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PURIFICATION AND CHARACTERIZATION OF (Na^+ + K^+)-ATPase FROM TOAD KIDNEY

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Summary

This report describes the partial purification and the characteristics of (Na^+ + K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) from an amphibian source. Toad kidney microsomes were solubilized with sodium deoxycholate and further purified by sodium dodecyl sulphate treatment and sucrose gradient centrifugation, according to the methods described by Lane et al. [(1973) J. Biol. Chem. 248, 7197—7200], Jørgensen [(1974) Biochim. Biophys. Acta 356, 36—52] and Hayashi et al. [(1977) Biochim. Biophys. Acta 482, 185—196]. (Na^+ + K^+)-ATPase preparations with specific activities up to 1000 μmol P_i/mg protein per h were obtained. Mg^{2+}-ATPase only accounted for about 2% of the total ATPase activity. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis revealed three major protein bands with molecular weights of 116 000, 62 000 and 26 000. The 116 000 dalton protein was phosphorylated by [γ-^{32}P]ATP in the presence of sodium but not in the presence of potassium. The 62 000 dalton component stained for glycoproteins. The K_m for ATP was 0.40 mM, for Na^+ 12.29 mM and for K^+ 1.14 mM. The K_i for ouabain was 35 μM. Temperature activation curves showed two activity peaks at 37°C and at 50°C. The break in the Arrhenius plot of activity versus temperature appeared at 15°C.

Introduction

Recently, (Na^+ + K^+)-ATPase has been purified to a high degree and biochemical characteristics have been determined in a variety of species, such as fish, birds and mammals [1—4] and the hypothesis of a coupled sodium and potassium transport effected by this enzyme has been consolidated on the basis of reconstitution experiments [5,6].

In contrast to the extensive study of the enzyme in the above mentioned

species, $(Na^+ + K^+)$-ATPase from amphibian tissues has received little attention. This fact is rather surprising in view of the importance played by these tissues as experimental models in the physiological approach of sodium transport problems.

A few data on the enzyme from toad and frog tissues are available [7—11] but the impure preparations with spec. act. ranging between 1.2 and 43 μmol P_i/mg protein per h in frog skin [11] and frog kidney [9], respectively, did not allow a conclusive characterization of the sodium pump in this species.

The principal aim of the present study consisted therefore in a maximal purification of $(Na^+ + K^+)$-ATPase from an amphibian tissue namely the toad kidney in order to establish the basis for a biochemical, biophysical and immunochemical characterization of the enzyme. These data will permit the comparison with the enzyme from other species and will provide the basis for a better definition of the relationship between the physiological and biochemical expression of sodium transport in amphibian epithelia.

Experimental

Tissue source and preparation of microsomes

Colombian male and female toads (Bufo marinus) (C.P. Chase and Co, Miami, FL) were killed by pithing and subsequently perfused through a heart puncture with oxygenated Ringer solution [12]. Kidneys were removed, weighed in homogenization medium and immediately frozen in liquid nitrogen. On average, 1 kidney weighed 0.5 g. Further steps were all carried out at 0—4°C. After thawing, the kidneys were minced with scissors and 1 g of tissue was added to 5 ml of homogenization medium containing 30 mM DL-histidine, 5 mM EDTA, 18 mM Tris and 200 mM sucrose, pH 7.4. After homogenization with 10 strokes at 2000 rev./min in a Teflon-glass Potter-Elvehjem homogenizer, the homogenate was centrifuged for 15 min at 5000 $\times g$ in the SS34 rotor of a Sorvall RC-2B centrifuge. The pellet was resuspended in one-half the original volume with 2 strokes of homogenization and centrifuged again at 5000 $\times g$ for 15 min. The pooled supernatants were then centrifuged at 48 000 $\times g$ in the SW 27.1 rotor of the Sorvall OTD-2 ultracentrifuge for 1 h and the resulting pellets (microsomal fraction) were resuspended in the homogenization medium to give a protein concentration of 10—15 mg/ml. This microsomal fraction was stored at —20°C or —60°C until used for subsequent treatment with sodium dodecyl sulphate (SDS) alone or in combination with deoxycholate.

Detergent treatment

Sodium dodecyl sulphate treatment. The method originally described by Jørgensen [4] and modified by Hopkins et al. [2] was adapted for the purification of the $(Na^+ + K^+)$-ATPase from the toad kidney. Microsomal material was incubated at a protein concentration of 1.4 mg/ml in a solution containing 3 mM Na_2ATP/2 mM EDTA/20 mM Tris-HCl at pH 7.5. Sodium dodecyl sulphate was added with constant stirring to final concentrations of 0.1—0.8 mg/ml. After standing for 30 min at 20°C, aliquots of 2.4 ml were layered on continuous or discontinuous sucrose gradient.

