

MOLECULAR CLONING AND STRUCTURAL ANALYSIS OF CANINE GASTRIC H⁺, K⁺-ATPase

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Gastric hydrogen-potassium ATPase (H⁺, K⁺-ATPase) is a heterodimeric protein which participates in the formation of hydrochloric acid. We cloned canine H⁺, K⁺-ATPase α and β subunit cDNAs from canine gastric cDNA libraries and the α subunit gene from a canine genomic library. The α subunit gene is 13 kb in length and contains 21 introns ranging from 77 to 1,076 bp. Its 5'-flanking region contains putative regulatory motifs for transcription that are similar to those found in H⁺, K⁺-ATPase genes from other species. The open reading frames of α and β subunit cDNAs are 3,500 and 870 bp in length and encode proteins of 1,034 and 290 amino acids, respectively. They are 80 - 90% homologous to corresponding cDNAs previously identified in porcine and rodent gastric tissues. © 1993 Academic Press, Inc.

Hydrogen-potassium ATPase (E.C. 3.6.1.36) functions as a proton pump in the secretion of hydrochloric acid by gastric parietal cells [1]. This enzyme resides in intracellular vesicles, is translocated upon cellular activation to secretory canaliculi by cytoskeletal elements, and through ATP hydrolysis catalyzes the electroneutral exchange of intracellular H⁺ for extracellular K⁺ [2]. A structurally-related H⁺, K⁺-ATPase which exhibits 63% amino acid identity with its gastric counterpart has been identified in distal colon where it is thought to play a role in transcellular K⁺ absorption [3]. Moreover, a renal H⁺, K⁺-ATPase exists which may contribute to K⁺-dependent acidification processes in the collecting duct [4]. An omeprazole-sensitive H⁺, K⁺-ATPase activity has also been detected in vascular smooth muscle cells [5]. H⁺, K⁺-ATPase is a member of the family of P-type ATPases which form an aspartyl phosphate intermediate during ATP hydrolysis. Na⁺, K⁺-ATPase and Ca²⁺-ATPase constitute other members of this enzyme class. Like Na⁺, K⁺-ATPase, H⁺, K⁺-ATPase is a membrane-spanning heterodimer composed of α and β subunits [6]. The 114 kDa α subunit functions in ATP hydrolysis and catalyzes the exchange of H⁺ for K⁺ with a 1:1 stoichiometry [2]. The highly-glycosylated, 35 kDa β subunit has no well-defined role, but may function in the membrane targeting of the enzyme [7-10] and in modulating the conformation of the catalytic α subunit [11, 12].

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Considerable information is available on the molecular biology of H⁺, K⁺-ATPase. Complementary DNAs encoding the α subunit of gastric H⁺, K⁺-ATPase have been cloned from rat [13], rabbit [14] and pig [15] gastric cDNA libraries, and the human gene encoding this subunit has been cloned, sequenced [16, 17] and mapped to chromosome 19q13.1 [18]. Complementary DNAs encoding the β subunit of gastric H⁺, K⁺-ATPase have been cloned from rat [19, 20], rabbit [21], cow [20], pig [22] and human [23] stomach cDNA libraries, and the mouse and rat genes have been identified and sequenced [24-27]. The gene encoding this subunit has been assigned to human chromosome 13q34 [28]. Despite this wealth of information, nothing is known about the structure or genetics of canine H⁺, K⁺-ATPase although canine gastric parietal cells have been used as a model for many physiological studies of acid secretion. Moreover, this cell preparation may be particularly useful for examination of the regulation of H⁺, K⁺-ATPase gene expression. Accordingly, we have undertaken these studies to isolate the canine H⁺, K⁺-ATPase α and β subunit cDNAs and the α subunit gene.

METHODS

MATERIALS. A 3.5 kb cDNA clone encoding rat gastric H⁺, K⁺-ATPase α subunit was a generous gift of Dr. Gary E. Shull (University of Cincinnati, OH). Canine, human and rat genomic libraries in the phage vector λ EMBL-3 were purchased from Clontech Laboratories, Inc. (Palo Alto, CA). Canine gastric cDNA libraries were constructed in λ gt11 and λ ZAP II vectors (Stratagene) as previously described [29].

