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A RECONSTITUTED Na^++K^+ PUMP IN LIPOSOMES CONTAINING PURIFIED (Na^++K^+)-ATPase FROM KIDNEY MEDULLA

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Summary

Liposomes containing either purified or microsomal (Na^+,K^+)-ATPase preparations from lamb kidney medulla catalyzed ATP-dependent transport of Na^+ and K^+ with a ratio of approximately $3Na^+$ to $2K^+$, which was inhibited by ouabain. Similar results were obtained with liposomes containing a partially purified (Na^+,K^+)-ATPase from cardiac muscle. This contrasts with an earlier report by Goldin and Tong (J. Biol. Chem. 249, 5907—5915, 1974), in which liposomes containing purified dog kidney (Na^+,K^+)-ATPase did not transport K^+ but catalyzed ATP-dependent symport of Na^+ and Cl^-. When purified by our procedure, dog kidney (Na^+,K^+)-ATPase showed some ability to transport K^+ but the ratio of Na^+ : K^+ was 5 : 1.

Introduction

Reconstitution of the sodium pump has been accomplished in a number of laboratories by incorporation of purified or semipurified (Na^+,K^+)-ATPase into liposomes (lipid vesicles). Initial studies reported only ATP-dependent Na^+ transport [1—3] but subsequently, coupled sodium, potassium transport has been reported using purified (Na^+,K^+)-ATPase from the shark rectal gland [4], and a partially purified brain microsomal preparation [5]. In contrast to this, Goldin and Tong [1] were unable to find potassium transport using a purified enzyme from canine kidney medulla but did observe ATP-dependent symport of Na^+ and Cl^-. They suggested that the sodium pump in kidney may transport

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Na^+ and Cl^- in vivo. In view of the fact that ATP hydrolysis by the enzyme from this source, as with enzymes from other sources [6], is stimulated by Na^+ and K^+ rather than Na^+ and Cl^-, this seemed intriguing and we have repeated this work with (Na^+,K^+)-ATPase purified from lamb kidney medulla.

Materials and Methods

Enzyme preparation

The sodium iodide-treated microsomal fraction and the glycerol enzyme fraction were prepared from the outer medulla of sheep or dog kidney as previously described [7]. The (Na^+,K^+)-ATPase activity of these two preparations was 140 and 520 μmol/mg per h respectively. The glycerol enzyme was shown to contain only two bands by gel electrophoresis corresponding to polypeptides with apparent molecular weights of 95 000 and 55 000 [7]. Cardiac (Na^+,K^+)-ATPase (citrate enzyme) was prepared as previously described [9].

Liposome preparation

The solubilization and dialyzing media were essentially the same as described by Hilden et al. [2] and contained 20 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 50 mM choline chloride, 1 mM EDTA, 1 mM cysteine and 30 mM imidazole-HCl, pH 7.0 (Solution A).

Liposomes containing microsomal (Na^+,K^+)-ATPase were prepared by the addition of 4 mg of enzyme to solution A containing 56 mg sodium cholate and 40 mg L-α-phosphatidylcholine (Type III-E, Sigma, supplied as a solution in hexane which was dried down under N_2) in a final volume of 2 ml. The mixture was centrifuged (100 000 $\times g$, 15 min) and the supernatant was dialyzed against solution A for 16 h at 4° C in Biofiber 50 minitubes (Bio Rad®) as described by Sweadner and Goldin [5].

Liposomes containing purified (Na^+,K^+)-ATPase (glycerol enzyme) were prepared by a slightly different procedure. The enzyme (2 mg) was solubilized in 1 ml of solution A containing 10 mg sodium cholate and centrifuged (100 000 $\times g$, 15 min). The supernatant was then added to 1 ml solution A containing 40 mg phosphatidylcholine, and 10 mg sodium cholate and dialyzed as described above. This procedure increased the amount of enzyme solubilized, but did not alter the characteristics of transport (i.e., the ratio of Na^+ : K^+ was unchanged). After reconstitution, the liposomes exhibited 10 to 15% of the original (Na^+, K^+)-ATPase activity. (In more recent experiments we have been able to reduce the loss of activity to about 10%).

Measurement of transport

Liposomes were incubated overnight at 0—4° C with either ^{22}Na (0.2 μCi/ml) or ^{86}Rb (4 μCi/ml) and then for 2 h at 25° C to ensure equilibration of the isotope. Preliminary trials were conducted to show that this length of time was adequate. ATP was added to aliquots of these liposomes in a final concentration of 5 mM and incubated at 25° C. After the desired time interval, an aliquot of the incubation mixture (60—120 μl) was transferred to a column of Sephadex G-50, medium grade (1 × 30 cm) and run at a flow rate of 2 ml/min. This is faster than can be obtained using the "fine" grade but gives adequate separa-

tion of the liposomes from the free isotope. The faster flow rate reduces loss of isotope from the liposomes which are eluted from the column within 8 min. Fractions (2 ml) were collected directly into scintillation vials, to which 10 ml of Bray's solution (New England Nuclear) was added and the vials were counted in a Beckman scintillation counter.

