Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 3541–3545, April 1992
Cell Biology

Selectively amplified expression of an isoform of the vacuolar H^+-ATPase 56-kilodalton subunit in renal intercalated cells

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Communicated by David M. Kipnis, December 18, 1991

ABSTRACT **The intercalated cells of the kidney collecting duct are specialized for physiologically regulated proton transport. In these cells, a vacuolar H^+-ATPase is expressed at enormous levels in a polarized distribution on the plasma membrane, enabling it to serve in transepithelial H^+ transport. In contrast, in most eukaryotic cells, vacuolar H^+-ATPases reside principally in intracellular compartments to effect vacuolar acidification. To investigate the basis for the selective amplification of the proton pump in intercalated cells, we isolated and sequenced cDNA clones for two isoforms of the ≈56-kDa subunit of the H^+-ATPase and examined their expression in various tissues. The predicted amino acid sequence of the isoforms was highly conserved in the internal region but diverged in the amino and carboxyl termini. mRNA hybridization to a cDNA probe for one isoform (the "kidney" isoform) was detected only in kidney cortex and medulla, whereas mRNA hybridization to the other isoform of the ≈56-kDa subunit and to the H^+-ATPase 31-kDa subunit was found in the kidney and other tissues. Immunocytochemistry of rat kidney with an antibody specific to the kidney isoform revealed intense staining only in the intercalated cells. Staining was absent from proximal tubule and thick ascending limb, where H^+-ATPase was detected with a monoclonal antibody to the 31-kDa subunit of the H^+-ATPase. This example of specific amplification of an isoform of one subunit of the vacuolar H^+-ATPase being limited to a specific cell type suggests that the selective expression of the kidney isoform of the ≈56-kDa subunit may confer the capacity for amplification and other specialized functions of the vacuolar H^+-ATPase in the renal intercalated cell.**

Vacuolar H^+-ATPases participate in a remarkably diverse variety of cellular functions. In the intracellular membrane compartments of eukaryotic cells, they acidify endosomes, lysosomes, and other components of the vacuolar system, serving in endocytosis and secretion (1). In cells specialized for H^+ transport, such as the renal intercalated cell (2, 3) and the osteoclast (4), vacuolar H^+-ATPases reside in high densities in a polarized distribution on the plasma membrane, effecting transcellular proton transport. How the vacuolar class of H^+-ATPases performs such diverse functions remains unknown. Accumulating evidence suggests that structural subsets of the vacuolar H^+-ATPases exist that may have unique roles. In prior studies, we reported that a vacuolar H^+-ATPase preparation isolated from bovine kidney microsomes could be resolved on an HPLC ion-exchange column as two peaks of activity that exhibited differences in the structure of their ≈56-kDa subunits on SDS/polyacrylamide gels (5). More recently, we found that H^+-ATPase purified from different membrane compartments in the mammalian kidney varied in their structural and functional properties (6). Again, differences in the structure of the ≈56-kDa polypeptide subunit were noted. Work from several laboratories has subsequently revealed at least two isoforms for the vacuolar H^+-ATPase ≈56-kDa subunit that are encoded by different genes (7–9). The present study examines how these isoforms might participate in the distinctive amplification and physiology of the vacuolar H^+-ATPase observed in mammalian kidney. We describe the complete predicted amino acid sequences for a human and bovine "brain" isoform and for a bovine "kidney" isoform of the ≈56-kDa subunit of the vacuolar H^+-ATPase.** We demonstrate that expression of one isoform is selectively amplified in the renal intercalated cells. These findings suggest that variable structural forms of the vacuolar H^+-ATPases, selectively amplified in distinct membrane compartments, may impart the capacity for specialized functions of the enzyme in different cells and tissues.