LIBRARY SCREENING. cDNA and genomic libraries were plated and the DNA in phage plaques was transferred to nitrocellulose filters. After baking for 2 h at 80° C, filters were prehybridized for 6 h at 65° C in 0.1 M HEPES (pH 7.5), 1x saline-sodium citrate (SSC), 5x Denhardt's solution and 100 μ g/ml salmon sperm DNA [30]. The rat H⁺, K⁺-ATPase cDNA probe for the α subunit was labelled by random-priming with ³²P-dCTP [31]. A fragment of canine H⁺, K⁺-ATPase β subunit cDNA was generated by polymerase chain reaction (PCR) using oligonucleotide primers which were based on the sequence of the rat gastric H⁺, K⁺-ATPase β subunit cDNA [19]. This PCR product was used as a probe in screening for the canine H⁺, K⁺-ATPase β subunit. Hybridization was performed for 12 - 16 h at 65° C in the same solution as used for prehybridization. Filters were washed for 60 min at 65° C in 2x SSC, for 15 min at 75° C in 1x SSC, and for 15 min at 85° C in 0.5x SSC. Positive plaques were detected by autoradiography using Kodak XAR-5 film with intensifying screens. The DNA from positive phage plaques was isolated and digested with *Bam* H I for α subunit DNA and *Kpn* I-*Xba* I for β subunit DNA and used for Southern blotting. Two DNA fragments from the α subunit gene (B2 - B3 and B4 - B5 on Fig. 1A), which were approximately 1.8 kb in length, could not be discriminated on the agarose gel because of their nearly identical mobilities. However, after subcloning into a sequencing vector the two fragments could be distinguished by hybridization to region-specific ³²P-labeled DNA probes.

DNA SEQUENCING. Restriction fragments of the α and β subunit DNA from positive clones were subcloned into either M13mp18 or M13mp19 sequencing vectors. They were sequenced in both directions by the dideoxynucleotide method [32]. Oligonucleotides used as sequencing primers were synthesized with an Applied Biosystems 380B oligonucleotide synthesizer. Computer analyses of nucleotide sequences were performed using the Genetics Computer Group Program (University of Wisconsin Biotechnology Center). Nucleotide sequences were translated into amino acid sequences using the IUB codes in the Genetics Computer Group program.

RESULTS AND DISCUSSION

Gene and cDNA Structure of Canine H⁺, K⁺-ATPase α Subunit. The gene encoding the α subunit of canine gastric H⁺, K⁺-ATPase was approximately 13 kb in length and was sequenced from two overlapping clones. The first clone, designated DHKG1, spanned an

