The (Na⁺, K⁺)ATPase of Rat Kidney: Purification, Biosynthesis, and Processing

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ABSTRACT. (Na⁺, K⁺)ATPase was purified from rat renal outer medulla by concanavalin A- and wheat germ agglutinin-lectin Sepharose affinity chromatographies. The antibody, which was raised in rabbits, markedly inhibited ATPase activity. The monospecificity of this antibody was assayed by the Ouchterlony double immunodiffusion and Western blotting tests. The endoplasmic reticulum (ER)-rich, and Golgi-rich subfractions were prepared from the rat kidney microsomal fraction by sucrose density gradient centrifugation. On the immunoblot, the molecular weight of the α subunit in both fractions was 95 kilodalton (Kd); whereas, that of the β subunit was 50 Kd in the ER-rich fraction and 54 Kd in the Golgi-rich fraction. When treated with endoglucosidase H, the 50 Kd component was converted to 38 Kd, but the 54 Kd component was endoglucosidase H resistant. These results suggest that the β subunit (38 Kd) is glycosylated cotranslationally in the ER (50 Kd) then is converted to the mature type subunit (54 Kd) in the Golgi apparatus.

(Na⁺, K⁺)ATPase is a plasma membrane protein which is composed of two non-covalently-linked subunits; a catalytic α subunit (~100 Kd) and a glycosylated β subunit (~50 Kd) of unknown function (15, 17). This ATPase has been purified from different tissues of various animals. Of these tissues, the outer medulla of mammalian kidney is the best for this enzymes purification, as reported by Jørgensen (12, 13).

Else where we reported purification of the α and β subunits of canine (Na⁺, K⁺) ATPase from the Jørgensen's ATPase fraction by WGA lectin-Sepharose affinity column chromatography (22). This method also has been used to purify the (Na⁺, K⁺)ATPase of the canine kidney outer medulla and the distribution of the ATPase on the cell surfaces of canine hepatocytes was analyzed quantitatively with mono-specific antibody against the purified (Na⁺, K⁺)ATPase (28).

In the study reported here we used the lectin-Sepharose affinity chromatography

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Abbreviations used: C₁₂E₈, octaethylene glycol dodecyl ether; Con A, concanavalin A; DTT, dithiothreitol; EDTA, ethylenediamine-tetraacetic acid; endo H, endo- β -N-acetylglucosaminidase H; ER, endoplasmic reticulum; IgG, immunoglobulin G; Kd, Kilo dalton; Pi, inorganic phosphate; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; WGA, wheat germ agglutinin.

to purify the (Na^+ , K^+)ATPase from the outer medulla of rat kidney and prepared the monospecific antibody against this enzyme. Using antibody, we investigated biosynthesis and processing of the ATPase of rat kidney.

This research was presented, in part, at the 63rd Annual Meeting of the Physiological Society of Japan (1).

MATERIALS AND METHODS

Materials. Trasylol was purchased from Bayer. Leupeptin and pepstatin were obtained from the Protein Research Foundation (Osaka, Japan). Con A-Sepharose 4B and WGA-Sepharose 6MB were the products of Pharmacia Fine Chemicals. Endo glycosidase H (Endo H) was purchased from Seikagaku Kogyo Co., Tokyo. ^{125}I -protein A was obtained from New England Nuclear. Nitrocellulose sheets (HAWP 304 FO) were purchased from Millipore Corporation. All the other chemicals used were of analytical grade and were obtained from various sources.

Animals. Male Sprague-Dawley rats weighing about 150 g were used. They were fed a commercial chow and water *ad libitum*.

