hemoglobin or glutathione and as yet unidentified, which account for the uptake of serum gold into the rbc's.

Summary

Definitive evidence has been obtained for the binding of gold complexes at cys-β-93 of hemoglobin. AuSTm, $Et_3PAuSAtg$, and $Et_3PAuSTg$ bind only at cys-β-93, while Et_3PAuCl binds there and also at additional weak binding sites. The correlation between thiol pK_{SH}, ^{31}P NMR chemical shift, and affinity of Au(I) for various thiols successfully rationalizes the extent of the reactions of auranofin and its analogues with proteins. The NMR correlation predicted the direction of the interprotein (hemoglobin to albumin) gold transfer reaction. Because gold(I) ligand exchange reactions are facile, this correlation should prove useful in elucidating the mechanism of action of gold(I) drugs and their distributions in vivo. Predicting of the position of equilibria using ^{31}P NMR chemical shifts will be especially helpful when pK_{SH} values are not available. The comparison between the high affinity of albumin and the low affinity of hemoglobin for gold(I) and the greater affinity of hemoglobin for AuSTm than for auranofin establish that the accumulation of gold metabolites of auranofin in red blood cells is not driven by thermodynamic factors related to hemoglobin-gold complex formation.

Registry No. Et_3PAuCl, 15529-90-5; Et_3PAuST_g, 34031-29-3; AuSTm, 112070-13-0; Au, 7440-57-5; Cys, 52-90-4; auranofin, 34031-32-8.

The Nigericin-Mediated Transport of Sodium and Potassium Ions through Phospholipid Bilayers Studied by ^{23}Na and ^{39}K NMR Spectroscopy

Frank G. Riddell,*† S. Arumugam,† Peter J. Brophy,‡ Brian G. Cox,† Mary C. H. Payne,† and Timothy E. Southon†§

Contribution from the Departments of Chemistry and Biological Sciences, The University of Stirling, Stirling FK9 4LA, Scotland. *Received December 11, 1986*

Abstract: Addition of nigericin to preparations of large unilamellar vesicles made from egg yolk phosphatidylcholine in sodium or potassium chloride solution gives rise to dynamic ^{23}Na and ^{39}K NMR spectra. The dynamic spectra arise from the nigericin-mediated transport of the metal ions through the membrane. The kinetics of the transport are followed as a function of metal ion and nigericin concentrations and are compatible with a model in which one nigericin molecule transports one metal ion. The data allow the extraction of the rate constants for the association and dissociation of the nigericin/metal complex in the water/membrane interface and the evaluation of the stability constants for complex formation in the interface. The rate-determining step in the transport process is dissociation of the complex. Although sodium on its own is transported more rapidly, the greater stability of the potassium complex means that potassium is preferentially transported when both metal ions are present.

The transport of materials through the limiting membranes of living cells is a process of fundamental importance that has attracted widespread attention.[1] Essential materials such as amino acids, nucleotides, sugars, and ions are transported by membrane-bound transport systems. Quantitatively the major metal ions in such transport processes are Na^+ and K^+. Many enzyme systems that transport organic substrates also require cotransport of these metal ions. Maintenance of the transmembrane gradients of Na^+ and K^+ is vital. This is ensured by the Na^+/K^+ pumping enzyme, NaKATPase.[2]

An alternative mode of transport of metal ions exists when ionophoric materials are present.[3-7] Many ionophores, notably monensin, nigericin, and valinomycin, are active as antibiotics[8] and also have marked physiological effects in mammals. These properties presumably result from the dissipation of transmembrane ion gradients. It is believed that these materials transport ions through membranes in the form of their complex with the metal.[9] The speeding up of the transport they induce cannot be compensated by the normal mechanisms available to the cell.

Ionophoric materials can be divided into two categories, anionic and neutral. The anionic ionophores have an ionizable group, generally a carboxylic acid, and can thus form a metal/ionophore complex which is electrically neutral. The molecular structures of these ionophores are characterized by a chain of tetrahydrofuran and tetrahydropyran rings terminated at one end by a carboxylic acid group and at the other by one or more hydroxyl groups which hydrogen bond round to the acid end in the metal ion complex. Examples include monensin and nigericin (1). Neutral ionophores have no ionizable group and thus form a charged complex with the alkali metal ion. Examples include valinomycin, enniatin, the crown ethers, and the cryptands. The rate at which neutral ionophores transport ions through membranes is potentially limited by charge buildup in the membrane or cotransport of anions. These limitations do not apply to anionic ionophores where the transporting species is neutral.