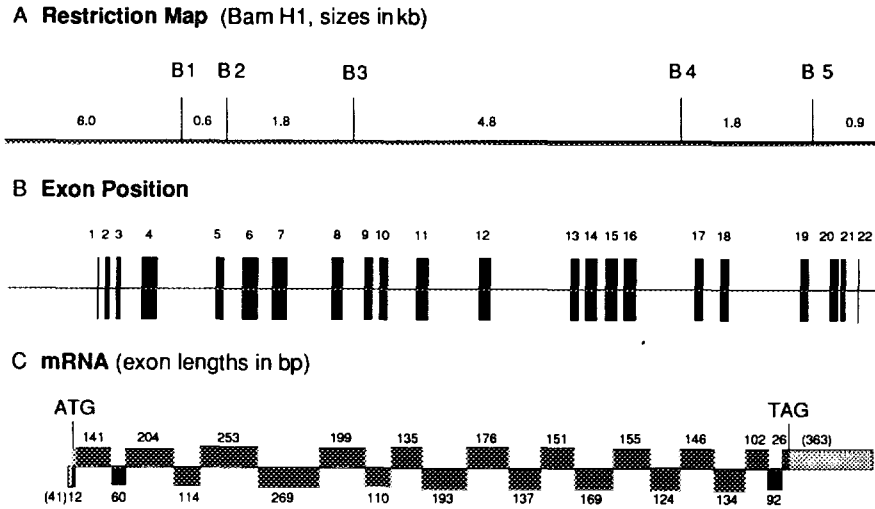


FIGURE 1. Gene structure of canine H^+ , K^+ -ATPase α subunit. (A) *Bam* H I (B1 - B5) restriction sites are shown with the sizes (in kb) of fragments subcloned into M13 sequencing vectors (see text for further details). (B) Relative positions of the 22 exons and 21 introns in the canine H^+ , K^+ -ATPase α subunit are depicted. (C) Lengths of exons are depicted by solid boxes and the locations of the initiation ATG codon and TAG termination codon are shown. The shaded portions of exon 1 (41 bp in length) and exon 22 (363 bp in length) indicate 5'- and 3'-untranslated regions, respectively.

area from the 5'-untranslated region to intron 19. The second clone, DHKG2, contained the entire gene except for the 5'-untranslated region. In addition to the protein-coding sequence, 2.2 kb of the 5'-flanking region and 400 bp of the 3'-flanking region were determined. As depicted in Fig. 1A, the gene contains five *Bam* H I restriction sites located in exons 6 (site B2) and 20 (site B5) and within introns 4 (site B1), 9 (site B3) and 17 (site B4). The protein-coding region of the gene is encompassed by 22 exons, ranging from 12 to 269 bp in length (Fig. 1, B and C). The 21 introns of the gene range in size from 77 to 1,076 bp. Examination of the exon-intron splice junctions of the gene revealed that the splice donor and acceptor sites conformed to standard convention and began with the nucleotides GT and terminated with AG [33].

The nucleotide sequence encompassing approximately 400 bp upstream from the initiation methionine codon shares >75% homology with corresponding regions of rat and human H^+ , K^+ -ATPase genes (Fig. 2) [17, 34]. Common motifs for transcriptional regulation shared by the canine, human and rat genes include a TATA box (-56 bp from ATG codon) and an AP4 binding motif (-102 bp). In addition, there are at least four motifs that are shared with human and rat genes (Fig. 2), the potential functions of which are not known. These sequence similarities suggest that the H^+ , K^+ -ATPase genes from various species are under similar transcriptional control.

Two canine parietal cell cDNA libraries were screened with a ^{32}P -labeled fragment of DNA encoding the α subunit of rat gastric H^+ , K^+ -ATPase DNA. After screening approximately 10^6 phage plaques, two full-length clones were identified from 19 positive clones. Upon sequencing, the canine H^+ , K^+ -ATPase α cDNA was 3.5 kb in length and encoded a deduced protein of 1,034 amino acids (Fig. 3). The protein-coding region exhibited 90 - 93% nucleotide identity with rat,