Preparation of crude (Na^+ , K^+) ATPase from rat kidney. Microsomal membranes were prepared from the outer medulla of rat kidneys by a slight modification of Jørgensen's procedure (22) described previously (12, 13): The tissues were homogenized in 0.25 M sucrose containing 30 mM histidine buffer (pH 7.2), 1 mM EDTA, 1 mM PMSF, 200 U/ml trasylol, 20 $\mu\text{g}/\text{ml}$ leupeptin and 20 $\mu\text{g}/\text{ml}$ pepstatin. The microsomal fraction (1.4 mg protein/ml) was sedimented by the centrifugation at 48,000 $\times$ g for 30 min then treated with SDS (0.54 mg/ml) at 20°C for 45 min in 50 mM imidazole buffer (pH 7.5) containing 2 mM EDTA, 3 mM ATP, 1 mM DTT, 200 U/ml trasylol, 20 $\mu\text{g}/\text{ml}$ leupeptin and 20 $\mu\text{g}/\text{ml}$ pepstatin.

The treated fraction was layered on a discontinuous sucrose density gradient that consisted of three layers; 25 ml of 29.4%, 15 ml of 15%, and 25 ml of 10% (W/V) sucrose in 25 mM imidazole buffer (pH 7.5 at 20°C) containing 1 mM EDTA. This gradient then was centrifuged in a Hitachi RP 42 rotor at 137,500 $\times$ g for 5 h at 4°C. The pellets obtained were resuspended at a concentration of 2–3 mg protein/ml in 50 mM sodium acetate buffer (pH 5.6) containing 1 mM EDTA. This suspension was diluted to a final concentration of 0.2–0.3 mg protein/ml by 9-fold dilution with 50 mM imidazole buffer (pH 7.5) containing 1 mM EDTA. The specific activity of this crude ATPase fraction was 700–800 $\mu\text{moles Pi released}/\text{mg}/\text{h}$.

Purification of (Na^+ , K^+)ATPase by Con A- and WGA-Sepharose affinity chromatography. Jørgensen's ATPase fraction was treated with C_{12}E_8 (4, 10, 21, 28) and subjected to Con A-Sepharose 4B affinity chromatography followed by WGA-Sepharose 6 MB chromatography as reported previously (28): 10% C_{12}E_8 solution was added to 3 ml of the Jørgensen's (Na^+ , K^+)ATPase fraction (2 mg protein/ml) containing 200 U/ml trasylol, 20 $\mu\text{g}/\text{ml}$ leupeptin and 20 $\mu\text{g}/\text{ml}$ pepstatin, at a weight ratio of protein to C_{12}E_8 of 1 : 10 (final concentration of C_{12}E_8 , 20 mg/ml). Solubility of the (Na^+ , K^+)ATPase at this concentration of C_{12}E_8 was greater than at the previous concentration (1 : 3), but ATPase activity was almost completely lost. The mixture was incubated at 0°C for 1 h then centrifuged at 100,000 $\times$ g for 1 h.

The supernatant formed was dialyzed at 4°C for 3 h against 10 mM Tris/HCl buffer (pH 7.5) containing 0.15 M NaCl and 0.01 % C_{12}E_8 . The dialyzed solution was incubated at 4°C overnight with Con A-Sepharose previously equilibrated with 10m M Tris/HCl buffer

(pH 7.5), containing 0.15 M NaCl and 0.01 % C₁₂E₈, then it was centrifuged. Its supernatant was loaded on a WGA-Sepharose column (0.5 × 5 cm) that had been equilibrated with the same buffer. The fraction that bound to the WGA-Sepharose was eluted with 0.2 M N-acetylglycosamine after washing the column with 10 column volumes of the equilibration buffer.

Antibody preparation. Purified (Na⁺, K⁺)ATPase in Freund's complete adjuvant was injected subcutaneously into rabbits (~200 µg protein/animal) at 30–40 sites; then, 3 and 6 weeks later the ATPase in Freund's incomplete adjuvant was injected the same way according to the method of Vaitukaitis (30). Nine weeks later, 100 µg of the ATPase was given as a booster. The IgG fraction was obtained by ammonium sulfate fractionation and DEAE cellulose (DE 52) column chromatography.

