_2 = (\pi\Delta\nu_{1/2})^{-1}$) of all the protons of the higher amine homologs were enhanced by interacting with the Gd : 2EDTA complex. A maximal enhancement was exerted on the α-methylene protons. For example, the $(T_1)^{-1}$ of the α-methylene protons and β-methyl protons of 0.5 M ethylamine was enhanced in the presence of 11 mM Gd · 2EDTA

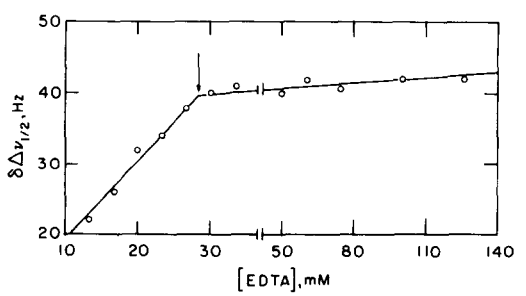

Fig. 1. The change in the linewidth of the methyl protons of methylamine (0.25 M) as a function of EDTA concentration, in the presence of 13.75 mM $GdCl_3$ ($p^2H = 7.1$). The arrow indicates the equivalence point for the formation of the methylamine-Gd^{3+}-2EDTA complex.

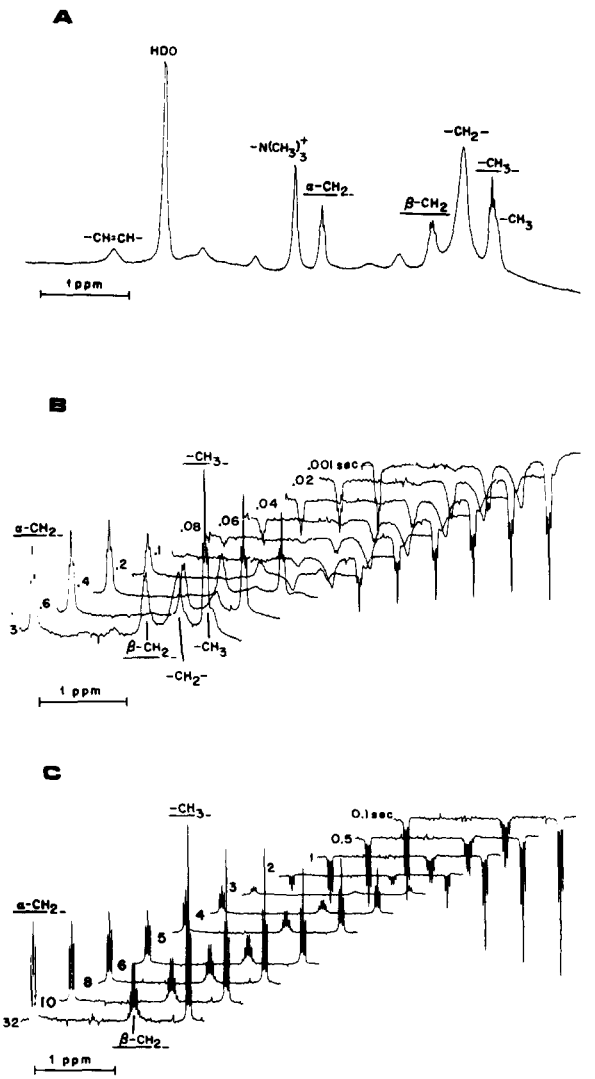

Fig. 2. 270 MHz ^1H spectra of *n*-propylamine (A) added to a suspension of phosphatidylcholine vesicles; (B) in a suspension of vesicles with entrapped Gd·2EDTA (50 mM); (C) in a control solution of (B). A, 0.25 M *n*-propylamine; B and C, 0.5 M *n*-propylamine. The vesicle suspension and the corresponding control solution were prepared as described in Materials and Methods. $p^2H = 6.7$.

(pH 7.1, 25°C) by a factor of 590 and 230, respectively.

The enhancement in the relaxation rates of the amine protons by the 1 : 2 chelates increased with increasing pH, similar to what has been observed previously for the 1 : 1 chelates [8,9].