METHODS

Screening of cDNA Libraries. A randomly primed bovine brain λgt10 cDNA library (Clontech) was screened at high stringency by plaque hybridization (10) with a 500-base-pair (bp) *Nco* I restriction fragment from the bovine kidney ≈56-kDa subunit cDNA clone (11) labeled with [^{32}P]dCTP by the random-priming method (12) to a specific activity of $>10^9$ dpm/μg (Multiprime, Amersham). The filters were incubated for 2 hr with sonicated salmon sperm DNA at 100 μg/ml in hybridization solution prior to probe addition. Probe was added to the hybridization solution at 1×10^6 dpm/ml. The hybridization was carried out for 12 hr at 42°C in 50% formamide/5× SSC (13)/0.2 M Tris, pH 7.6/5× Denhardt's solution (13)/10% dextran sulfate/1% SDS. The filters were washed twice with 2× SSC/0.1% SDS at 42°C for 30 min and once with 0.2× SSC/0.1% SDS at 50°C for 30 min. Of 240,000 recombinant plaques, one 1.4-kilobase (kb) insert was obtained. This clone was ligated into the *Eco*RI site of pBluescript KS (Stratagene). An oligo(dT)-primed bovine brain λgt11 cDNA library (Clontech) was screened by plaque hybridization as described above; 27 clones were obtained that shared common restriction fragment sizes. The longest clone was ligated into pBluescript KS for DNA sequencing. Similarly, an adult human kidney λgt10 cDNA library (a gift from Graham Bell, University of Chicago) was screened by the same methods. A single full-length clone was obtained and ligated into pBluescript KS for DNA sequencing.

Polymerase Chain Reaction (PCR) Cloning of 5′ Ends. Nested anti-sense oligonucleotides were synthesized from the 5′ nucleotide sequence of the longest bovine brain cDNA clone (boldface letters) with *Eco*RI restriction-site tails added (lightface letters). The first oligonucleotide was 5′-

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Abbreviation: mAb, monoclonal antibody.

**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X62949 for the human brain isoform; M88690 for the bovine brain isoform; and M88691 for the bovine kidney isoform).

CGGAATTCCTAGAACTTGCCCACTCCG-3′ and the second was 5′-CGGAATTCCTAAGATCACTAGTGGACCATT-3′. Two successive amplifications with PCR (14) were performed with these anti-sense oligonucleotides in combination with oligonucleotide primers complementary to the regions of the λgt10 arms flanking the cloning site. An aliquot of a randomly primed bovine kidney cDNA library in λgt10 prepared by using a library kit (Stratagene) was used as a template. The PCR product was cleaved with *Eco*RI and ligated into pBluescript KS for sequencing.

DNA Sequence Determination. The DNA sequence was determined by the dideoxy chain-termination method (15) with *Taq* DNA polymerase (TaqTrack sequencing kit; Promega). The sequence on both strands was determined by sequencing exonuclease III-generated nested deletions (Erase-a-Base; Promega) or oligonucleotide primers. The sequence was assembled and analyzed with PC GENE (Intelligenetics).

RNA Blots of Total RNA. Total RNA was prepared by the method of Chomczynski and Sacchi (16). RNA blots were performed according to published methods (13). RNA (10 μg) was separated by electrophoresis in 1% agarose gels containing 0.22% formaldehyde, transferred to a nylon membrane (Nytran; Schleicher & Schuell), and allowed to hybridize separately with three different cDNA probes: a 1.3-kb *Eco*RI fragment (bases 217–1527) from the coding region of the bovine brain ≈56-kDa subunit, a 1.6-kb *Eco*RI fragment (bases 445–1464) from the coding region of the bovine kidney ≈56-kDa subunit (11), and a 1.4-kb full-length clone for the bovine 31-kDa subunit (17). The probes had 75.8% identity over a region of 921 bases, but neither probe cross-hybridized with synthetically prepared RNA from the nonidentical isoform under the same conditions used in the tissue RNA blots (not shown). Probes were labeled with ^{32}P by random priming (Multiprime kit). After preincubation with sonicated salmon sperm DNA at 100 μg/ml in hybridization solution to inhibit nonspecific DNA binding, the hybridization was carried out for 12 hr at 42°C in 50% formamide/5× SSPE (13)/2× Denhardt's solution/50% dextran sulfate/0.1% SDS. The membranes were washed twice for 30 min with 2× SSC at 42°C, then for 30 min at 42°C in 0.2× SSC, and then for 30 min at 55°C in 0.2× SSC. Autoradiography was performed by exposing the membranes to XAR film (Kodak) for 1–4 days at −70°C. The size of the hybridizing transcripts was determined by comparison to RNA size standards.

