J. Membrane Biol. 108, 187–195 (1989)

The Journal of
Membrane Biology
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Isoforms of Na,K-ATPase in *Artemia salina*: II. Tissue Distribution and Kinetic Characterization

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Summary. To characterize the molecular properties conveyed by the isoforms of the α subunit of Na,K-ATPase, the two major transepithelial transporting organs in the brine shrimp (*Artemia salina*), the salt glands and intestines, were isolated in pure form. The α isoforms were quantified by ATP-sensitive fluorescein isothiocyanate (FITC) labeling. The salt gland enzyme exhibits only the $\alpha 1$ isoform, whereas the intestinal enzyme exhibits both the $\alpha 1$ and the $\alpha 2$ isoforms. After 32 hours of development, Na,K-ATPase activity [in μmol P_i/mg protein/hr (1 u)] in whole homogenates was 32 ± 6 in the salt glands and 12 ± 3 in the intestinal preparations (mean ± SEM). The apparent half-maximal activation constants ($K_{1/2}$) of the salt gland enzyme as compared to the intestinal enzyme were 3.7 ± 0.6 mM *vs.* 23.5 ± 4 mM ($P < 0.01$) for Na^+, 16.6 ± 2.2 mM *vs.* 8.29 ± 1.5 mM for K^+ ($P < 0.01$), and 0.87 ± 0.8 mM *vs.* 0.79 ± 1.1 mM for ATP (NS). The apparent K_i's for ouabain inhibition were 1.1×10^{-4} M *vs.* 2×10^{-5} M, respectively. Treatment of whole homogenates with deoxycholic acid (DOC) produced a maximal Na,K-ATPase activation of 46% in the salt gland as compared to 23% in the intestinal enzyme. Similar differences were found with sodium dodecyl sulfate (SDS). The two distinct forms of Na,K-ATPase isolated from the brine shrimp differed markedly in three kinetic parameters as well as in detergent sensitivity. The differences in $K_{1/2}$ for Na^+ and K^+ are more marked than those reported for the mammalian Na,K-ATPase isoforms. These differences may be attributed to the relative abundances of the α subunit isoforms; other potential determinants (e.g. differences in membrane lipids), however, have not been investigated.

Key Words Na,K-ATPase isoforms · Na,K-ATPase kinetics · *Artemia salina* · brine shrimp · fluorescein-isothiocyanate · salt glands · intestines · ouabain inhibition

Introduction

The principal mechanism responsible for active extrusion of Na^+ from the cellular compartment against large differences in electrochemical potential is the Na,K-ATPase (E.C.361.3) found in the plasma membrane of virtually all animal cells (Skou, 1988). Indirectly, the pump also regulates the cellular concentrations of Ca^{2+}, H^+, glucose and amino acids, the transport of each of which is coupled to the flux of Na^+. A growing body of evidence indicates that passive and active Na^+ and K^+ fluxes are stimulated transiently during cell growth and replication (Koch & Leffert, 1980; Leffert & Koch, 1980).

The kinetic properties of Na,K-ATPase and its inhibition by ouabain have been extensively investigated (Akera, 1981). The affinity of the enzyme for ouabain differs between species, among tissues within the same species and among cells within the same tissue (Lane et al., 1973; Sweadner, 1979; Akera, 1981; Resh, 1982; Fambrough & Bayne, 1983; Specht & Sweadner, 1984; Doucet & Barlet, 1986; Pressley & Edelman, 1986). One explanation for these observed differences, is the existence of isoforms of the catalytic α subunit, i.e., designated α and $\alpha+$ in mammalian tissues, with $\alpha+$ being more sensitive to ouabain (Sweadner, 1979, 1985; Lytton, Lin & Guidotti, 1985). It should be noted that Peterson et al. (1978) were the first to discover the existence of α isoforms in analyzing the composition of Na,K-ATPase of brine shrimp nauplii. And Churchill et al. (1984) detected a significant difference in sensitivity of the $\alpha 1$ and $\alpha 2$ isoform-containing enzymes to cardiotonic steroids. α isoform peptides of varying relative abundance have been reported in partially purified enzyme preparations from brine shrimp nauplii, rat brain, mouse and lamb kidney, goldfish, duck salt gland and electroplax of the eel (Peterson et al., 1978; Sweadner, 1979, 1985; Matsuda, Iwata & Cooper, 1984; Siegel, Desmond & Ernest, 1986).

Recently Shull, Greeb and Lingrel (1986) cloned 3 cDNA isoforms of the catalytic subunit,

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designated, α, $\alpha+$ and αIII, characterized by 15% differences in amino acid sequence. The three isoforms are derived from three distinct genes (Shull & Lingrel, 1987). At least five genes (two of which may be pseudogenes) homologous to the α subunit were identified in man (Sverdlov et al., 1987).

The existence of isoforms of the catalytic subunit (and designated α1 and α2 by Peterson et al., 1982) in brine shrimp nauplii has been demonstrated by the following: (i) SDS-PAGE shows two discrete bands, the α2 traveling slightly further than the α1; (ii) their sensitivity to several biochemical probes, such as proteases, cross-linking reagents, N-ethylmaleimide differ significantly (Peterson et al., (1982); and (iii) differing N-terminal sequences (Morohashi & Kawamura, 1984). Since the mammalian counterpart of the brine shrimp α1 and α2 is not established, the original designation of Peterson et al. (1978) is used in the present manuscript, and to avoid confusion the earlier notation, α, $\alpha+$ and αIII, will be used to designate the mammalian isoforms.

