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TEMPERATURE DEPENDENCE OF *N*-PHENYL-1-NAPHTHYLAMINE BINDING IN EGG LECITHIN VESICLES

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Summary

The temperature dependence of the binding of $PhNapNH_2$ (*N*-phenyl-1-naphthylamine) to vesicles of egg phosphatidylcholine has been determined. The Arrhenius plot of the association constant exhibits a discontinuity at 20.9 °C, some 30 °C above the broad phase transition region of the phospholipid. In the temperature range above 20 °C, $\Delta H^0 = -6100$ cal·mol^{-1} and $\Delta S^0 =$ 9.7 e. u.; in the temperature range below 20 °C, $\Delta H^0 = 0$ cal·mol^{-1} and $\Delta S^0 =$ 30.4 e. u. These values are consistent with the view that there are well ordered lipid-lipid bonds below 20 °C which are significantly less important above this temperature. The order in the temperature range of 5 to 20 °C, though significantly greater than that above 20 °C, is still significantly less than that in the crystalline state.

Lee et al. [1] have studied the partitioning of the spin label, TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) in aqueous phospholipid dispersions and have presented evidence that there is a break in the Arrhenius plots of TEMPO solubility in dioleoyllecithin at a temperature of about 30 °C. This discontinuity is approximately 50 °C above the liquid crystalline to crystalline phase transition temperature of —22 °C. In connection with experiments designed for another purpose, we have measured the temperature dependence of $PhNapNH_2$ (*N*-phenyl-1-napthylamine, $M_r = 220$) binding in vesicles of egg phosphatidylcholine. We have also found a discontinuity in the Arrhenius plot at 20 °C, some 30 °C above the broad phase transition region [2] of —15 °C to —5 °C.

Vesicles of egg phosphatidylcholine were prepared by a slight modification of the Huang method [3] which yields single-walled vesicles, greater than 95% homogeneous with outside diameters ≈ 210 Å. The vesicles were suspended in a medium containing 0.1 M Tris·HCl (pH 7.40) and 0.05 M NaCl to produce a final lipid concentration in the range of 10—400 μM. To this suspen-

sion a solution of $PhNapNH_2$ in methanol ($1 \cdot 10^{-6}$ mol $PhNapNH_2$/ml methanol) was added to produce $PhNapNH_2$ concentrations in the range of 0 to approx. 40 μM. $PhNapNH_2$ fluorescence was measured in a Perkin-Elmer fluorescence spectrophotometer, model MPF 2A, using an exciting wavelength of 340 nm and a detecting wavelength of 420 nm. Though fluorescence of the $PhNapNH_2$ in the aqueous phase is minimal, appropriate corrections were made. When a Scatchard plot [4] was made of the bound/free $PhNapNH_2$ against the bound $PhNapNH_2$, a single straight line was obtained indicating that there is a single homogeneous class of binding sites for the dye in the lecithin vesicles (Ting and Solomon, unpublished work). Scatchard plots were made at each temperature to determine the association constant, $K = [PhNapNH_2]_b/[L][PhNapNH_2]_f$ in which $[PhNapNH_2]_b$ is the concentration of bound probe, [L] is the free lipid concentration in the same units as $[PhNapNH_2]_b$ and $[PhNapNH_2]_f$ is the concentration of free dye in the system in M so that K has units of M^{-1}. The quantum yield was also estimated by measuring the fluorescence intensity as a function of lipid concentration at a given dye concentration and extrapolating to infinite lipid concentration. At very high lipid concentrations bound dye concentration approaches total dye concentration, which allows us to obtain a measure of the quantum yield expressed in terms of C, which is (fluorescence intensity/bound dye concn) with units of μM^{-1}. $[PhNapNH_2]_b$ is corrected for the temperature dependence of fluorescence intensity in the vesicles. A great deal of evidence [5—7] supports the conclusion that $PhNapNH_2$ is located in the hydrocarbon region of the vesicle.

Fig. 1 shows the dependence of the natural logarithm of K on T^{-1}. A line has been drawn through the four points above 20 °C with a slope of $3.08 \cdot 10^3$ degrees calculated by variance weighted least squares. Since the point at 20 °C is aberrant, weighted least squares slopes have been computed both for the three points at the lower temperatures (slope = $0.298 \cdot 10^3$ degrees and for the same three points in addition to the 20 °C point (slope = $-0.238 \cdot 10^3$ degrees). We have taken the slope as zero which is close to the average and drawn the horizontal line in Fig. 1 through the intersection of the two least squares lines. This procedure places the discontinuity at 20.9 °C. The quantum yield is shown in Fig. 2 as a function of T^{-1} and the flattening of the curve at the lower temperatures indicates that the polarity of the vesicles becomes relatively independent of temperature as the temperature is lowered. This corresponds reasonably well with the very small slope of the Arrhenius plot of K in the same temperature range.