The detailed model we employ for the transport of a metal ion (M^+) through a membrane is based on the classical mobile carrier system of Willbrandt and Rosenberg[10] as presented by Painter and Pressman.[9] For an anionic ionophore the transport through a phospholipid bilayer, e.g., phosphatidylcholine (PC), can be broken down into three distinct phases, each of which may in its turn be more complex. These steps are illustrated in Figure 1, and the validity of this model for transport is investigated in this

† Department of Chemistry.
‡ Department of Biological Sciences.
§ Present address: Department of Plant Sciences, University of Oxford, Parks Road, Oxford OX1 3PF, England.

(1) Wright, J. K.; Seckler, R.; Overath, P. *Annu. Rev. Biochem.* **1986**, *55*, 225-248.
(2) Glynn, I. M.; Karlish, S. J. D. *Annu. Rev. Physiol.* **1975**, *37*, 13-55.
(3) See, for example, articles in *Top. Curr. Chem.*, Vols. 98, 101, and 121.
(4) Lehn, J. M. *Struct. Bonding (Berlin)* **1973**, *16*, 1-69.
(5) Simon, W.; Morf, W. E.; Meier, P. C. *Struct. Bonding (Berlin)* **1973**, *16*, 113-160.
(6) Pressman, B. C. *Annu. Rev. Biochem.* **1976**, *45*, 501-530.
(7) Ovchinnikov, Y. A. *Eur. J. Biochem.* **1979**, *94*, 321-336.
(8) See, for example, articles in: *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Decker: New York, 1985; Vol. 19.
(9) For a review see: Painter, G. R.; Pressman, B. C. *Top. Curr. Chem.* **1982**, *101*, 83-110.
(10) Wilbrandt, W.; Rosenberg, T. *Pharmacol. Rev.* **1961**, *13*, 109-183.

0002-7863/88/1510-0734$01.50/0

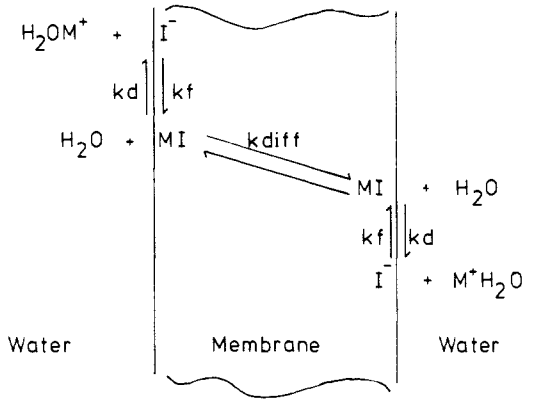


Figure 1. Model used for anionic transport.

paper. The ionophore is initially held in the membrane surface with its ionized group projecting into the aqueous medium and the rest of the molecule in the interior of the membrane. There is the possibility of the ionized group (COO^-) being associated with any positively charged groups (e.g., NMe_3^+) in the phosphatidylcholine–water interface.

In the first phase of the transport the metal ion, M^+, approaches and is complexed by the ionophore. This is undoubtedly a multistep process as each water molecule solvating the metal ion must be replaced by an oxygen atom in the ionophore. As each successive water is substituted, the metal ion gets more encapsulated by the ionophore and goes deeper into the interior of the membrane. The overall rate constant for this process is designated k_f. The neutral complex diffuses to the opposite surface in the second phase of the transport. The complex then releases M^+ to the aqueous medium on the second surface in a multistep process with overall rate constant k_d. The metal ion is resolvated by water during this phase and the ionophore is now trapped on the opposite surface.

To return to the original state with the ionophore on the first surface, a reversal of the sequence must occur and a similar or a different ion, N^+, is transported in the opposite direction. The overall process is, therefore, electrically neutral with no disruption of membrane potential. Neutral ionophores such as valinomycin can diffuse back to the initial surface in an uncomplexed form. Therefore, they do not necessarily maintain electrical neutrality across the membrane which can lead to disruption of the membrane potential causing metabolic chaos. Neutral ionophores are, therefore, potentially more toxic than anionic ionophores.

Chemically significant questions that can be asked about this model include: does the transport process proceed by a 1:1 metal ion:ionophore complex, is the rate-determining step one of the surface processes (presumably k_d) or is it the diffusion step, and how does the stability of the complex ($K = k_f/k_d$) affect the transporting ability?

We have previously shown that the use of large unilamellar phospholipid vesicles and aqueous shift reagents for ^{23}Na NMR allowed a study of the monensin-mediated transport of sodium ions through phospholipid bilayers.[11] The method relies upon the rate of exchange between equimolar concentrations of Na^+ inside and outside the vesicles giving rise to dynamic NMR spectra. We now report an extension of this work to the nigericin-mediated transport of Na^+ and K^+ through the walls of egg yolk PC vesicles. Nigericin gives rise to dynamic ^{23}Na, ^{39}K, ^{87}Rb, and ^7Li spectra under similar conditions to those we reported earlier. Quantification of the Rb and Li results is not easy, however, and we report here only the ^{39}K and ^{23}Na results.