Preparation of Peptide–Protein Conjugates. The peptide Cys-Pro-Gln-Asp-Thr-Glu-Ala-Asp-Thr-Ala-Leu from the carboxyl terminus of the bovine kidney isoform of the ≈56-kDa subunit (Fig. 1) was synthesized by using the RAMPS multiple peptide synthesis system (DuPont). The peptide was purified by reverse-phase HPLC on a C_{18} column (Vydac) by gradient elution with acetonitrile containing 0.1% trifluoroacetic acid. The identity of the major peak was verified by amino acid analysis. One milligram of the purified peptide was added to 10 mg of keyhole limpet hemocyanin (Calbiochem) and 100 μg of tuftsin (Calbiochem) in 500 μl of phosphate-buffered saline (PBS). Glutaraldehyde (25%) was added to a final concentration of 1%, and the mixture was incubated with rotation at 22°C for 1 hr. Then 2 M NH_4Cl was added to a final concentration of 200 mM. After 20 min at 22°C, the mixture was dialyzed in PBS with 0.01% Thimerosal overnight at 4°C.

Polyclonal Rabbit Antibodies. One milligram of the keyhole limpet hemocyanin–peptide conjugate was emulsified in 500 μl of Freund's adjuvant for each immunization. New Zealand

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1 Bovine "brain"  isoform   MALRAMRGIVNGAAPELPVPTSGLA-GSREQALAVSRNYL 39
2 Human  "brain"  isoform   MALRAMRGIVNGAAPELPVPTGGPAVGSREQALAVSRNYL 40
3 Bovine "kidney" isoform        MAAEVDSRPRGLPGGGASLGAAREHVQAVTRNYI 34
4 Human  "kidney" isoform        MAMEIDSRPGGLPGSSCNLGAAREHMQAVTRNYI 34
                                          *          ::**   **:***:

1       SQPRLTYKTVSGVNGPLVILDHVKFPRYAEIVHLTLPDGTKRSGQVLEVSGSKAVVQVFE 99
2       SQPRLTYKTVSGVNGPLVILDHVKFPRYAEIVHLTLPDGTKRSGQVLEVSGSKAVVQVFE 100
3       THPRITYRTVCSVNGPLVVLDQVKFAQYAEIVNFTLPNGTQRSGQVLEVSGTKAIVQVFE 94
4       THPRVTYRTVCSVNGPLVVLDRVKFAQYAEIVHFTLPDGTQRSGQVLEVAGTKAIVQVFE 94
        : **:**:**  ******:** ***::****** ****** ******** *:**:*****

1       GTSGIDAKKTSCEFTGDILRTPVSEDMLGRVFNGSGKPIDRGPVVLAEDFLDIMGQPINP 159
2       GTSGIDAKKTSCEFTGDILRTPVSEDMLGRVFNGSGKPIDRGPVVLAEDFLDIMGQPINP 160
3       GTSGIDAQKTTCEFTGDILRTPVSEDMLGRVFNGSGKPIDKGPVVMAEDFLDINGQPINP 154
4       GTSGIDARKTTCEFTGDILRTPVSEDMLGRVFNGSGKPIDKGPVVMAEDFLDINGQPINP 154
        ******* **:*****************************:****:**************

1       HFRIYPEEMIQTGISAIDGMNSIARGQKIPIFSAAGLPHNEIAAQICRQAGLVKKSKDVV 219
2       QCRIYPEEMIQTGISAIDGMNSIARGQKIPIFSAAGLPHNEIAAQICRQAGLVKKSKDVV 220
3       HDRIYPEEMIETGISPIDVMNSIARGQKIPIFSAAGLPHNEIAAQICRQAGLVKKSKAVL 214
4       HSRIYPEEMIQTGISPIDVMNSIARGQKIPIFSAAGLPHNEIAAQICRQAGLVKKSKAVL 214
          ********:****:** ************************************ *:

1       DYSEENFAIVFAAMGVNMETARFFKSDFEENGSMDNVCLFLNLANDPTIERIITPRLALT 279
2       DYSEENFAIVFAAMGVNMETARFFKSDFEENGSMDNVCLFLNLANDPTIERIITPRLALT 280
3       DYHDDNFAIVFAAMGVNMETARFFKSDFEQNGTMGNVCLFLNLANDPTIERIITPRLALT 274
4       DYHDDNFSIVFAAMGVNMETARFFKSDFEQNGTMGNVCLFLNLANDPTIERIITPRLALT 274
        ** ::** **********************:**:* *************************

1       TAEFLAYQCEKHVLVILTDMSSYAEALREVSAAREEVPGRRGFPGYMYTDLATIYERAGR 339
2       TAEFLAYQCEKHVLVILTDMSSYAEALREVSAAREEVPGRRGFPGYMYTDLATIYERAGR 340
3       TAEFLAYQCEKHVLVILTDMSSYAEALREVSAAREEVPGRRGFPGYMYTDLATIYDRAGR 334
4       TAEFLAYQCEKHVLVILTDMSSYAEALREVSAAREEVPGRRGFPGYMYTDLATIYERAGR 334
        ********************************************************:****