Deoxycholate/sodium dodecyl sulphate treatment. The procedure for the

deoxycholate/sodium dodecyl sulphate treatment was essentially the same as described by Lane et al. [3] and modified by Hayashi et al. [13]. Aliquots of the microsomal fraction (5 mg/ml) were incubated with 3.5—5.5 mg deoxycholate/ml in 30 mM DL-histidine, 1 mM Tris/EDTA, 500 mM NaCl, 40 mM KCl and 292 mM sucrose (pH 6.9) at 0°C for 30 min and then centrifuged at 100 000 × *g* in the Sorvall OTD-2 ultracentrifuge. Glycerol (final concn. 20%, v/v) was added to the supernatant. After standing for 15 min on ice with constant stirring, an equal vol. of 30 mM DL-histidine, 1 mM Tris/EDTA (pH 7.5) was added to the mixture. After an additional 45 min of incubation on ice, the suspension was centrifuged at 100 000 × *g* for 90 min at 20°C. The higher temperature reduced the viscosity of the suspension thus aiding the sedimentation of the material without having a harmful effect on the enzyme. The resulting pellets were washed in 30 mM DL-histidine, 1 mM Tris/EDTA (pH 7.5) by centrifugation at 100 000 × *g* for 90 min at 4°C and then resuspended in homogenization medium. The deoxycholate enzyme was further treated with sodium dodecyl sulphate as described above with the following modifications: protein concentrations were adapted to 1 mg/ml and final sodium dodecyl sulphate concentrations ranged between 0.9 and 1.3 mg/ml.

Sucrose gradients

2.4 ml of sodium dodecyl sulphate or deoxycholate/sodium dodecyl sulphate-treated enzyme preparations were layered on 11 ml of a continuous linear sucrose gradient formed from 15% (w/v) and 45% (w/v) sucrose in 20 mM Tris/1 mM EDTA, pH 7.5. Gradients were centrifuged in the SW 40.1 rotor of the Sorvall OTD-2 ultracentrifuge at 105 000 × *g* for 210 min at 4°C. 14 fractions were collected and directly analysed for activity and protein content. Peak activities were found in fractions with densities of 1.08—1.10 g/ml. A second, less pronounced activity peak was recovered at sucrose solution densities around 1.13 mg/ml.

Routinely, a discountinuous sucrose gradient consisting of 5.5 ml of 29.4% (w/v), 3.3 ml of 15% (w/v) and 2.2 ml of 10% (w/v) sucrose was used as proposed by Jørgensen [4]. The layer between 15 and 29.4% sucrose was collected, diluted 5 times with homogenization medium and sedimented. The resulting pellets were resuspended in homogenization medium to a protein concentration of 3—7 mg/ml and stored at —20°C or —60°C. These preparations are referred to as sodium dodecyl sulphate enzyme (SDS enzyme) or deoxycholate/SDS enzyme.

Protein measurements

Protein content was determined by the method of Lowry et al. [14].

Assay of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activity

$(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities were measured as described previously [15]. 1—20 μg protein of the various enzyme fractions were used for enzyme assay. Liberated inorganic phosphate was determined by the method of Fiske and Subbarow [16]. The conditions used gave linear rates of phosphate production up to 20 min and linear increase in phosphate liberation with protein concentrations used.

Determination of V and K_m

K_m and V for ATP of the SDS enzyme were determined in the presence of an ATP-regenerating system containing 18 mM *p*-enolpyruvate and 1.0 I.U. of pyruvate kinase [17]. The kinetic parameters were calculated from a Hofstee plot.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out by the procedure described by Maizel [18] and Laemmli [19]. Linear gradients produced from 30 ml 5% (w/v)/0.133% (w/v) and 30 ml (w/v)/0.346% (w/v) acrylamide/bis solutions were used in slab gel electrophoresis while 7.5% (w/v)/0.2% (w/v) and 10% (w/v)/0.266% (w/v) acrylamide/bis were used in tubes (length 8 cm, inner diameter 0.5 cm). The gels were stained with Coomassie Blue for protein detection or with periodic acid-Schiff [20] for glycoprotein detection. For the identification of the catalytic subunit, 150 μg of proteins containing the SDS enzyme were phosphorylated with [γ-^{32}P]ATP [21,33]. Samples were subjected to slab gel electrophoresis as described above for 3 h at a constant current of 30 mA at 12—15°C. In order to minimize phosphate hydrolysis, gels were stained and fixed within 1 h and discoloured within another hour. This procedure allowed the detection of the phosphorylated catalytic subunit on the Coomassie blue stained gel by exposure of the dried gel to a Kodak X-Ray Film for 60 h in the dark. The autoradiography was scanned with a Vitatron densitometer.

Results

Purification of toad kidney ($Na^+ + K^+$)-ATPase

A first approach to purify ($Na^+ + K^+$)-ATPase from the toad kidney involved the application of the procedure proposed by Jørgensen [4] using sodium dodecyl sulphate as detergent. In order to obtain maximal purity of the enzyme, optimal conditions for the incubation of the microsomes with the detergent were required; these were found by varying protein (0.2—1.4 mg/ml) as well as sodium dodecyl sulphate (0.05—1.2 mg/ml) concentrations. In such experiments maximal activation of the enzyme was observed with very low protein concentrations. By incubation of 0.2 mg protein/ml the maximal activity of the enzyme, obtained at low sodium dodecyl sulphfte concentrations, was about 55% higher than by incubation of 1.4 mg protein/ml (Fig. 1). The low protein condition could unfortunately not be exploited for practical purposes because the enzyme was very sensitive to sodium dodecyl sulphate inhibition under these conditions. Higher concentrations of protein prevented the inactivation of the enzyme by sodium dodecyl sulphate over a much wider range of detergent concentrations.

For standard SDS enzyme preparations, concentrations of 1.4 mg protein/ml were therefore used and a detergent concentration of 0.45 mg/ml which was shown in preliminary experiments to yield maximally purified enzyme with most microsomal preparations.

