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PROPERTIES OF A HIGH SPECIFIC ACTIVITY, (Na^+-K^+)-STIMULATED ATPASE FROM RAT INTESTINAL MUCOSA

JAMES P. QUIGLEY AND GERALD S. GOTTERER

Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Md (U.S.A.)

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SUMMARY

A high specific activity, membrane-bound, (Na^+-K^+)-stimulated ATPase, isolatable in good yield from rat intestinal mucosal cells was examined for its enzymatic properties. The stimulated enzyme activity required the combined presence of Na^+ and K^+ in physiological concentrations and exhibited high sensitivity to K^+ and an absolute requirement for Na^+. A Mg^{2+}/ATP ratio of 1.0 was necessary for optimal activity and the K_m for ATP was 0.1 mM. ATP was the only nucleotide triphosphate hydrolyzed. The effect of amino acids, pH, ouabain, phloridzin and fluoride ion on the enzymatic activity was also examined.

INTRODUCTION

The importance of (Na^+-K^+)-stimulated ATPase activity in active transport processes has become increasingly evident since the initial suggestions of SKOU[1]. The properties of such an enzyme in the intestinal mucosal cell is of special significance, since it may play a role not only in the transport of cations, but also perhaps in an indirect manner in the active transport of various organic compounds[2–4]. The previous paper[5] describes the isolation of a membrane-bound (Na^+-K^+)-stimulated ATPase from rat intestinal mucosa in high yield and with very high specific activity. The present paper describes the enzymatic properties of this ATPase.

MATERIALS AND METHODS

Preparation

Membrane fraction M-1 was isolated by discontinuous sucrose gradient centrifugation after aging of Fraction II, as described in the previous paper[5]. The isolated M-1 membrane preparation was washed with 1 mM EDTA (pH 7.1), again placed on a 20 % + 30 % discontinuous sucrose gradient and centrifuged in a Spinco SW 25.1 swinging-bucket rotor at 25000 rev/.min for 90 min in a Spinco Model L ultracentrifuge. The fraction which banded at the 20–30 % interface of the sucrose gradient was reisolated, suspended in 50 mM Tris buffer (pH 7.1) and used for all enzyme assays. This purified M-1 fraction had very little glucose-6-phosphatase or alkaline phosphatase

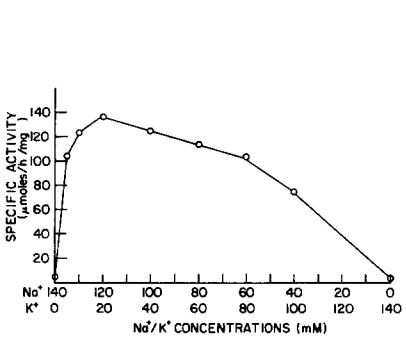
and no detectable invertase or cytochrome oxidase activity. The specific activity of the (Na^+-K^+)-stimulated ATPase represented a 34-fold purification over the original cellular homogenate.

Enzyme assays and reagents

The enzyme assays and protein determination were performed as described in the previous paper[5]. Unless indicated otherwise, the enzyme was assayed in the presence of 5.0 mM ATP, 10 mM $MgCl_2$, 120 mM NaCl, 20 mM KCl and 60 mM Tris–imidazole buffer (pH 6.8). All inorganic cations were added exclusively as chloride salts. Nucleotides were purchased from Sigma Chemical Co. and were used as the sodium salts. Tris–ATP was substituted when sodium-free media were required.

RESULTS

The M-1 membrane ATPase is stimulated by the combined presence of Na^+ and K^+. Fig. 1 shows that under conditions when the total $Na^+ + K^+$ concentration is kept constant, maximum activity is obtained when the Na^+ and K^+ concentrations are 120 and 20 mM, respectively. The enzyme is especially sensitive to K^+, with a K_m of 1 mM in the presence of either 20 mM or 120 mM Na^+ (Fig. 2). The affinity for Na^+ appears to be sensitive to the K^+ concentration, as seen from the experiment in Fig.3.



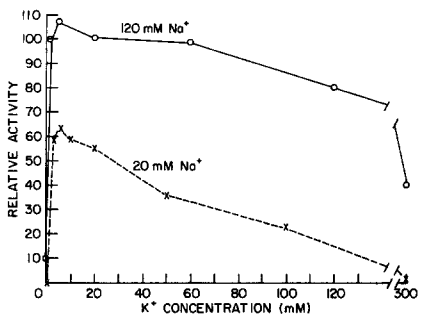


Fig. 1. Effect of Na^+/K^+ ratio on ATPase activity. Total monovalent cation concentration was kept constant; ratio of Na^+ and K^+ was varied as indicated.

Fig. 2. Effect of K^+ concentration on ATPase activity. O—O, 120 mM Na^+, ×---×, 20 mM Na^+.

