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# Structure of the $\alpha_1$ subunit of horse Na,K-ATPase gene

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Genomic DNA for Na,K-ATPase $\alpha_1$ subunit was obtained from libraries of horse kidney genomic DNA in Charon 4A and in EMBL3 bacteriophages by screening with the full sized cDNA probe of the $\alpha_1$ subunit of rat Na,K-ATPase as probe. The gene spans 30 kb and consists of 23 exons and 22 intervening sequences. Intron-exon boundaries were analyzed. The protein-coding nucleotide sequence encodes 1016 amino acids with an $M_r$ of 112 264. The putative amino acid sequence of horse $\alpha_1$ is 96–97% homologous to those of other mammalian species.

ATPase, (Na+K)-; Subunit, $\alpha_1$; DNA; Gene structure; Primary structure; (Horse)

## 1. INTRODUCTION

Na,K-ATPase is the plasma membrane protein responsible for active transport of $Na^+$ and $K^+$ across the cell membrane in most animal cells. It consists of at least two subunits, $\alpha$ and $\beta$. The primary structures deduced from cDNAs of $\alpha_1$ subunits from rat [1–3], human [4], sheep [5], pig [6], chicken [7] and *Torpedo* [8] are similar to each other. Isoforms of the catalytic unit, $\alpha_2$ ($\alpha^+$) [1,3] and $\alpha_3$ ($\alpha$III) [1,2], in rat brain have been identified by cDNA cloning and sequencing. Expression of isoform genes has been studied by determining the mRNA levels in various tissues and in different stages of development [3,9–15]. Multiplicity of human Na,K-ATPase genes encoding isoforms has been suggested [16,17] on the basis of studies on genomic DNA. Four genes including $\alpha_1$ and $\alpha_2$ have been identified by screening a human leucocyte genomic library [17]. In a genomic library from human placenta, 5 genes including $\alpha_1$, $\alpha_2$ and $\alpha_3$ have been isolated [16]. Isoforms are products of separate genes and the total length of an $\alpha$ gene is reported to be 20 to 25 kb [17]. Most of the intron-exon boundaries of the $\alpha_3$ subunit of human gene have been analysed [18]. Many of the introns are located at the boundaries of functional domains.

Horse kidney Na,K-ATPase has been used to determine the minimal structural unit, $\alpha\beta$ monomer, possessing enzyme activity [19,20] and a monoclonal antibody against the horse kidney $\alpha_1$ subunit which recognizes an extracellular domain has been prepared and characterized [21]. However, the primary structure of horse Na,K-ATPase has not been studied. We have obtained the genomic DNA that corresponds to the $\alpha_1$ subunit. The present study was undertaken to determine the complete intron-exon organization of $\alpha_1$ subunit DNA of horse Na,K-ATPase and at the same time to analyse the deduced amino acid sequence in relation to the characteristics of the enzyme.

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## 2. MATERIALS AND METHODS

### 2.1. *Construction of genomic library*

Genomic DNA was isolated from horse kidney outer medulla [22]. The first library was made as follows. The DNA fragments of 12–22 kb in length prepared by partial *Eco*RI digestion and elution from agarose gels were ligated to Charon 4A arms and packaged using packaging extract (Amersham). Amplification was done in LE392 cells. Approximately $3 \times 10^6$ plaques transferred to filters were screened with cloned full size cDNA of the rat brain $\alpha_1$ subunit (kidney type) of Na,K-ATPase [2] as probe. Then the hybridization-positive plaques were further screened with a synthetic oligonucleotide probe GGCTGGGT-GGAGAAGGAGACCTACTACTAG corresponding to the C-terminus of $\alpha$-subunit of sheep kidney Na,K-ATPase [5]. The second library was made of 9–23 kb *Sau*3AI fragments in EMBL3 lambda replacement vector. DNA was packaged using Gigapack Gold (Stratagene) and the phages were grown in P2392 cells. LE392 cells were used for plaque purification. Approximately $3 \times 10^6$ plaques were screened, without amplification, using as probe a 0.5 kb *Eco*RI-*Hinc*II fragment from near the 5′-end of clone 6 (designated as probe 1 in fig.1) and also using a 215 bp *Pst*I-*Hae*III fragment coding for the amino-terminus of rat $\alpha_1$ subunit (nucleotide number −45 to 170 [2]) to search for genomic DNA containing amino-terminal exons. A plaque containing insertion DNA spanning from clone 6 to clone 41 was probed with a 0.5 kb *Hinc*II-*Xba*I fragment of horse genomic DNA excised from the carboxyl-terminus of clone 6 (probe 2 in fig.1).

### 2.2. *Hybridization*

The synthetic probe of 30 nucleotides was 5′-end-labeled with [$\gamma$-$^{32}$P]ATP (spec. act. 25.9 TBq, 700 Ci/mmol, ICN) by kination and other probes were labeled with [$\alpha$-$^{32}$P]dCTP (spec. act. 111 TBq, 3000 Ci/mmol, ICN) by nick-translation (nick-translation system or multiprime DNA labeling systems, both from Amersham) and passed through a Sephadex column (NAP-25 from Pharmacia). Hybridization of the plaques on nylon filters (Hybond N, Amersham) with the 30 nucleotide probe and with the rat $\alpha_1$ amino-terminal 215 bp probe was performed at 42°C and hybridization with other probes was at 65°C. Hybridization solution contained 6 × SSC (1 × SSC = 150 mM NaCl and 50 mM sodium citrate), 5 × Denhardt's solution (1 × Denhardt's = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone and 0.02% Ficoll), 0.1% SDS, and 100 $\mu$g of denatured salmon sperm DNA/ml. Washings were performed in 2 × SSC and 0.1% SDS. Southern blot hybridization of phage DNAs and their subcloned DNAs was performed principally under the same conditions as plaque hybridization.