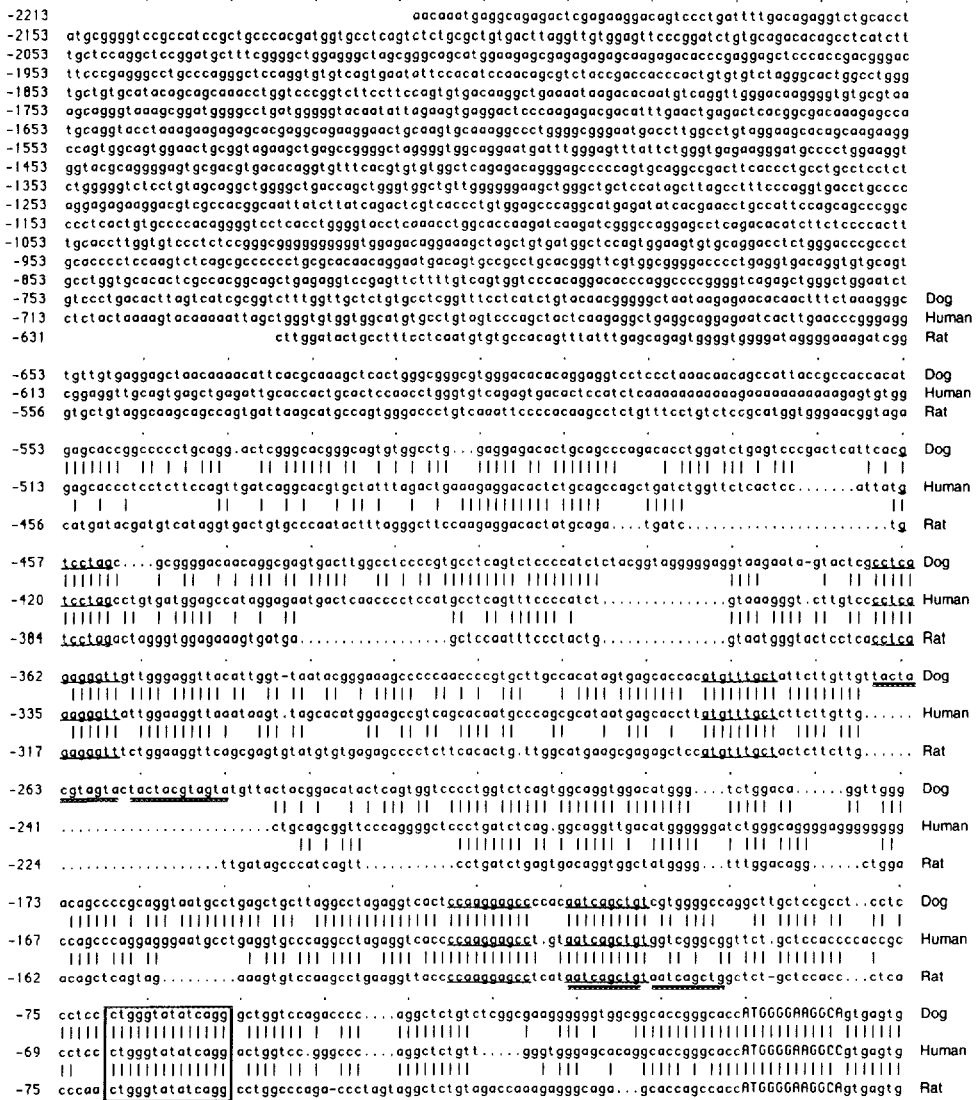


FIGURE 2. The 5'-regulatory region of the canine H⁺, K⁺-ATPase gene (α subunit) and its comparison to human and rat nucleotide sequences. The protein-coding sequences in exon 1 are indicated by upper-case letters and numbered with the ATG site at position +1. Dotted lines indicate gaps in nucleotide sequences among species. The putative cap site is indicated by an arrow. The TATA consensus motifs for RNA polymerase II binding are boxed. Potential sequence motifs for transcriptional control are lightly underlined; darkly underlined sequences represent tandem repeats in dog and rat genes.

pig, and human H⁺, K⁺-ATPase cDNAs. Moreover, the canine gastric H⁺, K⁺-ATPase α contains a lysine-rich region in its N-terminal cytoplasmic domain (amino acids 28 - 55, Fig. 3) which may function to confer specificity for H⁺ transport by H⁺, K⁺-ATPase [35] or promote enzyme targeting to the apical membrane [36]. The Asp³⁸⁶ residue found in exon 8 may function as a phosphorylation site common to P-type ATPases. Two lysine residues, Lys⁴⁹⁷ encoded in

(EX 1) ↓ (EX 2)

-17 TGGCGGCACCGGGCACATGGGGAAGGCAGAGAAATACGAGATGACTCGGTAGAGCTGGGCTCGGCCCTGGGGGGACATGGCGCAAGATGAGCAAGAAGAGCGGGGCAAGGGAG
(1) M G K A E N Y E M Y S V E L G P G P G G D M A A K M S K K K A G K G G ↓ (EX 3)

104 GAGGCAAGAAGAAGGAGAACTGGAGAACATGAAGAAGGAGATGGAGATCAACGACCACAGCTCGGTGGCGGAGCTGGAACAGAAATACCAAGACCAAGTGGCAGAAAGGGCTGCTGTG
(36) G K K K E K E I N D H Q L S V A E L E Q K Y Q T S A T V K G L S A ↓ (EX 4)