The objectives of this study were to isolate salt glands and intestines in essentially pure suspensions, to identify the isoforms of Na,K-ATPase expressed in each, and to characterize the kinetic properties of these preparations. The expectation that this approach would provide a means of accomplishing these aims was based on the earlier brine shrimp studies, including cytochemical localization of Na,K-ATPase, as summarized by Conte (1984).

Materials and Methods

Isolation of the Salt Glands and Intestines

Brine shrimp, *Artemia franciscana*, Great Salt Lake (GSL), dehydrated cysts were obtained from Artemia Inc. and stored at −20°C until use. *Artemia franciscana* (GSL) is one of many subspecies of *Artemia salina*. Large-scale (450 g cysts) batches were hydrated and grown in full strength artificial seawater (Instant Ocean) at 29°C as described by Salon et al. (1989), and salt glands and intestines were isolated by a modification of the method of Lowy and Conte (1985). Time zero was designated as the time of addition of the hydrated cysts to the artificial seawater. At the end of the incubation (usually 24 hr), the nauplii were collected on miracloth sieves (Calbiochem), extensively washed with a sterile "salt gland removal" solution (SGRM) consisting of (in mM) 100 NaCl, 5 KCl, 5 $NaHCO_3$, 5 $MgCl_2$, 100 diabasic and monobasic PO_4, pH 7.6. Aliquots of 100 g wet nauplii were transferred to 4-liter Erlenmeyer flasks containing 2.5 liters of "salt gland basic" medium (SGBM), consisting of (in mM) 120 NaCl, 5 KCl, 5 $NaHCO_3$, 5 $MgCl_2$, 10 dibasic and monobasic PO_4, pH 7.6, and incubated at 37°C in a Brunswick shaking incubator at setting 4–5 for 8 hr. At the end of this second incubation, the nauplii from each flask were collected individually over miracloth and washed with sterile ice-cold (4°C) SGBM several times. The nauplii were then suspended at a concentration of 1 g/8 ml SGBM in 4-liter Erlenmeyer flasks, and 10 ml aliquots were taken for microscopy and enzyme assay. The flasks were then swirled for 5 sec and SGBM was added to a final volume of 3 liters. The slurry was passed successively through 149 and 74 μm nylon mesh screens. The 74-μm screen retains both the salt glands and intestines, which were collected by back washing with SGBM and then passed through 149-μm nylon mesh and 74-μm nylon mesh in series. The meshes were washed 5–6 times with SGBM, and the tissue on the 74-μm mesh was finally collected by back washing and suspended in 200 ml SGBM. A small aliquot was checked by light microscopy and usually consisted mainly of salt glands and intestines with little other naupliar debris. The mixture was then passed though a 2.5 × 30 cm glass bead (1 mm in diameter) column. The salt glands were collected in the effluent on a 74-μm nylon mesh screen. The glass bead column was continuously washed until intestines start to appear in the effluent, at this point the salt glands (free of intestines) were collected by back washing the nylon mesh in 50 cc of ice-cold SGBM and kept on ice. The intestines were eluted off the upper 10 cm of the glass bead column with 100 ml of ice-cold SGBM. The salt glands and intestines were pelleted by centrifugation in a clinical centrifuge at 500 × g and washed two times with "shrimp homogenizing" solution (SHM), consisting of (in mM) 250 sucrose, 2 EDTA, 50 imidazole, 2 β-mercaptoethanol, pH = 7.2. After the last washing, the aliquots were examined by light microscopy and the preparations immediately frozen in liquid nitrogen and stored at −20°C.

SDS-PAGE, FITC Labeling, and Na,K-ATPase Assay

Whole nauplii, salt glands or intestines were suspended in 2.5× volumes of SHM (ml SHM)/(g tissue) at 4°C and homogenized with 10 strokes in a glass-Teflon homogenizer, on ice. Protein was determined by the method of Lowry et al. (1951), as modified by Peterson (1977). Whole nauplii and intestines were diluted to 6 mg protein per ml and salt gland homogenates to 2–3 mg protein per ml and kept in aliquots at −20°C. FITC labeling and further purification by zonal centrifugation according to Jorgenson (1974) were performed on salt gland and intestinal preparations as described by Salon et al. (1989). The FITC-labeled α subunits were resolved by SDS-PAGE and quantified by densitometry, as described by Salon et al. (1989).

The rate of enzymatic hydrolysis of ATP was measured by monitoring the release of ^{32}P from (γ-^{32}P)ATP. The free P_i was separated from the resulting ADP and unreacted ATP with molybdate, and the ^{32}P-molybdate complex was extracted into isobutanol, as described by Muallem and Karlish (1983) and modified by Pressley et al. (1986). The enzyme assay was performed in varying concentrations of Na,K and ATP, and ouabain: The complete mixture consisted of (mM) 150 NaCl, 50 KCl, 10 $MgCl_2$, 50 imidazole, pH 7.2, in the presence and absence of 2 × 10^{-2} M ouabain. Choline chloride was substituted for Na^+ or K^+ in some experiments to maintain osmolarity constant, but there was no difference when omitted, hence results of all experiments were pooled. Mg-ATPase was measured either in the absence of Na^+ and K^+, or in the presence of 2 × 10^{-2} M ouabain and substracted from the total ATPase activity, to give the computed Na,K-ATPase activity.

