Cloning of the H,K-ATPase β Subunit

TISSUE-SPECIFIC EXPRESSION, CHROMOSOMAL ASSIGNMENT, AND RELATIONSHIP TO Na,K-ATPase β SUBUNITS*

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We have isolated cDNA clones encoding the bovine and rat gastric H,K-ATPase β subunit. A bovine abomasum α11 cDNA library was screened with a monoclonal antibody raised against the rabbit H,K-ATPase β subunit. A single positive phage clone containing an ~900-base pair cDNA insert was identified as reactive with the antibody. The identity of the cDNA was established by comparing the deduced amino acid sequence with sequences of cyanogen bromide fragments of the porcine H,K-ATPase β subunit. Polymerase chain reaction and rapid amplification of cDNA ends were used to generate a cDNA fragment encoding the carboxyterminal portion of the rat gastric H,K-ATPase β subunit. A rat stomach cDNA library was screened with the polymerase chain reaction product, and several full-length β subunit cDNA clones were identified. The open reading frame predicts a protein of 294 amino acids with a molecular weight of 33,689. The rat H,K-ATPase β subunit shows 41% amino acid sequence identity to the rat Na,K-ATPase β2 subunit and shares a number of structural similarities with Na,K-ATPase β subunit isoforms. By analyzing the segregation of restriction fragment length polymorphisms among recombinant inbred strains of mice, we localized the H,K-ATPase β subunit to murine chromosome 8. Northern and Western blot analysis reveals that this gene is expressed exclusively in stomach. Our results suggest that the H,K-ATPase and Na,K-ATPase β subunits evolved from a common ancestral gene and may play similar functional roles in enzyme activity.

The oecytine cell of the gastric mucosa contains a membrane-associated enzyme, the H,K-ATPase, that provides the driving force for HCl secretion into the stomach (1). H,K-ATPase couples the electroneutral exchange of extracellular K⁺ and intracellular H⁺ to the hydrolysis of ATP (2), thereby generating an extremely high transmembrane proton gradient (3). The enzyme has been shown to consist of a major polypeptide component of M₀ ~ 100,000 (4) that contains the catalytic site for ATP hydrolysis (5). cDNA clones encoding the catalytic subunit of the rat and hog stomach H,K-ATPase have been isolated and characterized (6, 7). The deduced amino acid sequence of the H,K-ATPase shares a number of structural similarities with other cation transport ATPases, including the Na,K-ATPase (8,9), the Ca⁺⁺-ATPase (10), and the H⁺-ATPase of yeast and Neurospora (11, 12). These similarities suggest that P-type ion transport proteins arose from a common evolutionary ancestor.

We have recently characterized an abundant microsomal glycoprotein (gp 60-80) which is found in H,K-ATPase-containing membranes of several animal species (13). A number of lines of evidence suggest that this glycoprotein may represent a β-like subunit of the gastric H,K-ATPase. First, lectin affinity chromatography and immunoprecipitation reveal that gp 60-80 is noncovalently associated with the H,K-ATPase and remains associated with the catalytic subunit even after exposure to low concentrations of SDS. Second, gp 60-80 biosynthesis and H,K-ATPase activity appear concomitantly with the development of HCl secretion in the frog (14). Third, gp 60-80 shares several structural features with Na,K-ATPase β subunits including (a) similarity in size of core proteins, (b) association with the cell membrane, (c) the presence of several N-linked glycosylation sites, and (d) a stoichiometry of 1:1 with the catalytic subunit (13).

Here we describe the isolation and characterization of cDNA clones encoding bovine and rat stomach gp 60-80 utilizing two complementary approaches. First, a monoclonal antibody raised against rabbit gp 60-80 was used to screen a bovine abomasum α11 cDNA library. We identified an antibody-reactive recombinant phage clone containing an ~900 base pair cDNA insert which encodes a portion of bovine gp 60-80. Second, primers based upon sequences obtained from the bovine cDNA and cyanogen bromide fragments of porcine gp 60-80 were used to amplify a fragment of rat stomach gp 60-80 cDNA using the rapid amplification of cDNA ends (RACE) procedure (15). The PCR-generated and bovine cDNA were then used to screen a rat stomach cDNA library, and several full-length cDNA clones were isolated and characterized. Analysis of the amino acid sequence deduced from rat gp 60-80 cDNA shows a striking degree of primary sequence and secondary structure similarity to Na,K-ATPase β subunit isoforms. These results suggest that gp 60-80 cDNA

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The abbreviations used are: H,K-ATPase, potassium-activated proton transport adenosine triphosphatase (EC 3.6.1.37); PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; SDS, sodium dodecyl sulfate; kb, kilobase(s); gp, glycoprotein; RI, recombinant inbred.

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encodes a β subunit of the gastric H,K-ATPase. The availability of a molecular probe for the H,K-ATPase β subunit should facilitate an understanding of the role of this polypeptide in H,K-ATPase biogenesis and enzyme function.