An example of the distribution of ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase activity and protein on a continuous linear sucrose gradient is shown in Fig. 2. Most

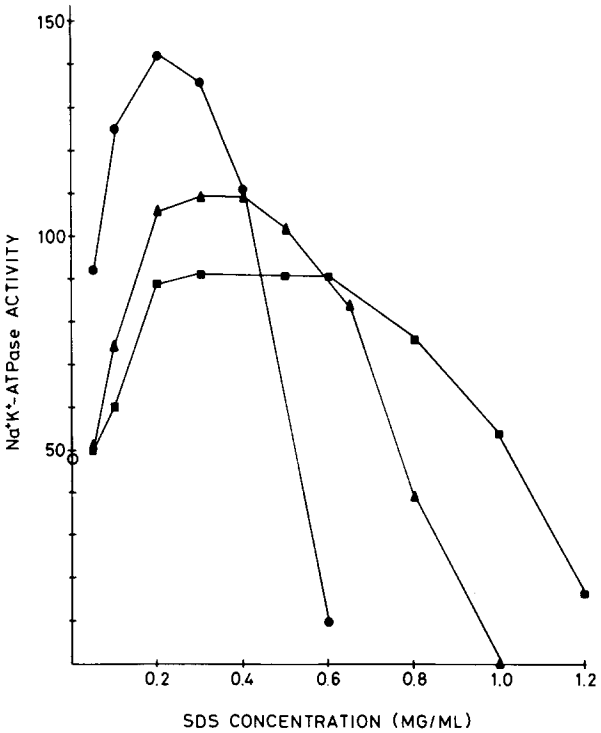

Fig. 1. The effect of the concentration of microsomal protein on the activation and inactivation of (Na^+ + K^+)-ATPase from toad kidney by various concentrations of sodium dodecyl sulphate. Aliquots of a microsomal fraction containing 0.2 mg (●), 0.6 mg (▲) and 1.4 mg (■) proteins were incubated with various concentrations of SDS in 20 mM Tris-HCl/1 mM EDTA/3 mM Na_2ATP in 1 ml at 20°C. After 30 min, aliquots were immediately tested for (Na^+ + K^+)-ATPase activity which is plotted in the graph as μmol P_i/mg protein per h. ○ = (Na^+ + K^+)-ATPase activity of untreated microsomal fraction which was 48 μmol P_i/mg protein per h.

of the protein remained in the sample zone while (Na^+ + K^+)-ATPase activity was highest in fractions with sucrose densities around 1.10 g/ml. A second but always smaller activity peak appeared in fractions of sucrose densities around 1.13 g/ml. Part of the Mg^{2+}-ATPase co-migrated with the lighter membrane portion. The various steps of the purification procedure with sodium dodecyl sulphate are documented in Table I for a representative preparation. The mean specific activity of the SDS enzyme collected from discontinuous sucrose gradients as the layer between the 29.4% and 15% sucrose concentrations amounted to 545 μmol P_i/mg protein per h (range 430—840 μmol P_i/mg protein per h, n = 10) and the protein recovery was on an average 1.6% of the total homogenate proteins. Mg^{2+}-ATPase still represented 21% (range 17—24%, n = 10) of the total ATPase activity.

In order to improve the purification of the enzyme, a combined method of deoxycholate solubilization [3] and sodium dodecyl sulphate treatment, together with sucrose gradient centrifugation [4,13], was applied. Again optimal conditions for incubation with deoxycholate were tested by varying protein and detergent concentrations (not shown). A protein concentration of 5

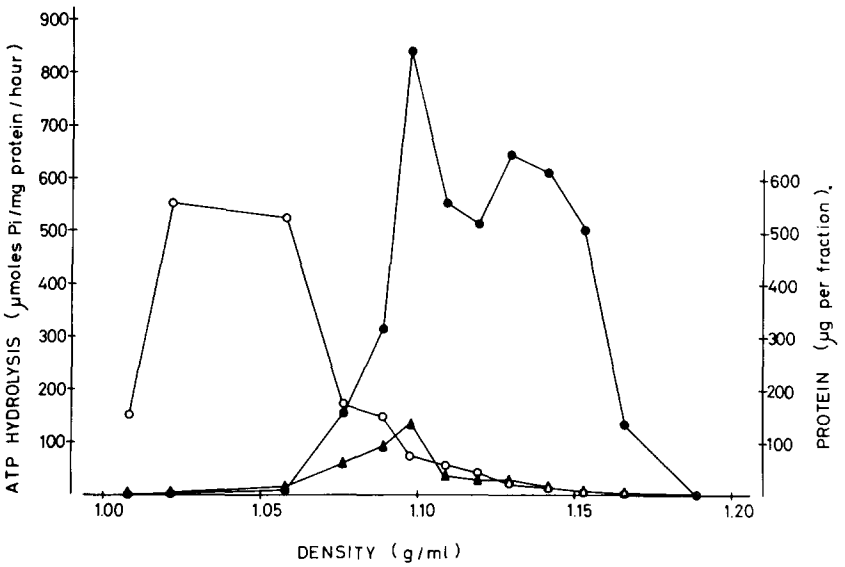

Fig. 2. The distribution of protein (○), ($Na^+ + K^+$)-ATPase (●) and Mg^{2+}-ATPase (▲) on a continuous linear sucrose gradient after centrifugation of a microsomal fraction from toad kidney treated with sodium dodecyl sulphate. 2.4 mg of microsomal protein were incubated for 30 min at 20°C with 0.5 mg SDS/ml in a total vol. of 2.4 ml. The incubation medium was the same as described in Fig. 1. The sample was layered on a sucrose gradient produced by a 15% and a 45% sucrose solution and centrifuged as described under Experimental.

mg/ml showed to be optimal for nearly all preparations while deoxycholate concentrations for maximal purification of the enzyme varied between 3.5 and 5 mg/ml.