The data were analyzed by a program run on a VAX/780 (Digital Equipment Corp., Maynard, MA) using PAR utility of

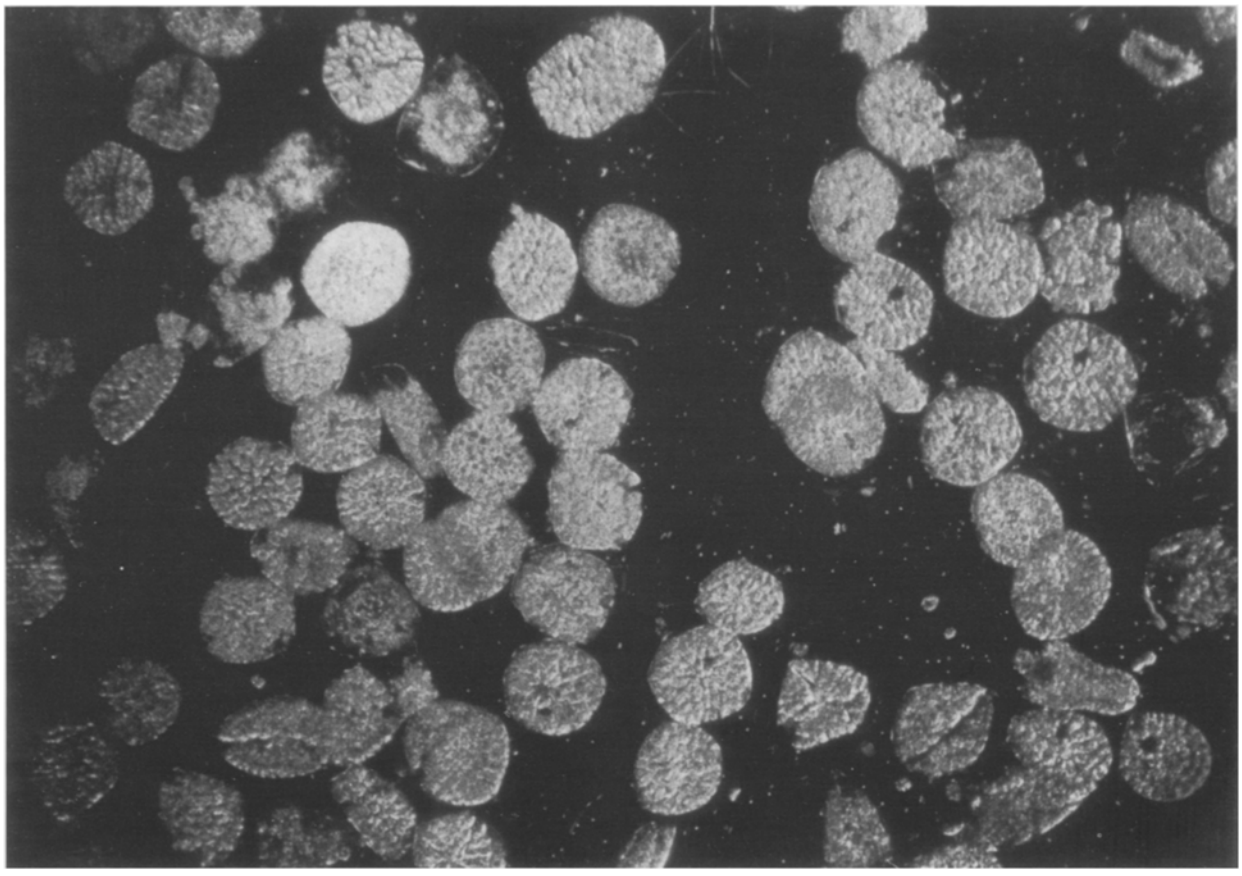

Fig. 1. Photomicrograph of isolated brine shrimp salt glands. Magnification (50×) under dark field displays intact salt glands free of other tissues

the biomedical Data Package (BMDP Software, Los Angeles, CA) that gave the best fit for a Hill equation and computed the apparent $K_{1/2}$'s. Single-site or two-site models were fitted to the ouabain inhibition data as described by Pressley and Edelman (1986).

Detergent Activation Curves

Whole homogenates or membrane fractions were incubated at a protein concentration of 1.5 mg/ml of SHM, with or without 3 mM ATP, for 15 min in ice water and then exposed to varying concentrations of SDS or DOC for 45 min at 20°C. Aliquots were then immediately transferred to the incubation mixes for Na,K-ATPase assay, with a 20-fold dilution of the detergent, and assayed for enzyme activity.

Results

The isolated salt glands and intestines were essentially homogeneous and structurally intact, by light microscopy, as shown in Figs. 1 and 2. The yields were 75–100 mg of salt glands and 400–600 mg of intestines from 400 g of dry *Artermia* cysts. The specific activity of Na,K-ATPase in whole homogenates of the salt glands was 32 ± 6 *u* (*u* = μmol P_i/mg protein/hr), which is 2.6-fold higher than the activity in homogenates of the intestines and 6.6-fold higher than that of whole nauplii. Na,K-ATPase comprised 85–90% of total ATPase in the salt gland preparation, 30–40% in intestines and 40–60% in whole nauplii. Na,K-ATPase in whole nauplii incubated for 24 hr in seawater at 29°C, and then for 8 hr in SGBM at 37°C, was 6.2 ± 1.3 *u*, which is not significantly different from the activity found in whole nauplii incubated in seawater at 29°C for 32 hr. This indicates that development continued in SGBM at 37°C. Na,K-ATPase activity remained constant in intestinal preparations, but in salt gland preparations increased from 19 to 32 *u* between 26 and 32 hr of development, and then dropped to 16 and 10 *u* by 40 and 50 hr. These changes are coincident with the growth and development of the salt gland. The basis for the drop in Na,K-ATPase activity seen in whole nauplii after 42 hr of development was not established by our studies, since the salt gland remains functional for up to 10 days after