MATERIALS AND METHODS

Sequencing of Porcine gp 60-80—Purified hog gastric microsomes were solubilized in dodecyltrimethylammonium bromide and subjected to wheat germ agglutinin affinity chromatography as previously described (13). The wheat germ agglutinin-binding fraction was eluted with 0.1 M NaCl, 1.5 mM sodium citrate, 1.3 mM KH₂PO₄, 0.1% SDS at 65 °C and exnosed to Kodak XRP film at -80 °C with an intensifying screen. For reprobing, the blots were washed two times at 95 °C for 15 min in 0.1 x SSCPE, 0.1% SDS.

Preparation of Membrane Fractions and Western Blotting—Crude cell membranes were isolated from various rabbit tissues and purified as described previously (13). Crude microsomal membrane fractions were prepared from rat stomach by the method of Jørgensen (22). Protein concentrations were determined as described by Bradford (23). Solubilized membrane proteins were fractionated on SDS-containing 10% polyacrylamide gels, transferred to nitrocellulose sheets (24), quenched (13, 25), and probed with an anti-gp 60-80 monoclonal antibody (16). Blots were rinsed and then incubated with horseradish peroxidase (Bio-Rad) or alkaline phosphatase (Kirkegaard and Perry Laboratories) conjugated goat anti-mouse IgG (secondary) antibody.

Chromosomal Localization—Recombinant inbred (RI) strains of mice (A/J, AKR/J, C57BL/6J, DBA/2J, C3H/J, C57L/J) were obtained from Jackson Laboratories (Bar Harbor, ME). Genomic DNA was digested to completion with TaqI, separated on a 1% agarose gel (containing 40 mM Tris acetate, pH 8.0, 1 mM EDTA), blotted onto a hybridization membrane, and probed with the 5' labeled 1.5 kb 3' RACE cDNA fragment (containing a portion of the coding and 3'-untranslated regions of rat gp 60-80 cDNA). Conditions for hybridization analysis were as described previously (20). The strain distribution pattern of the TaqI polymorphism in RI strains derived from an AKR/J × C57L/J cross (AXKL) was determined and is charted in Table II.

RESULTS

Isolation and Characterization of gp 60-80 cDNA Clones—A bovine abomasum Agt11 cDNA library (provided by Dr. Mark Williams, Salk Institute, San Diego) was screened with an anti-gp 60-80 monoclonal antibody (16) according to established protocols (17). Approximately 300,000 independent recombinants were screened and one positive phage clone containing an ~900 base pair cDNA insert was isolated and characterized. The cDNA insert was used to rescreen the library and two additional clones were isolated. The cDNA inserts were subcloned in pUC18 and sequenced using a Sequenase kit (U. S. Biochemical Corp.).

cDNA Cloning, Polymerase Chain Reaction, and RACE—A bovine abomasum Agt11 cDNA library (provided by Dr. Mark Williams, Salk Institute, San Diego) was screened with an anti-gp 60-80 monoclonal antibody (16) according to established protocols (17). Approximately 300,000 independent recombinants were screened and one positive phage clone containing an ~900 base pair cDNA insert was isolated and characterized. The cDNA insert was used to rescreen the library and two additional clones were isolated. The cDNA inserts were subcloned in pUC18 and sequenced using a Sequenase kit (U. S. Biochemical Corp.).

cDNA clones encoding rat gp 60-80 were generated utilizing PCR and RACE (15). For the 3' RACE, first strand cDNA was synthesized from 1.5 μg of total rat stomach RNA. A (dT)₃₀ primer (55 pmol) (15) served as primer for avian myeloblastosis virus reverse transcriptase (Stratagene). First strand rat stomach cDNA was subjected to PCR using the adaptor primer (25 pmol) and a 16-fold degenerate primer (100 pmol) derived from the amino acid sequence, PYPRTDYQDF, of porcine gp 60-80 peptide B (Table I). Second strand cDNA synthesis and PCR were carried out using Taq polymerase (Cetus) as described by Frohman et al. (15), except that the composition of the PCR buffer was that recommended in the Cetus GeneAmp kit. The PCR was run for 40 cycles (30 s at 94 °C, 1 min at 40 °C, 3 min at 72 °C) using a Coy thermocycler. Aliquots of the amplification products were electrophoresed through agarose gels, transferred to nitrocellulose filters (Schleicher and Schuell), and probed with radiolabeled bovine gp 60-80 cDNA. A DNA fragment of the expected size (~1.15 kb) gave a positive hybridization signal. This fragment was gel-purified using a GeneClean II kit (Bio 101 Inc.), and reamplified for 30 cycles under the conditions described above. The PCR products were phosphorylated using polynucleotide kinase (Stratagene). First strand rat stomach cDNA was subjected to PCR using the adaptor primer (25 pmol) and a 16-fold degenerate primer (100 pmol) derived from the amino acid sequence, PYPRTDYQDF, of porcine gp 60-80 peptide B (Table I). Second strand cDNA synthesis and PCR were carried out using Taq polymerase (Cetus) as described by Frohman et al. (15), except that the composition of the PCR buffer was that recommended in the Cetus GeneAmp kit. The PCR was run for 40 cycles (30 s at 94 °C, 1 min at 40 °C, 3 min at 72 °C) using a Coy thermocycler. Aliquots of the amplification products were electrophoresed through agarose gels, transferred to nitrocellulose filters (Schleicher and Schuell), and probed with radiolabeled bovine gp 60-80 cDNA. A DNA fragment of the expected size (~1.15 kb) gave a positive hybridization signal. This fragment was gel-purified using a GeneClean II kit (Bio 101 Inc.), and reamplified for 30 cycles under the conditions described above. The PCR products were phosphorylated using polynucleotide kinase (Boehringer Mannheim), repaired with T4 DNA polymerase (Boehringer Mannheim), inserted into pUC18 or Bluescript (Stratagene) vectors, and sequenced.