Experimental Section

Large unilamellar vesicles (LUV) were prepared from various samples of phosphatidylcholine (PC) by a modification of the dialytic detergent removal technique introduced by Reynolds and co-workers.[12] In a typical preparation 25–30 μmol of PC together with 15 equiv of *n*-octyl-β-glucopyranoside (0.45 mmol) were dissolved in 1.5 cm^3 of 100 mM NaCl. This solution was dialyzed at 40 °C against ca. 2 L of 100 mM NaCl for 12 h. This dialysis was repeated three times. The dialysis medium was then changed to 50 mM NaCl/10 mM Na_5PPPi/20 mM choline chloride and two further dialyses were performed. All dialyses of egg PC were performed under a constant stream of bubbled oxygen-free nitrogen. All such dialysis solutions were presaturated with nitrogen at 40 °C for at least 12 h before use. For potassium the final dialysis medium was solely composed of K_5PPPi and choline chloride. All aqueous solutions were prepared from distilled water.

Table I. Rate Constants for Sodium Transport

$[Na^+]$ (M)	k_2[a]	std dev	corr coeff
0.025	5.71×10^4	2.6×10^3	0.991
0.050	3.82×10^4	1.7×10^3	0.991
0.100	2.23×10^4	0.7×10^3	0.994
0.150	1.70×10^4	0.5×10^3	0.996
0.200	1.52×10^4	0.8×10^3	0.958
0.050 Na^+ 0.050 K^+	0.63×10^4	0.24×10^3	0.980

[a] Equation 4a: mol of PC·mol of nigericin^{-1} s^{-1}.

The resulting typical sample of LUV's thus had 100 mM Na^+ inside and outside. The internal anion was chloride and the external anions were chloride and tripolyphosphate. The choline chloride was added to balance the total ionic concentration on both sides of the membrane. Since the preparations resulted in equal concentrations of Na^+ (or K^+) on each side of the membrane, the transport experiments involve no net movement of metal ion. A slight contraction of volume generally occurred upon dialysis and typically 1.45 ± 0.1 cm^3 of vesicle suspension was recovered.

This suspension of LUV's was taken, and a sufficent amount of a 1 M solution of $DyCl_3$ (Na^+ vesicles) or $Tb(NO_3)_3$ (K^+ vesicles) (typically a few microliters to give 1–5 mM solutions in lanthanide) to create a chemical shift difference of ca. 12 ppm (Na) or 9 ppm (K) was added. Vesicle suspensions prepared at this higher temperature consistently gave a higher proportion of internal M^+ (~9.5%) than those prepared at 4 °C (~2.6%).

Electron microscopy of freshly prepared vesicles was performed on a JEOL 100C transmission electron microscope using freshly prepared formvar grids pretreated with bacitracin using uranyl acetate or ammonium molybdate as a negative stain. LUV's with radii between about 150 and 800 nm were observed with mean radius 380 nm and standard deviation 180 nm based on observations of 188 objects.

Commercial sodium tripolyphosphate was used containing ca. 85% triphosphate and ca. 15% diphosphate. The errors in M^+ concentrations arising from this composition are less than 2%. Potassium tripolyphosphate was prepared by neutralizing a known quantity of KOH with tripolyphosphoric acid prepared from sodium tripolyphosphate on a cation-exchange column in its H^+ form. ^{23}Na NMR revealed negligible amounts of sodium salts in these preparations. Nigericin was supplied by Sigma as 90–95% pure and no further purification was attempted. Nigericin was used as standard solutions in methanol, typically ca. 5×10^{-3}M. Egg yolk phosphatidylcholine was supplied by Lipid Products.

Sodium transport studies were carried out on a Bruker WP80 FT NMR spectrometer in Stirling operating at 21.19 MHz. Potassium transport studies were carried out on a Bruker WH360 FT NMR spectrometer in Edinburgh University operating at 16.8 MHz. In all cases the spectrometer was field/frequency locked on the ^2H resonance of ^2H_2O in the inner compartment of a coaxial tube. All measurements were performed at 303 K. Spectra were line broadened, typically by 2 Hz (Na) or 5 Hz (K), to improve the signal-to-noise ratio. For ^{23}Na typically 4000 transients were collected into 512 data points with a spectral width of 1000 Hz, zero filled and transformed in 4K data points. For ^{39}K typically 40 000 transients were collected into 256 data points with a 2000 Hz spectral width, zero filled and transformed in 16K data points. Pulse widths of ~90° were used throughout with typical recycle times 0.128 s (Na^+) and 0.064 s (K^+). In the absence of exchange, T_1 values were typically ~0.020 s, ^{39}K; 0.30 s, ^{23}Na.