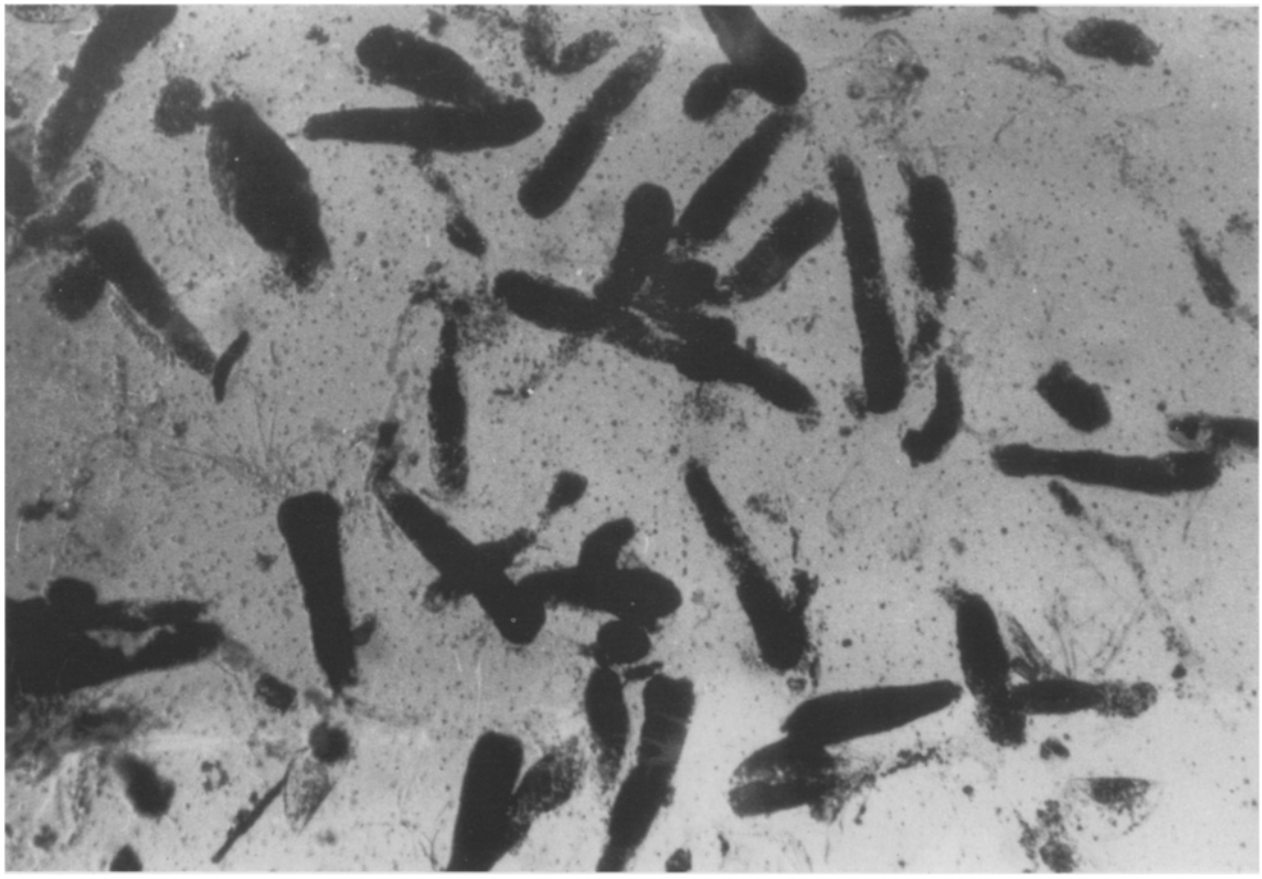

Fig. 2. Photomicrograph of isolated brine shrimp intestines. Magnification (50×) displays both intact and partially disrupted full-length intestinal tracts

hatching (Conte, 1984). Partially purified enzyme was prepared from both salt glands and intestines by treating the microsomes with 0.45% SDS, as described by Jorgensen (1974). The membrane fraction was obtained by centrifugation at 250,000 × *g* in 50% glycerol for 2½ hr. Na,K-ATPase activity was enriched to 290 *u* in the salt gland preparation and 210 *u* in the intestinal preparation. Coomassie blue-stained SDS-PAGE of these two preparations revealed that the salt gland expresses one isoform, the slower migrating $\alpha 1$, while the intestinal enzyme exhibits $\alpha 1$ and $\alpha 2$ as shown in Fig. 3. To eliminate the possibility of differential loss of one isoform during purification, crude homogenates of whole nauplii, intestines and salt glands, with 9, 15 and 30 *u* of enzyme activity, respectively, were labeled with 10 μM FITC, in the presence and absence of 5 mM ATP. The FITC fluorograms confirm that the salt gland expressed only $\alpha 1$, whereas whole nauplii and intestinal preparations expressed both $\alpha 1$ and $\alpha 2$ isoforms (Fig. 4).

To assess the properties of each of the isoforms, Na,K-ATPase was assayed with varying concentrations of ouabain, Na^+,K^+ and ATP. The enzyme was incubated with or without ouabain for 1 hr prior to the initiation of the reaction by the addition of (γ-^{32}P)ATP. Ouabain inhibition curves revealed that the salt gland enzyme is less sensitive, ($K_i = 1.1 \times 10^{-4}$ M) than the whole naupliar or intestinal enzymes ($K_i = 10^{-5}$ and 2×10^{-5} M, respectively) (Fig. 5).