. It is important to note that egg phosphatidylcholine vesicles are impermeable to the EDTA chelates of the trivalent lanthanides [7,9]. This permits the use of these chelates as shift and relaxation reagents in NMR permeability studies.

Permeability of alkylamine

A typical ^1H spectrum of a vesicle suspension containing an alkylamine is shown in Fig. 2A. The features of the membranal signals are not affected by the addition of the amine. The signal due to the methylene protons is well separated from the membranal signals and can therefore be studied directly.

The T_1 relaxation rate of the α-methylene protons of the amine in a vesicle suspension containing entrapped relaxation reagent was faster than that in the corresponding control solution (see example in Fig. 2B and C). Such a difference is expected if the exchange rate across the vesicles contributes to the measured relaxation rate. This was confirmed by studying the temperature dependence of the T_1 relaxation rate of the α-methylene protons in vesicle suspensions and in solutions that mimic the composition of the intra- and extravesicular (control) media (Figs. 3 and 4).

The temperature dependence of the T_1 relaxation rate of the α-methylene protons exhibits in the vesicle suspensions three regions (Fig. 3): (i) slow exchange at low temperatures, (ii) kinetic region where the exchange rate is of the same order of magnitude as the nuclear relaxation rate and (iii) fast exchange at high temperatures where the measured relaxation rate is the weighted average of the relaxation at each environment. Since the fraction of the intravesicular fast-relaxing amine protons is small (5%), T_1^{-1} can be ap-

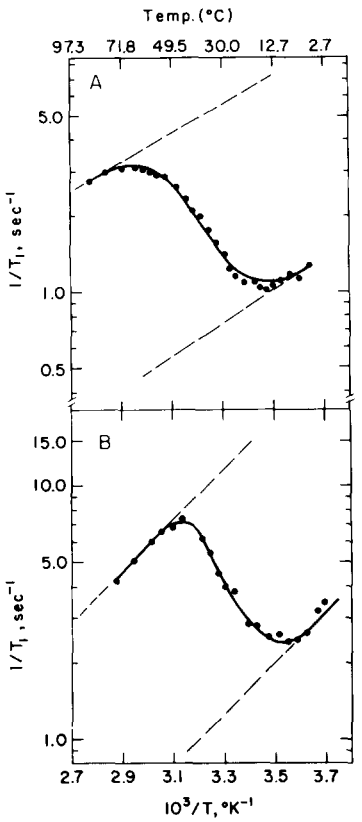

Fig. 3. Temperature dependence of T_1 relaxation rate of the α-protons of methylamine (A) and *n*-propylamine (B) in a suspension of egg phosphatidylcholine vesicles. The vesicles contained entrapped Gd·2EDTA and were prepared as described in Materials and Methods. The full curve is a best computer fit to Eqn. 1. The upper and lower dashed lines present the extrapolated curves from which the relaxation without exchange in the intra- and extravesicular media were determined.

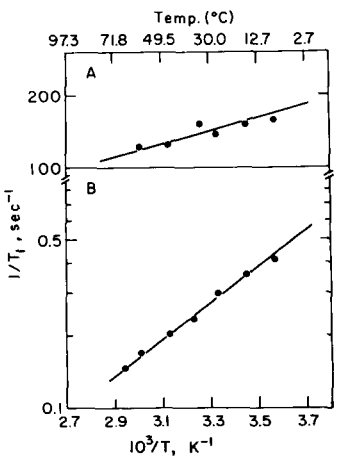

Fig. 4. Temperature dependence of T_1 relaxation rate of the α-methylene protons of *n*-propylamine. (A) In a solution comprising of 0.5 M *n*-propylamine/50 mM Gd·2EDTA, p^2H = 6.7; (B) in a control solution with a composition as in Fig. 2C.