Inhibition of rat kidney (Na⁺, K⁺)ATPase activity by the antibody. As described elsewhere (22), 0–200 µl of antiserum or non-immunized rabbit serum, which previously had been dialyzed extensively against water, and 10 µl of Jørgensen's (Na⁺, K⁺)ATPase fraction (0.4 mg/ml) were added to 10 : 9 concentrated buffer (3 mM MgCl₂, 130 mM NaCl, 20 mM KCl, 30 mM histidine, pH 7.5) with or without 1 mM ouabain at a final volume of 0.45 ml. This mixture was incubated at 37°C for 20 min, after which 50 µl of 30 mM ATP was added. After another 5 min, the reaction was stopped by adding 50 µl of 50% trichloroacetic acid and cooling the sample to 0°C. The amount of Pi released was determined by the method of Pavin and Roberts (23).

Subfractionation of the microsomes. The microsomal fraction prepared by the method of Jørgensen (12, 13) was resuspended in 0.25 M sucrose containing 50 mM histidine buffer (pH 7.2) to a final volume of 2 ml (~10 mg protein). This suspension was loaded on a continuous sucrose density gradient of 0.8 M–1.4 M sucrose containing 50 mM imidazole buffer, pH 7.5. The gradient was centrifuged at 199,000 × g for 4 h in a Hitachi RPS 40 T rotor then was fractionated with a gradient fractionator by the continuous collection of 25 drops per tube from the top of the gradient. The fractionated samples were diluted with 0.25 M sucrose solution, recentrifuged at 105,000 × g for 1 h then resuspended in 0.5 ml of 50 mM histidine buffer (pH 7.2). The various enzyme activities of the samples were assayed, after which a part of each was put through Western blotting analysis according to Burnette (2).

Endo H treatment of the cell subfractions. The subfractions were dialyzed at 4°C overnight against 0.1 M sodium citrate buffer (pH 5.5) containing 1 mM PMSF, 200 U/ml trasylol, 20 µg/ml leupeptin and 20 µg/ml pepstatin, after which they were treated with 0.05 U of endo H at 37°C for 24 h in 0.1 M sodium citrate buffer (pH 5.5), containing 0.5 mM PMSF, 0.04% sodium azide and 0.2% SDS. Next, the solution was adjusted to pH 6.8 by adding 1 M Tris/HCl buffer (pH 8.0) diluted with an equal volume of the SDS-PAGE sample buffer containing 2% SDS, 20 mM phosphate buffer (pH 7.0), 8 M urea, 4% 2-mercaptoethanol and 20% glycerol. The samples were heated at 100°C for 5 min then subjected to SDS-PAGE.

Cell-free translation of poly(A)-containing mRNA from rat kidney. Poly(A)-containing mRNA was isolated from rat kidney according to Geregori *et al.* (9) and translated in a rabbit reticulocyte lysate system as described in the legend to Fig. 8.

Other procedures. Amino acid analysis of the α and β subunits was done with a LKB amino acid analyser (Model 4,000). Protein was determined by the method of Lowry *et al.* (20) with crystallized bovine serum albumin as the standard. SDS-PAGE was done with a 10% polyacrylamide gel according to the method of Laemmli (18). Glucose-6-phosphatase was determined by the procedure of Leskes *et al.* (19), galactosyltransferase by that of Fleischer *et al.* (7), 5'-nucleotidase by that of Emmelot *et al.* (3), and acid phosphatase by

that of Pricer and Ashwell (24).

RESULTS

Purification of (Na^+ , K^+)ATPase. Crude (Na^+ , K^+)ATPase was prepared from the outer medulla by SDS treatment and sucrose density gradient centrifugation according to a slight modification of Jørgensen's procedure. The specific activity of this ATPase fraction ranged from 600–800 μmole of Pi released/mg/h, and the apparent molecular weights of the α and β subunits were 95 Kd, and 54 Kd. This preparation, however, contained several impurities (Fig. 1, lane 1, small arrows) as reported previously (28). The (Na^+ , K^+)ATPase from this fraction was further purified by treating it with C_{12}E_8 followed by Con A- and WGA-Sepharose affinity chromatographies.

Treatment of the crude enzyme with 2% C_{12}E_8 solution was very effective making this enzyme soluble as reported previously (28), and the component which migrated more rapidly than the β subunit (Fig. 1, lane 1, lower arrow), was removed by this procedure; but, (Na^+ , K^+)ATPase activity was lost. The band which migrated more slowly than the α subunit (Fig. 1, lane 1, upper arrow) was effectively removed by treatment with Con A-Sepharose.