The 3' RACE and bovine gp 60-80 cDNAs were labeled by the random priming method (18) and used as probes to screen a rat stomach cDNA library prepared in λ Zap (Stratagene). Duplicate filters with a total of 600,000 phage from the cDNA library were differentially hybridized with the rat and bovine probes, and 12 cDNA clones were selected based on positive hybridization to both probes. These clones were transduced into Bluescript plasmid vectors using the helper phage R408 (Stratagene). The clone containing the longest insert (~8 kb) was sequenced on both strands. The DNA sequence was compiled and analyzed using the programs from the University of Wisconsin Genetics Computer Group (19).

Northern Blot Analysis of H,K-ATPase β Subunit mRNA—Total RNA was isolated from rat tissues by the guanidine thiocyanate method of Chomczynski and Sacchi (20). RNA samples were fractionated by electrophoresis through a formaldehyde-agarose (1%) gel, transferred to an Immobilon P filter (Millipore), and stained with Coomassie Blue. Strips containing peptide fragments were excised from the blot and subjected to N-terminal sequence analysis on an Applied Biosystems 1470 gas phase sequencer.

PCR products were utilized as probes to screen a rat stomach cDNA library as described (26). The strain distribution pattern of the TaqI polymorphism in RI strains derived from an AKR/J × C57L/J cross (AXKL) was determined and is charted in Table II.

We next used PCR and RACE (15) to amplify gp 60-80 cDNAs from rat stomach mRNA according to the strategy schematized in Fig. 1. To carry out 3' RACE, a fully degenerate 5' primer oligonucleotide (sense primer) was synthesized that encodes a portion of the amino acid sequence of porcine gp 60-80 CNBr peptide B (Table I). The 3' (antisense) primer was a 35 base oligonucleotide composed of a stretch of 17 dT residues and an adaptor sequence (15). Total RNA was isolated from rat stomach, and first strand cDNA was synthesized using the 3' oligonucleotide as primer. The cDNA fragment spanning the primers was then amplified by PCR using the sense and adaptor primers (15), and the first strand rat stomach cDNA as template according to Fig. 1. The PCR products were analyzed by Southern blotting using radiolabeled bovine gp 60-80 cDNA, and a DNA band of the expected size (~1.15 kb) was identified as reactive with the probe. The amino acid sequence deduced from this PCR-generated cDNA agreed very well with the sequence predicted from bovine gp 60-80 cDNA (data not shown). Taken together, these results suggest that the 5' end of gp 60-80 cDNA is unlikely to be represented in the library.

The 3' RACE and bovine gp 60-80 cDNAs were labeled by the random priming method (18) and used as probes to screen a rat stomach cDNA library prepared in λ Zap (Stratagene). Duplicate filters with a total of 600,000 phage from the cDNA library were differentially hybridized with the rat and bovine probes, and 12 cDNA clones were selected based on positive hybridization to both probes. These clones were transduced into Bluescript plasmid vectors using the helper phage R408 (Stratagene). The clone containing the longest insert (~8 kb) was sequenced on both strands. The DNA sequence was compiled and analyzed using the programs from the University of Wisconsin Genetics Computer Group (19).

Nonlinear Blot Analysis of H,K-ATPase β Subunit mRNA—Total RNA was isolated from rat tissues by the guanidine thiocyanate method of Chomczynski and Sacchi (20). RNA samples were fractionated by electrophoresis through a formaldehyde-agarose (1%) gel, and transferred to a Zetabind (AMF Cuno) filter. Conditions for hybridization analysis were as described previously (20). Blots were washed to a final stringency of 0.1 x SSCPE (1 x SSCPE is 0.15 M NaCl, 1.5 mM sodium citrate, 1.3 mM KH₂PO₄, 100 μM EDTA, 0.1% SDS at 65 °C and exposed to Kodak XRP film at -80 °C with an intensifying screen. For reprobing, the blots were washed two times at 95 °C for 15 min in 0.1 x SSCPE, 0.1% SDS.
Molecular Characterization of the Gastric H,K-ATPase α Subunit