With higher concentrations of deoxycholate, the enzyme activity was markedly reduced. Treatment with sodium dodecyl sulphate was routinely performed with a protein concentration of 1 mg/ml and a detergent concentration of 1.1 mg/ml after preliminary concentration studies (not shown). Compared to the sodium dodecyl sulphate treatment, the combined method yielded an enzyme with higher mean spec. act. of 710 μmol P_i/mg protein per h (range

TABLE I

PURIFICATION OF ($Na^+ + K^+$)-ATPase FROM TOAD KIDNEY WITH SODIUM DODECYL SULPHATE

Purification procedure as described under Experimental. For this experiment, a homogenate with 660 mg of proteins was used. Conditions for the incubation with detergent were 1.4 mg protein/ml and 0.45 mg SDS/ml.

	($Na^+ + K^+$)-ATPase		Mg^{2+}-ATPase (% of total ATPase activity)	Protein recovery (%)
	Specific activity (μmol P_i/mg protein per h)	Total activity (μmol P_i per h)		
Homogenate	12	7908	70	100
Microsomal fraction	52	5500	64	15
SDS enzyme	703	6305	19	1.3

TABLE II

PURIFICATION OF $(Na^+ + K^+)$-ATPase FROM TOAD KIDNEY WITH A COMBINED DEOXYCHOLATE AND SODIUM DODECYL SULPHATE TREATMENT

Purification procedure as described under Experimental. For this experiment, a homogenate with 1333 mg of proteins was used. Conditions for the incubation with deoxycholate were 5 mg protein/ml and 4 mg deoxycholate/ml. Conditions for the incubation with SDS were 1 mg protein/ml and 1.1 mg SDS/ml.

	$(Na^+ + K^+)$-ATPase		Mg^{2+}-ATPase (% of total ATPase activity)	Protein recovery (%)
	Specific activity (μmol P_i/mg protein per h)	Total activity (μmol P_i per h)		
Homogenate	12	16 000	67	100
Microsomal fraction	58	11 495	53	17
Deoxycholate enzyme	336	8 336	4	2.2
Deoxycholate/SDS enzyme	964	1 643	2.6	0.15

530—970 μmol P_i/mg protein per h, $n = 5$) (Table II). Deoxycholate treatment reduced markedly the portion of Mg^{2+}-ATPase to a mean of 2% (range 1.4—2.6%, $n = 5$) of the total ATPase activity. Protein recovery from homogenate material was about 10 times less than with sodium dodecyl sulphate treatment alone. The increase in total $(Na^+ + K^+)$-ATPase activity after deoxycholate treatment, as described by Lane et al. [3] and Hayashi et al. [13] was not observed, and sodium dodecyl sulphate after deoxycholate treatment, no longer induced an activation of the enzyme.

SDS-polyacrylamide slab gels as well as cylindrical gels were run in order to test the purity of the detergent treated enzymes. Considerable elimination of protein bands could be achieved by the detergent treatment of the microsomal fraction (Fig. 3). Both treatments, sodium dodecyl sulphate alone or in combination with deoxycholate yielded a preparation which showed three major bands on SDS-polyacrylamide gel electrophoresis: a prominent high molecular band with a mol. wt. of 116 000 as determined on slab gradient gels with protein standards of known molecular weights, an intermediate band of 62 000 daltons and a low molecular weight band of 26 000 daltons. The heavy band was identified as the catalytic subunit by subjecting the phosphorylated intermediate formed by [γ-^{32}P]ATP in the presence of sodium to slab gel electrophoresis and by subsequent autoradiography (Fig. 4). No phosphorylation occurred in the presence of potassium. Traces of labelling were also found in two slow moving bands. Phosphorylation experiments were carried out with SDS enzyme which frequently yielded high molecular weight bands of about 200 000 daltons on SDS-polyacrylamide gel electrophoresis. Therefore, the slight phosphorylation of these proteins might be an indication that these bands are primary aggregates formed by the subunits of $(Na^+ + K^+)$-ATPase which were not attacked by sodium dodecyl sulphate treatment, rather than contaminants.

The 62 000 dalton band was identified as the glycoprotein subunit by staining with periodic acid-Schiff (Fig. 5). Considerable periodic acid-Schiff staining

was observed with the protein of 26 000 daltons, suggesting that this band is of glycoprotein nature.