224 CCAGCTGGCGCAGACAGCTGCTGCTGGCGGACGGGCCAACGCCCTGAGCGCGCGGTGGTACCCCGAGTATGTCAAGTTCGCCCGCAGCTGGCAGGTGGTTCGATGCTCATGT
(76) S L A A D L L L R D G P N A L R P P R G T P E Y V K F A R Q L A G G L Q C L M W ↓ (EX 5)

344 GGGTGGCTGCTGCCATCGCTCATTGCCTTTGCCATCCAGGCCAGTGGGGTGACCTCACCACCGCAGCAATCTGTACCTGGCAGCTGGCCCTCATCGCCCTGGTGGTCCACGGCT
(116) V A A A I C L I A F A I Q A S E G D L T T D D N L Y L A L A L I A V V V V T G C ↓ (EX 6)

464 GCTTTGGCTACTACCGAAATTAAGACACCAACATCATCGCCAGCTTCAAGAACCTGGTGGCCAGCAAGCACTGTGATCCGAGATGGGGACAAGTCCAGATCAACGCAGCAGC
(156) F G Y Y Q E F K S T N I I A S F K N L V P Q Q A T V I R D G D K F Q I N A D Q L

584 TGCTGGTGGCGACCTGGTGGAGATGAAGAGCGGGGACCGAGTCCAGCCGACATCAGGATCCTCAGGCCCGAGGCTGCAAGTGGACACTCTCGCTGACCGGAGATCCGAGCCCG
(196) V V G D L V E M K G G D R V P A D I R I L Q A Q G C K V D N S S L T G E S E P Q ↓ (EX 7)

704 AGACCCGCTCGCCGAGTACACACGAGAGCCCGCTGGAGACCGCAACATCGCCTTCTTCCACCATGTGCTTGGGGCAGCAGCAAGCCCTGGTGGTGAACCGGGTGACCGCA
(236) T R S P E C T H E S P L E T R N I A F F S T M C L E G T A Q G L V V N T G D R T

824 CCATCATCGGGCGCATGCTGCTGGCTGGAGTGAAGAATGAGAAGACCCCACTGGATTAAGAAATCGAAATTTGTGGACATCTGCAGCAGGCTGGCAGCCCTTTCGGCGCCA
(276) I I G R I A S L A S G V E N E K T P I A I E I E H F V D I I A G L A I L F G A T ↓ (EX 8)

944 CGTTTTTGTGGTGGCATTGTGATGGTACACCTTCTCGGGCCATGGTCTTCTTCAATGGCATTGGTGGCTATGTGCCCGAGGGGCTGGTGGCAGTGTACCGTCTGCTGTG
(316) F F V V A M C I G Y T F L R A M V F F M A I V V A Y V P E G L L A A T V T V C L S

1064 CCTGACGCGCAAGCGCTGGCTGGCTGAGTGTGTAGTCAAGAACCTGGAGACACTGGGCTCCAGCTCAGTGTCTGCTCAGCAGGACAGGACCTTGTACTCAGAAC
(356) L T A K R L A S K N C V V K N L E A V E T L G S T S V I C S D K T G T L T Q N R ↓ (EX 9)

1184 GCATGACTGTGCCATCTCTGGTTCGCAACACACACCGCTGACACCCAGGAGCAGTCAAGGCGAGCCTTTCAGCAGTCTCGGAGACCTGGCGGGCGCTATGCCCGGTGC
(436) M T V S H L R V L G F C Q L L S E K D Y P F G Y A F D V E A M N F P T S G L C F ↓ (EX 10)

1304 TCACCTCTGCAACCGGGCGCCCTTAAGTCGGGCCAGGACGCTGTCGGGTGCCCAAGCGCATCTGTGATCGGGGACCGCTCCGAGAGCGCGCTGCTCAAGTTCGAGAGCTGACCCCTCG
(436) T L C N R A A F K S G Q D A V P V P K R I V I G D A S E T A L L K F S E L L T L G ↓ (EX 11)