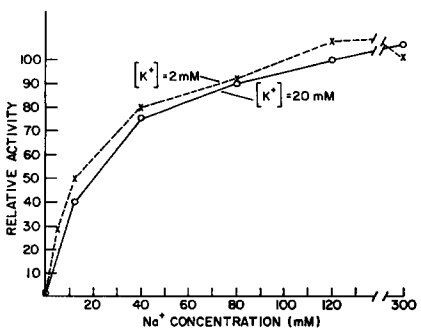


Fig. 3. Effect of Na^+ concentration on ATPase activity. ×---×, 2 mM K^+; O—O, 20 mM K^+.

In the presence of low concentrations of K^+ (2 mM), the K_m for Na^+ is 12 mM. By increasing the K^+ concentration 10-fold to its optimum concentration of 20 mM, the K_m for Na^+ increases to 29 mM. High concentrations of K^+ will inhibit the cation-stimulated activity, and the inhibition is reversed by increasing the Na^+ concentration (Fig. 2).

Other cations can not replace Na^+, but the K^+ requirement is less specific (Table I). The effectiveness of the various monovalent cations is in the order: $K^+ > Rb^+ > NH_4^+ > Cs^+ > Li^+$; choline is completely ineffective.

TABLE I

EFFECT OF MONOVALENT CATIONS ON ATPase ACTIVITY

KCl (mM)	*NaCl (mM)*	*Other additions*	*Cation concn. (mM)*	*Specific activity (μmoles/h per mg)*
—	—	—		20
20	120	—		156
20	—	—		28
20	—	Li^+	20	27
20	—		120	27
20	—	NH_4^+	20	30
20	—		120	25
20	—	Cs^+	20	27
20	—		120	32
20	—	Rb^+	20	33
20	—		120	30
20	—	Choline	20	36
20	—		120	32
—	120	—		30
—	120	Li^+	20	41
120	—		120	57
—	120	NH_4^+	20	136
120	—		120	146
—	120	Cs^+	20	102
120	—		120	118
—	120	Rb^+	20	143
120	—		120	129
—	120	Choline	20	34
120	—		120	32

A recent paper by MOHRI *et al.*[6] indicated that certain amino acids activate the (Na^+-K^+)-stimulated ATPase of HeLa cells and the activation is ouabain-inhibitable. In the intestine, the transport of sugars and certain amino acids has been shown to be Na^+ dependent and inhibited by ouabain[3,7]. The effect of glycine and lysine on the intestinal M-1 membrane (Na^+-K^+)-stimulated ATPase was therefore investigated, and the results are shown in Table II. Glycine and lysine at two concentrations can not further activate the intestinal ATPase in the presence of optimal Na^+ and K^+ concentrations and the same amino acids are not able to replace either Na^+ or K^+.

The ATPase exhibits an absolute requirement for a divalent cation. Fig. 4 illustrates the effect of magnesium concentration on the ATPase activity at fixed ATP concentration. The results show that equimolar concentrations of Mg^{2+} and ATP are needed for optimal activity and that there is no inhibition by Mg^{2+} at concentrations up to twice that of the ATP concentration. In the presence of optimal Mg^{2+} concentrations

other divalent cations inhibit both the basal Mg^{2+}-ATPase and the (Na^+-K^+)-stimulated ATPase (Table III). Only Mn^{2+} is able significantly to replace Mg^{2+} in the (Na^+-K^+)-stimulated ATPase.

TABLE II

EFFECT OF AMINO ACIDS ON ATPase ACTIVITY

NaCl (mM)	*KCl (mM)*	*Amino acid*	*Concn. (mM)*	*Specific activity (μmoles/h per mg)*
120	20	—		115
120	—	—		18
120	—	Lysine	20	17
120	—	Lysine	120	22
120	—	Glycine	20	18
120	—	Glycine	120	21
—	20	—		10
—	20	Lysine	20	10
—	20	Lysine	120	10
—	20	Glycine	20	11
—	20	Glycine	120	9
120	20	Lysine	20	118
120	20	Lysine	120	114
120	20	Glycine	20	120
120	20	Glycine	120	114

TABLE III

EFFECT OF DIVALENT CATIONS ON ATPase ACTIVITY

Mg^{2+} concn. (mM)	*Addition*	*Concn. (mM)*	*Specific activity (μmoles/h per mg)*	
			Mg^{2+}-ATPase	*(Na^+-K^+)-ATPase*
5	—		15	122
5	Mn^{2+}	5	6	39
5	Ca^{2+}	5	8	20
5	Zn^{2+}	5	4	<1
5	Pb^{2+}	2.5	6	<1
—	Mn^{2+}	5	6	44
—	Ca^{2+}	5	9	2
—	Zn^{2+}	5	4	19
—	Pb^{2+}	5	2	5

The effect of ATP concentration on the activity of both the Mg^{2+}-ATPase and the (Na^+-K^+)-stimulated ATPase is presented in Fig. 5. Under conditions where the Mg^{2+}/ATP ratio is maintained at 2, both enzymes exhibit typical Michaelis–Menten kinetics and a double-reciprocal plot of the Lineweaver–Burke type gives a K_m of 0.12 mM for the (Na^+-K^+)-stimulated ATPase and 0.03 mM for the Mg^{2+}-ATPase.

