Immunocytochemical Localization of Na\(^+\), K\(^+\)-ATPase in the Rat Kidney *

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Summary. To determine if rat kidney Na\(^+\), K\(^+\)-ATPase can be localized by immunoperoxidase staining after fixation and embedding, we prepared rabbit antiserum to purified lamb kidney medulla Na\(^+\), K\(^+\)-ATPase. When sodium dodecylsulfate polyacrylamide electrophoresis gels of purified lamb kidney Na\(^+\), K\(^+\)-ATPase and rat kidney microsomes were treated with antiserum (1:200), followed by \([^{125}\text{I}]\)-Protein A and autoradiography, the rat kidney microsomes showed a prominent radioactive band coincident with the \(\alpha\)-subunit of the purified lamb kidney enzyme and a fainter radioactive band which corresponded to the \(\beta\)-subunit. When the Na\(^+\), K\(^+\)-ATPase antiserum was used for immunoperoxidase staining of paraffin and plastic sections of rat kidney fixed with Bouin's, glutaraldehyde, or paraformaldehyde, intense immunoreactive staining was present in the distal convoluted tubules, subcapsular collecting tubules, thick ascending limb of the loops of Henle, and papillary collecting ducts. Proximal convoluted tubules stained faintly, and the thin portions of the loops of Henle, straight descending portions of proximal tubules, and outer medullary collecting ducts did not stain. Staining was confined to basolateral surfaces of tubular epithelial cells. No staining was obtained with preimmune serum or primary antiserum absorbed with purified lamb kidney Na\(^+\), K\(^+\)-ATPase, or with osmium tetroxide postfixation. We conclude that the basolateral membranes of the distal convoluted tubules and ascending thick limb of the loops of Henle are the major sites of immunoreactive Na\(^+\), K\(^+\)-ATPase concentration in the rat kidney.

Introduction

Our knowledge of Na\(^+\), K\(^+\)-ATPase localization in the kidney has been derived principally from biochemical and cytochemical methods which are based upon

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functional properties of the enzyme (Ernst 1975; Shaver and Stirling 1978; Katz et al. 1979). Immunocytochemistry offers an alternative approach to localizing Na$^+$, K$^+$-ATPase, and has the advantage of depending on the protein’s antigenic properties rather than its enzymatic characteristics. However, immunocytochemical detection of kidney Na$^+$, K$^+$-ATPase has been previously accomplished only with a technically difficult method involving ferritin-labeled antibodies on ultrathin frozen sections (Kyte 1976a, b). Because immunoperoxidase staining of fixed and embedded kidney offers a simpler and more convenient approach to localization of this enzyme, we developed an antiserum to lamb kidney Na$^+$, K$^+$-ATPase and used it to determine the feasibility of localizing Na$^+$, K$^+$-ATPase in the fixed and embedded rat kidney (Baskin and Stahl 1981). The present article reports our experiments which demonstrated the specificity of the antiserum for rat kidney Na$^+$, K$^+$-ATPase and its use to localize Na$^+$, K$^+$-ATPase-like immunoreactive material in rat kidney tubules by immunoperoxidase staining.

Materials and Methods

Purification of Na$^+$, K$^+$-ATPase. The enzyme was purified from lamb kidney medulla as described by Lane et al. (1979) and had a specific activity of 1,100–1,300 μmoles P$_i$ released per mg protein per h, assayed at 37°C (Stahl 1968). Purification involved preparation of sodium iodide-treated lamb kidney microsomes, washing with deoxycholate, solubilization with detergent, precipitation with glycerol, and dialysis to yield a particulate enzyme which was stable for several months at 4°C. Purity was established by analytical sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Ames (1974), using a Studier-type apparatus (Studier 1973; Reid and Bieleski 1968) and the discontinuous buffer system of Laemmli (1970). For visualization of protein, gels were stained in a solution containing 25% (v/v) 2-propanol, 10% (v/v) glacial acetic acid, and 0.1% (w/v) Coomassie brilliant blue, and destained by diffusion in the above solution without the dye. Standard protein molecular weight markers included in each run were: β-galactosidase (130,000), phosphorylase a (94,000), bovine serum albumin (68,000), and ovalbumin (43,000).

Production of Antiserum to Na$^+$, K$^+$-ATPase. Rabbits were immunized with the purified lamb kidney medulla Na$^+$, K$^+$-ATPase by the procedure of McCans et al. (1975). Antiserum used in the present study was obtained six weeks following initial injection of the antigen in Freund’s complete adjuvant. In a standard biochemical assay of Na$^+$, K$^+$-ATPase activity (Stahl 1968), this antiserum reduced purified lamb kidney enzyme activity by 42% when diluted 1:20 and by 20% when diluted 1:200.

Specificity of Na$^+$, K$^+$-ATPase Antiserum. The immunological reactivity of the antiserum to purified lamb kidney Na$^+$, K$^+$-ATPase subunits and to proteins present in rat kidney microsomes, prepared as described elsewhere (Schellenberg et al. 1981), were assessed by separation on analytical SDS-PAGE on slab gels, followed by autoradiography with $[^{125}I]$-protein A. Gels were placed in direct contact with nitrocellulose filter paper and were held in a sandwich of porous polyethylene and Whatman 3 MM filter paper. The sandwich was placed in an electrophoresis chamber constructed according to Bittner et al. (1980) containing 25 mM phosphate buffer, pH 6.5. The peptides were electrophoretically transferred from the gel to the nitrocellulose paper at 100 mA for 16 h at room temperature. These sheets were then immersed and incubated in a solution containing 0.9% NaCl, 10 mM Tris-HCl, pH 7.4, (TBS) containing 5% (w/v) bovine serum albumin (BSA) for 90 min at 40°C. The sheets were washed with fresh TBS/BSA containing antiserum (1:200 final dilution) and were incubated for 90 min at room temperature. They were washed for 10 min in approximately 200 ml TBS without BSA, for 20 min in 2 changes (200 ml each) of TBS containing 0.05% NP-40 detergent, and for 10 min in 200 ml of TBS. Sheets were immersed in fresh TBS/BSA containing 2–5 × 10$^5$ cpm/ml of $[^{125}I]$-protein A (New England Nuclear). After 60 min incubation at room temperature, the radioactive solution was aspirated and the nitrocellulose sheet was again rinsed