Addition of small aliquots (microliter amounts) of the standard solution of nigericin in methanol caused a dynamic broadening of both the in and out lines from the vesicle preparation. Lifetimes (τ) were measured either by following the line broadening of the M^+_{in} peak in the slow exchange limit using the relationship $(1/\tau) = \pi$(line broadening) or by calculation of the complete lineshape. Good agreement was obtained between the lifetimes obtained by both methods. Results quoted are from

(11) Riddell, F. G.; Hayer, M. K. *Biochim. Biophys. Acta* **1985**, *817*, 313–317.

(12) Mimms, L. T.; Zampighi, G.; Nozaki, Y.; Tanford, C.; Reynolds, J. A. *Biochemistry* **1981**, *20*, 833–840.

Table II. Rate Constants for Potassium Transport

[K+] (M)	k_2[a]	std dev	corr coeff
0.050	1.64×10^4	1.0×10^3	0.992
0.075	1.18×10^4	1.1×10^3	0.974
0.100	0.90×10^4	0.4×10^3	0.992

[a] Equation 4a: mol of PC·mol of nigericin^{-1} s^{-1}.

Table III. Temperature Variation of Sodium Transport Rates[a,b]

temp (K)	k (s^{-1})[c]	temp (K)	k (s^{-1})[c]
283	3.8	313	16.9
293	8.0	323	24.5
303	11.5	328	32.2

[a] Conditions: egg PC, 100 mM Na^+, sufficient nigericin added to give a rate constant of 11.5 s^{-1} at 303 K. [b] These rate constants give E_a = 8.21 ± 0.28 kcal·mol^{-1}. [c] $k = 1/\tau_{M^+,in}$.

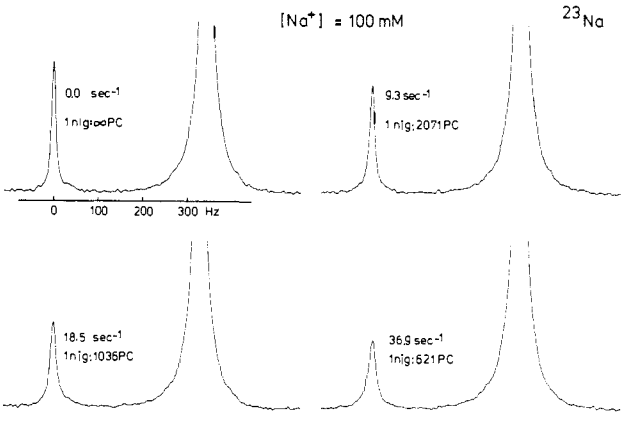


Figure 2. Typical dynamic ^{23}Na NMR spectra.

measurements of line broadenings. Rate constants (k) for efflux were determined from plots of $1/\tau$ versus nigericin/PC molar ratio. Results are given in Tables I–III. Ratios of nigericin to phosphatidylcholine used in this work range between 1:12000 and 1:300. This corresponds to a concentration of nigericin in the membrane of ca. 1.3×10^{-4} to 5.5×10^{-3} M. Figure 2 shows typical dynamic ^{23}Na NMR spectra with an indication of exchange rate and nigericin/phosphatidylcholine ratio.

Results and Discussion

The model presented earlier and drawn in Figure 1 can be used in conjunction with steady-state kinetic analysis to derive the following rate equation describing the lifetime (τ) of a metal ion (M^+) inside a lipid vesicle of volume V_{in} and surface area A with a total ionophore concentration (complexed + uncomplexed) $[L]_T$:[13]

$$\frac{1}{\tau_{M^+,in}} = \frac{A}{V_{in}} \frac{k_{diff} k_d [L]_T}{(k_d + 2k_{diff})([M^+] + k_d/k_f)} \quad (1)$$

Two limiting forms of this equation can be identified according to whether diffusion through the membrane interior or dissociation at the membrane surface is the rate-limiting process. For diffusion being rapid ($k_{diff} \gg k_d$) eq 1 reduces to:

$$\frac{1}{\tau_{M^+,in}} = \frac{A}{V_{in}} \frac{k_d [L]_T}{2([M^+] + k_d/k_f)} \quad (2)$$

and for diffusion being rate-determining ($k_d \gg k_{diff}$):

$$\frac{1}{\tau_{M^+,in}} = \frac{A}{V_{in}} \frac{k_{diff} k_d [L]_T}{[M^+] + k_d/k_f} \quad (3)$$

Equation 1 can be rewritten as:

$$\frac{1}{\tau_{M^+,in}} = \frac{A}{V_{in}} \frac{V_m [L]_T}{K_m + [M^+]} \quad (4)$$

where $K_m = k_d/k_f = K_s^{-1}$ (K_s is the stability constant of the membrane bound ligand metal complex). The value of V_m depends upon the relative values of k_{diff} and k_d.