TABLE I

A. Sequences of hog CNBr peptide B (residues 5-13), 5' PCR amplification primer, and rat gp 60-80 cDNA

| Peptide B (partial) | PCR primer | Rat gp 60-80 cDNA |
|---------------------|------------|------------------|
| ProTyrThrProAspTyrGlnAspGln | 5' CCTTAYACCCGCAIGATVACAGACA 3' |
| ProTyrThrProAspTyrGlnAspGln | 5' CCTACACCCCCGATCCAGGACCA |

B. Comparison of amino acid sequences deduced from bovine and rat gp 60-80 cDNAs to hog CNBr and proteolytic peptides

| Peptide A | Cow gp 60-80 | Rat gp 60-80 |
|-----------|--------------|--------------|
| XRIVKFLPGD | GKCPIIKMNRIKVFPGN |
| Cow gp 60-80 | GKCPIIKMNRIKVFPGN |
| Rat gp 60-80 | XXKKFIIKKMNHIKFL |
| Peptide B | Rat gp 60-80 |
| XTIDYPYTPYDYPQDLKLPGVTLDPFVYXKGL | MQTIDYPYTPYDPQDLKPSGVTLDPFVYGERGL |
| Peptide D | Cow gp 60-80 | Rat gp 60-80 |
| EYPAPADGTYSLLHYPPYGGKAQPHYNSPLV | EYPAPADGTYSLLHYPPYGGKAQPHYNSPLV |
| QYPAPADGTYSLLHYPPYGGKAQPHYNSPLV |

* Sequences of hog CNBr peptides A and B were determined as described under "Materials and Methods." The portion of peptide B used to design the 5' PCR primer is boxed.
| I, inosine; Y, C + T|
| Hog peptides C and D are sequences of V8 fragments C and A/B as described in Ref. 27.

Fig. 1. Strategy for rat gp 60-80 RACE. A restriction map of rat gp 60-80 cDNA is shown at the top. The stippled box indicates the open reading frame and the open boxes represent 5' (left) and 3' (right)-untranslated regions. Boxes designated B and A (middle) denote regions of homology to hog CNBr peptides B and A. A fragment of rat gp 60-80 cDNA (open box, bottom) was amplified using the RACE procedure (15). First strand cDNA was primed using a 3' dT/adaptor primer (stippled box, right). The fragment was then amplified using the adaptor primer and a degenerate 5' primer (stippled box, left) derived from the amino acid sequence, PYTPYDYPQDQQLLKPGVTLDPFVYSXKGL.

Structure of Rat Stomach gp 60-80—The complete nucleotide sequence of rat gp 60-80 cDNA and the deduced amino acid sequence of the predicted protein are shown in Fig. 2. The open reading frame defines a protein of 294 amino acids with a molecular weight of 33,689. The predicted protein starts at nucleotide position 176 and terminates at nucleotide position 1057, followed by 426 nucleotides of 3'-untranslated sequence and a poly(A) tail. The hydropathy profile (Fig. 3) indicates that rat gp 60-80 contains a polar cytoplasmic amino terminus followed by a single hydrophobic transmembrane domain of 27 amino acids and a 228-residue-long extracellular carboxyl-terminal domain including seven potential N-linked glycosylation sites (asterisks).

gp 60-80 is Related to Na,K-ATPase α Subunit Isoforms—The deduced amino acid sequence of rat gp 60-80 was compared with all sequences currently in the National Biomedical Research Foundation (NBRF) database (Release 24.0). Interestingly, rat gp 60-80 showed significant similarity only to Na,K-ATPase α subunit isoforms. A comparison of the amino acid sequence of rat gp 60-80 with the rat Na,K-ATPase α1 (28) and α2 (29) subunits is shown in Fig. 4. The amino acid sequence deduced from rat gp 60-80 cDNA showed 41% identity with the rat α2 subunit and 35% identity with the rat α1 subunit. Rat gp 60-80 consists of 294 amino acid residues, whereas the rat α2 and α1 subunits consist of 290 and 304 amino acid residues, respectively. There are 6 cysteine residues (positions 131, 152, 162, 178, 201, and 266) within the presumed extracellular domain of gp 60-80. In the computer aligned sequences, these cysteine residues appear to be highly conserved among gp 60-80 and the Na,K-ATPase α2 and β subunits. The asparagine residues marked with asterisks (Fig. 2) represent potential sites of N-linked glycosylation.
are seven such sites in gp 60-80, seven in the rat β2 subunit, and three in the rat β1 subunit polypeptide. One of the predicted N-linked glycosylation sites found in gp 60-80 (position 161) is exactly conserved relative to one of the predicted N-linked glycosylation sites found in the rat β1 and β2 subunits, whereas a second (position 193) is located 3 residues from a predicted N-linked glycosylation site which is conserved between the β1 and β2 subunits. A third asparagine residue (position 256) found in gp 60-80 is also exactly conserved relative to the position of a predicted N-linked glycosylation site found in the β1 and β2 subunits. However, this asparagine residue is not contained within a consensus glycosylation sequence (19). A putative transmembrane segment is located between residues 40 and 60 in gp 60-80 and 40 and 67 in the Na,K-ATPase β2 subunit. Of the 27 amino acid residues compared in this region, 11 are identical and 9 are conservative substitutions (30) between gp 60-80 and the β2 subunit. When analyzed by the method of Chou and Fasman (31), the predicted secondary structures of gp 60-80 and the Na,K-ATPase β1 and β2 subunits appear to be virtually identical. Taken together, these results indicate that rat gp 60-80 is a polypeptide related to the Na,K-ATPase β1 and β2 subunits. We have therefore termed this polypeptide the H,K-ATPase β subunit.