The final purification of the rat kidney (Na^+ , K^+)ATPase was made by WGA-Sepharose chromatography as described by Takemura *et al.* (28). Our purified ATPase was exclusively composed of α and β subunits with the apparent molecular weights of 95 Kd and 54 Kd (Fig. 1, lane 2). The specific activity during the purification procedures was not shown, because ATPase activity had been lost when the Jørgensen's ATPase fraction was treated with C_{12}E_8 . The yield of protein was $6.3 \pm 2.1\%$ ($n=6$), that of Jørgensen's ATPase fraction.

Fig. 1. SDS-PAGE patterns of rat kidney (Na^+ , K^+)ATPase preparations. The (Na^+ , K^+)ATPase fraction prepared by SDS treatment (0.54 mg/ml) according to the method of Jørgensen (12, 13) was dissolved in 2% C_{12}E_8 solution then centrifuged. The supernatant was chromatographed on a Con A-Sepharose column then on a WGA-Sepharose column.

Lane 1: SDS-treated rat kidney (Na^+ , K^+)ATPase fraction.

Lane 2: WGA-Sepharose-bound fraction eluted with 0.2 M N-acetylglucosamine.

Amino acid composition of the subunits. The α and β subunits resolved by SDS-PAGE were extracted separately from the gel, pooled, and their amino acids analyzed. Table I shows the amino acid compositions of the α and β subunits of rat kidney (Na⁺, K⁺)ATPase together with those of the canine kidney ATPase previously

Fig. 2. Ouchterlony double immunodiffusion analysis of the purified rat kidney (Na⁺, K⁺)ATPase. The enzymes was analysed in 1% agarose containing 0.1% Triton X-100, 10 mM sodium phosphate buffer (pH 7.2), 0.15 M NaCl, 0.02% NaN₃. Well 1; anti(Na⁺, K⁺)ATPase antibody, Well 2, 3; purified (Na⁺, K⁺)ATPase.

Fig. 3. Inhibition of rat kidney (Na⁺, K⁺)ATPase activity by rabbit antibody against rat (Na⁺, K⁺)ATPase. The Jørgensen's (Na⁺, K⁺)ATPase fraction prepared from rat kidney first was incubated with various amounts of nonimmunized rabbit serum (●) or antiserum (○) in the presence or absence of 1 mM ouabain at 37°C for 20 min then incubated with ATP. The amount of Pi liberated was measured as described in MATERIALS AND METHODS. An addition of 200 μl of antiserum markedly inhibited enzyme activity (85% inhibition).

reported from this laboratory (22). Our present results are in general agreement with those for canine kidney ATPase.

Antibody specificity shown by the Ouchterlony test, inhibition assay and Western blot. The antibody against purified (Na^+ , K^+)ATPase was raised in rabbits. It formed a single precipitation line with the purified antigen on Ouchterlony double immunodiffusion analysis (Fig. 2). Similar result was obtained with the microsomal membranes in the presence of 0.1% Triton X-100. We then examined the inhibition of kidney (Na^+ , K^+)ATPase by this antibody. Fig. 3 shows that the antiserum effectively inhibited this enzymes activity.

The specificity of the antibody also was tested by Western blotting analysis. In Fig. 4, lanes 1 and 2 show SDS-PAGE patterns (10% polyacrylamide gel) of the original and the SDS treated-microsomal fractions. Lanes 3 and 4 are immunoblots of the same samples. In the latter two fractions, the antibody bound exclusively with two components that correspond to the α (95 Kd) and β (54 Kd) subunits. The upper band (arrow) above the α subunit presumably represent 1 : 1 complex of α and β subunits.

Subfractionation studies. *In vivo* processing of the α and β subunits was investigated with this monospecific antibody against the enzyme. The microsomal fraction prepared from the outer medulla of rat kidney was subfractionated by sucrose

Fig. 4. Characterization of the antibody by immunoblotting. Lanes 1, 2: SDS-PAGE patterns of the microsomal fraction (lane 1) (50 μg protein) and the Jørgensen's crude (Na^+ , K^+)ATPase fraction (lane 2) (20 μg protein).