1       VEGRNGSITQIPILTMPNDDITHPIPDLTGYITEGQIYVDRQLHNRQIYPPINVLPSLSR 399
2       VEGRNGSITQIPILTMPNDDITHPIPDLTGYITEGQIYVDRQLHNRQIYPPINVLPSLSR 400
3       VEGRGGSITQIPILTMPNDDITHPIPDLTGFITEGQIYVDRQLHNRQIYPPINVLPSLSR 394
4       VEGRGGSITQIPILTMPNDDITHPIPDLTGFITEGQIYVDRQLHNRQIYPPINVLPSLSR 394
        **** ************************ ****************************

1       LMKSAIGEGMTRKDHADVSNQLYACYAIGKDVQAMKAVVGEEALTSDDLLYLEFLQKFEK 459
2       LMKSAIGEGMTRKDHADVSNQLYACYAIGKDVQAMKAVVGEEALTSDDLLYLEFLQKFER 460
3       LMKSAIGEGMTRKDHGDVSNQLYACYAIGKDVQAMKAVVGEEALTSEDLLYLEFLQKFEK 454
4       LMKSAIGEGMTRKDHGDVSNQLYACYAIGKDVQAMKAVVGEEALTSEDLLYLEFLQKFEK 454
        ***************:*******************************:************:

1       NFITQGPYENRTVYETLDIGWQLLRIFPKEMLKRIPQSTLSEFYPRDSAKH           510
2       NFIAQGPYENRTVFETLDIGWQLLRIFPKEMLKRIPQSTLSEFYPRDSAKH           511
3       KFINQGPYEKRSVFESLDLGWKLLRTFPKEMLKRIPQNIIDEFFSREGAPQDTEADTAL   513
4       NFINQGPYENRSMFESLDLSWKLLRIFPKEMLKRIRQAVIDEFYSREGRLQDLAPDTAL   513
         ** ***** *:::*:**: * *** ********* *  : **: *:
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FIG. 1. Comparison of the amino acid sequences in single-letter code of the bovine and human isoforms of the ≈56-kDa subunits. Identical amino acids are indicated by asterisks, and conserved amino acids are indicated by colons. The shaded amino acids indicate amino acid residues that differ between isoforms. The amino- and carboxyl-terminal domains discussed in the text are indicated in boldface type. The double underline shows the sequence of the synthetic peptide used for immunization of rabbits. A partial sequence of the human brain isoform has been reported (7). The full-length cDNA reported here was isolated from a human kidney cDNA library as described in *Methods*. The first ATG site has been designated as the initiation codon [which has an A in the −4 position and a G in the −3 position and adheres to the loose consensus sequence for initiation (18)] and results in a deduced amino acid sequence of 511 amino acids ending at a TAG stop codon. The same criteria were used for translation of the bovine brain isoform as indicated for the human "brain" isoform. The sequence of the human kidney isoform is from ref. 8.

White rabbits were immunized initially with the mixture in complete adjuvant and thereafter every 3 weeks with the mixture in incomplete adjuvant. Antisera were tested for reactivity with affinity-purified bovine kidney H^+-ATPase as described below.

Immunoblotting. Preparation of affinity-purified H^+-ATPase and electrophoresis and transfer of protein samples to membranes were as described (6). Microsomes from bovine tissues were prepared as described for rat tissues (17). Microsomal protein (50 μg) from each tissue indicated was applied to a lane in a $NaDodSO_4$/10% polyacrylamide gel; the samples were separated by electrophoresis and transferred electrophoretically to polyvinylidene membrane (Immobilon; Millipore). The lanes were cut into strips, incubated in 5% ovalbumin/20 mM Tris chloride, pH 7.4, for 5 min and then incubated with the primary antibody for 2 hr. The primary antibody was either rabbit antiserum to the kidney isoform of the ≈56-kDa subunit diluted 1:100 in PBS or E11 hybridoma culture supernatant containing mouse monoclonal antibody (mAb) to the 31-kDa subunit (3, 19, 20). Strips were washed with PBS containing 0.1% Tween three times for 15 min and then with either goat anti-rabbit or goat anti-mouse immunoglobulin labeled with horseradish peroxidase. Strips were washed six times for 15 min with PBS/Tween and then incubated for 1 min with the enzymatic chemiluminescent ECL substrate solution (Amersham) diluted 1:1 in water following the manufacturer's recommended procedure. Strips were then exposed to XAR film for 1–10 min to detect antibody binding. Antibody specificity was confirmed by demonstrating that binding was eliminated by preincubation of the antibody with 1 mM of the synthetic peptide used to generate the antibody (not shown).