TABLE I

THE KINETIC AND THERMODYNAMIC PARAMETERS FOR AMINE TRANSFER ACROSS PHOSPHATIDYLCHOLINE VESICLES AT 25°C

Amine	p^2H	Exchange rate ($1/\tau^i$, s^{-1})	Permeability coefficient, P ($cm \cdot s^{-1}$)($\times 10^6$)	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/(mol·deg))
Methyl	6.7	4.6± 0.5	1.0	18±2	23± 7
Ethyl	6.7	3.4± 0.5	0.7	21±2	32± 7
Propyl	6.7	20.0± 4	4.3	20±2	33± 7
Butyl	6.7	240.0± 30	52	20±2	38± 7
Pentyl	6.7	1850.0±200	400	21±3	45±13
Ethyl	8.9	300.0± 50	65		

proximated by [10]:

$$T_1^{-1} = (T_1^e)^{-1} + \frac{F^i}{F^e}(T_1^i + \tau^i)^{-1} \quad (1)$$

where e and i designate extra- and intravesicular protons, respectively. F, the fraction of amine molecules in each environment was determined from the fractional volume [10]. T_1^e and T_1^i, the relaxation times without exchange, were determined by extrapolation of the slopes at the slow and fast exchange, respectively (Ref. 10, Fig. 3).

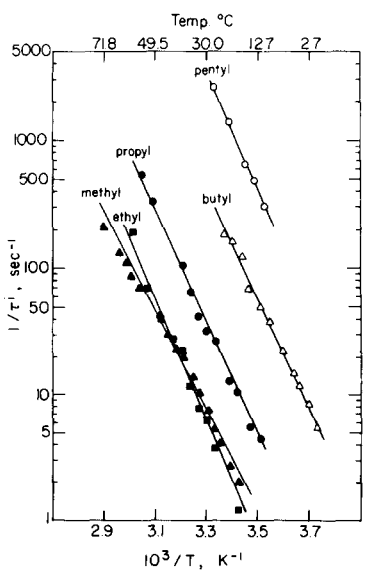

Fig. 5. Arrhenius plots of the rate of exchange of the first five *n*-alkyl homologs across egg phosphatidylcholine vesicles.

Analysis of the results assuming an Arrhenius behavior and using a nonlinear least-squares fit of the data yielded the mean intravesicular lifetime (τ^i) and the enthalpy and entropy of activation (Fig. 5, Table I). Permeability coefficients (Table I) were calculated as previously described [10].

Discussion

A general mechanism for the diffusion of nonelectrolytes across membranes is provided by the solubility/diffusion model of Diamond et al. [11]. In the limiting case, the membrane is assumed to be homogeneous and symmetrical. The membrane resistance to permeation of the solute ($1/P$) is then the sum of the two interfacial resistances (r) and the diffusional resistance of the membrane interior:

$$1/P = 2r + X_0/KD \quad (2)$$

where K is the solute partition coefficient into the membrane, D the diffusion coefficient and X_0 the membrane thickness. For the five amines studied, the interfacial resistance exerted on the amine group should be approximately the same at the same pH (their ammonium ion dissociation constant is similar: $2 \cdot 10^{-11}$), the interfacial resistance exerted on the alkyl part should increase with increasing chain length and the diffusion coefficient should decrease with increasing chain length. Thus, changes in the interfacial resistance and the diffusion coefficient in the ascending amine series should lead to a reduction in the permeability.

However, the observed large increase in the permeability with increasing chain length indicates that the transfer is dominated by the partition into the membrane.

It is reasonable to assume that only the neutral amine is actually partitioned into the membrane, and the apparent equilibrium partition coefficient is therefore:

$$K = \frac{[RNH_2]_m}{[RNH_2 + RNH_3^+]_w} = \frac{[RNH_2]_m}{[RNH_2]_w\left(1 + \frac{[H^+]}{K_b}\right)} \qquad (3)$$

where m and w designate the membrane and water phase, respectively. For $K_b \ll [H^+]$, the partition coefficient and therefore the permeability, should be inversely proportional to the acidity of the water solution. This has been shown to occur by measuring the exchange rate of ethylamine at pH 6.7 and 8.9 (Table I).

