THE STIMULATION OF DIFFUSION OF ADENINE NUCLEOTIDES ACROSS BIMOLECULAR LIPID MEMBRANES BY DIVALENT METAL IONS

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SUMMARY

The diffusion rates of [^{3}H] adenine nucleotides across bimolecular lipid membranes were shown to be directly related to their organic/water partition coefficients, the order being ATP > ADP > AMP. Nucleotide diffusion was stimulated by divalent metal ions with the order of stimulation being Cu^{+2} >> Zn^{+2} > Mg^{+2}. The ability of a divalent metal ion to stimulate diffusion appears to be related to its ability to bind to the N-7 of the adenine ring. The divalent metal ions increase adenine nucleotide diffusion both by complexing with the nucleotide thus decreasing the charge on the nucleotide and by increasing the permeability of the lipid bilayer.

INTRODUCTION

The mechanism of nucleotide transport is currently under debate. Three general types of mechanisms have been suggested: 1) a transphosphorylation mechanism (1); 2) a specific transferase or permease (2); and 3) a diffusion mechanism (3,4). Support for the diffusion mechanism centers around the fact that uptake of adenine nucleotides into mitochondria is proportional to the external ATP concentration (3). In addition, nucleotides taken up by the mitochondria can be readily washed out with isotonic media (4). Although the exact nature of the diffusing species is unknown it may well involve a nucleotide-metal complex. Such complexes have been studied for several years but the exact structural relationship between nucleotide and metal has yet to be unequivocally proven.

Several conclusions about metal-nucleotide complexes have been drawn, based primarily on NMR studies (5,6,7). In the case of nucleotide triphosphates, it was found that Mg^{+2}, Ca^{+2} and Zn^{+2} bind at the β-γ phosphates while Mn^{+2}, Ni^{+2} and Co^{+2} bind at all 3 phosphates. Cu^{+2} binds preferentially

to the α-β phosphates. In addition, divalent metals have varying ability to bind to the nucleotide base. The bond is probably indirect from the metal through a water molecule to the N-7 of the adenine ring. The order of binding to the ring nitrogen was shown to be $Cu^{+2} > Cd^{+2} > Zn^{+2} > Mn^{+2} > Ni^{+2} > Co^{+2} > Mg^{+2}$ (8,9). The binding of metals to nucleotides decreases the negative charge on the nucleotide and should therefore increase the ability of the nucleotide to enter and cross the membrane.

Bimolecular lipid membranes (BLM)* have been shown to be an excellent model for studying numerous membrane-related phenomena including transport (10,11). Klein et al. (12) and Stillwell and Winter (13) have shown that amino acid diffusion across BLM is directly related to the organic/water partition coefficient. Bean and Shephard (14,15) found a similar relationship for a series of indoles. By decreasing the charge on a nucleotide the metal ion should increase both the lipophilicity of the molecule and its diffusion rate across BLM.

Petkau and Chelak (16) have measured the diffusion of [^{3}H]ATP across BLM in the presence of Na^+ and Mg^{+2}. They noticed a small increase in diffusion in the presence of 0.1 M NaCl and 0.5 mM $MgCl_2$. They observed no lag time and so concluded that the cations were binding to the nucleotide rather than binding the nucleotide to the membrane. Their model system was in close agreement with measurements made on erythrocytes.

In this paper the rates of diffusion of [^{3}H]AMP, [^{3}H]ADP and [^{3}H]ATP were measured and compared to their water/organic partition coefficients. The effect of the divalent metal ions Mg^{+2}, Zn^{+2} and Cu^{+2} on stimulating adenine nucleotide diffusion is also measured and compared with their mode of binding to the nucleotides.

MATERIALS AND METHODS

The tritium-labelled adenine nucletides (all 8-^{3}H) AMP (10 Ci/mM), ADP (18.4 Ci/mM) and ATP (18.3 Ci/mM) were purchased from Schwarz-Mann. The

*Abbreviation used: BLM, Bimolecular Lipid Membrane.

purity was checked by chromatography on silica gel thin layer plates in the solvent 3M LiCl: 1N acetic acid 1/4 v/v. The nucleotides had reasonable radiochemical purity ([^{3}H]AMP, 98.2%; [^{3}H]ADP 99.3%; and [^{3}H]ATP, 97.1%). The small amount of radioactive impurity appeared to be the same in each case. The labelled adenine nucleotides were sequestered inside liposomes composed of egg lecithin and cholesterol. The buffer used was 0.1 M KCl-0.025 M tris, pH 7.5. The metal chlorides at concentrations of either 0.5 mM or 5.0 mM were added to 1 ml of the buffer containing 5-10 μCi of the [^{3}H] nucleotides. Metal concentrations much greater than 5.0 mM caused precipitation of the phospholipids and concentration of much less than 0.5 mM had no noticeable effect on diffusion. The liposomes were formed by mixing the buffer with the dried phospholipid-cholesterol mixture in a conical flash on a vortex mixer and then sonicating gently in a water bath sonicator for 30 minutes (13). The unsequestered label was removed from the label trapped inside the liposome by chromatography on Sephadex G-25 (Pharmacia) followed by dialysis for 3 hours against the KCl-tris buffer. The efflux of the [^{3}H] nucleotides from the purified liposomes was measured by determining the radioactivity in the diffusate after a second dialysis of 24 hours. Results are expressed as the percentage of label originally trapped inside the liposomes which diffused out after 24 hours.

