The Activation of Sodium-Plus-Potassium Ion-Dependent Adenosine Triphosphatase from Marine Teleost Gills by Univalent Cations

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The apparent affinity constants for the binding of Cs^+ , Rb^+ , K^+ , Li^+ , Tl^+ and NH_4^+ to $(\text{Na}^+ + \text{K}^+)$ -dependent adenosine triphosphatase from teleost gills were measured and the values discussed in terms of the ion-selectivity isotherm described by Eisenman & Krasne (1975) [in *MTP International Review of Science: Biochemistry Series One* (Fox, C. F., ed.), vol. 2, pp. 27–59, Butterworths University Park Press, Baltimore]. The ion selectivity of the present enzyme is remarkably similar to that from nerve and brain.

The $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase* (EC 3.6.1.3) from a number of tissues, including nerve, brain, kidney and the erythrocyte, can be activated by various univalent cations (Skou, 1957, 1960; Bader & Sen, 1966; Britten & Blank, 1968; Inturrisi, 1969; Skulskii *et al.*, 1973). The degree of activation, however, is not a simple monotonic function of ionic radius (Eisenman, 1962; Diamond & Wright, 1969). The concept of 'ion-selectivity fingerprints' has been introduced by Eisenman & Krasne (1975) to describe several properties of univalent cations, including their enthalpies of solvation in different solvents, their affinities for various ionophores and enzymes, and their permeabilities through the nerve membrane. This approach revealed a remarkable similarity in the 'selectivity fingerprints' for activation of the $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase of nerve, cation-exchange reactions across the cell membrane in *Chlorella*, and cation permeability in the resting nerve membrane (Eisenman & Krasne, 1975). The present report establishes that a similar ion selectivity holds for the $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase in the salt-transporting gill epithelium of a marine teleost.

Materials and Methods

Chemicals

Ouabain, imidazole, sodium deoxycholate and the disodium salt of ATP were purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. All other chemicals were purchased as AnalaR grade where possible from BDH Chemicals Ltd., Poole, Dorset, U.K. Alkali-metal chlorides not available as AnalaR salts were purchased at the highest grade of purity available from BDH. These salts were prepared in glass-distilled water.

Animals

Sexually mature eels (*Anguilla anguilla*) were netted in a freshwater loch (Loch of Skene) near Aberdeen,

* Abbreviation: ATPase, adenosine triphosphatase.

immediately transported to a seawater aquarium at 8–12°C and kept there for at least 1 month in full-strength seawater before being killed.

Preparation of enzyme fraction

Fish were killed by decapitation and the gills immediately perfused *in situ* with chilled 0.9% NaCl. All subsequent operations were carried out at 0–2°C. The gill epithelium was scraped off the underlying cartilage with a scalpel and homogenized in a glass/Teflon homogenizer (0.125–0.175 mm clearance; 12 complete strokes at 500 rev./min) with 9 vol. of 0.32 M-sucrose/0.1% sodium deoxycholate/1 mM-di-sodium EDTA, the final pH being adjusted to 8.0 with 1 M-NaOH at 20°C. The homogenate was centrifuged at 225 000 g_{av} -min and the pellet discarded. The supernatant was centrifuged at 600 000 g_{av} -min and the resulting microsomal pellet suspended by homogenizing in 5 mM-imidazole/HCl/0.32 M-sucrose/1 mM-disodium EDTA, pH 7.0, to give a final protein concentration (measured by the method of Lowry *et al.*, 1951) of about 1 mg/ml. The microsomal suspension was stored at –20°C.

Enzyme assays

The enzyme was assayed at 30°C by the automated method of Dale *et al.* (1975) under conditions where the reaction rate was proportional to time and enzyme concentration. Rates were measured over the first 12 min of the reaction, during which time the enzyme was maximally activated by ATP under all conditions studied. The basic assay contained 6 mM-MgCl₂, 4 mM-Na₂ATP, 100 mM-NaCl and 30 mM-imidazole/HCl, pH 7.0 at 30°C. The assay contained in addition one of the ions Tl^+ , Rb^+ , K^+ , NH_4^+ , Cs^+ or Li^+ , added as their chloride salts, over the range 0–100 mM. Enzyme activity was calculated from the mean difference between triplicate assays carried out in the absence and presence of 0.1 mM-ouabain. The baseline value for ouabain-insensitive ATPases was low (specific activity 0.08 μmol of ATP hydro-

Table 1. *Kinetic parameters for the activation of (Na⁺+K⁺)-dependent ATPase by different univalent cations*

Assays were carried out in the presence of fixed concentrations of Na⁺ and Mg²⁺ and at various concentrations of the ions shown below as detailed in the Materials and Methods section. The Hill coefficients (*h*) and apparent affinity constants (*K*_{0.5}) were calculated from Hill plots of reaction velocity against cation concentration. The mean value of *K*_{0.5} was used for calculating $-RT\ln K_{0.5}$. Naked ionic radii (*r*) are those quoted in the *Handbook of Chemistry and Physics* (45th edn., The Chemical Rubber Co., Cleveland, Ohio), except that for NH₄⁺, from *Advanced Inorganic Chemistry* (Cotton, F. A. & Wilkinson, G., Interscience Publishers, New York).