The permeability of methylamine, the smallest molecule in this series does not follow the general trend observed from ethyl to pentyl amine (Fig. 1, Table I). This distinct behavior of the smallest molecule in a homologous series was previously detected for methanol transfer across toad urinary bladder [12] and for amide transfer across red blood cells [13] and lipid vesicles [14,15]. The faster transfer of the small molecules suggest that for these molecules the membrane does not act as a barrier only by virtue of its partitioning properties, but also by its size-sieving properties. For example, the small molecules can jump through vacancies formed in the membrane by the internal motions such as the kinks. A similar mechanism was previously proposed for water diffusion across lipid membranes [16]. The smaller increase in the entropy for methylamine transfer (Table I) serves as an additional indication that this molecule perturbs the least the state of order of the hydrocarbon chains.

The enthalpy of activation for the transfer of the alkylamine was found to be high, 20 kcal/mol, and similar for all derivatives studied (Table I). This indicates that the enthalpy changes are mainly due to the energy involved in the dehydration of the amine moiety and its solubilization in the lipid phase. A similar behavior was observed for the transfer of *n*-alkylamides in lipid liposomes [17] although the activation energy was much lower. High activation enthalpies were determined for the transfer of alcohols across toad bladder [12] and of amides across human red blood cells [18]. In the latter transfers, the activation enthalpy increased with increasing chain length [12,18].

The increase in the permeability with increasing chain length is mainly due to the change in the entropy term $T \cdot \Delta S^{\ddagger}$ of 1.2 kcal/mol per -CH_2-. A similar behavior with a larger positive incremental entropy change was observed in the biological membranes mentioned above [19]. However, a negative incremental entropy change was determined for the partition in dimyristoylphosphatidylcholine liposomes [19,20]. The differences in the mode of diffusion reflect the variability in the composition, structure and order of the various systems studied. The egg phosphatidylcholine bilayer vesicles used in our study seem to present an adequate model system for studying diffusion across lipid membranes ordered in the liquid crystalline phase.

References

1 Deamer, D.W., Prince, R.C. and Crofts, A.R. (1972) Biochim. Biophys. Acta 274, 323–335
2 Rottenberg, H., Grunwald, T. and Auron, M. (1972) Eur. J. Biochem. 25, 54–63
3 De Benedetti, E. and Garlaschi, F.M. (1977) J. Bioenerg. Biomembranes 9, 195–201
4 Quintanilha, A.T. and Mehlhorn, R.J. (1978) FEBS Lett. 91, 104–108
5 Fiolet, J.W.T., Van der Erj-ter Haar, L., Kraayenhot, R. and Van Dam, K. (1975) Biochim. Biophys. Acta 387, 320–334
6 Searle, G.F.W., Barber, J. and Mills, J.D. (1977) Biochim. Biophys. Acta 461, 413–425
7 Degani, H. and Bar-On, Z. (1981) Period. Biol. 83, 61–68
8 Elgavish, G.A. and Reuben, J. (1977) J. Am. Chem. Soc. 99, 1762–1765
9 Degani, H. and Elgavish, G.A. (1978) FEBS Lett. 90, 357–360
10 Lipshitz-Farber, C. and Degani, H. (1980) Biochim. Biophys. Acta 600, 291–300
11 Diamond, J.M., Szabo, B. and Katz, K. (1974) J. Membrane Biol. 17, 148–152
12 Bindslev, N. and Wright, E.M. (1976) J. Membrane Biol. 29, 265–288
13 Sha'afi, R.I., Gary-Bobo, C.M. and Solomon, A.K. (1971) J. Gen. Physiol. 58, 238–258
14 Posnansky, M., Tong, S., White, P.C., Milgram, J.M. and Solomon, A.K. (1976) J. Gen. Physiol. 67, 45–66

15 Cohen, B.E. and Bangham, A.D. (1972) Nature 236, 173–174
16 Trauble, H. (1971) J. Membrane Biol. 4, 193–208
17 Cohen, B.E. (1975) J. Membrane Biol. 20, 205–234
18 Galey, W.R., Owen, J.D. and Solomon, A.K. (1973) J. Gen. Physiol. 61, 727–746
19 Wright, E.M. and Bindslev, N. (1976) J. Membrane Biol. 29, 289–312
20 Diamond, J.M. and Katz, Y. (1974) J. Membrane Biol. 17, 121–154