Lanes 3, 4: Protein from lanes 1 and 2 was transferred electrophoretically to a nitrocellulose sheet by the method of Burnette (14). The sheet was allowed to react with the antibody solution (0.1 mg/ml in 50 mM Tris/HCl buffer, pH 7.6, containing 0.9% NaCl, 0.05% NaN_3 , and 5% bovine serum albumin). It then was incubated with ^{125}I -protein A, after which it was checked by autoradiography.

density gradient centrifugation. The submicrosomal fractions collected were characterized by assays with several marker enzymes, after which they were analyzed by Western blotting.

The specific activities of glucose-6-phosphatase, galactosyltransferase, 5'-nucleotidase and acid phosphatase used as the respective marker enzymes of the ER, Golgi apparatus, plasma membrane and lysosomes, are shown in Fig. 5.

Most of the galactosyl transferase activity was mostly concentrated in fraction 3 and showed a marked decrease in the direction of both the lighter and heavier fractions, whereas, glucose-6-phosphatase activity had a bimodal distribution, the higher peak occurring in fraction 8 and the other peak in fraction 4. The acid-phosphatase activity was greatest in fraction 7, whereas the activity of 5'-nucleotidase was spread over all the fractions and showed no peaks. Number 3 is the Golgi-rich and number 8 the ER-rich fractions.

Western blot analysis of the subfractions. Lane 1 of Fig. 6 shows the immunoblot of fraction 8 (ER-rich) and lane 2 that of fraction 3 (Golgi-rich). In the Golgi-rich fraction, the antibody bound with the 95 Kd and 54 Kd components, whereas, in the ER-rich fraction it bound mainly with the 95 Kd and 50 Kd components. In the

Fig. 5. Subfractionation of rat kidney microsomes by sucrose density gradient centrifugation. The microsomes (~10 mg protein) were centrifuged for 4 h at 199,000 x g on a 0.8-1.4 M linear sucrose gradient then fractionated. The subfractions obtained were pelleted and resuspended (0.5 ml each) assayed as described in MATERIALS AND METHODS.

Fig. 6. Western blotting the Golgi-rich and ER-rich fractions. Fraction 3 (lane 2) has the highest galactosyltransferase activity and fraction 8 (lane 1) the highest glucose-6-phosphatase activity. They were designated the Golgi-rich and ER-rich subfractions. These two fractions were assayed by SDS-PAGE followed by immunoblotting and autoradiography.

latter fraction, a small amount of the 54 Kd component also was detected.

These findings suggest that, whereas the molecular weight of the α subunit did not change in the two subfractions, the β subunit that first was synthesized as a 50 Kd glycoprotein in the ER was converted to a mature 54 Kd form in the Golgi apparatus. A small amount of the 54 Kd component in the Golgi rich fraction is presumably to be due to contamination of the Golgi or post Golgi membranes.

Endo H treatment of the ER-rich and Golgi-rich subfractions. We examined whether the 50 Kd form of the β subunit in the ER-rich fraction (Fraction 8) contains high-mannose oligosaccharides of the N-glycoside type. We used endo H to digest both the ER-rich and Golgi-rich subfractions then analyzed the digests by SDS-PAGE and Western blotting.

The β subunit (50 Kd) in the ER-rich subfraction was sensitive to endo H and was converted to a 38 Kd form (Fig. 7); whereas the β subunit (54 Kd) in the Golgi-rich subfraction was resistant to endo H (data not shown).

The difference in the amount of the α subunits present before and after endo H digestion (Fig. 7) is presumed to be due to proteolytic cleavage during this endo H digestion the α subunit being much more sensitive to protease digestion than the β subunit (22). This protease activity was most probably derived from the endo H preparation, which were not inhibited by any protease inhibitors commercially available.