The (Na^+-K^+)-stimulated ATPase shows high substrate specificity with an almost absolute requirement for ATP, while the basal activity can hydrolyze all the nucleotides studied with at least 50 % of the activity exhibited towards ATP (Table IV).

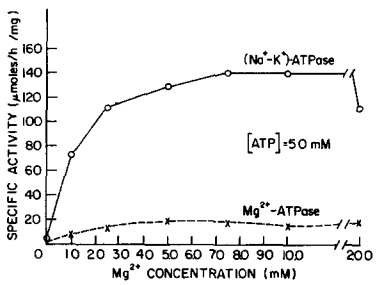
A pH–activity curve (Fig. 6) illustrates the broad pH range of the Mg^{2+}-ATPase, while the (Na^+-K^+)-stimulated activity exhibits a sharp optimum at pH 6.8. The cardiac glycoside, ouabain, is a characteristic specific inhibitor of the (Na^+-K^+)-stimulated ATPase and also an inhibitor of active transport in the intestine. The effect of ouabain concentration on the inhibition of the intestinal membrane (Na^+-K^+)-stimulated ATPase was examined. The K_i calculated from such studies is $1 \cdot 10^{-4}$ M.

TABLE IV

SUBSTRATE SPECIFICITY OF TRIPHOSPHATASE ACTIVITY

All nucleotides were assayed at a concentration of 5 0 mM.

Nucleotide	*Specific activity (μmoles/h per mg)*	
	Mg^{2+} activity	*(Na^+-K^+)-stimulated activity*
ATP	15	140
CTP	10	< 1
ITP	14	8
GTP	8	2
UTP	11	2



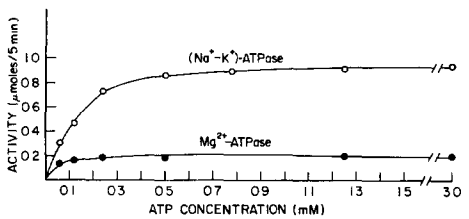
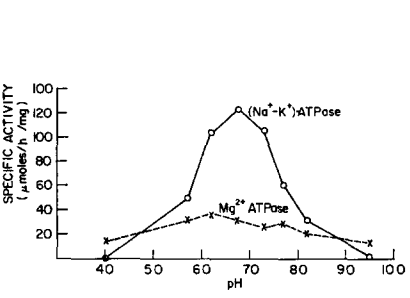


Fig. 4. Effect of Mg^{2+} concentration on ATPase activity. O—O, (Na^+-K^+)-stimulated ATPase; ×---×, Mg^{2+}-ATPase.

Fig. 5. Effect of ATP concentration on ATPase activity Mg^{2+}/ATP ratio was 2 for all points O—O, (Na^+-K^+)-stimulated ATPase; ●—●, Mg^{2+}-ATPase



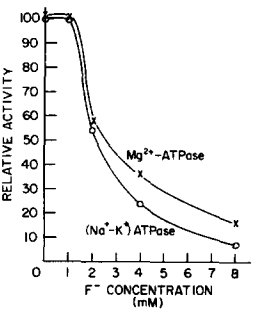


Fig. 6. Effect of pH on ATPase activity. O—O, (Na^+-K^+)-stimulated ATPase; ×---×, Mg^{2+}-ATPase.

Fig. 7. Fluoride inhibition of ATPase activity. Fluoride was added as the K^+ salt. The levels of K^+ added had no effect on the Mg^{2+}-ATPase. ×—×, Mg^{2+}-ATPase; O—O, (Na^+-K^+)-stimulated ATPase.

Nonspecific inhibitors of the M-1 membrane ATPase are fluoride ion (K_i = $2.2 \cdot 10^{-3}$ M) and phloridzin (K_i = $2 \cdot 10^{-4}$ M). Fig. 7 demonstrates that fluoride inhibits both the Mg^{2+}-ATPase and the (Na^+-K^+)-stimulated ATPase; the (Na^+-K^+)-stimulated activity is slightly more sensitive to the fluoride inhibition. Phloridzin also inhibits both ATPase activities, and is also somewhat more effective against the (Na^+-K^+)-stimulated ATPase than against the Mg^{2+}-ATPase.