In order to measure ouabain inhibition of transport, ouabain was added to an aliquot of the liposomes which had been preincubated with either ^{22}Na or ^{86}Rb (as indicated) to the final concentration indicated in the figures ($5 \cdot 10^{-4}$ or 10^{-3} M). The mixture was then incubated for 4 h at 25° C prior to the addition of ATP, to allow ouabain to diffuse into the liposomes. (Na^+,K^+)-ATPase activity was determined as previously described [9] and protein was determined by the method of Lowry et al. [10], after elimination of turbidity due to excess lecithin by centrifugation at 100 000 × *g* for 20 min.

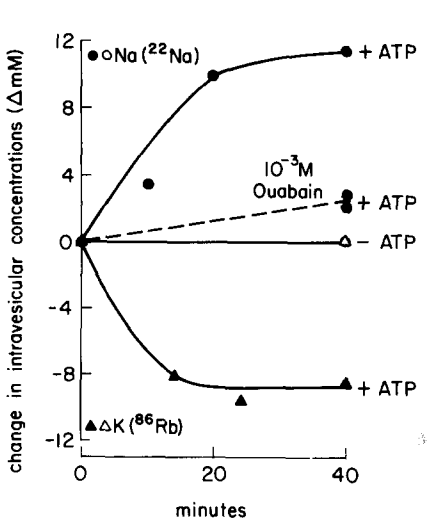
Results and Discussion

In view of the fact that a semi-purified microsomal (Na^+,K^+)-ATPase preparation from brain [5] catalyzed sodium vs. potassium transport while a purified kidney medulla preparation [1] did not, it seemed possible that the purification procedure could have resulted in loss of the ability to transport potassium ions. Accordingly, in our first experiment, we used a sodium iodide-treated microsomal preparation from lamb kidney medulla which was of similar purity to the brain microsomal preparation of Sweadner and Goldin [5]. Both were microsomal preparations of relatively low specific activity (140 μmol/mg per h for our preparation and 69 μmol/mg per h for that of Sweadner and Goldin). This preparation catalyzed ATP-dependent sodium vs. potassium transport as shown in Fig. 1. In all these studies, we have used ^{86}Rb rather than ^{42}K because the half-life of the latter is inconveniently short. Many studies have documented the fact that Rb^+ can replace K^+ in all functions of the (Na^+,K^+)-ATPase and sodium pump [6] and that the two isotopes give identical results for liposome transport [4].

In these experiments, the incubation mixture did not contain unlabelled RbCl, so that the ion actually transported was K^+. Preliminary experiments confirmed the findings of Hilden and Hokin [4] that the same results are obtained if unlabelled RbCl is added.

Three Na^+ were transported for 2.5 K^+, a ratio which compares well with values obtained by Sweadner and Goldin [5] with dog brain (Na^+,K^+)-ATPase and by Hilden and Hokin [4] with the enzyme from shark rectal gland, and is close to the value of 3 : 2 obtained with red cell ghosts [11]. We have also obtained a similar ratio (3 : 1.8) with liposomes containing beef cardiac (Na^+,K^+)-ATPase (Fig. 2). The heart enzyme incorporated into these liposomes had a specific activity of 263 μmol/mg per h and was approximately 30% pure as judged by gel electrophoresis [9] assuming that the pure enzyme is composed of two polypeptides.

In a second study, we incorporated a purified (Na^+,K^+)-ATPase preparation from lamb kidney medulla into liposomes and found that this too, was able to catalyze ATP-dependent transport of both Na^+ and K^+ with a ratio of $3Na^+$ transported to $2.6K^+$ in this experiment (Fig. 3). In other experiments an aver-



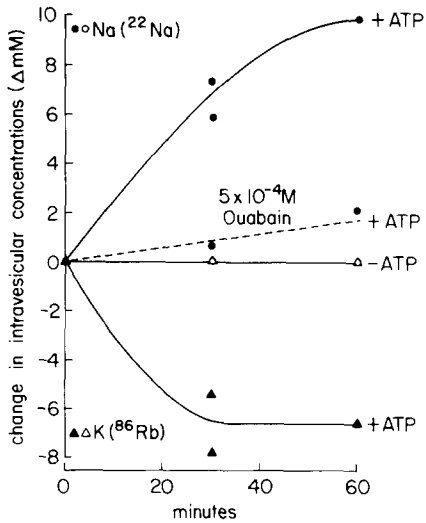
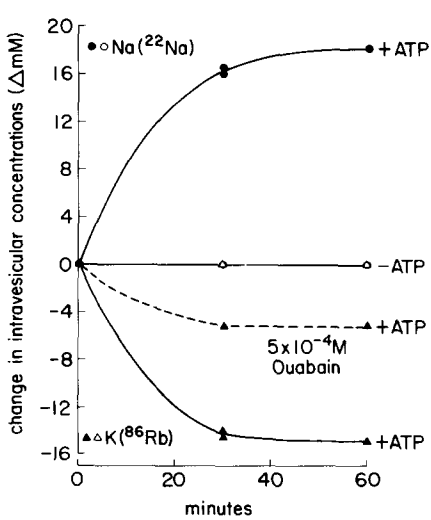