Cell-free translation of poly(A)-containing mRNA from rat kidney. Poly(A)-containing mRNA was prepared from rat kidney and translated in a rabbit reticulocyte cell-free system. The products were immunoprecipitated with the antibody against $(\text{Na}^+, \text{K}^+)\text{ATPase}$ or the control immunoglobulin from non-immunized rabbit serum then subjected to SDS-PAGE. A comparison of lanes 1 and 3 of Fig. 8, shows that the antibody against $(\text{Na}^+, \text{K}^+)\text{ATPase}$ immunoprecipitated a 38 Kd component in lane 1 (arrow head). This cell-free translation system, however, did not show the ~ 100 Kd component which corresponds to the α subunit of the ATPase. Presumably the α subunit was degraded during cell-free translation because it is

Fig. 7. Endo H sensitivity of the ER-rich subfraction. The ER-rich subfraction ($\sim 50 \mu\text{g}$ protein) was treated without (lane 1), or with (lane 2), 0.05 U of endo H as described in MATERIALS AND METHODS, after which it was assayed by SDS-PAGE followed by immunoblotting and autoradiography.

Fig. 8. Cell-free translation of poly(A)-containing mRNA from rat kidney. Poly(A)-containing mRNA was translated *in vitro* (0.08 or 0.24 $A_{260}/50 \mu\text{l}$) in a reticulocyte lysate containing (^{35}S) methionine ($50 \mu\text{Ci}$). After incubation for 60 min at 30°C , the translation mixtures were cooled on ice then diluted ten-fold with the lysate buffer (containing 1% Triton X 100, 0.1% SDS, 10 mM Tris-HCl buffer, pH 7.3, 0.2 M NaCl, and 5 mM EDTA). After the additions of 0.1 mM PMSF and 1 mM dithiothreitol, the mixtures were incubated at 4°C for 1 h with *Staphylococcus Aureus* then centrifuged. The supernatants obtained were allowed to react with antiserum against rat (Na^+ , K^+) ATPase (lane 1) or with normal rabbit serum (lane 3) for 1 h at room temperature, then to react with $50 \mu\text{l}$ of protein A (50% in PBS), followed by stirring for 1 h at 4°C . After washing the precipitated proteins with the above buffer, they were dissolved in 1% SDS, 10 mM sodium phosphate buffer (pH 7.0), 10% glycerol, 4 M urea and 2% mercaptoethanol, after which they were heated for 5 min at 100°C then assayed by SDS-PAGE followed by fluorography. Lane 2 showed that the immunoprecipitated component with the antibody (lane 1) could be immunocompeted by the addition of the β subunit ($100 \mu\text{g}$). The arrowhead indicates the translation product with an apparent molecular mass of $\sim 38 \text{ Kd}$ which corresponds to the core polypeptide of the β subunit.

much more sensitive to protease digestion than is the β subunit.

DISCUSSION

(Na^+ , K^+)ATPase has been prepared from a variety of tissues from different

TABLE I. AMINO ACID COMPOSITION OF THE α AND β SUBUNITS OF RAT KIDNEY (Na^+ , K^+) ATPASE. VALUES GIVEN IN MOL PER 100 MOL AMINO ACIDS

	α subunit		β subunit	
	Rat Kidney	Dog Kidney*	Rat Kidney	Dog Kidney*
Aspartic acid	8.8	11.0	9.2	9.3
Threonine	5.4	6.3	4.9	3.8
Serine	6.7	5.8	6.0	4.5
Glutamic acid	11.6	11.5	11.6	13.5
Proline	7.4	5.0	6.3	6.4
Glycine	9.9	8.1	11.5	9.4
Alanine	7.1	8.9	6.0	3.2
Half-cystine	0.5	1.3	1.7	1.2
Valine	6.9	6.4	5.4	5.2
Methionine	1.9	1.7	1.4	1.4
Isoleucine	5.6	6.2	4.6	5.9
Leucine	8.1	9.0	8.9	7.7
Tyrosone	3.0	2.5	2.9	6.3
Phenylalanine	4.3	4.1	6.7	5.3
Lysine	4.3	5.2	5.9	9.6
Histidine	1.21	1.5	1.6	0.1
Tryptophan	—	0.7	—	1.3
Arginine	6.4	5.1	4.1	5.9

Data of Omori *et al.* (21).

animals. Of the mammalian tissues, kidney has provided the only sources of pure (Na^+ , K^+)ATPase (14), (Na^+ , K^+)ATPase having been prepared from the kidneys of various mammals including the rat (16, 26, 27).