Cation	<i>h</i>	<i>K</i> _{0.5} (mM)	$-RT\ln K_{0.5}$		<i>r</i> (nm)	1/ <i>r</i> (nm ⁻¹)
			(kJ·g-ion ⁻¹)	(kcal·g-ion ⁻¹)		
Cs ⁺	1.78	13.0	10.9	2.61	0.167	5.98
	1.33	13.1				
Tl ⁺	1.46	0.28	20.6	4.92	0.148	6.76
Rb ⁺	1.59	1.61	16.2	3.88	0.147	6.80
	1.32	1.47				
NH ₄ ⁺	1.33	14.3	11.1	2.65	0.143	6.99
	1.71	9.6				
K ⁺	1.40	1.73	15.5	3.70	0.133	7.52
	1.30	2.49				
Li ⁺	1.10	55.6	7.6	1.82	0.068	14.70
	1.51	40.6				

lysed/min per mg of enzyme protein), compared with the value for (Na⁺+K⁺)-dependent ATPase fully activated with 20mM-K⁺ (specific activity 0.80 μmol/min per mg) (cf. Sargent & Thomson, 1974). The former value (0.08) was constant under all assay conditions used, indicating that the (Na⁺+K⁺)-dependent ATPase was consistently and completely inhibited by ouabain. Plots of reaction velocity against metal-ion concentration were determined at least twice for a given ion, except for Tl⁺, by using ten different concentrations of the ion in each case.

Calculation of kinetic parameters

Plots of reaction velocity against cation concentration were sigmoidal for all cations studied. For this reason the Hill equation was fitted to the data on the ICL 4-75 computer at the Edinburgh Regional Computing Centre by a modified Fibonacci search method (Atkins, 1973). The points were weighted equally because the variance of velocity was independent of its magnitude. Apparent affinity constants were determined as *K*_{0.5}, the cation concentration at half-maximum velocity (*V*), where $K_{0.5} = K^{1/h}$, *K* being the substrate constant of the Hill equation, and *h* being the Hill coefficient.

Results and Discussion

The kinetic parameters *h* and *K*_{0.5} for the univalent cations tested in the present study are shown in Table 1. All the Hill coefficients (*h*) are greater than unity, consistent with our initial observations that plots of reaction velocity against cation concentration were sigmoidal. Apart from Li⁺, all the ions had Hill coefficients in the region of 1.5. The (Na⁺+K⁺)-

dependent ATPase is thought to exist in the bio-membrane as a dimer, in which each monomer consists of two different subunits (Stein *et al.*, 1973; Kyte, 1975; Giotta, 1976). A value of *h*=1.5 is consistent with a limited interaction between dimers, and we can conclude that all the cations tested here, except possibly Li⁺, permit co-operation between monomers with essentially the same efficiency. The values of *K*_{0.5} in Table 1 reveal that the enzyme here is activated by group-1A cations in the sequence Rb⁺ ≈ K⁺ > Cs⁺ > Li⁺, since Rb⁺ and K⁺ are not significantly different (0.2 < *P* < 0.3). We note that the selectivity series for ion transport across the gall bladder frequently has Rb⁺ ≈ K⁺ (Moreno & Diamond, 1974), which is interpreted as either series III (Rb⁺ > K⁺) or series IV (K⁺ > Rb⁺) (Eisenman, 1962).

Table 1 also presents apparent affinity constants, *K*_{0.5}, in the form of apparent energies of interaction, $RT\ln K_{0.5}$ (Eisenman & Krasne, 1975). We emphasize that the latter are apparent values, since the affinity constants as measured are almost certainly not true constants, but are more probably the sum of a complex collection of separate constants. Nevertheless we note that the relationship of $RT\ln K_{0.5}$ to the reciprocal of the (naked) ionic radius, which is the ion-selectivity isotherm of Eisenman & Krasne (1975), is the same for the enzyme here and the enzyme from nerve and brain (Eisenman & Krasne, 1975). In particular, Tl⁺ and NH₄⁺ have unusually high and low apparent activation energies respectively when compared with ions of similar ionic radius (Rb⁺ and K⁺). According to Eisenman & Krasne (1975), these deviations arise because neither Tl⁺ nor NH₄⁺ behaves as a simple rigid charged sphere. Tl⁺ has an

electron distribution that can be markedly distorted by its immediate environment so as to yield, in certain cases, excess energies of interaction, and NH_4^+ has a tetrahedral configuration that sterically influences its interaction with ligands.

One implication of these considerations is that the ligand sites which bind non-hydrated cations are the same in the enzymes from gills, nerve and brain. This indicates that there is a high degree of structural and functional unity among $(\text{Na}^+ + \text{K}^+)$ -dependent ATPases present in different animal cells with specialized function. It is further noteworthy that the $(\text{Na}^+ + \text{K}^+)$ -dependent ATPases in nerve and brain yield 'selectivity fingerprints' very similar to that given by the permeabilities of these cations through nerve membranes (Eisenman & Krasne, 1975). This finding is consistent with the ligand-binding sites on the enzyme being the same as those involved in permeability of ions through the nerve membrane. Much evidence exists that $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase is involved in the secretion of a hyperosmotic saline across marine teleost gills (see Maetz, 1974). It remains to be tested, however, whether there is a precise correlation between apparent cation affinities for the enzyme and cation permeabilities across the gills.

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