Equations 1–4 predict the following:

$$\frac{1}{\tau_{M^+,in}} = k_2 [L]_T \quad (4a)$$

i.e., first-order kinetics in [L], where

$$k_2 = \frac{A V_m}{V_{in}(K_m + [M^+])}$$

$$\frac{1}{\tau_{M^+,in}} \propto \frac{A}{V_{in}} \propto \frac{1}{r}$$

where r is the radius of the vesicles

$$\tau_{M^+,in} = \frac{K_m}{V_m [L]_T} + \frac{[M^+]}{V_m [L]_T} \quad (5)$$

Equation 5 indicates that a plot of $\tau_{M^+,in}$ versus $[M^+]$ at constant $[L]_T$ (i.e., 1/(first-order rate constant for efflux)) should be linear with slope $1/V_m[L]_T$ and intercept $K_m/V_m[L]_T$. Thus for this graph the ratio of slope/intercept is the stability constant K_s for the membrane bound complex. The slope and intercept will be proportional to $1/k_d$ and $1/k_f$, respectively, if diffusion is rapid. For slow diffusion the graph will have slope proportional to $1/k_{diff}$ and intercept proportional to $k_d k_{diff}/k_f$. For intermediate situations the functions describing slope and intercept will be somewhat more complex.

As we previously observed for monensin, adding small aliquots of nigericin to the vesicle preparation broadened both lines in the spectra. The line broadenings are consistent with a dynamic exchange process between the M^+(in) and M^+(out) populations. The rates of exchange are recorded in Tables I and II. For every concentration of M^+ studied the transport rate in the direction in → out varies linearly with nigericin concentration which is completely consistent with our model. This variation indicates a first-order relationship between nigericin concentration and transport rate which is consistent with the postulated 1:1 complex between M^+ and the transporting species. Furthermore, this is in agreement with the most recent results obtained electrochemically by Antonenko and Yaguzhinsky,[14] but at variance with earlier electrochemical measurements which suggested a bimolecular model.[15,16]

The inverse relationship of the transport rate to the radius of the vesicles predicted by our model was tested by preparing smaller egg yolk PC vesicles containing 200 mM NaCl by an ethanol injection technique[17] and concentrating them by vacuum dialysis. These vesicles were found both by electron microscopy and by NMR determination of the proportion of Na^+ enclosed to have ca. 25% of the diameter of the vesicles prepared in our previous work.[11] When treated with monensin the transport rate, predicted to be ca. 4 times faster, was found to be 4.8 times faster, in satisfactory agreement with our model.

The question as to the nature of the rate-determining step was tackled in two ways: application of the model to examine the differences between Na^+ and K^+ transport for which k_{diff} should be almost identical but k_d should be different, and altering the interior of the membrane from a less to a more rigid state which should retard the diffusion step.

A quantitative investigation of the variation of the exchange rate ($\tau^{-1}_{M^+,in}$) with $[M^+]$ suggests that dissociation and not diffusion is the rate-limiting step. Figure 3 shows a comparison of $1/k$ versus $[M^+]$ for K^+ and Na^+. It can be seen that both graphs

(13) For a general discussion of steady-state equations for mediated ion transport, see: Stein, W. D., In *Membrane Transport*; Bonting, S. L., de Pont, J. J. H. H. M., Eds.; Elsevier: 1981; pp 123–157.

(14) Antonenko, Y. N.; Yaguzhinsky, L. S. *FEBS Lett.* **1983**, *163*, 42–45.
(15) Toro, M.; Gomez-Lojero, C.; Montal, M.; Estrada, O. S. *J. Bioenerg.* **1976**, *8*, 19–26.
(16) Markin, V. S.; Sokolov, V. S.; Boguslavsky, L. I.; Yaguzhinsky, L. S. *Biofizika* **1977**, *22*, 48–53.
(17) Kremer, J. M. H.; v. d. Esker, M. W. J.; Pathmamanoharan, C.; Wiersema, P. J. *Biochemistry* **1977**, *16*, 3932–3935.

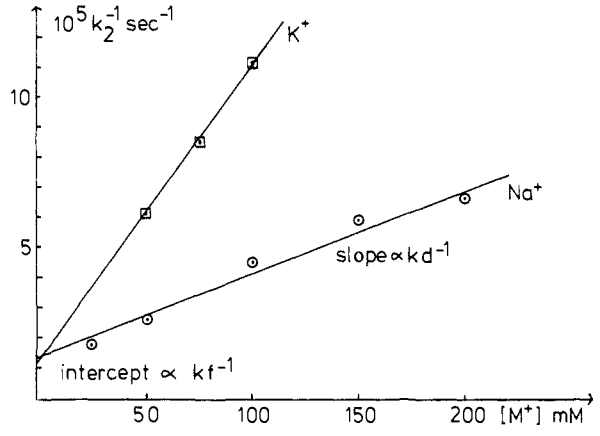


Figure 3. Graph of k_2^{-1} vs [M^+] for Na^+ and K^+.

