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$(Mg^{2+}$-Na^{+}-$K^{+})$-ATPase activity of liver from tumor bearing rats

The occurrence of a (Mg^{2+}- Na^{+}- K^{+})-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) in rat liver has previously been demonstrated[1-4] but very little work has been done on the kinetics of the effects of Na^{+} and K^{+}. This is due in part to the low increase in activity produced by addition of Na^{+} and K^{+} compared with the ATPase activity seen in the presence of Mg^{2+} alone. The present communication describes a method for greatly reducing the relative contribution of the Mg^{2+}-stimulated ATPase activity so that a kinetic examination of the Na^{+} and K^{+} interaction is made possible.

Livers from Sprague–Dawley rats bearing the ascitic form of Novikoff hepatoma for 10 days were perfused with cold saline, removed, homogenized in 25 ml 0.3 M cold sucrose and centrifuged at 10 000 $\times g$ for 10 min. The sediment was discarded and to the supernatant was added 10 ml 0.8 M sodium citrate, pH 4.1. The mixture was centrifuged at 6000 $\times g$ for 10 min, then washed once more with distilled water and finally, the sediment was resuspended in 10 ml distilled water. The enzyme preparation retained its activity at 1° for 12 h, but lost 40% of its activity after being kept frozen overnight. The basic reaction mixture consisted of 0.5 ml protein precipitate, pH 4.1, 6.0 mM ATP, 60 mM Tris, pH 7.8, and 1.8 M urea in a final volume of 3.0 ml. Na^{+} and K^{+} concentrations were as described in the Table I and Figs. 1–3. In experiments where urea was used the samples were preincubated for 20 min before ATP was added because it was found that urea inhibition of Mg^{2+}-dependent ATPase activity reaches a peak after this time and then remains steady. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid and P_i was determined by the method of FISKE AND SUBBAROW[5].

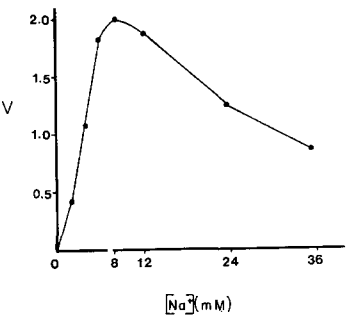
TABLE I

ATPASE ACTIVITY OF pH 4.1 PROTEIN FRACTION

Each figure is the average of five experiments. Conditions: temperature as indicated in table. Gas phase: air.

Additions	*Temp.*	*ATPase activity**
Nil	37°	0.2
120 mM Na^{+}	37°	0.5
6.0 mM K^{+}	37°	0.2
120 mM Na^{+}, 6.0 mM K^{+}	37°	0.4
6.0 mM Mg^{2+}	37°	8.3
6.0 mM Mg^{2+}, 6.0 mM K^{+}	37°	8.6
6.0 mM Mg^{2+}, 120 mM Na^{+}	37°	8.8
6.0 mM Mg^{2+}, 6.0 mM K^{+}, 120 mM Na^{+}	37°	12.8
6.0 mM Ca^{2+}	37°	7.8
6.0 mM Ca^{2+}, 6.0 mM K^{+}, 120 mM Na^{+}	37°	7.7
6.0 mM Mg^{2+}, 0.2 mM ouabain	37°	8.4
6.0 mM Mg^{2+}, 6.0 mM K^{+}, 120 mM Na^{+}, 0.2 mM ouabain	37°	10.3
6.0 mM Mg^{2+}, 1.8 M urea	37°	3.0
6.0 mM Mg^{2+}, 6.0 mM K^{+}, 120 mM Na^{+}, 1.8 M urea	37°	8.0
6.0 mM Mg^{2+}, 1.8 M urea	45°	1.7
6.0 mM Mg^{2+}, 6.0 mM K^{+}, 120 mM Na^{+}, 1.8 M urea	45°	7.6

* ATPase activity expressed as μg P_i liberated per mg protein per 10 min.



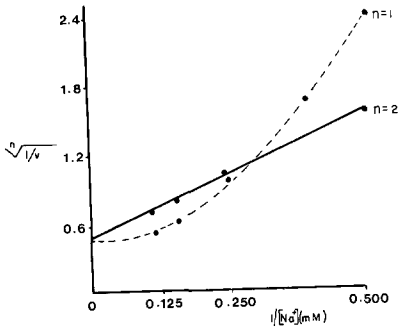


Fig. 1. Effect of Na^+ concentration on the (Mg^{2+} — Na^+ — K^+)-dependent ATPase activity. v represents the μg P_i liberated per mg protein per 10 min. Each point represents the average of four experiments. Assay conditions: 0.5 mM K^+, remainder as described in text. Temp.: 45°; gas phase: air.