There are several conclusions to be drawn from Fig. 1. At 10 °C, $K = 8.5 \cdot 10^4$ M^{-1}, corresponding to $\Delta G^0 = -6350$ cal·mol^{-1}, whereas at 35 °C, $K = 4.8 \cdot 10^4$ M^{-1}, corresponding to $\Delta G^0 = -6600$ cal·mol^{-1}. In the temperature range above 20 °C, the slope of the curve corresponds to $\Delta H^0 = -6100$ cal·mol^{-1}. Thus, increasing temperature causes a decrease in solubility of $PhNapNH_2$ in the lipid vesicles.

It is possible that the change in slope in Fig. 1 might be ascribed to interaction of $PhNapNH_2$ with the vesicles. Träuble and Overath [6] considered this possibility when they used $PhNapNH_2$ fluorescence to measure phase transitions in dispersions of *Escherichia coli* lipids, which are primarily composed of phosphatidylethanolamine (82 mol %). Träuble and Overath found

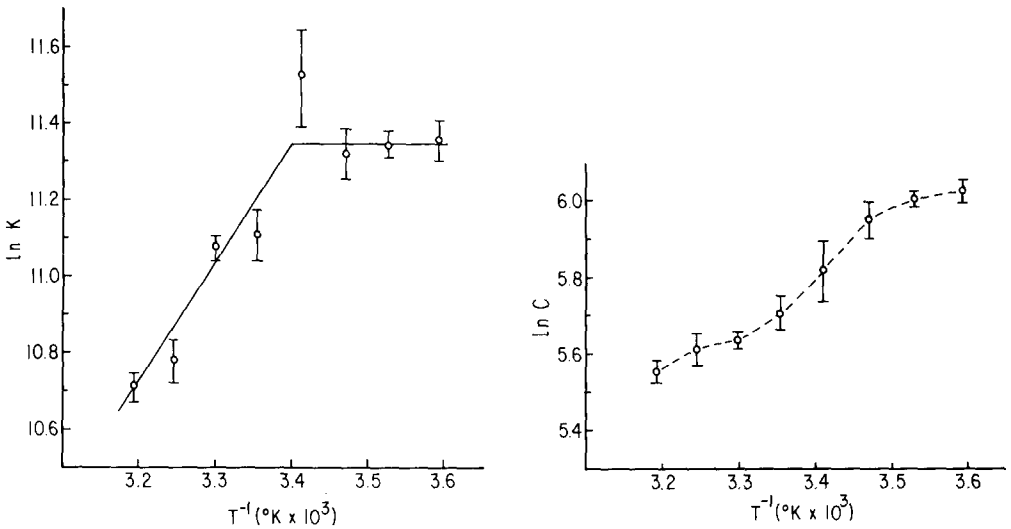

Fig. 1. Arrhenius plot showing the logarithmic dependence of the association constant $K = ([PhNapNH_2]_b/[L][PhNapNH_2]_f)$ on inverse temperature. The lines have been drawn according to the method of variance weighted least squares as described in the text.

Fig. 2. Dependence of ln C (fluorescence intensity/bound dye concn) on inverse absosute temperature. The lines have been drawn to connect the points.

that the temperature of the phase transition decreased as $PhNapNH_2$ concentration was increased above 10 μM $PhNapNH_2$ for 50 μM lipid. Though the incorporation of larger amounts of $PhNapNH_2$ "fluidizes" the membrane, it does not affect the fluorescence amplitude change of $PhNapNH_2$ at the transition, which is linearly related to $1/PhNapNH_2$ concn up to 150 μM $PhNapNH_2$. We used $PhNapNH_2$ concentrations of 0—40 μM, with lipid concentrations of 10—400 μM, using the higher $PhNapNH_2$ concentrations at the higher temperatures for which the association constant is lower. The linearity of the Scatchard plots leads us to conclude that our value of K is independent of $PhNapNH_2$ concentration under our experimental conditions.

For small nonpolar solutes dissolved in nonpolar solvents increasing temperature increases solubility and ΔH^0, which is an index of the temperature coefficient of solubility, is positive as is the case for ethane solubility in benzene, discussed by Tanford [8]. However as the solute gets larger, ΔH^0 decreases and then changes in sign. A positive temperature coefficient has been reported by Katz and Diamond [9] for short-chain monohydric alcohol partition from water into dimyristoyl lecithin liposomes, though the temperature coefficient decreases with alcohol chain length. For the transfer of alkanes from water to pure liquid hydrocarbon, ΔH^0 decreases from 2500 $cal \cdot mol^{-1}$ for ethane to 800 $cal \cdot mol^{-1}$ for propane and changes sign to -600 $cal \cdot mol^{-1}$ for butane [8], so that butane solubility decreases with increasing temperature. This fits with the observation of Diamond and Katz [10] that increasing the number of methylene groups in hydrophobic solutes leads to a change in sign in ΔH^0 for partition into dimyristoyl lecithin liposomes.

