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Primary structure of avian H^+/K^+-ATPase β-subunit

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Abstract

A cDNA encoding a β-subunit of the avian H^+/K^+-ATPase was cloned from a chicken stomach cDNA library, and its nucleotide sequence determined. A comparison between all the available sequence data for the β-subunits of P-type ATPases reveals several evolutionarily conserved regions. Overall identity was 66% when compared with mammalian H^+/K^+-ATPase β-subunits, 34% identity when compared with the Na^+/K^+-ATPase $\beta 2$-subunits, and 33% identity when compared with the Na^+/K^+-ATPase $\beta 1$-subunits.

Key words: ATPase, Na^+/K^+-; ATPase, H^+/K^+-; Beta subunit; Isoform; Evolution; (Chicken)

The H^+/K^+-ATPase and the Na^+/K^+-ATPase consist of two subunits: the catalytic α-subunit and the glycoprotein β-subunit. Several lines of evidence indicate that the proper functional expression of the α-subunit requires its assembly with the β-subunit [1,2]. Recently, it was shown that the β-subunit of the rabbit H^+/K^+-ATPase can assemble with the α-subunit of the *Xenopus* [3] and *Torpedo* Na^+/K^+-ATPase [4], suggesting the existence of evolutionarily conserved domain(s) within both subunits. The critical domains of the α-subunit required for such assembly reside in a 160 amino acid stretch within the carboxy terminus of the α-subunit [5]. The corresponding domains of the β-subunit required for assembly have not been identified, although it has been suggested that these domains likely reside in the extracellular regions [6]. Recent cDNA cloning and sequence studies have produced amino acid sequences for mammalian and other vertebrate β-subunits [7–16]. In this study, we add an avian H^+/K^+-ATPase β-subunit sequence to this family. Multiple sequence alignment in this family suggests common sequences preserved through evolution.

Total RNA was isolated from adult chicken stomach using the acid/phenol extraction method [17]. Polyadenylated RNA was purified through two rounds of oligo-(dT)-cellulose chromatography, converted to cDNA by oligo(dT)-primed reverse transcription, and ligated into the *Eco*RI site of a phage, λ-Zap II (Stratagene). This cDNA library was screened using random-primed cDNA fragments encoding the rat gastric H^+/K^+-ATPase β-subunit [8] under a moderate stringency (hybridization in the presence of 20% formamide at 42°C and washing in $3 \times$ SSPE at 42°C). The longest cDNA clone among several overlapping ones was analyzed using standard DNA-sequencing methods [18].

Fig. 1 shows the nucleotide sequence of the cDNA and its deduced amino acid sequence. The encoded protein (299 amino acids) contains a single hydrophobic region, which can provide a unique transmembrane domain (M1) that is close to the amino-terminus. In addition, the protein contains six conserved Cys and several potential Asn-linked glycosylation sites between M1 and the carboxy-terminus. These features are found in all known P-type ATPase β-subunits, although the number and the positions of potential Asn-linked glycosylation sites vary among β-subunit isoforms (see text below and Fig. 4). The encoded protein shares 65.9% amino acid identity with the human H^+/K^+- and 65.6% identity with the rat H^+/K^+-ATPase β-subunit. As expected, it shares less identity with the

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chicken Na^+/K^+-ATPase β-subunits (34% and 33% with the β1- and β2-subunits, respectively). Thus, the cloned cDNA encodes a chicken H^+/K^+-ATPase β-subunit.

To explore the evolutionary relationships between the P-type ATPase β-subunits, we constructed a multiple alignment of the 12 amino acid sequences (Fig. 2) and examined the phylogeny of these β-subunits using several programs for constructing evolutionary trees. Both distance and parsimony methods produced the same tree (Fig. 3). This tree suggests that there are four divergent families of β-subunits; the H^+/K^+-ATPase β-subunit, the Na^+/K^+-ATPase β1-subunit, the mammalian Na^+/K^+-ATPase β2-subunit, and the non-mammalian Na^+/K^+-ATPase β2-(chicken)/β3-(*Xenopus*) subunits. The phylogenetic analysis groups the ion-specific subunits and the different β-subunit isoforms as expected. However, it is interesting to note that the *Xenopus* β3-subunit is most closely related to the chicken β2-subunit, and that these two subunits are not significantly more closely related to the mammalian β2-subunits than they are to the other ATPase β-subunits. This fact implies a few alternative possibilities. One possibility is that there is no mammalian-type β2-subunit in birds and frogs, and the non-mammalian β2/3-isoform is the counterpart of the mammalian-type β2-subunit in certain cell-types of chicken and *Xenopus* (as suggested by mRNA distribution [16]). Another possibility is that the chicken β2-subunit might be more appropriately called a β3-type, and that one might expect to find additional amphibian and avian 'β2'-subunits, as well as mammalian 'β3'-subunits. Interestingly, it has been suggested that at least three different Na^+/K^+-ATPase β-subunits exist in the chicken [22].