The results of kinetic analysis of the salt gland and intestinal enzymes are shown in Fig. 6, and summarized in the Table. The salt gland enzyme has higher affinity for Na^+ ($K_{1/2} = 3.7 \pm 0.6$ mM *vs.* 23.5 ± 1.5 mM) and a lower affinity for K^+ ($K_{1/2} = 16.6 \pm 2.0$ mM *vs.* 8.3 ± 1.5 mM) than the intestinal preparation. Their respective affinities for ATP ($K_m = 0.87 \pm 0.8$ mM *vs.* 0.79 ± 1.1 mM) were indistinguishable.

Peterson et al. (1978) reported that enzyme activity in zonal fractions of DOC-treated microsomal membranes from whole nauplii sedimented bimodally in two sets of fractions. The optimal $Na^+ + K^+$ concentration (at 4 : 1 ratio) for Na,K-ATPase activity was different for the enzyme in each of these

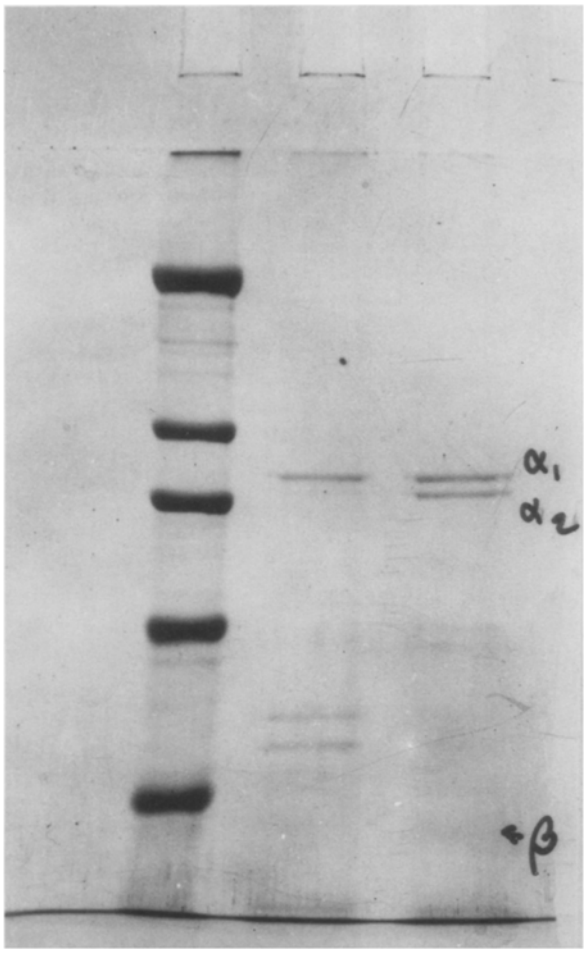

Fig. 3. Coomassie blue-stained SDS-PAGE. Lanes from left to right, mol wt standards: myosin, 200,000; β-galactosidase, 116,000; phosphorylase *b*, 92,000; bovine serum albumin, 66,000; ovalbumin, 45,000; and carbonic anhydrase, 31,000; partially purified enzyme were from salt glands (290 *u*) and from intestines (210 *u*). The units are indices of the high degree of purification. The subunits (α1, α2 and β) are identified by the appropriate symbols

sets of fractions. To investigate this further, Na,K-ATPase activity was measured at various $Na^+ + K^+$ concentrations, keeping the Na/K ratio constant. The intestinal enzyme was tolerant to a wider range of ionic strengths than the salt gland enzyme (Fig. 7). This is particularly evident at high ionic strengths. To eliminate the possibility that unexposed enzyme may have contributed to these differences, whole homogenates (1.5 mg protein/ml) of salt gland and intestines were incubated for 30 min with DOC in concentrations ranging from 0–2 mg/ml and immediately assayed for Na,K-ATPase activity. At 0.4 mg of DOC per ml, the salt gland enzyme was activated by 46% and the intestinal enzyme by only 23% (Fig. 8). There was no change in kinetic parameters (i.e., $K_{1/2}$ for Na^+ and K^+, and K_m for ATP) in the presence of DOC, and were indistinguishable from the values in the Table. A similar pattern was seen with SDS activation but the concentrations resulting in maximal activation differed for each preparation when whole nauplii, salt glands and intestines were compared (Fig. 9). Our results are in accord with those of Peterson et al. (1978) except that they attributed the narrow monovalent cation activation profile to a dominance of the α2 isoform. Since our studies on the salt gland provided an α1 isoform free from admixture with the α2 enzyme, it appears that their zonal fraction (pool 2) was dominated by the α1 isoform even though the α2 isoform was present in as high or higher abundance in this preparation.

These results suggested that the isoform-specific enzymes could be separated by appropriate use of detergent concentrations. A mixture of 60% salt glands and 40% intestines was homogenized and the microsomal membranes were treated with 0.57 mg/ml SDS for 45 min in the presence of ATP, and then fractionated by zonal centrifugation through a continuous sucrose gradient of 15 to 45%. Fifty fractions were collected, 14 of which had Na,K-ATPase activity. Fractions 16 and 17 were markedly enriched in the α1 containing enzyme, with specific activities of 1070 and 1210 *u*, respectively (Fig. 10). The activity curve was bimodal, suggesting that most of the α2 isoform activity was in fractions 10–11.