1424 GCAACCGCATGGGCTACCGCGAGCGCTTCCCAAAAGTTCGCGAGATCCCTTCAACTCCACCAAGTTCAGCTGTCATCCACACCGCTGGAGGACCGCGGCAACCCCGGCGACCTGTC
(476) N A M I G Y R E R V L K V C E I D T R I N K F Q L S F L Y D R A L R C H V L ↓ (EX 12)

1544 TGTTGATGAAGGGCGCCCGAGCGCTGCTGGAGCGCTGTAGTCCATCTCATCAAGGCGCAGGAGCTGCCGCTGGAGAGCAATGGCGGGAGCCCTCCAGACTGCCTACTTATAGCC
(516) V M K G A P E R V L E R C S S I L I K G Q E L P L D E Q W R E A F Q T A Y L S L ↓ (EX 13)

1664 TGGGGGGCTGGGGGAACCGCTGCTCGGTTCTGCTGAGTGTACCTGAGTGAAGAAGGACTCCCGCTGGCTATGCTTGTGATGGAGCCATCAACTCCCAACTAGTGGCTGTGCT
(556) G G L G E R V L G F C Q L L S E K D Y P F G Y A F D V E A M N F P T S G L C F ↓ (EX 14)

1784 TTGGGGACTTGTATCATGATAGACCCCGCTCGGGCCACCGTCCCTGATGCTGCTCAAGTGGCCACCGCGGGCATCCGGGTGATCATGTTAGAGGGTGCACCCCATCAGGCCA
(596) A G L V S M I D P P R A T V P D A V L K C R T A G I R V I M V T G D H P I T A K ↓ (EX 15)

1904 AGGCCATTCACGCTGGGCACTATTTCGGAGGGCAGCAGACAGTGGAGACACTCCGCCCGCCCTCGGGTGGCTGGAACCAAGTAAATAGGAAGCATGCCCGGCTGCTCA
(636) A I A G S I V G S E S E T V E D A I A R L R V P V D Q V N R K D A R A C V I ↓ (EX 16)

2024 TCAATGGCATGACGCTGAAGGACATGACCCGCTCCGAGCTGGTGGAGCGCTGGGACTCACCCGAGATGGTGTTCGCTGACAGTCCCGCAGCAAGCTGGTGTGCTGGAGAGCT
(676) N G M Q L K D M P S E L V E A L R T H P E A M V F A R T S P Q K L V I V E S C ↓ (EX 17)

2144 GCCAGAGACTGGTGGCATGTGGCGGTGACCGGGTGAACGACTCCCGGCCCTGAAGAAGGCCGACATCGGGCTGGCCATGGCCATTCGCCGCTGGAGCTGCCAAAAATG
(716) Q R L G A I V A V T G D V N D S P A L K K A D I G V A M G I A G S D A A K N A ↓ (EX 18)

2264 CAGCGACATGATCTGCTGGATGACAATTTGCTCCATGCTGACAGGGCTGGAGCGGCTGATCTTTGATAACCTGAAAAGTCCATGCCTACAGCTGACCAAGAACATCC
(756) A D M Q L L D D N F A S I V T G V E Q G R L I F D N L K K S I A Y T L P K N I P ↓ (EX 19)

2384 CCGAGCTGACCGCTACCTCATCATACACCGCTGACGCTGCCCTGCCCTCGGGTGCATACCATCTCTTCATAGAGCTCTGACCGCAGATTTCCCGTGGGTCTCCCTGGGTATG
(796) E L T P Y L I Y I T V S V P L P L G C I T I L F I E L C T D I F P S V S L A Y E ↓ (EX 20)

2504 AQAAGCGGAGAGTGATCATGACCTGCGCCCAAGCAACCAAGAGACCGATGGTCAACAGCCCGCTGGCTGCTACTCTTCCAGATCGGTGCCATCCAGTCACTGATCTGCT
(836) K A E S D I M H L R P R N P K R D R L V N E P L A A Y S Y I G A I Q S F A G ↓ (EX 21)