In a previous paper, we showed that Jørgensen's (Na^+ , K^+)ATPase fraction prepared from canine kidneys contained impurities (28). We therefore further purified the ATPase from this fraction using the selective solubility of this enzyme in C_{12}E_8 then lectin-Sepharose chromatography (28).

As with canine kidney, the fraction from rat kidney contained impurities which could be eliminated by selective solubility of the enzyme in C_{12}E_8 and by lectin-Sepharose chromatography (Fig. 1). The purified enzyme is composed exclusively of α and β subunits with apparent molecular weights of 95 and 54 Kd. No other impurities were detected in SDS-PAGE analysis. We then prepared the antibody against the purified (Na^+ , K^+)ATPase. This antibody was shown to be specific by double diffusion and immunoblot analyses and it markedly inhibited (Na^+ , K^+)ATPase activity.

Biosynthesis of (Na^+ , K^+)ATPase has been studied exclusively in cultured cells such as MDCK cells (25), amphibian cells (8), chicken myogenic cells and sensory neurons (5, 29). We now have reported on the biosynthesis and processing of the α and β subunits of (Na^+ , K^+)ATPase in rat kidney *in vivo*.

Considerable difference exist in previous results obtained from studies of the biosynthesis of the α subunits. Sabatini *et al.* (25) reported that the α subunit is synthesized by free polysomes and inserted post-translationally into membranes. Hiatt *et al.* reported finding a 96 Kd-product obtained by cell-free translation of the

mRNA from free polysomes (29). Geering *et al.* (8) investigated these problems and suggested that both the α and β subunits of (Na⁺, K⁺)ATPase are integrated cotranslationally into the ER membrane, probably with different time courses. Tamkun and Fambrough (29) investigated the biosynthesis and intracellular transport of the (Na⁺, K⁺)ATPase of chick sensory neurons in culture. They suggested that α and β subunit syntheses are concurrent in the rough ER and that subunit assembly takes place during, or immediately after, polypeptide synthesis.

In the experiment reported here the molecular weights of the α subunit in both the ER-rich and Golgi-rich fractions were the same (95 Kd), and endo H treatment did not change the mobility of the α subunit in the ER-rich fraction. We previously showed (22) that the carbohydrate content of the α subunit is negligible. These results are strong evidence that the α subunit is transported from the ER to the Golgi apparatus without glycosylation. This conclusion agrees with reports that the treatment of cultured cells with tunicamycin produced no change in the size of the α subunit (5, 8, 25, 29) and that the mobility of the α subunit of rat kidney (Na⁺, K⁺) ATPase was not changed by treatment with neuraminidase or endoglycosidase F (27).

We found that the molecular weight of the β subunit was 50 Kd in the ER-rich fraction and 54 Kd in the Golgi-rich fraction. Endo H treatment, did not change the molecular weight of the β subunit in the Golgi-rich fraction, but in the ER-rich fraction it decreased from 50 to 38 Kd. The major polypeptide chains synthesized by cell-free translation of poly(A)-containing mRNA was 38 Kd, which suggests that the core polypeptide of the β subunit, with a molecular weight of $\sim$ 38 Kd is glycosylated cotranslationally in the ER to a high mannose glycoprotein of $\sim$ 50 Kd, after which it is transported and converted to the mature complex type (54 Kd) in the Golgi region.

In previous research, we found the carbohydrate content of the canine β subunit to be 33% (22). The core polypeptide of the β subunit therefore is thus estimated as $\sim$ 38 Kd. This value agrees with the molecular weight of the endo H-treated rat β subunit (38 Kd) and that of the product (38 Kd) of the cell-free translation system. These results on the processing of the β subunit also are consistent with results recently reported by Tamkun and Fambrough for cultured cells and for an *in vitro* translation system (29).

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