The H,K-ATPase β Subunit Is a Glycosylated Polypeptide Expressed Exclusively in Stomach.—A panel of rat tissues was examined for the presence of H,K-ATPase β subunit mRNA. The pattern of expression of β subunit mRNA is shown in Fig. 5 (β panel). Of the rat tissues analyzed (brain, heart, lung, kidney, liver, spleen, and stomach), the β subunit mRNA was detected only in stomach. The H,K-ATPase β subunit gene encodes two transcripts, ~1.7 and ~3.7 kb in size. The 1.7-kb mRNA is the predominant species and is ~20-fold more abundant than the 3.7-kb transcript. When the blot was reprobed with a cDNA specific for a portion of the H,K-ATPase catalytic (α) subunit (Fig. 5, α panel), two mRNA species ~4.0 and ~4.2 kb in size were detected which appeared to be expressed exclusively in stomach. These results indicate that the H,K-ATPase catalytic (α) and β subunit genes exhibit an identical tissue-specific pattern of expression.

To determine the tissue distribution of H,K-ATPase β subunit polypeptides, we probed Western blots of rabbit cellular and rat microsomal membrane fractions with the anti-gp 60-80 monoclonal antibody (16). As shown in Fig. 6A, the antibody reacted with a broad band of ~60 to ~80 kDa in rabbit fundus. In contrast, H,K-ATPase β subunits were undetectable in a variety of other rabbit tissues including duodenum, intestine, proximal and distal colon, liver, pancreas, and brain. We also used the monoclonal antibody to probe a Western blot of crude rat microsomes (Fig. 6B). A broad band of 60–80 kDa was detected in rat stomach, whereas the antibody failed to show immunoreactivity with kidney, brain, and heart microsomes. These results suggest that H,K-ATPase β subunits are expressed exclusively in stomach of at least two animal species.

We next analyzed the species distribution of the H,K-ATPase β subunit. A Western blot of microsomes prepared from hog, cow, rabbit, rat, and mouse stomach was probed with the anti-gp 60-80 monoclonal antibody. The results are shown in Fig. 7. In each species, the mature form of the β subunit migrated as a broad band with an apparent molecular weight of 60,000–80,000. Digestion of the β subunit with N-glycosidase F revealed that in each species, the β subunit core protein migrated with an apparent molecular weight of 32,000. This is very close to the value of 33,689 for the rat β subunit predicted from cDNA cloning. (In the cow, rabbit, and mouse lances, several intermediate bands were generated, indicating that the enzyme did not digest to completion.) These data suggest that the H,K-ATPase β subunit is expressed in stomach of a wide variety of mammals.

Chromosomal Localization of the H,K-ATPase β Subunit Gene.—We have used segregation of restriction fragment length polymorphisms among RI strains of mice to identify the chromosomal location of the mouse gene encoding the β subunit of the gastric H,K-ATPase (Atp4b). Mouse genomic DNA sequences were identified by hybridizing Southern blots to radiolabeled 3′ RACE-generated rat H,K-ATPase β subunit cDNA. As shown in Fig. 8, this probe hybridizes to three

2 The mouse gene encoding the H,K-ATPase β subunit has been named according to the rules for mouse gene nomenclature (40).
FIG. 5. Expression of H,K-ATPase β and catalytic subunit mRNAs in rat tissues. RNA was prepared from the adult rat tissues indicated. Total cellular RNA (25 μg) was fractionated by electrophoresis through a 1% agarose-containing formaldehyde gel, transferred to a Zetabind filter, and sequentially hybridized with cDNA probes specific for the rat H,K-ATPase β subunit (β panel) and catalytic (α) subunit (α panel). The α subunit probe was generated by PCR amplification of rat stomach cDNA utilizing primers derived from the published rat α subunit cDNA sequence (6). The positions of the 28 and 18 S markers are indicated at the left and right, respectively.