2624 GCTTACCGACTACTTACCGGCCATGGCCAGGAGGGCTGGTCCCGTGTGCTGTGGGCTCGCGCGTACTGGGAGAACCACCACTACAGGATCTGCAGGACAGCTACGCCAGG
(876) F T D Y F T A M A Q E G W F P L L C V G L R P Y W E N H H L Q D L Q D S Y G Q E ↓ (EX 22)

2744 ACTGACGTTTCGGCGAGCGCTGTACCAGCAGTACACCTGCTACACCGTGTCTTTCATCAGCATCGAGATGTGCCAGATCGTGCAGCTCTCATCCGCAAGACCGCCCGCTGTGCTGCT
(916) W T F G Q R L Y Q Q Y T C Y T V F I S I E M C Q I A D V L I R K T R R L S A F ↓ (EX 23)

2864 TCCAGCAGGCTTCTTCAGGAACAGGATCTGGTGAACGATCGTGTCCAGGTGTCATCGGCTGCTTCTGCTGCTACTGCCCGGAATGCCCAACTCTTCAACTCATGCCATTC
(956) Q Q G F F R N R I L V I A I V F Q V C I G C F L C Y C P G M P N I F N F M P I R ↓ (EX 24)

2984 GGTACCAGTGGTGGCTGCTCCCATGCGCTTTGGCTCCTCATCTTCTGTATGATGAGATTCGGAACCTGGAGTTCGCTGTTGCCCGAGGAGCTGGTGGGATCAGGAGCTTACTATT
(996) Y Q W W L V P M F Y L L I F V Y D E I R K L G V R C C P G S W W D Q E L Y Y * ↓ (EX 25)

3104 AGAGGAACCATCCCTGCACCCCGCAGCAGAGGTGGGGCATGGGACCGCTGGGCGCCACTGGAACATCAAAGGACCAAGGAGCCCTGCCACCTGTGCTGAGCTCTGCAGCCACG
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3224 GCGCCCGACTTGGAGGCGCTCCCGCTCCCTTCCCATCCACAGAGACCCACCCGTCAGGGCGTCCCTGGCCAGGCTCTGGTCAAGAGTGGGAGGGGGGTGGTGGCAG

3344 GAGTGGGCGCTGCTGCTGCTGAGCGGGTCTGCATCGGGTCCCGAGAGGCTCCCGAGGCTACTGCTCAATACACACTGTCCGCGGTAGTATATAACCACTACACTGA

3464 CGAAAAAAAAAAAAAAAAA

FIGURE 3. cDNA and deduced amino acid sequences of canine H⁺, K⁺-ATPase α subunit. Nucleotide sequences are numbered sequentially in the left column from the initiation ATG codon (+1) through the polyadenylated tail. Numbers in parentheses on the left column denote amino acid sequence position; amino acids (in the single letter code) appear below the nucleotide sequence. Exon boundaries are indicated by arrows. The stop codon and polyadenylation signal sequence begins at nucleotides 3103 and 3444, respectively.