Low molecular weight contaminants were frequently observed with both

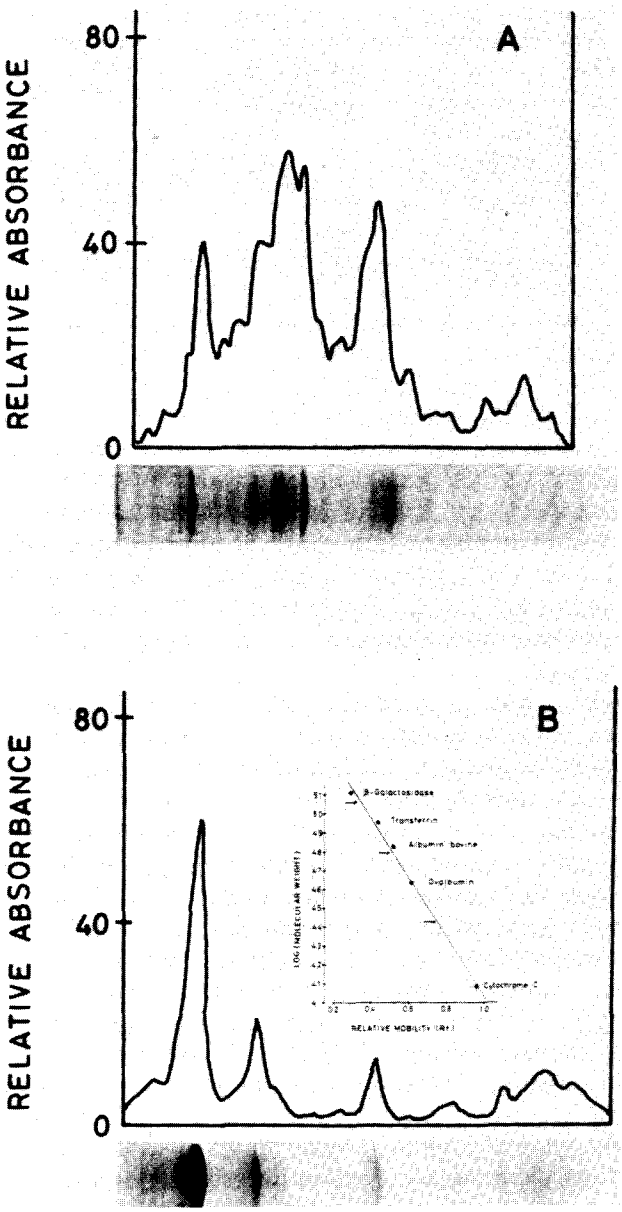

Fig. 3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of preparations of toad kidney ($Na^+ + K^+$)-ATPase. 75 μg of protein were loaded on the gels, electrophorized and scanned as described under Experimental. Panel A, microsomal fraction. Panel B, deoxycholate/sodium dodecyl sulphate-treated enzyme (deoxycholate/SDS enzyme). Inset, calibration of sodium dodecyl sulphate-polyacrylamide gels with proteins of known molecular weights. Arrows indicate the relative mobilities of the three major bands of the deoxycholate/SDS enzyme.

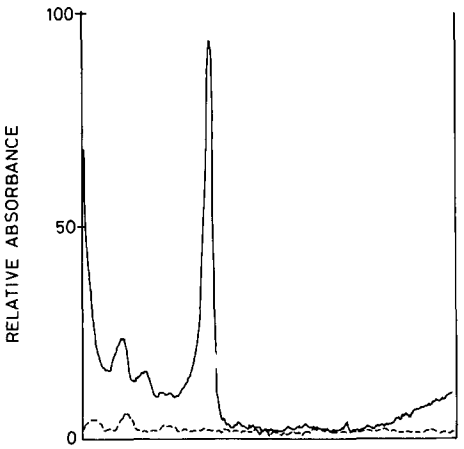

Fig. 4. Scans of an autoradiography of a sodium dodecyl sulphate-polyacrylamide slab gel loaded with 150 μg phosphorylated, purified $(Na^+ + K^+)$-ATPase from toad kidney. Phosphorylation was carried out with [γ-^{32}P]ATP in the presence of 100 mM NaCl (———) or 20 mM KCl (- - - - -) as described under Experimental. The labelled peak corresponds to a protein band with a mol. wt. of 116 000.

SDS and deoxycholate/SDS enzyme (Fig. 3). The catalytic and glycoprotein subunit of the deoxycholate/SDS enzyme make up about 55% of the total absorbance area as determined by scanning of a Coomassie Blue stained gel.

Kinetic data of $(Na^+ + K^+)$-ATPase from toad kidney

The mean K^+ concentration for half-maximal activation ($K_{1/2}$) was 1.14 mM (1.20, 1.26, 0.96 mM, $n = 3$) in the presence of a Na^+ concentration of 100 mM. The mean $K_{1/2}$ value for Na^+ was observed at 12.29 mM (10.25, 14.43,

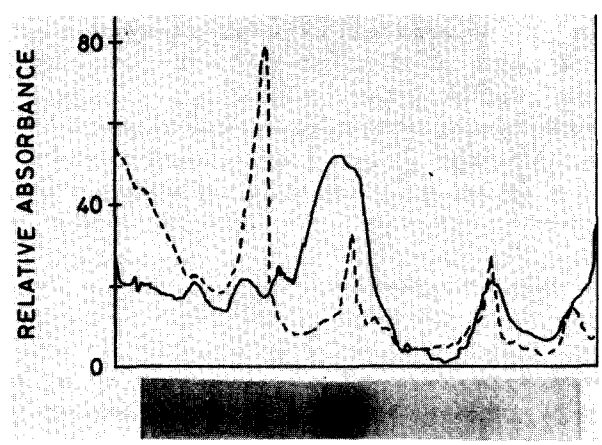

Fig. 5. Scan of a periodic acid-Schiff stained gel (———) compared to a scan of a Coomassie Blue stained gel (- - - - -). The corresponding periodic acid-Schiff stained gel loaded with 150 μg of deoxycholate/sodium dodecyl sulphate-treated enzyme is shown. Electrophoresis of purified $(Na^+ + K^+)$-ATPase was carried out on 7.5% sodium dodecyl sulphate-polyacrylamide gels as described under Experimental.