Partition coefficients were determined by adding small quantities (about 1 μ Ci) of the labelled nucleotide to a flask containing 5.0 ml of the aqueous buffer and 5.0 ml of n-octanol with continuous vigorous stirring. After 1 to 3 days the phases were allowed to separate and samples of each phase were withdrawn. The aqueous phase was then repartitioned with fresh n-octanol to assure that the 1-2% of radioactive contaminant was not so highly lipid soluble as to affect the partition coefficients. The repartitioned label gave results similar to the original partitioning. All samples were counted in aquasol (New England Nuclear) in a Beckman LS-150 liquid scintillation counter.

TABLE 1

The Diffusion of [^{3}H] Adenine Nucleotides Across Egg Lecithin-Cholesterol Membranes in the Presence of Divalent Metals

Experiment	Nucleotide	Divalent Metal Added			
		none	Mg^{+2}	Zn^{+2}	Cu^{+2}
A	AMP	2.92	2.82	2.90	3.01
	ADP	3.28	2.84	3.40	4.90
	ATP	3.38	3.15	4.60	10.40
B	AMP	2.92	3.20	3.15	40.60
	ADP	3.28	4.60	13.40	57.00
	ATP	3.38	7.70	19.60	68.20

The nucleotide-metal complexes were trapped inside the liposome and the percentage of the label diffused after 24 hours was reported. All liposomes were made in a buffer of 0.1 M KCl-0.025 M Tris, pH 7.5. To this buffer was added either 0.5 mM metal chloride (exp. A) or 5.0 mM metal chloride (exp. B). Results are expressed as percent of total radioactivity trapped in the liposome which diffused out after 24 hours.

RESULTS AND DISCUSSION

The rate of efflux of the [^{3}H] adenine nucleotides from liposomes was shown to be related to the partition coefficient. The small increase in diffusion rate from AMP to ATP (Table 1) correlates with the decrease in the H_2O/n-octanol partition coefficient (Table 2). The metal ions were shown to stimulate nucleotide diffusion with the order of stimulation being Cu^{+2} >> Zn^{+2} > Mg^{+2} (Table 1). This order is the same as the order of binding of the

TABLE 2

The Partition Coefficient of [^{3}H] Adenine Nucleotides

Nucleotide	H_2O/n-octanol	H_2O-Cu^{+2}/n-octanol
AMP	64.8	52.0
ADP	52.1	40.3
ATP	26.4	19.4

Water phase contains 0.1 M KCl-0.025 M Tris, pH 7.5 buffer. The Cu^{+2} is 5.0 mM.

metals to the N-7 of the adenine ring (8,9). The relative abilities of the metals to stimulate diffusion was the same for AMP, ADP and ATP. To see if the large stimulation in diffusion caused by Cu^{+2} was due to its binding affecting the lipid solubility, the water/n-octanol partition coefficients of the adenine nucleotides in the presence of Cu^{+2} were measured (Table 2). Although Cu^{+2} did lower the water/organic partition for the nucleotides it did not appear to cause a large enough change to account entirely for the large increase in diffusion rates. For this reason the effect of the metal ion on the rate of efflux of [^{14}C] glucose, which diffuses only very slowly across BLM (11), was measured. Glucose alone was shown to diffuse 4.5% in 24 hours while 5.0 mM Mg^{+2} increased this to 7.1%, 5.0 mM Zn^{+2} increased it to 10.2% and 5.0 mM Cu^{+2} increased the diffusion to 18.2%. Although the presence of Cu^{+2} obviously made the membranes more permeable to glucose it did not increase the diffusion enough to account for the large diffusion rate (68.2%) noted for ATP. Also if the effect of Cu^{+2} was only on the membrane it would be expected that the diffusion of AMP, ADP and ATP would all be the same. Obviously the diffusion rate of the nucleotides are different.

The effect of Cu^{+2} (and presumably the other divalent metals) on diffusion appears to be twofold. First it binds to the nucleotide and thereby decreases the charge and changes the conformation of the nucleotide

and secondly, it somehow effects the membrane making it more permeable to solutes.

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