Fig. 1. ATP-dependent transport of ^{22}Na and ^{86}Rb catalyzed by liposomes containing a sodium iodide-treated microsomal fraction from sheep kidney medulla.

Fig. 2. ATP-dependent transport of ^{22}Na and ^{86}Rb catalyzed by liposomes containing a partially purified "citrate enzyme" fraction from beef heart prepared as previously described [9].

age ratio of 3 : 1.9 was obtained. These results are very similar to those obtained with purified (Na^+,K^+)-ATPase from shark rectal gland [4] and with a microsomal enzyme from beef brain [5], but are quite different to those obtained with purified enzyme from dog kidney [1], in which ATP-dependent symport of Na^+ and Cl^- was observed, but with no significant transport of K^+.



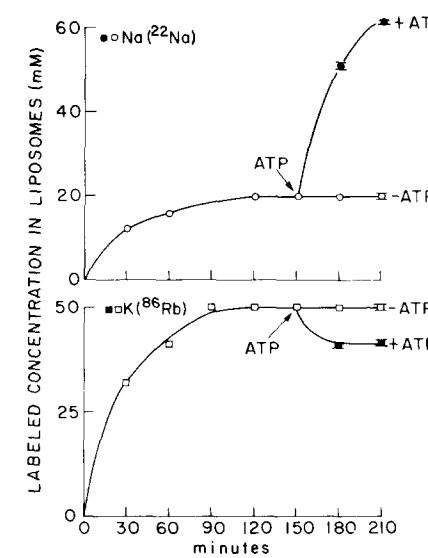


Fig. 3. ATP-dependent transport of ^{22}Na and ^{86}Rb catalyzed by liposomes containing a purified glycerol enzyme fraction from sheep kidney medulla.

Fig. 4. ATP-dependent transport of ^{22}Na and ^{86}Rb catalyzed by liposomes containing a purified glycerol enzyme fraction from dog kidney medulla.

The difference between the results obtained with the sheep and dog kidney preparations could possibly be explained either by a species difference or by a difference in the procedures used to purify the enzymes. To test this second possibility, we purified the enzyme from the outer medulla of dog kidney by the same procedure that was used for sheep kidney. The purified glycerol enzyme was shown by sodium dodecyl sulfate (SDS)-gel electrophoresis to contain only the catalytic and glycoprotein subunits, but had a specific activity of only 560 μmol/mg per h. Although preparations from this source usually exhibit much higher specific activities (900—1500 μmol/mg per h by our procedure), this preparation was selected because its specific activity was similar to that employed by Goldin and Tong. When incorporated into liposomes, this preparation did exhibit ATP-dependent antiport of Na^+ and K^+ (Fig. 4), but the ability to transport K^+ was much lower than with the sheep kidney preparation. The ratio of Na^+ to K^+ was 3 : 0.6. Clearly, when prepared by our procedure, dog kidney (Na^+,K^+)-ATPase does have some ability to transport K^+.

Comparison of our results with those of Goldin and Tong [1] suggests that the ability of the dog kidney enzyme to transport K^+ depends on the procedure used to purify the enzyme. Possibly the K^+ transport mechanism in this enzyme is more readily denatured (or modified) than in enzymes from other sources and, if sufficiently gentle techniques could be devised, it might be possible to obtain Na^+,K^+ transport with a ratio of 3 : 2. It is of interest that our purification procedure [7] uses a lower ratio of detergent to protein (0.55 mg deoxycholate/mg protein) than that employed by Goldin and Tong which was reported as 2.4 mg deoxycholate/mg protein [8], and high concentrations of this detergent are known to denature the enzyme [12]. It is also possible that the loss of ability to transport K^+ may have occurred during incorporation of the enzyme into the liposomes. This would be equally compatible with our suggestion that the dog kidney enzyme is more susceptible to denaturation resulting in the loss of ability to transport K^+ than is the sheep kidney enzyme.

Goldin and Tong [1] have suggested that the sodium pump in kidney may, in fact, catalyze symport of Na^+ and Cl^- in contrast to the antiport of Na^+ and K^+ observed in other tissues, and this report has subsequently been cited as evidence that the sodium pump in kidney differs from that in other tissues [3]. Our data show that this is not true for the kidney in general.

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