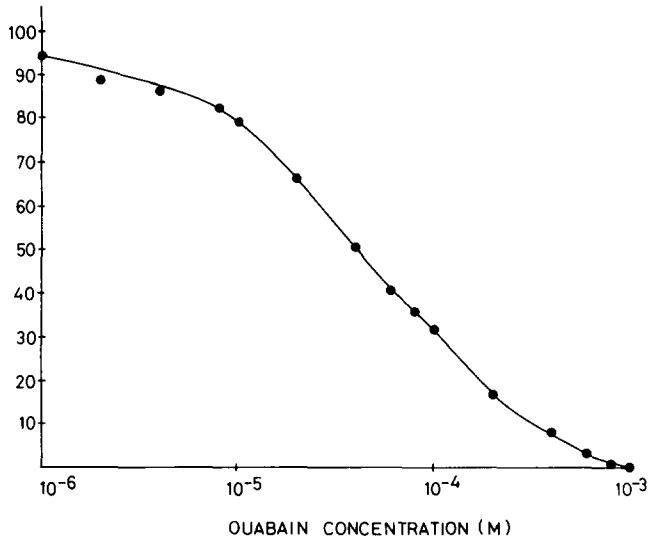

Fig. 6. Inhibition of $(Na^+ + K^+)$-ATPase by ouabain. Standard assay conditions were used (see Experimental) with ouabain concentrations varying from 1—200 μM.

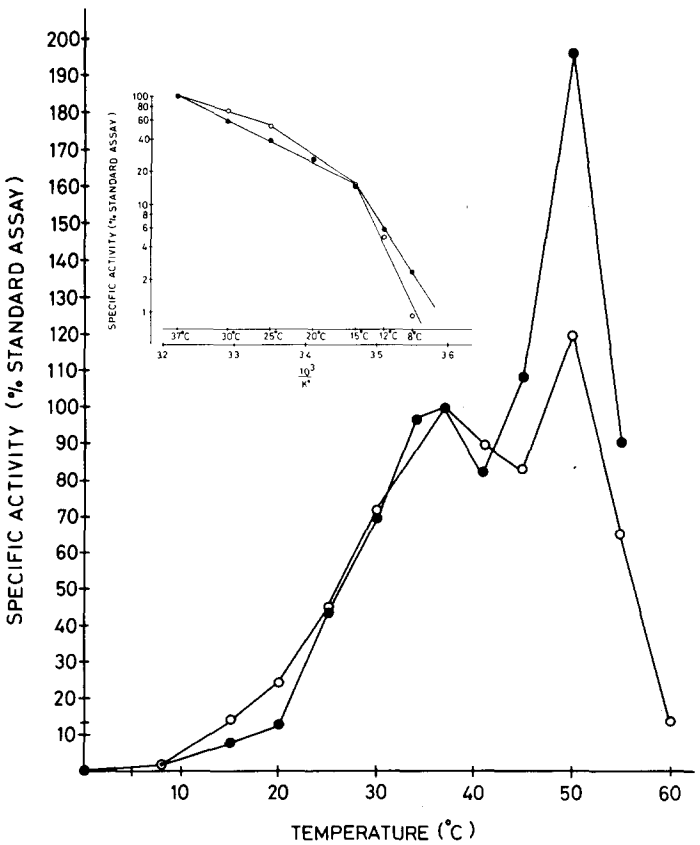

Fig. 7. Temperature dependence of $(Na^+ + K^+)$-ATPase from toad kidney. Inset, Arrhenius plot of $(Na^+ + K^+)$-ATPase activities between 8°C and 37°C. Standard incubation conditions were used (see Experimental) with constant pH of 7.4. ●, microsomal fraction; ○, purified enzyme.

12.19 mM, $n = 3$) in the presence of a K^+ concentration of 20 mM. This value corresponds well to the value of 11.8 mM reported by Cortas and Walser [8] for the toad bladder enzyme. Enzyme activities observed in the presence of 5 and 9 mM Na^+ did not fit the Hofstee plot. A discontinuity of the Na^+ activation curve has already been described by Cortas and Walser [8] for the toad bladder enzyme. They explained this phenomenon by a cooperative interaction of binding sites.

ATP concentrations for half-maximal activity (K_m) in the presence of a Mg^{2+} concentration of 3 mM, a Na^+ concentration of 100 mM and a K^+ concentration of 20 mM was 0.4 mM (0.39, 0.43, 0.38 mM, $n = 3$).

Half maximal inhibition (K_i) of the SDS enzyme by ouabain was observed at a concentration of 35 μM while complete inhibition occurred at 1 mM (Fig. 6).

The influence of temperature on ($Na^+ + K^+$)-ATPase activity was measured over a temperature range of 0—60°C. At each temperature the total ATPase activity was corrected for ATPase activity measured either in the presence of ouabain or in the absence of Na^+ and K^+, in order to control the temperature dependence of the ouabain inhibition [22].