Immunocytochemistry of Rat Kidney Tissue Sections. Immunocytochemistry of paraffin sections of rat kidney was performed as described (19). The double-antibody labeling was done by incubating sections first with rabbit antiserum to the kidney isoform of the ≈56-kDa subunit diluted 1:50 in the blocking solution, followed, after washing, with the fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin second antibody (Fisher). The sections were washed in PBS for 15 min, then stained with E11 hybridoma culture supernatant containing mouse mAb to the 31-kDa subunit, washed again, and then stained with Texas red-labeled goat anti-mouse immunoglobulin (Fisher). The sections were mounted in 50% (vol/vol) glycerol with 0.1% paraphenylenediamine and were photographed with an epifluorescent microscope (Zeiss Axiophot) equipped with fluorescein and Texas red filters.

RESULTS

Partial cDNA clones for two isoforms of the ≈56-kDa subunit of the vacuolar H^+-ATPase were isolated from bovine and human kidney and bovine brain cDNA libraries as detailed in *Methods*. A comparison of the predicted amino acid sequences for the published human vacuolar H^+-ATPase 58-kDa subunit (8), a human ≈56-kDa subunit, and the two corresponding homologous bovine clones is shown in Fig. 1. The comparison supports the existence of two isoforms of the ≈56-kDa subunit. A partial human cDNA clone has been reported for the first isoform (7), whose nucleotide sequence is identical to our human clone. This isoform is referred to as the brain isoform. The second isoform corresponds to our bovine kidney clone and a published human kidney 58-kDa subunit clone (8). This isoform is referred to as the kidney isoform. The differences in nucleic acid sequence between the kidney and brain isoforms of either species were scattered throughout the entire mRNA sequence (data not shown) with no regions of nucleic acid identity longer than 22 bases, suggesting that they were not the product of alternative mRNA splicing, but are likely to be encoded by two separate genes.

A comparison of both isoforms in both species revealed that the middle 469 amino acids of the coding region were highly conserved. In contrast, the sequences at the amino and carboxyl-terminal ends of the two isoforms were entirely different as indicated in Fig. 1. In distinction to the variations in amino acid sequence at the amino and carboxyl-terminal ends between the different isoforms, the terminal sequences were highly conserved between species. Hence, it appears that the ≈56-kDa subunit has a "constant" domain that, by functional analogy with the α subunit of the F_0F_1 H^+-ATPases, may represent a segment participating in catalytic activity. The amino and carboxyl termini represent "variable" domains whose sequences are highly conserved and may determine the specialized functions of the isoforms.

As discussed above, we described previously several lines of evidence suggesting that H^+-ATPases in different membrane compartments of the kidney had different enzymatic and structural properties. To determine the location and potential function of the two different isoforms of the ≈56-kDa subunit, we analyzed their distribution in total RNA samples from a panel of bovine tissues. The bovine brain cDNA probe hybridized to a 3.0-kb transcript in all tissues tested, with the highest amounts of hybridization found in brain and adrenal medulla (Fig. 2*B*). In contrast, the bovine kidney cDNA probe hybridized to a 3.0-kb transcript in the kidney cortex and kidney medulla (Fig. 2*A*). Finally, hybridization to the probe for the 31-kDa subunit was found in all tissues, with the highest amount found in the kidney, brain, and adrenal medulla (Fig. 2*C*). This distribution is consistent with published results for the 31-kDa subunit (17) and correlates well with recently published distribution of the 15-kDa subunit (21).