Discussion

Analysis of the basis for differing kinetic properties of various Na,K-ATPase should take into account the presence of the particular α isoforms with respect to (i) The affinity of each form for Na^+ and K^+, (ii) the maximum enzyme rate, (iii) the degrees of cooperativity in the kinetics, and (iv) the affinity of the pump for ATP. These criteria were used as the guides for the present study.

In brine shrimp nauplii, two isoforms have been described at the protein level, α1 and α2, by SDS-PAGE (Peterson et al., 1982). In mammals, evidence for the existence of a third α isoform was provided by Shull and colleagues (Shull et al., 1986; Shull & Lingrel, 1987) by cDNA cloning. Similar results were reported by Sverdlov et al. (1987). At the amino acid level, the mammalian α isoforms are approximately 85% homologous. Since the brine shrimp α isoforms have not yet been extensively sequenced, the evolutionary relationships between the mammalian and brine shrimp isoforms have not been defined.

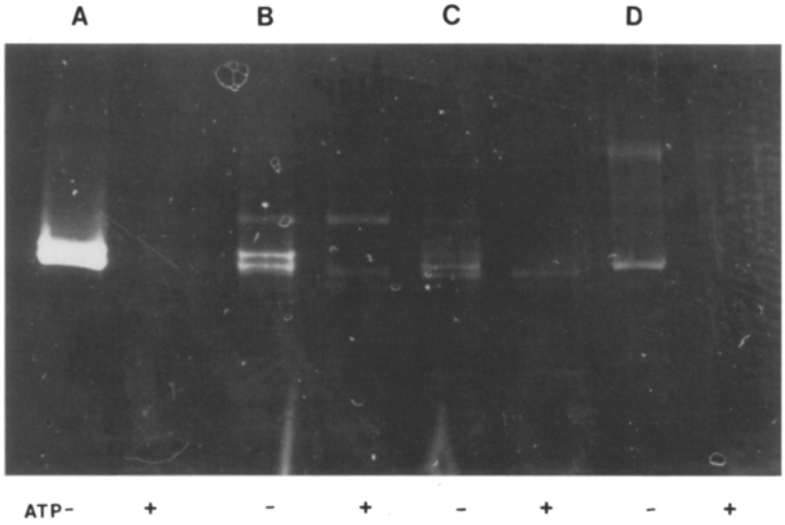

Fig. 4. Comparison of FITC-labeled α subunits of Na,K-ATPase from brine shrimp nauplii, salt glands and intestines. Equal amounts of protein were labeled with FITC and resolved by SDS-PAGE. (*A*) Whole nauplii partially purified Na,K-ATPase (200 *u*), (*B*) intestinal homogenates (15.7 *u*), (*C*) whole naupliar homogenates (9.0 *u*) and (*D*) isolated salt gland homogenates (38 *u*). The units indicate the degrees of purification. The +/− ATP designates the presence or absence of 5 mM ATP during the exposure to FITC

Table. Kinetic parameters of Na,K-ATPase isolated from salt glands (SG) and intestines (*I*) of the brine shrimp at 32 hr of development

	Na^+		K^+		ATP	
	(SG)	(*I*)	(SG)	(*I*)	(SG)	(*I*)
K_m	3.7 ± 0.06	23.5 ± 4	16.6 ± 2.0	8.3 ± 1.5	0.87 ± 0.8	0.79 ± 1.1
V^+_{max}	29.3 ± 3.6	11.3 ± 1.4	35.7 ± 5.8	11.6 ± 2.7	32 ± 6	14 ± 2
n(Hill)	1 ± 0.01	1.16 ± 0.02	1.4 ± 0.08	1.5 ± 0.25	1 ± 0.01	1 ± 0.2

Values are means ± SEM for seven experiments. The salt gland has only the α1 subunit, whereas the intestine has both α1 and α2 subunits. The K_ms were computed by curve fitting (*see* Figs. 5 and 6). $P < 0.01$ except for ATP where $P > 0.1$ for the K_ms. All of the differences in V_{max}'s were statistically significant ($P < 0.01$). All of the Hill coefficients (n) had values close to 1.0. Thus, cooperative interactions in the enzyme mechanism were not evident.

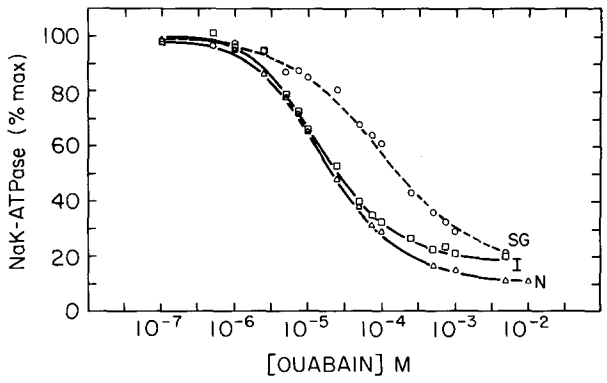

Fig. 5. Comparison of ouabain sensitivities of whole homogenates of brine shrimp nauplii, intestines and salt glands. All Na,K-ATPase activities were normalized to V_{max} of the ouabain-sensitive activity. Salt gland (*SG*) is denoted by –○–, intestines (*I*) by –□–; whole nauplii (*N*) by –△–

To assess the relative abundance of the α1 and α2 isoforms of Na,K-ATPase in the salt gland and intestine, partial purification was performed by the discontinuous gradient method of Jorgensen (1974). In the salt gland, only the slower migrating α1 subunit was detected by Coomassie blue staining while the intestinal enzyme exhibited both α1 and α2 bands. To eliminate the possibility of differential loss of one of the subunits with purification, homogenates of whole nauplii, salt glands and intestines were labeled with FITC, with the same results. From the kinetic data, α1 in the brine shrimp is similar to α in mammalian tissue, while α2 corresponds to α+. However, in SDS-PAGE of the brine shrimp enzyme, α1 is the slower migrating band and α2 is the faster one. In mammalian preparations, the slower migrating α+, has a lower molecular weight than the faster migrating α, as deduced from cDNA sequencing (Shull et al., 1986).