The pH of the incubation medium was adjusted to 7.4 at each temperature. Both the microsomal and the purified enzyme showed two activity peaks, one at 37°C and an even higher one at about 50°C (Fig. 7). Arrhenius plots of activities versus temperature between 4°C and 37°C revealed a break at 15°C with both the microsomal and the SDS enzyme and with both incubation conditions.

Discussion

The procedure described for the purification of an amphibian ($Na^+ + K^+$)-ATPase yielded a preparation with spec. act. up to 1000 μmol P_i/mg protein per h. This enzyme activity is not as high as those described for the most pure preparations at present available from fish, avian and mammalian sources [1—4] but the 15—20 fold increase in activity of the enzyme attained with respect to the initial specific activity of microsomes is well comparable to that of highly active ($Na^+ + K^+$)-ATPase preparations from mammalian kidneys treated with sodium dodecyl sulphate [4] and from the electric organ of *Electrophorus* treated with Lubrol [1].

The detergent-treated enzyme preparations subjected to SDS-polyacrylamide gel electrophoresis show a considerable enrichment in the catalytic and glycoprotein subunits compared to the microsomal fraction (Fig. 3). The area of absorbance peaks of the two subunits which was calculated to account for about 55% of the total absorbance area of a Coomassie Blue stained gel is probably an underestimate of the real enrichment considering the poor and nonlinear staining of the glycoprotein with this dye [13,23]. In addition, the observation of a faint Na^+ dependent phosphorylation of high molecular weight proteins occurring on SDS-polyacrylamide gel electrophoresis suggests that these bands might be stable aggregates of the subunits of the enzyme rather than contaminants (Fig. 4). The formation of such complexes may be attributed either to sodium dodecyl sulphate treatment [23,24] or to electrophoresis at pH 9 as described by Kyte [25].

The detergent-treated enzyme preparations still contain some low molecular weight contaminants, a fact which points to a resistance of some membrane proteins of toad kidney to conventional detergent solubilization. One might imagine that the low activation of $(Na^+ + K^+)$-ATPase sites in microsomal fractions by sodium dodecyl sulphate and deoxycholate is an expression of this phenomenon. Incubation with sodium dodecyl sulphate increases the specific activity of enzyme preparations from nasal salt glands of the duck 6.5 fold [2] and from the outer medulla of rabbit kidney 5 fold [4] while sodium dodecyl sulphate-activated enzyme from toad kidney only exhibits a 1.5-fold increase in activity compared to the untreated microsomes under the same conditions (Fig. 1).

Deoxycholate treatment resulted in a 1.8-fold [13] or a 2.4-fold increase in total activity in preparations from canine kidneys while preparations from toad kidneys showed under the same conditions a decrease in total activity of about 28% (Table II).

Activation of $(Na^+ + K^+)$-ATPase is attributed to the unmasking of latent pump sites not accessible for the substrate [24]. It has been suggested that this process might be due to a fragmentation of microsomal vesicles by detergents [13]. The fact that the level of sodium dodecyl sulphate activation in our preparations augments with a decreasing protein concentration in the incubation medium makes unlikely an explanation based on the existence of a smaller number of latent pump sites in this tissue, as has been proposed by Jørgensen [24] for cold blooded animals. It is more likely that unfavourable vesicle conformation hinders the optimal detergent action. This phenomenon could be related to an unusual lipid content of the kidney preparation, a hypothesis which would also explain the migration of the purified enzyme on sucrose gradients to densities of 1.10 g/ml (Fig. 2) compared to the recovery of $(Na^+ + K^+)$-ATPase from other sources [2,4,13] at densities of 1.12—1.14 g/ml.

A special lipid composition of the toad kidney preparations could also explain the two biochemical peculiarities of the $(Na^+ + K^+)$-ATPase from this source. For instance, the occurrence of two activity peaks at 37°C and 50°C (Fig. 7) could indicate the presence of particular lipids which undergo fluidity changes at high temperatures. Such alterations would modulate the $(Na^+ + K^+)$-ATPase activity, as has been proposed for activity breaks in Arrhenius plots at temperatures around 20°C [26,27].

The second diverging property of the toad $(Na^+ + K^+)$-ATPase compared with the enzyme from most other sources is its well known insensitivity to ouabain. Reported K_i values for the toad $(Na^+ + K^+)$-ATPase range between 1.6 mM for the skin enzyme [10], 0.1 mM for the bladder enzyme [8], 0.03 mM for the intestinal enzyme [28] and 0.035 mM for the kidney enzyme described in this study (Fig. 6). The values for the renal and intestinal enzyme of the toad are still greater by 1—2 orders of magnitude than those reported for most other preparations (Table III, and ref. 29). While Allen and Schwartz [30] explain the ouabain insensitivity of certain animal species by a higher dissociation rate of the ouabain-enzyme complex, Cortas and Walser [31] propose, on the basis of deoxycholate treatment, that in the toad bladder a specific protein-phospholipid interrelationship is responsible for the ouabain insensitivity.