Fig. 2. Kinetic analysis of the effect of Na^+ concentration on (Mg^{2+} — Na^+ — K^+)-ATPase activity. Legend as in Fig. 1.

Table I shows that the ATPase activity seen in the presence of Mg^{2+} is further stimulated only upon the addition of both Na^+ and K^+, neither of these ions alone having much effect. Ca^{2+} could not substitute in the (Na^+-K^+)-ATPase system for Mg^{2+} and the complete system showed an inhibition by ouabain. Urea decreased the Mg^{2+}-stimulated ATPase activity but had no effect on the Na^+, K^+ stimulation. Increasing the temperature to 45° in the presence of urea inhibited the Mg^{2+}-stimulated ATPase activity while the level of the (Mg^{2+} — Na^+ — K^+)-ATPase remained unaltered. Our system gives a ratio of Mg^{2+} — Na^+ — K^+ activity to Mg^{2+} activity of 4.5, a ratio which makes kinetic analysis possible.

The effect of increasing concentrations of Na^+ on the ATPase activity in the presence of a constant concentration of K^+ is shown in Fig. 1. The ATPase activity reaches a maximum when the Na^+ concentration corresponds to a Na^+/K^+ ratio of 16. Enzyme activity then falls with increasing Na^+ concentration.

AHMED *et al.*[6] have shown using brain tissue that, at a constant K^+ concentration, the effective number of Na^+ required to activate the (Mg^{2+} — Na^+ — K^+)-dependent

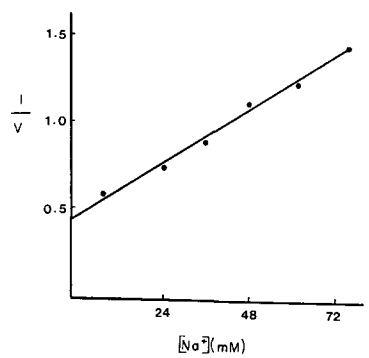


Fig. 3. The effect of inhibitory concentrations of Na^+ on the (Mg^{2+} — Na^+ — K^+)-dependent ATPase activity. Legend as in Fig. 1.

ATPase may be obtained by plotting $1/[Na^+]$ against $\sqrt[n]{-1/v}$, where the numerical value of n giving a linear relationship is equivalent to the number of Na^+ required to activate the enzyme. Fig. 2 shows that a straight line is obtained when n equals 2 but not when n equals 1. This result suggests that two Na^+ and one K^+ are required to activate the enzyme, a result similar to that seen with brain[6]. The fact that maximum activity occurs at a Na^+/K^+ ratio of 16 and it requires only two Na^+ to one K^+ to activate the enzyme suggests that K^+ has a much higher affinity for the active site of the enzyme.

The inhibition of ATPase activity by higher concentration of Na^+ was further investigated over the range 12–72 mM. The results obtained, plotted by the method of DIXON[7], are shown in Fig. 3. The linear relationship obtained indicates that the site of inhibition by Na^+ is probably the same as that involved in the activation by K^+.

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Activity against a synthetic substrate by a preparation of extracellular proteinase from *Serratia marcescents*

Preparations of extracellular proteinase from several strains of the bacterium *Serratia marcescens* have been found to digest a wide range of proteins and with these substrates showed optimal activity at about pH 9 (refs. 1–5). Purified proteinase was found to split carboxymethylated B chain of insulin at 22 bonds and peptides containing not less than 3 residues were formed[4]. The biological activity of kinin-9 (a nonapeptide) was destroyed by the enzyme[5] but benzoyl arginine ethyl ester[4], benzoyl tyrosine ethyl ester[1], peptides ranging from diglycine to hexaglycine[4], polylysine[2] and polyaspartic acid[2] were unhydrolysed. In the present work proteinase isolated from *S. marcescens* NCIB 10351 proved able to hydrolyse Z–Gly–Pro–Gly–Gly–Pro–Ala at the Gly–Gly bond. This observation could facilitate characterisation of the enzyme.