From an evolutionary perspective, each isoform is simply in a different branch of the tree and the four branches are approximately equally distant from one another. Nevertheless, a recent observation on heterologous subunit assembly between the mammalian H^+/K^+-ATPase β-subunit and the amphibian and teleost Na^+/K^+-ATPase α-subunits [3,4] suggests the existence of a common mechanism of P-type ATPase subunit assembly. Our avian H^+/K^+-ATPase β-subunit also assembles with the avian Na^+/K^+-ATPase α1-, α2- and α3-subunits (manuscript in preparation). We applied a PLOTSIMILARITY program to the deduced amino acid sequences, and identified three major conserved regions (Fig. 4); Region I including the single hydrophobic domain (position Gly^{31}-Pro^{86}),

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5'.....GAATTCGGCACGAGA.GGCAGTGATCTCTTCAACCTTTAGTGGAGGGGAAGGTATAAAAACCAAAGGCAGTCTGCTTGGGCAACTCTTTGAGCCCC

TGTTGAATTTCTTCATTTCTTCATTTCCGAGGAAGACGCTTTTTGGGGAAATTTTGTGGCAAA ATG GCA ACT TTA AAT GAA AAG AAG ACC TGC AGT
                                                                  Met Ala Thr Leu Asn Glu Lys Lys Thr Cys Ser

GAA CGG ATG GAA AAT TTC CGC CGT TTT GTC TGG AAT CCA GAA ACT AAG CTG TTT ATG GGA AGG ACC TTG ATT AAC TGG GTG
Glu Arg Met Glu Asn Phe Arg Arg Phe Val Trp Asn Pro Glu Thr Lys Leu Phe Met Gly Arg Thr Leu Ile Asn Trp Val

TGG ATC AGC CTG TAC TAC CTG GCC TTC TAC GTG GTG ATG ACT GGG ATC TTC GCC CTC TCC ATA TAC TCC CTA ATG AGG ACG
Trp Ile Ser Leu Tyr Tyr Leu Ala Phe Tyr Val Val Met Thr Gly Ile Phe Ala Leu Ser Ile Tyr Ser Leu Met Arg Thr

GTC AAT CCC TAC GAG CCA GAT TAC CAA GAC CAG CTC AAG TCC CCA GGT GTA ACG TTA CGA CCG GAT GTG TAT GGG CAC AGA
Val Asn Pro Tyr Glu Pro Asp Tyr Gln Asp Gln Leu Lys Ser Pro Gly Val Thr Leu Arg Pro Asp Val Tyr Gly His Arg

GGG CTG CAA ATC TAC TAC AAT GCA TCT GAC AAC AAG ACC TGG GAG GGG CTG GTG ACA ATG CTC CAG ACG TTT CTG ACA GCA
Gly Leu Gln Ile Tyr Tyr Asn Ala Ser Asp Asn Lys Thr Trp Glu Gly Leu Val Thr Met Leu Gln Thr Phe Leu Thr Ala

TAT AGC CCA GCT GCT CAG CAT CTG AAC ATC AAC TGC ACA AGC AAC ACA TAC TTC ATC CAG AAC ACC TTT GAT GGC CCA AAT
Tyr Ser Pro Ala Ala Gln His Leu Asn Ile Asn Cys Thr Ser Asn Thr Tyr Phe Ile Gln Asn Thr Phe Asp Gly Pro Asn

AAC ACA AAA CTG TCC TGC AAG TTT ACA TCA GAT ATG CTT CAA AAC TGC TCT GGC ATC ACA GAT CCT ACT TTT GGA TTT CCA
Asn Thr Lys Leu Ser Cys Lys Phe Thr Ser Asp Met Leu Gln Asn Cys Ser Gly Ile Thr Asp Pro Thr Phe Gly Phe Pro