With respect to other biochemical data the enzyme from the toad kidney is

TABLE III

COMPARISON OF BIOCHEMICAL CHARACTERISTICS OF ($Na^+ + K^+$)-ATPase FROM DIFFERENT ANIMAL SPECIES

Authors	Enzyme source	Detergent treatment	Specific activity (μmol P_i/ mg protein per h)	$K_{1/2}$(mM)		K_m(mM) ATP	K_i(μM) Ouabain
				Na	K		
Dixon and Hokin [1]	electric organ electric eel	Lubrol	1200	15	2	0.29	1.2
Ratanabanankoon et al. [32]	rectal gland dogfish	Lubrol	1500			0.28	
Present study	toad kidney	SDS	500	12.2	1.14	0.4	35
Lo et al. [33]	rat kidney	deoxy-cholate	169	20.4	1.59	0.65	
Kline et al. [34]	beef brain	Lubrol	450—750			0.1	0.62
Braughler and Corder [35]	human kidney	deoxy-cholate	50—200	16	1.5	0.38	1.8

very similar to purified preparations from a variety of animal species (Table III). This fact supports the statement of Dixon and Hokin [1] that the enzyme has undergone little alteration with evolution and that the considerable variations reported in other publications are probably due to the impure state of the enzymes used.

Despite the fact that ($Na^+ + K^+$)-ATPase from toad kidney has not been purified to homogeneity in this study, an enzyme preparation was obtained which allowed the extensive characterization of an amphibian ($Na^+ + K^+$)-ATPase. A sufficiently pure enzyme is now available to serve in studies aiming to relate the physiological and biochemical expressions of sodium transport in amphibian tissues.

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References

1 Dixon, J.F. and Hokin, L.E. (1974) Arch. Biochem. Biophys. 163, 749—758
2 Hopkins, B.E., Wagner, H. and Smith, T.W. (1976) J. Biol. Chem. 251, 4365—4371
3 Lane, L.K., Copenhaver, J.H., Lindenmayer, G.E., Jr. and Schwartz, A. (1973) J. Biol. Chem. 248, 7197—7200
4 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36—52
5 Hokin, L.E. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E., eds.), pp. 374—388, Springer Verlag, Berlin-Heidelberg-New York, FEBS-Symposium No. 42
6 Goldin, S.M. (1977) J. Biol. Chem. 252, 5630—5642
7 Bonting, S.L. and Canady, M.R. (1964) Am. J. Physiol. 207, 1005—1009
8 Cortas, N. and Walser, M. (1971) Biochim. Biophys. Acta 249, 181—187

9 Asano, Y., Tashima, Y., Matsui, H., Nagano, K. and Nakao, M. (1970) Biochim. Biophys. Acta 219, 169—178
10 Park, Y.S. and Hong, S.K. (1976) Am. J. Physiol. 231, 1356—1363
11 Siegel, G.J., Tormay, A. and Candia, O.A. (1975) Biochim. Biophys. Acta 389, 557—566
12 Rossier, B.C., Wilce, P.A. and Edelman, I. S. (1977) J. Membrane Biol. 32, 177—194
13 Hayashi, Y., Kimimura, M., Homareda, H. and Matsui, H. (1977) Biochim. Biophys. Acta 482, 185—196
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Hokin, L.E., Dahl, J.L., Deupree, J.D., Dixon, J.F., Hackney, J.F. and Perdue, J.F. (1973) J. Biol. Chem. 248, 2593—2605
16 Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375—400
17 Asano, Y., Liberman, U.A. and Edelman, I.S. (1976) J. Clin. Invest. 57, 368—379
18 Maizel, J.V., Jr. (1971) in Methods in Virology (Maramorosch, K. and Koprowski, H., eds.), Vol. 5, pp. 179—246, Academic Press, New York
19 Laemmli, U.K. (1970) Nature 227, 680
20 Matthieu, J.M. and Quarles, R.H. (1973) Anal. Biochem. 55, 313—316
21 Post, R.L. and Sen, A.K. (1967) Meth. Enzymol. 10, 773—776
22 Gruener, N. and Avi-Dar, Y. (1966) Biochem. J. 100, 762—767
23 Giotta, G.J. (1976) J. Biol. Chem. 251, 1247—1252
24 Jørgensen, P.L. (1975) Quart. Rev. Biophys. 7, 239—274
25 Kyte, J. (1971) J. Biol. Chem. 246, 4157—4165
26 Priestland, R.N. and Whittam, R. (1972) J. Physiol. 220, 353—361
27 Kimelberg, H.K. (1975) Biochim. Biophys. Acta 413, 143—156
28 Robinson, J.W.L. (1970) J. Physiol. 206, 41—60
29 Bonting, S.L. (1970) in Membranes and Ion Transport (Billar, E.E., ed.), Vol. 1, pp. 257—363, Wiley Interscience, London
30 Allen, J.C. and Schwartz, A. (1969) J. Pharmacol. Exp. Therap. 168, 42—46
31 Cortas, N. and Walser, M. (1971) Fed. Proc. 30, 331 (abst. 782)
32 Ratanabanankoon, K., Dixon, J.F. and Hokin, L.E. (1973) Arch. Biochem. Biophys. 156, 342—349
33 Lo, C.S., August, T.R., Liberman, U.A. and Edelman, I.S. (1976) J. Biol. Chem. 251, 7826—7833
34 Kline, M.H., Hexum, T.D., Dahl, J.L. and Hokin, L.E. (1971) Arch. Biochem. Biophys. 147, 781—787
35 Braughler, J.M. and Corder, C.D. (1977) Biochim. Biophys. Acta 481, 313—327