Kwant and Seeman [11] have shown that the partition coefficient for

chlorpromazine from aqueous buffer into the membrane of red cell ghosts decreases with increasing temperature; ΔH^0 varies from about −500 cal·mol^{-1} at about 13 °C to about −3000 cal·mol^{-1} at about 30 °C. Similarly valinomycin solubility in egg lecithin liposomes has been found by Blok, de Gier and van Deenen [12] to decrease with increasing temperature. Thus, the negative sign of ΔH^0 that we have observed for binding of $PhNapNH_2$ to egg lecithin vesicles in the temperature range above 20 °C is not at all unusual. The magnitude of our value of ΔH^0 however, is appreciably larger than that of ΔH^0 for chlorpromazine, a molecule of somewhat larger size (M_r = 319) which is bound more tightly to human red cell ghost membranes than $PhNapNH_2$ is to egg lecithin vesicles.

Removal of $PhNapNH_2$ from an aqueous environment and its insertion into the hydrocarbon moiety of a lecithin vesicle at temperatures above 20 °C is accompanied by a significant decrease in the enthalpy of the system. This means that the transfer of $PhNapNH_2$ to the vesicle is energetically favored. The enthalpy lost when bonds are formed in the interaction of $PhNapNH_2$ with the vesicles is less than that lost due to the cage effect, when $PhNapNH_2$ is removed from the aqueous environment.

The unusual feature of Fig. 1 is the discontinuity in ΔH^0 at 20 °C. In other systems ΔH^0 is a continuous function varying smoothly with temperature, as for ethane solubility in benzene reported by Tanford [8] and for the partition coefficients between water and decane for butanol, diethyl ether and chloroform studied by Johnson and Bangham [13] over the temperature range of 21 °C—51 °C. Since there is no evidence for a discontinuity in ΔH^0 in simple systems involving partition out of water into nonpolar solvents such as benzene or decane, we may conclude that the discontinuity we have observed must be associated with events in the vesicle and not in the aqueous environment. Since ΔH^0 is ≈ 0 for $PhNapNH_2$ binding by egg lecithin vesicles below 20 °C the bond energy gained (or enthalpy lost) by removal of $PhNapNH_2$ from the water and its introduction into the vesicles is balanced by bond-energy losses in another part of the system. This finding would be consistent with the presence of well-ordered association between neighboring hydrocarbon chains of the lipid vesicles. When $PhNapNH_2$ is introduced, these hydrocarbon-hydrocarbon interactions would have to be broken and replaced with $PhNapNH_2$-hydrocarbon chain interactions, thus providing the balance in bond energy. Above 20 °C, however, the order could be significantly less than that at the lower temperature. Then, ordered lipid-lipid bonds would be weaker and less important, so that the introduction of the $PhNapNH_2$ molecule would be accompanied by a net gain in bond energy. This view is entirely consistent with the suggestions of Lee et al. [1].

It is necessary to separate the entropy of mixing in order to express the entropy change for $PhNapNH_2$-lipid binding in terms which include only solute-solvent interactions. For this purpose, the association constant must be computed in mol fraction units [8], rather than M^{-1} units as in Fig. 1. Using mol fraction units, ΔG^0 = −9000 cal·mol^{-1} at 25 °C and −8800 cal·mol^{-1} at 15 °C leading to values of ΔS^0 of 9.7 e.u. and 30.4 e.u. at these two temperatures. The value of ΔS^0 of 9.7 e.u. above 20 °C is of the right sign though lower in magnitude than the figures of 21—23 e.u. given by Tanford for the

transfer of the alkanes (ethane through butane) from water to pure liquid hydrocarbon; and of 13—19 e.u. for the similar transfer of benzene and its methyl and ethyl derivatives. Thus, our value of ΔS^0 above 20 °C is generally consistent with the usual attribution of entropy changes of this kind to cage effects in the aqueous solution, though the entropy change in our system is appreciably smaller.

Below 20 °C the entropy change rises discontinuously to 30.4 e.u. In this temperature range the disorder introduced by the removal of the $PhNapNH_2$ from the aqueous environment appears to be supplemented by the disorder introduced by inserting the $PhNapNH_2$ into the lipid vesicle. In the case of butyramide solubility in dimyristoyl lecithin, Diamond and Katz [10] have found that ΔS^0 for partition increases from 8.2 e.u. above the transition temperature to 77.1 e.u. below. Thus, the order in our egg lecithin vesicle system in the temperature range of 5—20 °C, though significantly greater than that above 20 °C, is still significantly less than that in the crystalline state. These conclusions derived from considerations of the entropy change are consistent with those from the enthalpy change. Both point to an important change in the properties of the lipid vesicle at a temperature some 30 °C above the transition from the crystalline to the liquid crystalline phase.

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