GAA GGA AAA CCT TGC TTT ATT GTA AAG ATG AAC AGG ATT ATC AAA TTT TAT CCT GGC AAC GGC ACC GCA CCG AGA GTG GAC
Glu Gly Lys Pro Cys Phe Ile Val Lys Met Asn Arg Ile Ile Lys Phe Tyr Pro Gly Asn Gly Thr Ala Pro Arg Val Asp

TGC AGC TAT GTA GGT GAC GAG TCC CGC CCG CTG GAG GTA GAA TAC TAC CCG GTG AAT GGA ACC TTC AAC CTG CAC TAC TTC
Cys Ser Tyr Val Gly Asp Glu Ser Arg Pro Leu Glu Val Glu Tyr Tyr Pro Val Asn Gly Thr Phe Asn Leu His Tyr Phe

CCC TAC TAT GGA AAG AAG GCA CAG CCC ACT TAC AGT AAT CCT TTG GTA GCT GTG AAA TTT CTC AAC ATC ACA AAG AAT GTA
Pro Tyr Tyr Gly Lys Lys Ala Gln Pro Thr Tyr Ser Asn Pro Leu Val Ala Val Lys Phe Leu Asn Ile Thr Lys Asn Val

GAA GTC CAG ATA GTG TGC AAG ATC ATT GGA GCC GGG ATT ACC TTC GAT AAC GTT CAT GAC CCA TAT GAA GGA AAA GTG GAA
Glu Val Gln Ile Val Cys Lys Ile Ile Gly Ala Gly Ile Thr Phe Asp Asn Val His Asp Pro Tyr Glu Gly Lys Val Glu

TTT AAA TTG AAA ATA GAG GAT GGA GCA GCG AGA GAC ACC TCT AAA AAG CAT GTG TGA AGA ACG ACT TAC CTGTTGTCACACTGA
Phe Lys Leu Lys Ile Glu Asp Gly Ala Ala Arg Asp Thr Ser Lys Lys His Val ***

CTGATATTTATTTTCTATGATTGCCATATGAATGTACTCAAATTACAGTGACAGTAGACATCTTCTACTGAGGGGTAACCCTATATAACTAAAAATCAGTACATCAC
TCAGCGTGAGAAGGAGATGAGAATTATCCCCAGTGTACACAGTCTAGTGTTCTGTGGCTACACTGTGCTTAAACCTCCGTGCAAATCCTTGTACAAAACATGTGTCT
TCTAACCCAAGCAGGTGTGCCTTCCCAGAAAAGGAAGTATGGGATCAAAACATTGTTCTTCATCATTTTTCGAACTCTAATAAATAACTGATGTATAATATTGAATA
CTGGATACTGTAGGAGCAATTAGGATAGCTAGAAAAATAAGTAGCTCAAAATACTGTGAGATAATAATACCCTTCTCGATCTGCCTTGTACAGGATGTTTAAACTTT
AAAGAGACAGCAAATTAACTGCATGCCAATGCATTGCTTTGTGTACAAGCCAGCCGAGTCTGACTTCAGATGAGAAATCACTCACTGTAAGAAGAAGAACTTGAAAG
GGGCCTGTAGCTTTTCTAATACCTAGCACCTGTGATCTCGTGCCGAATTG.....3'
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Fig. 1. Nucleotide and deduced amino acid sequences of a chicken H^+/K^+-ATPase β-subunit cDNA. Potential N-linked glycosylation sites and unique transmembrane domain are underlined.

Region II between Cys[158]-Arg[192] and Region III between Leu[256]-Lys[320]. These regions might be involved in subunit assembly, since the regions of the β-subunit isoforms that interact with the α-subunits are possibly conserved throughout evolution. There are several N-linked glycosylation sites that are conserved within isoforms; 7–8 in the H^+/K^+-ATPase β-, 3–4 in the Na^+/K^+-ATPase β1-, 8 in the mammalian Na^+/K^+-ATPase β2-subunits, and 4 in non-mammalian Na^+/K^+-ATPase β2-/β3-subunits, respectively. It is interesting to note that almost all the N-linked glycosylation sites reside within the variable domains (Fig. 4), and the blocking of in vivo Asn-linked glycosylation by tunicamycin does not prevent subunit assembly [23–25].