Since steady-state RNA levels do not necessarily reflect the abundance of proteins in tissues, immunoblots on microsomes

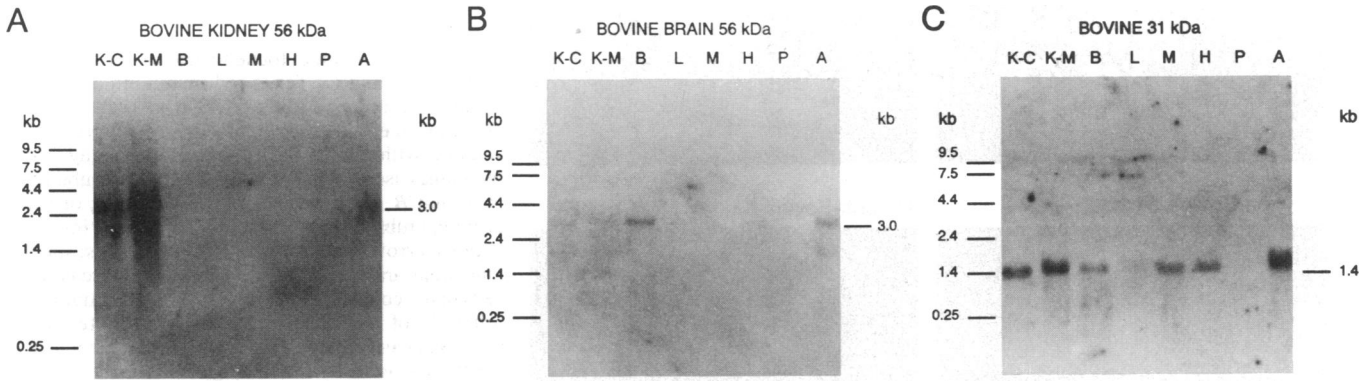

FIG. 2. RNA blots of bovine tissue total RNA. Tissues in lanes are: K-C, kidney cortex; K-M, kidney medulla; B, brain; L, liver; M, skeletal muscle; H, heart ventricle; P, pancreas; A, adrenal. (*A*) RNA probed with the bovine kidney ≈56-kDa subunit probe. (*B*) RNA probed with bovine brain ≈56-kDa subunit probe. (*C*) RNA probed with the bovine 31-kDa subunit probe.

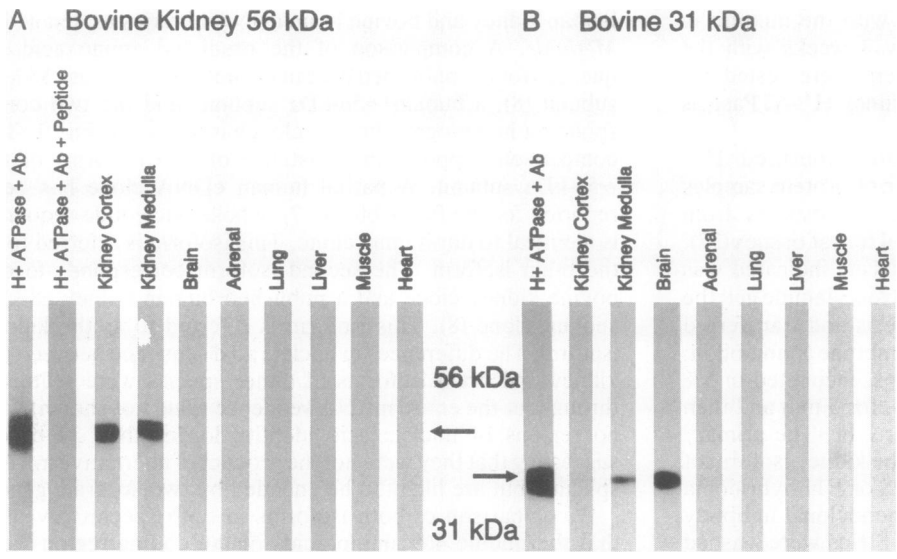

FIG. 3. Immunoblots of bovine tissue nicrosomes. (*A*) Microsomes probed vith polyclonal rabbit antiserum against he bovine kidney ≈56-kDa subunit. (*B*) Microsomes probed with the mAb to the 31-kDa subunit of the H^+-ATPase.

from bovine tissues were performed by using a polyclonal antibody from a rabbit immunized with a peptide from the carboxyl-terminal sequence of the kidney isoform of the ≈56-kDa subunit. The sequence of the peptide to which the antibody was prepared is shown in Fig. 1. In Fig. 3*A*, immunoblots on microsomes from various tissues probed with this polyclonal antibody reacted with a polypeptide at ≈56 kDa only in kidney cortex and kidney medulla. In contrast, Fig. 3*B* shows immunoblots of the same samples probed with a mAb (E11) to the carboxyl terminus of the 31-kDa subunit. Its distribution has been shown to reflect the distribution of total vacuolar H^+-ATPase (19, 20). The antibody reacted with a 31-kDa polypeptide in the kidney, brain, and adrenal medulla. These results show that the kidney isoform of the ≈56-kDa subunit is amplified selectively in the kidney, whereas the vacuolar H^+-ATPase, assayed by immunoreactivity of the 31-kDa subunit, is readily detected in other tissues besides the kidney.