Separation and isolation of salt glands and intestines, in pure suspensions, provided enough material for significant purification of Na,K-ATPase from both organs. As described above, salt glands exhibited only the α1 isoform, whereas the intestine exhibited both. To characterize the enzyme ob-

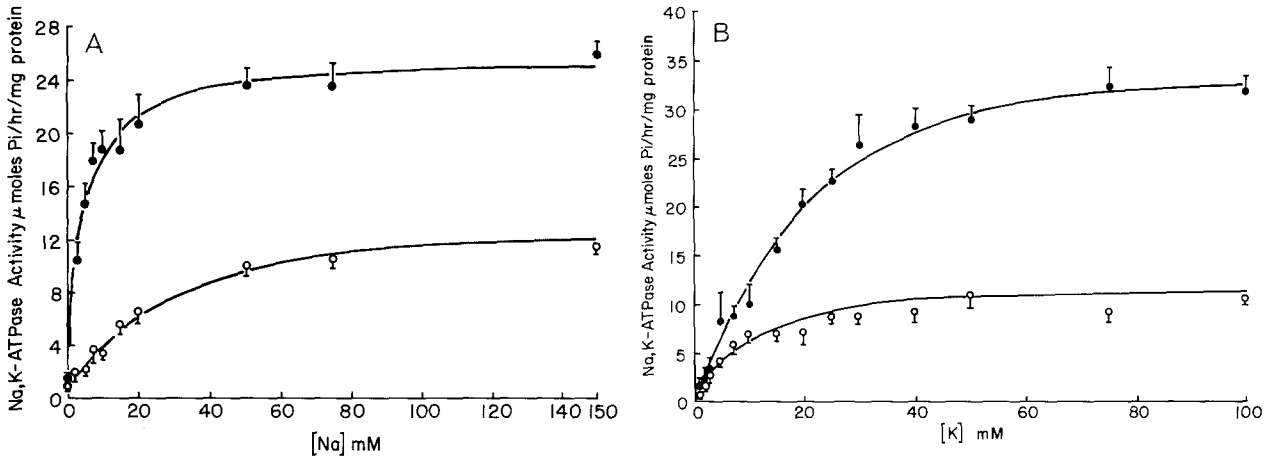

Fig. 6. Substrate activation curves for Na^+ (*A*) and K^+ (*B*) salt gland (--●--) and intestinal homogenates (--○--). The $K_{1/2}$s for Na^+ and K^+ activation were computed by nonlinear least squares curve fitting (*see* the Table). Values are means ± SEM. $n = 7$

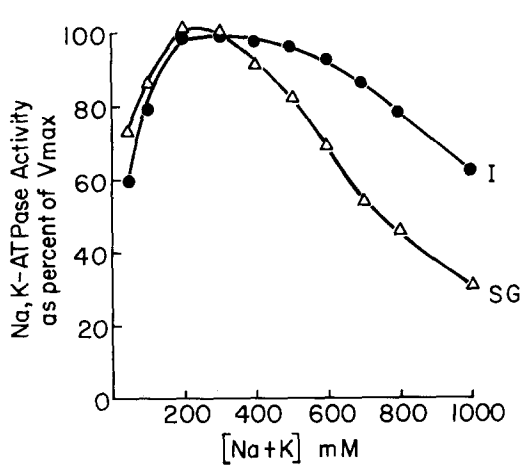

Fig. 7. Comparison of the tolerances of salt gland (*SG*, -△-) and intestine (*I*, --●--) Na,K-ATPase to the $Na^+ + K^+$ concentrations. The ratio of Na^+/K^+ in the medium was maintained constant at 4. Whole homogenates were incubated for 45 min at each salt concentration

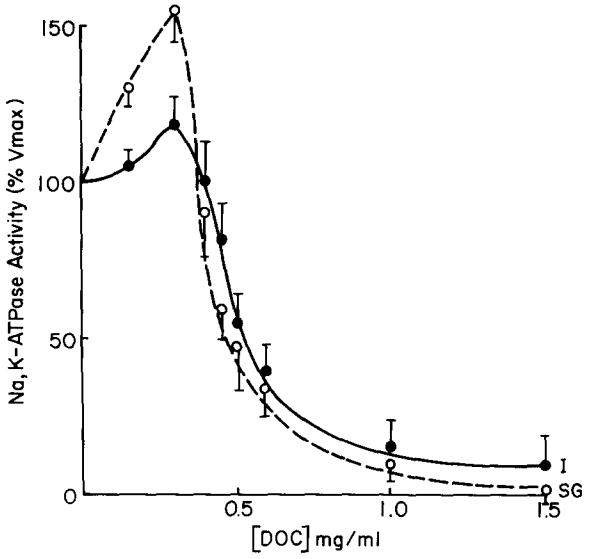

Fig. 8. Comparison of the effects of DOC on Na,K-ATPase activity of salt glands (*SG*, --○--) and intestines (*I*, --●--). Whole homogenates were incubated at a protein concentration of 1.5 mg/ml with or without 3 mM ATP for 15 min and then exposed to a varying concentration of DOC for 45. The curves were drawn by eye

tained from each organ, the affinities for Na^+,K^+ and ATP were determined. In the salt gland, the $K_{1/2}$ for Na^+ was sixfold lower than in the intestinal preparation. The difference is more marked than that described by Lytton (1985) in the mammalian rat adipocytes. In contrast to Na^+, the affinity for K^+ was twofold higher in the intestinal preparation than in the salt gland. The affinity for ouabain was fivefold lower in the salt gland enzyme. The intestinal enzyme tolerated a wider range of osmolarities than the salt gland enzyme, as shown in Fig. 7. The responses to both DOC and SDS also differed: The intestinal preparation was inactivated at significantly lower concentrations of these detergents.