The general characteristics of the primary structures described here should be useful for further experimental design to identify important functional domains within the P-type ATPase β-subunits.

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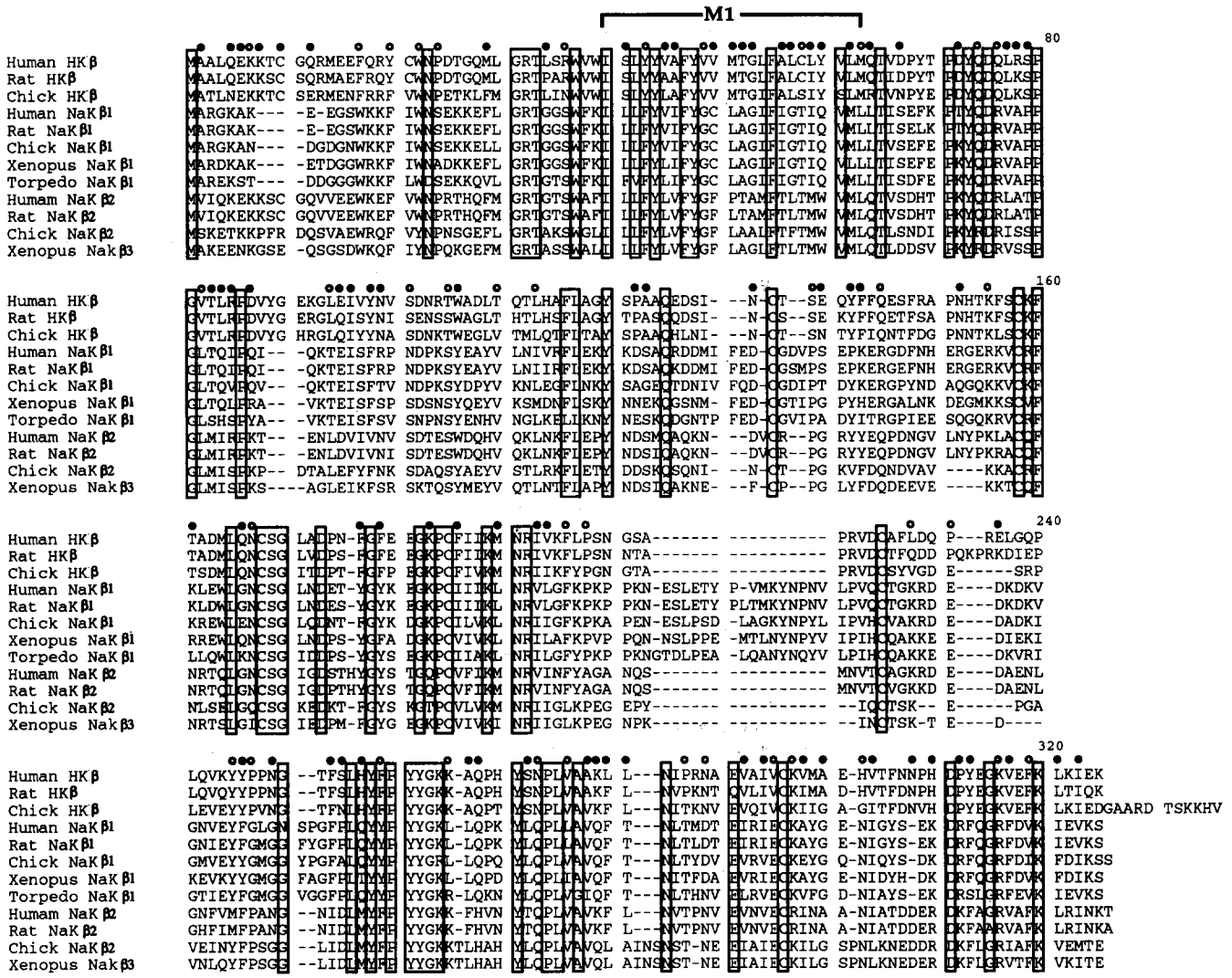