DISCUSSION

The properties of the (Na^+-K^+)-stimulated ATPase of rat intestinal mucosal cell membranes are similar to the enzyme system first reported by SKOU in crab nerve[1]. The activation by sodium and potassium (Fig. 1) is identical to that of a rat brain preparation[8] which like the M-1 preparation has a very high specific activity for (Na^+-K^+)-stimulated ATPase and is also similar to the specific cation activation reported for other intestinal preparations[9–11] of much lower specific activity. The sensitivity to K^+ (Fig. 2) is characteristic of this enzyme system, and the K_m for K^+ reported corresponds to that found for the rat liver[12], heart[13] and brain[8] enzymes. High concentrations of K^+ inhibit the stimulated enzyme, and this is compatible with the suggestion that K^+ can competitively displace Na^+ from its activation site on the enzyme[14]. The order of effectiveness of replacement of K^+ with other monovalent cations found with the M-1 intestinal preparation also agrees with the results reported for other (Na^+-K^+)-stimulated ATPase preparations[10,15]. It is perhaps significant that the inhibition by high concentrations of K^+ and the same relative effectiveness of the various monovalent cations have also been found in studies *in vitro* of active transport of sugars in the intestine[16].

The requirement for Mg^{2+} (Fig. 4), the ability of Mn^{2+} to replace Mg^{2+} and the inhibition by Zn^{2+} and Ca^{2+} agree with results obtained by BERG[10] with an intestinal membrane enzyme of lower specific activity. However, the data in Table III indicate that the high specific activity preparation reported in this paper exhibits absolute substrate specificity, while the (Na^+-K^+)-stimulated ATPase isolated by BERG[10] hydrolyzes CTP at 34% the rate of ATP hydrolysis. RENDI AND UHR[15] and POST *et al.*[17] similarly report absolute specificity towards ATP in the (Na^+-K^+)-stimulated ATPase found in the kidney and erythrocyte, respectively. They also found that the Mg^{2+}-ATPase shows broad specificity for nucleotide triophosphates, as reported for the intestinal preparation in this paper.

The pH curve (Fig. 6) confirms the finding of WHITTAM AND WHEELER[18] in the kidney, that the (Na^+-K^+)-stimulated ATPase is more sensitive to pH, while the Mg^{2+}-ATPase exhibits a broad pH optimum. The optimum pH for the M-1 (Na^+-K^+)-stimulated ATPase (pH 6.8) is lower than that found with other intestinal (Na^+-K^+)-stimulated ATPases of lower specific activity[9–11].

Ouabain, the characteristic inhibitor of cation-stimulated ATPase and active transport, specifically inhibits the M-1 (Na^+-K^+)-stimulated ATPase and has no effect on the Mg^{2+}-ATPase. The K_i ($1 \cdot 10^{-4}$) agrees with the intestinal studies of BERG[10], who also points out that resistance to ouabain inhibition is greater in rat tissues than in corresponding tissues from other species. Phloridzin at low concentrations is a specific inhibitor of active sugar transport in the intestine[19,20], but at higher concentrations a rather nonspecific inhibitor of ATP-related membrane enzymes[20–22].

The (Na^+-K^+)-stimulated ATPase of M-1 is inhibited by phloridzin with a K_i of $2 \cdot 10^{-4}$ M, in agreement with that found for the enzyme isolated from kidney[15]. This concentration of phloridzin is higher than that required for specific inhibition of sugar transport (10^{-6} M) and therefore presumably represents nonspecific inhibition of the phosphohydrolase reaction.

Fluoride ion, an inhibitor of active ion transport[23], has been shown to inhibit the (Na^+-K^+)-stimulated ATPase[24,25]. YOSHIDA *et al.*[25], working with a guinea pig brain preparation treated with NaI, have shown an irreversible inhibition by fluoride of not only the (Na^+-K^+)-stimulated ATPase, but also a K^+-stimulated phosphatase which had been shown to have many properties in common with the (Na^+-K^+)-stimulated ATPase. The results presented in this paper show that the Mg^{2+}-ATPase as well as the (Na^+-K^+)-stimulated ATPase of rat intestine are inhibited by fluoride, with roughly comparable sensitivity. The fluoride inhibition of the (Na^+-K^+)-stimulated ATPase would appear to be an example of the nonspecific inhibition of metal-requiring enzymes shown by fluoride[26].

These results indicate that the (Na^+-K^+)-stimulated ATPase isolated from rat intestinal mucosal cells has properties very similar to those found for similar enzymes isolated from other sources. The ability to isolate this enzyme from the intestine in high yield and with high specific activity will facilitate further studies of the enzyme's physical and biochemical properties and thereby provide a framework from which to evaluate the enzyme's role in the active transport processes which take place in the intestinal mucosal cell.

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