Fig. 6. Expression of H,K-ATPase β subunit polypeptides in rabbit and rat tissues. Membrane fractions were prepared from adult rabbit and rat tissues. Solubilized membrane proteins were fractionated by electrophoresis through SDS-containing 10% polyacrylamide gels, transferred to nitrocellulose filters, and probed with an anti-gp 60-80 monoclonal antibody (16). A, rabbit tissues: Fundus (50 μg), duodenum (100 μg), intestine (200 μg), proximal colon (97 μg), distal colon (200 μg), liver (156 μg), pancreas (99 μg), brain (165 μg). B, rat tissues (80 μg/lane). The apparent molecular weight of gp 60-80 is indicated.

major genomic fragments, 5.0, 3.2, and 2.4 kb long, in TaqI-digested mouse DNA. The 3.2-kb fragment is common to the six strains tested, whereas the 5.0-kb fragment is specific to A/J, AKR/J, C57BL/6J, C3H/J, and DBA/2J mice, and the 2.4-kb fragment is specific to C57L/J mouse DNA. The strain distribution pattern of the TaqI polymorphism in AKXL RI strains (described under “Materials and Methods”) is presented in Table II. Analysis of the strain distribution pattern of AKXL RI strains reveals linkage of Atp4b with mouse chromosome 8 markers. Among 18 strains examined, there were no recombinants between Atp4b and Xmu-26 (32) and 2 with Xmu-12 (32). The maximum likelihood estimate places Atp4b very tightly linked to Xmu 26 (95% confidence interval 0-6.9 centimorgans (cM) and at a distance of ~3 cM from Xmu-12 (95% confidence interval 0.3-18 cM) (33). However, no gene order could be inferred from our analysis.

FIG. 7. Species distribution and N-glycanase F digestion of the H,K-ATPase β subunit. Membrane fractions were prepared from adult hog, cow, rabbit, rat, and mouse stomach. Solubilized (−) and N-glycanase F (+) treated membrane proteins were electrophoresed through an SDS-containing 10% polyacrylamide gel, transferred to nitrocellulose, and probed with the anti-gp 60-80 monoclonal antibody. Hog (10 μg/lane), cow (15 μg/lane), rabbit (50 μg/lane), rat (45 μg/lane), mouse (40 μg/lane). The apparent molecular weight of the glycosylated and deglycosylated β subunits are indicated at the left.

FIG. 8. Restriction fragment length polymorphism for the H,K-ATPase β subunit. Genomic DNA from A/J (lane 1), AKR/J (lane 2), C57BL/6J (lane 3), C3H/J (lane 4), C57L/J (lane 5), and DBA/2J (lane 6) inbred mouse strains. Genomic DNA was digested to completion with TaqI, separated on a 1% agarose gel, blotted onto a hybridization membrane, and hybridized to a radiolabeled H,K-ATPase β subunit cDNA probe as described under “Materials and Methods.” Conditions for hybridization analysis were as described previously (26). Positions of size markers (HindIII fragments of λDNA) in kilobases are shown at the left. Arrows indicate the position of polymorphic hybridizing fragments.

DISCUSSION

The isolation of cDNA clones for each of the subunit components of the H,K-ATPase represents an initial step in elucidating the structure and function of this enzyme. Analysis of cDNAs for the H,K-ATPase catalytic subunit (6, 7) has revealed that the encoded polypeptide is highly homologous (~60%) to the Na,K-ATPase α subunit. The organization of the H,K-ATPase (34) and Na,K-ATPase (35) α subunit genes is also strikingly similar. The data presented in this report describe the cloning of a cDNA for the H,K-ATPase β subunit. The amino acid sequence deduced from the cDNA reveals a polypeptide which is remarkably similar to Na,K-ATPase β subunits.

The rat H,K-ATPase β subunit exhibits 41% amino acid sequence identity to the rat Na,K-ATPase β subunit (29) and shows significant similarity only to Na,K-ATPase β subunits when compared to all sequences currently listed in the NBRF data base. Hydrophathy analysis of the H,K-ATPase β subunit suggests a structure similar to that of Na,K-ATPase β subunits; it contains a short intracellular amino-terminal region, a transmembrane segment, and a large extracellular carboxyl-terminal domain. The high degree of homology between the H,K-ATPase and Na,K-ATPase β subunits suggests that they comprise a family of related P-type ion trans-
port protein subunits. The positions of the 6 cysteine residues in the extracellular portion of the rat H,K-ATPase β subunit are highly conserved relative to the positions of 6 cysteine residues in the rat Na,K-ATPase β1 and β2 subunits. These residues could play an important role in maintaining β subunit structure, possibly forming several folded subdomains each cross-linked by disulfide bonds (36). The H,K-ATPase β subunit, like the Na,K-ATPase β1 and β2 subunits (25), is a glycosylated polypeptide. Two of the predicted N-linked glycosylation sites within the rat H,K-ATPase β subunit are highly conserved relative to the positions of two potential N-linked glycosylation sites in the rat Na,K-ATPase β2 (28) and β (29) subunits. These structural similarities strongly indicate that the H,K-ATPase and Na,K-ATPase β subunits evolved from a common ancestral gene.