Fig. 2. Alignment of amino acid sequences of P-type ATPase β-subunits. To maximize alignment, minimum numbers of space (–) were added. 12 sequences are shown; human H^+/K^+-β [7], human Na^+/K^+-β1 [10], human Na^+/K^+-β2 [9], rat H^+/K^+-β [8], rat Na^+/K^+-β1 [11], rat Na^+/K^+-β2 [9], chicken Na^+/K^+-β1[12], chicken Na^+/K^+-β2 [16], *Torpedo* Na^+/K^+-β1 [14], *Xenopus* Na^+/K^+-β1 [15], and *Xenopus* Na^+/K^+-β3 [15]. Evolutionary conserved regions in which 11 of 12 sequences are identical are boxed. 58 amino acids (17% of the total of 336 positions) are conserved throughout β subunit-isoform evolution. 39 amino acids (open circles (○)) are conserved between three isoforms (e.g., Pro-247 for the H^+/K^+-β, mammalian Na^+/K^+-β2 and non-mammalian Na^+/K^+-β2/3 subunits), and 78 amino acids (filled circles (●)) are conserved between two isoforms (e.g., Gln268 for the H^+/K^+-β and Na^+/K^+-β1 subunits). Among them, 16 amino acids are preserved in an ion-pump specific manner; the amino acids in the H^+/K^+-and the Na^+/K^+-ATPase are distinct but conserved within the ion-pumps (e.g., Gln-76 (H^+/K^+-β) and Arg-76 (Na^+/K^+-β)). Within variable regions (not marked), there are 27 amino acids that are preserved only within isoforms (e.g., Phe-305 (H^+/K^+-β), Thr-305 (mammalian Na^+/K^+-β2), Asn-305 (non-mammalian Na^+/K^+-β2/3) and Tyr-305 (Na^+/K^+-β1)).

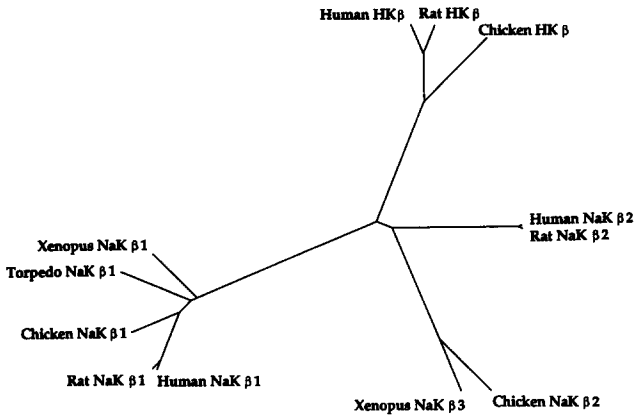

Fig. 3. Evolutionary relationships among the P-type ATPase β-subunits. Each of the sequences in Fig. 2 was compared to each of the others using a global alignment algorithm [19] The resulting distances were used to build an evolutionary tree using the neighbor-joining program [20] from the PHYLIP package [21]. Analysis of the data using other programs for constructing distance trees and the PROTPARS program all found the same tree topology.

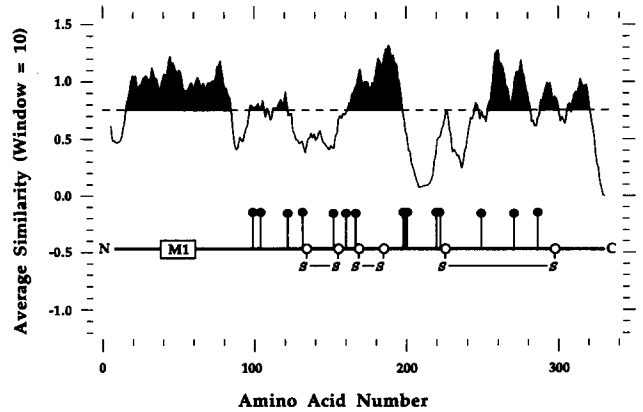

Fig. 4. Distribution of conserved regions in the P-type ATPase β-subunits. The average identity within a ten residue window for the alignment shown in Fig. 2 was calculated with the PLOTSIMILARITY program of the Genetics Computer Group sequence analysis package. (Inset) Three conserved S–S bonds (○) and the potential transmembrane domain (M1) are indicated. All potential N-linked glycosylation sites (●) identified in all β-subunit isoforms are also shown.

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