The existence of isoform-related differences in ouabain sensitivity have also been noted by Churchill et al. (1984) in brine shrimp nauplii and Sweadner (1979) in α isoform enzymes obtained from axolemma and astrocytes of rat brain. Our results, however, differ from those of Churchill et al. (1984) in that they inferred that the α1 isoform had a higher rather than a lower affinity for digitalis glycoside. Churchill et al. (1984) used a K^+-sensitive dephosphorylation assay rather than the equilibrium enzyme inhibition assay used in our studies. Since our

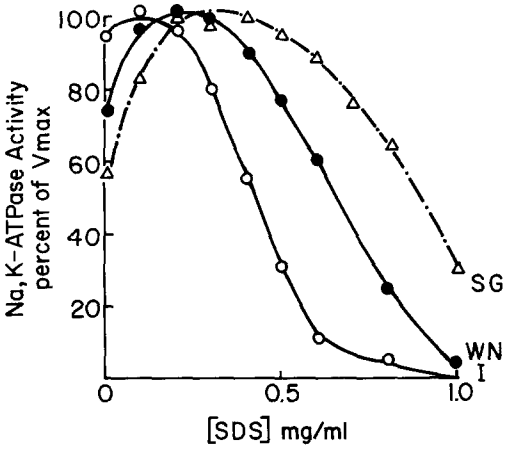

Fig. 9. Comparison of the effects of SDS on Na,K-ATPase activity of salt glands (*SG*, --△--), whole nauplii (*WN*, --●--) and intestines (*I*, --○--). Homogenates were incubated as in Fig. 8 with SDS replacing DOC. The curves were drawn by eye

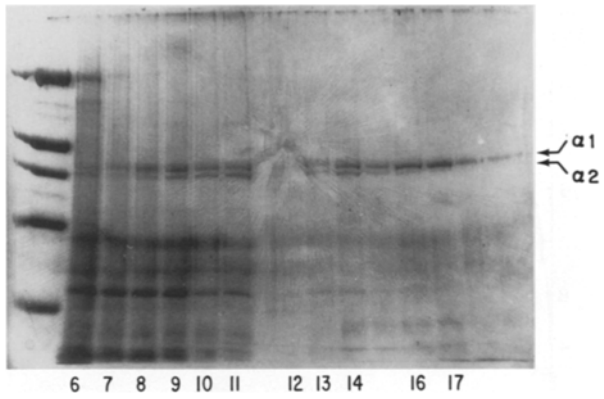

Fig. 10. Coomassie blue-stained SDS-PAGE of zonal fractions 6–20 obtained from microsomal membranes of a mixture of salt glands and intestines. Left lane represents mol wt standards. Lanes 6–17 represent the fractions obtained by rate zonal centrifugation in 15–45% sucrose, as described by Jorgensen (1974). Fractions 16–17 contained only the $\alpha 1$ isoform and had a mean Na,K-ATPase activity of 1140 *u*. Fractions 10–11 contained both isoforms with mean Na,K-ATPase activity of 670 *u*

results indicate substantial differences in the isoform affinities for K^+ as well as for ouabain, the differences in findings may reflect differences in the role of K^+ concentrations in the two assays.

The high affinity of the salt gland preparation for Na^+ suggests that the enzyme may be saturated with this ion, under physiological conditions, since the intracellular Na^+ concentration may be well in excess of the $K_{1/2}$. In contrast, the lower affinity for K^+, in the face of a hemolymph K^+ concentration of ~10 mM, implies that the pump rate may be regulated by external K^+ under physiological conditions.

The presence of multiple isoforms with differing kinetic properties may account for the observations that different segments of the renal tubules have different K_i's for ouabain (Doucet & Barlet, 1986) and the biphasic ouabain-binding curve in cardiac muscle (Kazazoglou et al., 1983), but other factors may also play a role in the response to ouabain. Na,K-ATPase isolated from toad bladder is more sensitive to ouabain when assayed with 0.1% deoxycholate, suggesting a role for lipid-protein interactions (Cortas & Walser, 1971). Treatment of plasma membranes from murine plasmacytoma cells with EDTA increased ouabain sensitivity by 1000-fold (Geny et al., 1982). The effect was reversed when the proteins solubilized with EDTA were treated with β-actinin.

In conclusion, Na,K-ATPase from the salt gland is composed predominantly of the $\alpha 1$ isoform and has a much higher affinity for Na^+, and lower affinities for K^+ and ouabain, than that of the intestinal tract. Intestinal Na,K-ATPase contains both α isoforms. Purification to homogeneity of the enzyme species should enable precise definition of the role of the α isoforms as determinants of their kinetic properties.

This work was financed by a grant-in-aid from U.S. Public Health Services, National Institutes of Health grant no. P01 DK31089.

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Received 14 September 1988; revised 3 January 1989