have the same intercept within the experimental error but that the slopes differ by a factor of 3.5. In the event that diffusion as rate limiting our model predicts that the slope of this graph should be given by $1/k_{diff}$. Since the nigericin complexes of Na^+ and K^+ have almost exactly the same molecular weight, have been shown by X-ray crystallography to have the same shape,[18,19] and also have virtually identical molecular volumes,[20] the diffusion rate should be virtually identical for both metals. Indeed for valinomycin- and trinactin-mediated transport of K^+, Rb^+, and Cs^+ through bilayer membranes, the diffusion rates for the complexes of the different cations vary by only 30%.[21,22] The three-to-four-fold difference in slope therefore argues strongly against diffusion being (wholly) rate determining in the transport process for the nigericin complexes.

The absolute values of the diffusion rate constants calculated on the assumption that diffusion is rate determining also appear unreasonably low in comparison with other systems. From the slopes of the lines in Figure 3 we obtain for the rate constant of diffusion k_{diff} ($=k_{diff}A/V$) = 2.5×10^3 s^{-1} (Na^+), and 7×10^2 s^{-1} (K^+) or diffusion times of 0.4 and 1.4 ms, respectively.[21,22] These diffusion coefficients are 5 to 100 times lower than corresponding values for neutral ligands or their cationic complexes in the case of valinomycin and trinactin (glycerol monooleate/*n*-decane bilayer membranes). Again these calculations suggest that dissociation not diffusion is rate determining.

Confirmation of the nature of the rate-determining step was obtained by studying the effect of cholesterol incorporation into the membrane. Cholesterol is widely believed to stiffen the interior of biological membranes[23] and should therefore slow the diffusion step. This would reduce the transport rate if diffusion were rate determining. When this experiment was performed with monensin as ionophore transporting sodium, no appreciable rate differences were found at cholesterol incorporations of up to 12.5%.

Our results differ from those reported for neutral ionophores in that for the neutral carriers the diffusion and dissociation rate constants are normally closely similar.[24] However, the overall rates, reported as turnover numbers (V_m, eq 4, or $A/V(1/k_{diff} + 2/k_d)^{-1}$, eq 1), are of similar magnitude for the two types of ligand. For valinomycin/K^+ turnover numbers varying between 2×10^{-4} s^{-1} (thylakoid membrane) to 2×10^{-3} s^{-1} (mytochondrial membrane) have been found compared with 2.5×10^{-3} s^{-1} (Na^+) and 7×10^{-2} s^{-1} (K^+) for nigericin in the present case.

The activation energy for the overall process of the nigericin-mediated transport of Na^+ was determined by following the temperature variation of the linewidth of the Na(in) signal. Sufficient nigericin was added to the vesicle preparation to establish a moderate rate (~ 12 s^{-1}) at 303 K. The line broadenings at the various temperatures were calculated from the observed linewidth minus the natural linewidth of the same sample at that temperature (Table III). Application of the Arrhenius equation to the results gives a small positive activation energy (8.2 kcal mol^{-1}). This activation energy cannot be interpreted in detail as the temperature dependence of k will have contributions from several individual rate constants (eq 1). However, activation energies for complex formation and dissociation of ionophores in homogeneous solution are small[25] and so this result is not unreasonable.

The ratio of slope to intercept gives the apparent stability constant for the ionophore/metal complex in the membrane surface. The values obtained are 22 M^{-1} (Na^+) and 96 M^{-1} (K^+). The K^+ complex of nigericin is therefore more stable in the membrane water interface than the Na^+ complex. This position also holds in homogeneous methanol solution, but the absolute magnitudes of the stability constants are much larger: log K_s(MeOH) = 4.68 (Na^+) and 5.66 (K^+). Furthermore, it is noticeable that at the membrane-water interface the difference in stability arises from a difference in the rate constants for dissociation, k_d, (1/slope) with the k_f values (1/intercept) for the complexes being almost identical. This is quite a general property of complexes of ionophores in homogeneous solution.[26]

The differences between the apparent stability constants in the membrane/water interface and those measured in methanol are interesting. The membrane/water interface values are around 4×10^3 times lower than those for methanol, but the stability order $K^+ > Na^+$ is the same. Even larger reductions (ca. 10^5) have been observed for the neutral ionophore valinomycin.[24] Part of the reduction may be attributed to the higher solvation energy of Na^+ and K^+ in water compared to methanol (ca. 6 kJ mol^{-1} or 1 log unit in K_s).[27] The remainder must come from stronger interaction between the carboxylate group or other ligand donor atoms and water molecules at the interface. The membrane potential may also be an important factor.

Nigericin is often pointed to as the archtypal potassium carrying carboxylic ionophore and monensin as its sodium carrying counterpart. An interesting comment on this generalization arises as a result of our work, for it is seen that nigericin transports sodium more rapidly than potassium under our experimental conditions. How are these results reconciled?