To establish the distribution of the isoform in the kidney, we performed immunocytochemistry using the antibody to the kidney isoform and the E11 mAb. We showed previously that the E11 mAb gave a pattern of reactivity indistinguishable from that of kidney stained with polyclonal antibody prepared to the H^+-ATPase holoenzyme (3, 19). The mAb stained the proximal tubule brush border microvilli and the subvillar invaginations, the apical pole of the thick ascending limb and the distal convoluted tubule, and the intercalated cells in the collecting duct—cells with both apical and basolateral polarization (Fig. 4 *A* and *C*). In contrast, the antibody to the kidney isoform of the ≈56-kDa subunit stained intensely only the plasma membrane of the intercalated cells (Fig. 4 *B* and *D*). No staining was observed in any part of the proximal tubule or in the thick ascending limb; weak-to-moderate staining was also observed in the apical membrane of the distal convoluted tubule and the apical membrane of the principal cells of the inner medullary collecting duct (data not shown). [However, no staining was detected for the 31- or 17-kDa subunits of the H^+-ATPase in the inner medullary principal cells (I. Sabolic, F. Wuarin, L.-B. Shi, A. Verkman, D. Ausiello, S.G., and D.B., unpublished data).] Staining was observed in both the apical and basolateral membranes of the intercalated cells, suggesting that the kidney isoform of the ≈56-kDa subunit may contain structural information important to plasma membrane targeting but does not contain structural determinants for differential targeting to apical or basolateral polarization.

DISCUSSION

The findings in this study provide direct evidence that different structural forms of the vacuolar H^+-ATPase are amplified selectively in distinct membrane compartments, where they may have specialized functions. This is an example of specific amplification of an isoform of one subunit of the vacuolar H^+-ATPase limited to a specific cell type.

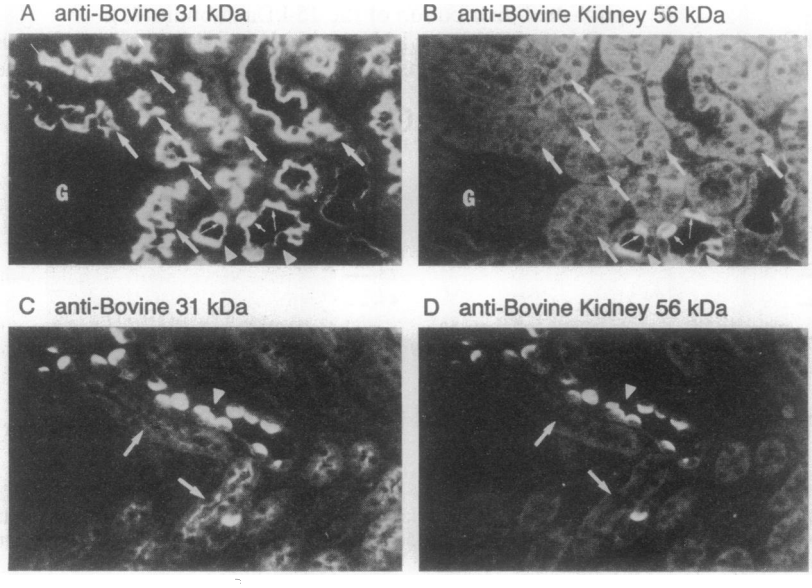

FIG. 4. Immunocytochemistry of rat kidney sections. (*A* and *C*) Texas red immunofluorescent staining with a mAb to the 31-kDa subunit. (*B* and *D*) Fluorescein isothiocyanate immunofluorescent staining with polyclonal rabbit antiserum against the kidney isoform of the bovine ≈56-kDa subunit. In *A* and *B*, large arrows indicate staining of the proximal tubule brush border and subapical invaginations; arrowheads indicate collecting ducts; small long-stem arrows indicate staining of intercalated cells in the collecting duct with H^+-ATPase staining primarily of the apical pole; small short-stem arrows indicate intercalated cells with apical and basolateral membrane staining; and G indicates a glomerulus. In *C* and *D*, arrows indicate staining of the apical pole of the thick ascending limb, and arrowheads indicate staining of intercalated cells within the collecting ducts.