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-40 CCCGTGTTCTGCAGGAGGGGACTCCAGGGGCCGGGACGATGGCAGCACTACAGGAGAAAA
      M A A L Q E K K      8
60 AGTCATGCAGCCAGCGGATGGAGGAGTTCACGCGCTACTGCTGGAACCCAGACACGGGGC
      S C S Q R M E E F Q R Y C W N P D T G Q      28
80 AGATGCTGGGCGGACACTGTCCCGGTGGGTGGGATCAGCCTCTACTACGTAGCCTTCT
      H L G R T L S R W V W I S L Y Y V A F Y      48
140 ACGTGGTGATGACTGGAATCTTTGCCCTGTGCATTTACACGCTGATGTGCCACTTGACC
      V V M T G I F A L C I Y T L M C T L D P      68
200 CATAACCGCCTGACTACCAAGACAGCTAAAATCACCAGGGGTGACCTTGAGGCCCGATG
      Y T P D Y Q D O L K S P G V T L R P D V      88
260 TCTATGGGAGAAAGGCTGGACATTTCCACACGCTCTGTGACAACAGGACCTGGGTAG
      Y G E K G L D I S Y N V S D N R T W V D      108
320 ACCTTGGAACATCCTCCACAACCTTGGAAAGGCTACTCGCCACATCCAGGAGGACA
      L V N I L H N F L E G Y S P T S Q E D N      128
380 ATATCACTGTACCTCTGAGAAGTACTTCTTCAGGACGTTTTTTGGGGCCCAATCACA
      I N C T S E K Y F F Q D V F G A P N H T      148
440 CCAAGTTCCTGCAAGTTCATGGCAGACATGCTGCAAAACTGCTACGGCCTGCAGAGACC
      K F S C K F M A D M L Q N C S G L T D P      168
500 CCAACTTCGGCTTTGCAGAAGAAAGCGGTATTTATTATAAATGAACAGGATCGTGA
      N F G F A E G K P C F I I K M N R I V N      188
560 ACTTCTCCCAAGCAACAGCACAGCGCCAGGGCCGACTGCACCTTCTGGACAGCACA
      F L P S N S T A P R A D C T F L D Q H K      208
620 AGGATGACCGCCCTGACAGTGAATATTACCCACCCACGGTACCTCCAGCCTGGCT
      D D R P L Q V E Y Y P P N G T F S L R Y      228
680 ATTTCCCTTACTACGGGAAAAGCGCAGCCCACTACAGCAACCCCTCTGGTCGCCCGCA
      F P Y Y G K K A Q P H Y S N P L V A A K      248
740 AGCTTCTCAATGTCAGAACACGGAAGTCTCATTGTGTGCAAGATCTGGCAGATT
      L L N V P R N T E V L I V C K I L A D Y      268
800 ATGTACCTTTGACAACCCCATGACCCCTATGAAGGGAAAGTGGAGTCAAGCTCACA
      V T F D N P H D P Y E G K V E F K L T I      288
860 TTCAGCAGTAGAGCAAGGAGGCTGCACCCCTGGCCCCATGACCCCTATGAAGGGAAAG
      Q Q ***      290
920 TGGAGTTCAAGCTCACCATTCAGCAGTAGAGAGCAAGGAGGCTGCACCCCTGGCTGCCAC
980 CCCCCTGCCATCCCTGTACTCTCTCTAGTCCCGTC

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FIGURE 4. cDNA and deduced amino acid sequences of canine H⁺, K⁺-ATPase β subunit. Nucleotide sequences are numbered on the left column with respect to the initiation ATG codon (+1). Numbers on the right column denote amino acid sequence position; amino acids are notated by the single letter code. The stop codon (TAG) is indicated by asterisks at nucleotides 268 to 270.

exon 10 and Lys⁵¹⁸ encoded in exon 11 may represent pyridoxal 5'-phosphate and ATP-protectable fluorescein isothiocyanate (FITC) binding sites respectively [2]. Both canine and rat H⁺, K⁺-ATPase cDNAs encode a -KKEK- amino acid sequence (residues 38 - 41 of canine protein) which is also contained in the cytoskeletal protein villin and may represent a potential actin binding site involved in tethering the α subunit to the apical membrane [37].

cDNA Sequence of Canine H⁺, K⁺-ATPase β Subunit. A single clone encoding the full length canine β subunit cDNA was isolated from a canine gastric cDNA library using the partial length PCR-generated canine H⁺, K⁺-ATPase β subunit. Its nucleotide sequence contained 870 bp which encoded 290 amino acids (Fig. 4). The amino acid sequence exhibited 80 - 85% identity with the H⁺, K⁺-ATPases encoded by cDNAs isolated from rat, rabbit, pig and human stomach [19-23]. Like its counterparts from other species, the canine H⁺, K⁺-ATPase β cDNA encodes an -FQRY- amino acid sequence (residues 17 - 20) located in the cytoplasmic domain which is similar, but opposite in orientation, to that found in the transferrin receptor [38]. Gottardi and Caplan [36] have speculated that this sequence may form a tight-turn recognition motif capable of acting as an orientation-independent signal for the rapid internalization of the β subunit into the preapical tubulovesicular compartment of parietal cells. This event may occur after the withdrawal of acid secretory signals and may result in a relative reduction in H⁺, K⁺-ATPase activity.

ACKNOWLEDGMENTS

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