The speed with which nigericin transports sodium relative to potassium is seen from our results to arise from a more rapid dissociation rate while the association rates are very similar. The consequences of this is a lower apparent stability constant for the sodium complex in the membrane surface. One can calculate, on the basis of our equilibrium constant data, that at 50 mM M^+ with no competing metal present 50% of the ionophore would be complexed as its sodium salt but 82% as its potassium salt. Furthermore, in the presence of a mixture of Na^+ and K^+ the greater stability of the K^+ complex will lead to a greater proportion of the ionophore being bound as its K^+ complex. Thus in a 50 mM Na^+/50 mM K^+ mixture it can be calculated that 69% of the ionophore will be found as its K^+ complex as opposed to 15% as the Na^+ complex. The transport of potassium will thus be barely slowed by the presence of competing sodium, but the transport of sodium should be reduced by about a factor of 3.5 by the competing potassium.

When this experiment was performed, it was found that the transport rate of Na^+ was reduced by a factor of ~ 6.0, qualitatively in agreement with our hypothesis. The result is more than acceptable as a demonstration of the greater stability of the

(18) Geddes, A. J.; Sheldrick, B.; Stevenson, W. T. J.; Steinrauf, L. K. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 1245–1251.
(19) Barrans, P. Y.; Alleaume, M.; David, L. *Acta Crystallogr., Sect. B* **1980**, *36*, 936–938.
(20) Pointud, Y.; Juillard, J.; Jeminet, G.; David, L. *J. Chem. Soc., Faraday Trans. 1* **1981**, *77*, 1–8.
(21) Benz, R.; Läuger, P. *J. Membr. Biol.* **1976**, *27*, 171–191.
(22) Benz, R.; Stark, G. *Biochim. Biophys. Acta* **1975**, *382*, 27–40.
(23) El-Sayed, M. Y.; Guion, T. A.; Fayer, M. D. *Biochemistry* **1986**, *25*, 4825–4832.
(24) Burgermeister, W.; Winkler-Oswatisch, R. *Top. Curr. Chem.* **1976**, *69*, 93–196.
(25) Cox, B. G.; Firman, P.; Schneider, H. *J. Am. Chem. Soc.* **1985**, *107*, 4297–4300.
(26) Cox, B. G. *Annu. Rep. Chem. Soc., Sect. C* **1985**, 43–80.
(27) Cox, B. G. *Annu. Rep. Chem. Soc., Sect. A* **1973**, 249–274.

membrane bound K^+ complex of nigericin. Quantitatively it is in fair agreement with our estimates of the stability constants of the Na^+ and K^+ complexes. It is noteworthy that when a similar experiment was performed with monensin, little effect was observed on the Na^+ transport rate when an equimolar amount of K^+ was present.[28] For monensin the Na^+ complex is observed to have the higher stability constant in nonaqueous solvents,[26] and one would therefore expect little effect on the Na^+ transport rate.

Acknowledgment. We thank the Science and Engineering Research Council for a grant to carry out this work and for an allocation of time on the high-field spectrometer in Edinburgh. We thank Dr. Stephen George of the NERC aquatic biochemistry unit of Stirling University for assistance with the electron microscopy.

Registry No. Na, 7440-23-5; K, 7440-09-7; nigericin, 28380-24-7.

(28) Hayer, M. K. Ph. D. Thesis, Stirling, 1984.

Dramatic Influence of the Nature of the Surfactant Aggregate on the Rate Constant for Hydrolysis of Phosphinate Esters in Aqueous Nematic Lyotropic Liquid Crystals

V. Ramesh and M. M. Labes*

Contribution from the Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122. *Received March 26, 1987*

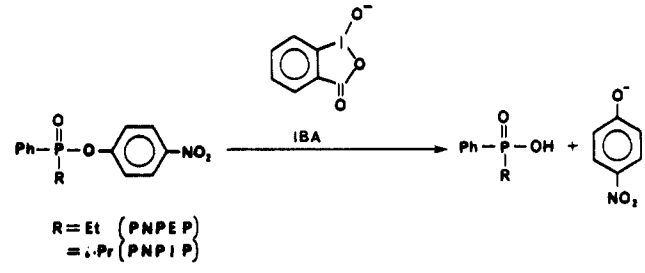
Abstract: The *o*-iodosobenzoic acid catalyzed hydrolysis of two organophosphinate esters has been studied in the lyotropic nematic phases of myrystyltrimethylammonium bromide in 1-decanol, ammonium bromide, and water. Depending on the concentration of these ingredients, three phases are observed at room temperature consisting of rod-like (N_C), disk-like (N_L), or sphere-like (I) aggregates. There is a three order of magnitude difference in the rate constant for hydrolysis between N_L and N_C phases. The N_L phase serves as a protective environment, preventing the hydrolysis of the ester, whereas the N_C phase allows the reaction to occur rapidly. Based upon infrared evidence, the phosphinate ester is solubilized in the hydrocarbon region of N_L aggregates, but in N_C and I aggregates the solute is nearer the aqueous interface.