Chromosomal mapping experiments have demonstrated that the Na,K-ATPase β1 and β2 subunits are encoded by separate genes located on murine chromosomes 1 and 11, respectively (26, 37). We have now localized the H,K-ATPase β subunit gene to mouse chromosome 8. The identification of separate chromosomal loci for each β subunit gene suggests that there are likely to be cis-acting control elements that determine the tissue-specific pattern of expression for each gene. In addition, the fact that each β subunit gene is located on a different chromosome suggests that correction mechanisms such as gene conversion did not participate in the evolution of the β subunit gene family.

Our data indicate that the H,K-ATPase β subunit is expressed in the stomach of a wide variety of mammalian species including rat, rabbit, hog, cow, and mouse. The polypeptide has also been detected in purified frog gastric microsomes (14), suggesting its potential existence in all vertebrate capable of gastric HCl secretion. Hybridization analysis reveals that the H,K-ATPase β subunit gene is expressed exclusively in rat stomach, and encodes two distinct mRNA transcripts (Fig. 5). All of the β subunit cDNA clones we isolated appear to represent a single class of cDNA, and N-glycosylation: F digestion of rat stomach microsomes (Fig. 7) indicates only one form of β subunit core polypeptide. These results suggest that the two β subunit mRNA species are likely to differ in untranslated regions and encode only one form of β subunit polypeptide. Interestingly, the H,K-ATPase α subunit gene also appears to encode two mRNA transcripts. We do not yet know whether these α and β subunit mRNAs arise by differential splicing, utilization of alternative polyadenylation signals or whether the larger β subunit mRNA represents an unprocessed nuclear precursor.

Identification of the gene encoding the β subunit of the gastric H,K ATPase raises important questions regarding the functional significance of β subunit isoforms. On one hand, the existence of a β subunit for the H,K-ATPase argues in favor of a common function for H,K-ATPase and Na,K-ATPase β subunits. For example, β subunits of the H,K- and Na,K-ATPases may play similar roles in cation transport, ATPase activity, and/or proper membrane orientation of their respective holoenzymes (36). Alternatively, chromosomal dispersion, amino acid sequence divergence, and tissue-specific expression of the β subunit genes suggest that the polypeptide encoded by each gene may have properties selected in response to different physiological demands. Consideration of the biology of the oxyntic cell may provide some insight into this question. In the oxyntic cell, H,K-ATPase is sorted to the apical tubulovesicular membrane compartment, whereas the Na,K-ATPase is located in the basolateral membrane (reviewed in Ref. 38). Thus it is possible that determinants involved in targeting H,K ATPase and Na,K ATPase to their proper membrane destinations are sequences residing within their respective β subunits. A genetic approach could prove very powerful in studying a phenomenon of this type.

The fact that both H,K-ATPase and Na,K-ATPase are expressed in the oxyntic cell raises an additional point of interest. Are there control mechanisms which govern the specific association of H,K-ATPase and Na,K-ATPase β subunits with their respective catalytic α subunits? Distribution of H,K-ATPase and Na,K-ATPase activities to separate membrane compartments (38) is consistent with the view that the H,K-ATPase β subunit does not associate with the Na,K-ATPase α subunit (and vice versa). It will clearly be of interest to determine how the sequence of each β subunit leads to specificity regarding interaction with the H,K- or Na,K-ATPase α subunit. The construction of chimeric cDNA molecules between H,K-ATPase and Na,K-ATPase β subunit cDNAs should permit identification of sites within a given β subunit that interact with the corresponding α subunit. This strategy should also be useful for identification of other functional domains within H,K- and Na,K-ATPase β subunits.