The functional significance of the isoforms of the vacuolar H^+-ATPase ≈56-kDa subunit is unknown; they may have different enzymatic or sorting capacities or may control the membrane density of the H^+-ATPase. The kidney collecting duct modifies its rate of H^+ transport to regulate acid–base balance while presumably maintaining the normal acidification of intracellular compartments required for constitutive function of the vacuolar system. Having isoforms with unique enzymatic properties located in different compartments of the cell would be one possible means of regulating proton pumps on the plasma membrane independently of intracellular compartments. In preceding studies we showed that vacuolar H^+-ATPase purified from different kidney compartments varied in enzymatic properties (6). Although this finding initially suggested that structurally distinct catalytic ATP-binding subunits might be present in the different H^+-ATPase preparations, our two-dimensional SDS/polyacrylamide gels of the enzyme in both preparations showed only a single 70-kDa polypeptide (6). To date, only one gene for this subunit has been found in the plant (22), fungal (23), and mammalian forms (M. Marushack, B. Lee, K.M., and S.G., unpublished data). These observations imply that another subunit of H^+-ATPase might affect the overall catalytic properties. We found that the two-dimensional SDS/polyacrylamide gels of H^+-ATPase from different kidney compartments had dissimilar patterns of polypeptide spots in the ≈56-kDa region, suggesting a potential role for the ≈56-kDa subunit in determining the differences in catalytic properties of the H^+-ATPase. If the ≈56-kDa subunit does influence the enzyme's catalytic properties, the "variable" amino-terminal and/or carboxyl-terminal domains might impart differences in enzymatic attributes.

The proton-transport apparatus of the intercalated cell is a specialized membrane system that inserts proton pumps into and removes them from the plasma membrane to maintain acid–base homeostasis. Cytoskeletal elements may mediate the trafficking of H^+-ATPase between the plasma membrane and other membrane compartments by interacting with the unique carboxyl- and amino-terminal domains. We have found that both the kidney and the brain isoforms are expressed in the cultured renal epithelial cell line MDBK and in HeLa cells (D. Underhill, R.D.N., and S.G., unpublished data), but the level of expression of the kidney isoform is far lower in these cell lines than in the kidney and is not targeted to the plasma membrane. Staining with the antibody to the kidney isoform of the ≈56-kDa subunit was also observed weakly in other epithelial cell types in the kidney—notably, inner medullary principal cells and cells of the distal convoluted tubule. It is conceivable that the intercalated cell proton-transport apparatus is a modification of an intracellular compartment present in all cells that functions in the shuttling of the kidney isoform of H^+-ATPase to the plasma membrane and that the kidney isoform of the ≈56-kDa subunit is a component crucial for this specialized function of the vacuolar H^+-ATPase.

As revealed in rapid-freeze electron micrographs (2), proton pumps of the intercalated cell proton-transport apparatus are densely packed in a nearly crystalline array, a property that has not been observed in endosomes, lysosomes, and other intracellular organelles (2, 24). Amplified expression of the kidney isoform may be a mechanism for achieving a high density of the H^+-ATPase in the plasmalemma-associated intercalated cell membranes without affecting other compartments. A comparable system was observed in UT-1 cells overexpressing hydroxymethylglutaryl-CoA reductase (25), in which expansion of the endoplasmic reticulum and formation of a microcrystalline array of the enzyme were observed. However, our results have not established that increased expression of the mRNA for the ≈56-kDa subunit kidney isoform is responsible for the increase in vacuolar H^+-ATPase in intercalated cells; additional studies are needed to examine this directly.

Aside from the function of the kidney isoform of the ≈56-kDa subunit, the high level of expression of this protein and its mRNA transcript is an important marker for intercalated cells that might be used in studies on the development and differentiation of the collecting duct.

We thank B. Bastani, B. Lee, and D. Underhill for valuable discussions and technical advice and Edna Major for assistance in preparation of the manuscript. R.D.N. was supported by a fellowship from the National Kidney Foundation. This investigation was supported in part by National Institutes of Health Grants DK38848, DK09976, and DK40535. S.G. is the recipient of an American Heart Association–Sandoz Pharmaceutical Company Established Investigator Award.

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