It has recently been reported that the disk-like (N_L), rod-like (N_C), and sphere-like (I) phase of nematic lyotropic liquid crystals differ dramatically as solvent media for organic reactions.[1-3] One case of a hydrolysis reaction has been studied in detail: catalyzed ester hydrolysis of *p*-nitrophenyl laurate in myristyltrimethylammonium bromide (MTAB) lyomesophases.[2] The rate constant for hydrolysis in the lamellar N_L phase was lower by a factor of ~5 than in the N_C phase. Since the solubility of organic molecules in these concentrated surfactant phases are about 100-fold greater than the dilute micellar phases of the same surfactants, and since reactivity control can be afforded by varying the surfactant medium only slightly, it seems appropriate to extend this study to other hydrolytic processes.

In particular, the process of detoxification of organophosphorus compounds by hydrolytic cleavage has been the subject of considerable investigation.[4-10] Recently, Moss et al.[6-9] and Mackay et al.[10] have demonstrated that micelle solubilized *o*-iodosobenzoic acid (IBA) and its derivatives, in their preferred 1-hydroxy-1,2-benziodoxolin-3-one valence tautomeric form, are very efficient catalysts for phosphate cleavage.

In this work, the IBA-catalyzed hydrolyses of *p*-nitrophenyl ethyl(phenyl)phosphinate (PNPEP) and *p*-nitrophenyl isopropyl(phenyl)phosphinate (PNPIP) are studied in the dilute micellar and the N_L, N_C, and I phases of MTAB/1-decanol/ammonium bromide/water (Scheme I). Utilizing FTIR spectroscopy the nature of the solute solubilization in the N_L, N_C, and I phases are also determined. A more than three-order of magnitude difference is found between the rate constants in the N_L and N_C phases, and an explanation for the reactivity differences is proposed.

Scheme I



Experimental Section

Materials. PNPEP and PNPIP were obtained from Ash-Stevens, Inc. and used as received. IBA and MTAB were obtained from Aldrich Chemical Co. IBA was used as received, whereas MTAB was recrystallized twice from 95% ethanol/water and dried under vacuum. Triply distilled deionized water was used in the preparation of all samples.

Kinetic Studies. Solutions (2.0×10^{-4} M) of IBA in the liquid crystalline solvents MTAB/water (N_C) and MTAB/water/1-decanol/NH_4Br (N_L) and the isotropic micellar solution were prepared by stirring together for 10 h weighed amounts of IBA and the components forming the phases[11-13] (Table I). The N_L–I and N_C–I transitions were depressed by approximately 2 °C in these solutions. Samples were prepared so that

(1) Ramesh, V.; Labes, M. M. *J. Am. Chem. Soc.* **1986**, *108*, 4643.
(2) Ramesh, V.; Labes, M. M. *Mol. Cryst. Liq. Cryst.* **1987**, *144*, 257.
(3) Ramesh, V.; Labes, M. M. *J. Am. Chem. Soc.* **1987**, *109*, 3228.
(4) Mackay, R. A. *Adv. Colloid Interface Sci.* **1981**, *15*, 131.
(5) *Catalysis in Micellar and Macromolecular Systems*; Fendler, J. H., Fendler, E. J., Eds.; Academic Press: New York, 1975.
(6) Moss, R. A.; Alwis, K. W.; Bizzigotti, G. O. *J. Am. Chem. Soc.* **1983**, *105*, 681.
(7) Moss, R. A.; Alwis, K. W.; Shin, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 2651.
(8) Moss, R. A.; Kim, K. Y.; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 788.
(9) Moss, R. A.; Chatterjee, S.; Wilk, B. *J. Org. Chem.* **1986**, *51*, 4303.
(10) Mackay, R. A.; Longo, F. R.; Knier, B. L.; Durst, H. D. *J. Phys. Chem.* **1987**, *91*, 861.
(11) Boden, N.; Radley, K.; Holmes, M. C. *Mol. Phys.* **1981**, *42*, 493.
(12) (a) Kuzma, M.; Skarda, V.; Labes, M. M. *J. Chem. Phys.* **1984**, *81*, 2925. (b) Skarda, V.; Labes, M. M. *Mol. Cryst. Liq. Cryst.* **1985**, *126*, 187. (c) Hui, Y. W.; Labes, M. M. *J. Phys. Chem.* **1986**, *90*, 4064.
(13) Forrest, B. J.; Reeves, L. W. *Chem. Rev.* **1981**, *81*, 1 and references therein.

0002-7863/88/1510-0738$01.50/0