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REFERENCES
1. Forte, J. G., Ganser, A. L., Beesley, R., and Forte, T M (1975) Gastroenterology 69, 175-189
2. Sachs, G., Chang, H. H., Rabon, E., Schackman, R., and Saccomani, G. (1976) J. Biol. Chem. 251, 7600-7608
3. Forte, J. G., and Lee, H. C. (1977) Gastroenterology 73, 921-926
4. Forte, J. G., Ganser, A. L., and Ray, T. K. (1976) in Gastric Hydrogen Ion Secretion (Kachekar, D. K., Sachs, G., and Rehm, W., eds) pp. 302-330, Marcel Dekker, New York
5. Farley, R. A., and Faller, L. D. (1985) J. Biol. Chem. 260, 3999-3001
6. Shull, G. E., and Lingrel, J. B. (1986) J. Biol. Chem. 261, 16788-16791
7. Maeda, M., Ishizaki, J., and Futai, M. (1988) Biochim. Biophys. 
Rev. Commun. 157, 203–209
8. Shull, G. E., Greer, J., and Lingrel, J. B. (1986) Biochemistry 25, 
8125–8132
9. Herrera, V. L., Emanuel, J. R., Ruiz-Opazo, N., Levenson, R., 
and Nadal-Ginard, B. (1987) J. Cell Biol. 105, 1855–1865
10. MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. 
(1988) Nature 316, 696–700
11. Serrano, R., Kielland-Brandt, M. C., and Fink, G. R. (1986) 
Nature 319, 689–693
12. Hager, K. M., Mandala, S. M., Davenport, J. W., Speicher, D. 
W., Benz, E. J., Jr., and Slayman, C. W. (1986) Proc. Natl. 
Acad. Sci. U. S. A. 83, 7693–7697
13. Okamoto, C. T., Karpilow, J. M., Smolka, A., and Forte, J. G. 
(1990) Biochim. Biophys. Acta 1037, 360–372
14. Rawley, R. C., and Forte, J. G. (1973) Biochim. Biophys. Acta 
307, 372–385
15. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) Proc. 
Natl. Acad. Sci. U. S. A. 85, 8098–8099
16. Chow, D., Okamoto, C., and Forte, J. G. (1989) FASEB J. 3, 
A873
17. Schneider, J. W., Mercer, R. W., Benz, E. J., Jr., and Levenson, 
R. (1988) Methods Enzymol. 156, 379–392
18. Feinberg, A., and Vogelstein, B. (1984) Anal. Biochem. 137, 266– 
269
19. Devereux, J., Haebirli, P., and Smithies, O. (1984) Nucleic Acids 
Res. 12, 387–395
20. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 
156–159
21. Emanuel, J. R., Garetz, S., Stone, I., and Levenson, R. (1987) 
Proc. Natl. Acad. Sci. U. S. A. 84, 9030–9034
22. Jørgensen, P. L. (1974) Biochim. Biophys. Acta 356, 36–52
23. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
24. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. 
Sci. U. S. A. 76, 4350–4354
25. Shyjan, A. W., Gottardi, C., and Levenson, R. (1990) J. Biol. 
Chem. 265, 5166–5192
26. Malo, D., Schurr, E., Levenson, R., and Gros, P. (1990) Genomics 
6, 697–699
27. Helli, K., Peres, G., Anderson, D., Gutierrez, C., Munson, K., 
Hersey, S. J., Kaplan, J. H., and Sachs, G. (1990) Biochemistry 
29, 701–706
28. Merc, R. W., Schneid, J. W., Savita, A., Emanuel, J., Benz, 
E. J., Jr., and Levenson, R. (1986) Mol. Cell. Biol. 6, 3884–3890
29. Martin-Vasallo, P., Dackowski, W., Emanuel, J. R., and Leven-
son, R. (1989) J. Biol. Chem. 264, 4613–4618
30. Dayhoff, M. O., Barker, W. C., and Hunt, L. T. (1983) Methods 
Enzymol. 91, 524–545
31. Chou, P. Y., and Fasman, G. D. (1978) Annu. Rev. Biochem. 47, 
251–276
32. Frankel, W. N., Stowe, J. P., Taylor, B. A., and Coffin, J. M. 
(1989) J. Virol. 63, 1763–1774
33. Silver, J. (1985) J. Hered. 76, 436–440
34. Maeda, M., Oshiman, K.-I., Tamura, S., and Futai, M. (1990) J. 
Biol. Chem. 265, 9027–9032
35. Ovchinnikov, Y. A., Monastyrskaya, G. S., Broude, N. E., Ushkar-
yov, Y. A., Melkov, A. M., Smirnov, Y. V., Malyshev, I. V., 
Allikmets, R. L., Kostina, M. B., Dulebova, I. E., Kiyatkin, N. 
I., Grishin, A. V., Modyanov, N. N., and Sverdlov, E. D. (1988) 
FEBS Lett. 239, 87–92
36. McDonough, A. A., Geering, K., and Farley, R. A. (1990) FASEB 
J. 4, 1598–1605
37. Kent, R. B., Fallowo, D. A., Geiseler, E., Glaser, T., Emanuel, J. 
R., Lalley, P. A., Levenson, R., and Housman, D. (1987) Proc. 
Natl. Acad. Sci. U. S. A. 84, 5389–5393
38. Forte, J. G., and Soll, A. (1988) in Handbook of Physiology 
(Schultz, S. G., and Forte, J. G., eds.) Vol. 3, pp. 207–228, 
American Physiological Society, Bethesda, MD
39. Kyle, J., and Doolittle, R. F. (1962) J. Mol. Biol. 157, 105–132
40. Levenson, R., and Davison, M. (1991) Mouse Newsletter, 89, in 
press
Cloning of the H,K-ATPase beta subunit. Tissue-specific expression, chromosomal assignment, and relationship to Na,K-ATPase beta subunits.
V A Canfield, C T Okamoto, D Chow, J Dorfman, P Gros, J G Forte and R Levenson
J. Biol. Chem. 1990, 265:19878-19884.

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