### 2.3. *Subcloning and sequencing*

Genomic DNAs in phages were analyzed by restriction mapping and subcloned into pUC18 or pUC19 plasmid in *E. coli* strain JM83. DNA fragments in pUC18 were subjected to nucleotide sequence analysis using [$\alpha$-$^{35}$S]thio-dCTP (spec. act. 18.5 TBq, 500 Ci/mmol, NEN) and Sequenase (United States Biochemical Corporation) by the dideoxy chain termination method [23]. Large fragments in pUC18 or pUC19 were deleted from one end by use of a deletion system (Takara Shuzo) to obtain appropriate sizes for sequencing.

## 3. RESULTS AND DISCUSSION

### 3.1. *Organization of the gene for Na,K-ATPase $\alpha_1$ subunit*

Screening of approximately $3 \times 10^6$ plaques of recombinant Charon 4A bacteriophages with a rat cDNA of the entire coding region of the $\alpha_1$ subunit as probe yielded 33 positive clones. Eighteen clones had identical restriction profiles (called clone 6). One clone, designated clone 41, hybridized with an oligonucleotide corresponding to the C-terminal probe. Clone 6 carried the majority of exons of the $\alpha_1$ subunit gene of Na,K-ATPase. The second gene library in EMBL3 was screened with probe 1 (fig.1) and 5 clones were obtained. Among them clone 107 was the longest extending to 5′-direction. By probing the second library with probe 2 (fig.1), clone 42, which overlapped with both clone 6 and clone 41, was obtained. The restriction map of the gene was shown in fig.1. Total length of the gene is approximately 30 kb.

### 3.2. *Nucleotide sequences*

Analysis of intron-exon distribution revealed that Na,K-ATPase gene consists of 22 intervening sequences and 23 exons ranging in size from 60 to 269 basepairs. Exon 1 has 12 bp coding region and continues to the 5′-noncoding region. As for the exon 23, 26 bp exon continues to 3′-region (fig.2). First exon which contains coding sequence for only 4 amino acids is 10.5 kb apart from the 2nd exon, whereas most introns are less than 2.30 kb long. This large intron does not seem to contain a region to code for exons of any other form of Na,K-ATPase protein (data not shown). However, it is not known if any promoter sequences exist in this intron. All of the intron junctions are completely consistent with the published consensus sequences for donor and acceptor sites [24]. Six out of 9 splice sites, which occurred within a triplet, were within glycine codons. The organization of the $\alpha_3$ subunit of the human Na,K-ATPase gene has been elucidated [16,18,25] for the most part except for the site of first intron and the splice site of the first intron was presented in the case of human $\alpha_2$ gene [26]. The intron-exon arrangement in the horse $\alpha_1$ gene corresponds to that in human $\alpha_3$ except that intron 10 in horse gene intervenes 2 bp upstream of that in human $\alpha_3$ [18]. The size of introns is variable. However, the organization of exons

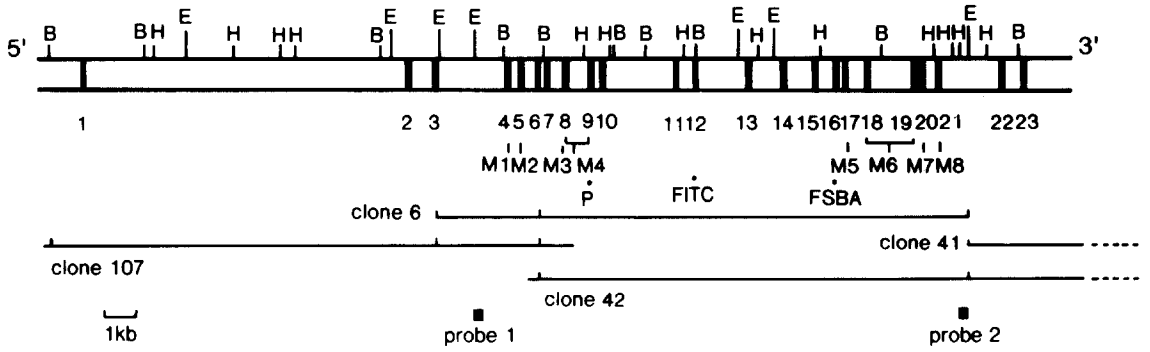


Fig.1. The map of horse Na,K-ATPase $\alpha_1$ subunit genomic DNA. Positions of exons are shown by vertical thick lines. Numbers from 1 to 23 represent exons. Clone 6 and clone 41 were obtained from the Charon 4A library and clone 107 and clone 42 were from the EMBL3 library. Restriction sites of *Eco*RI (E), *Bam*HI (B) and *Hin*dIII (H) are indicated.

seems to be conserved among species and even among isoforms. The horse $\alpha_1$ Na,K-ATPase DNA sequence, composed of 1–23 exons which correspond to the open reading frame of cDNA, has the homology values, rat $\alpha_1$ [2] 87.9%, human $\alpha_1$ [4] 91.2%, sheep $\alpha_1$ [5] 90.6%, pig $\alpha_1$ [6] 90.9%, chicken $\alpha_1$ [7] 80.5%, *Torpedo* $\alpha_1$ [8] 75.2%, rat $\alpha_2$ [1] 75.6% and rat $\alpha_3$ [1] 76.1%. It is interesting that the 5′-noncoding sequence of $\alpha_1$ from horse is similar to those from human [4] and other species [7], but there is no resemblance with $\alpha_2$ from human [26] or rat [1], or with $\alpha_3$ from human [18] or rat [1]. The same features are: the similarity of those of $\alpha_2$ from human [26] and rat [1] and $\alpha_3$ from human [18] and rat [1], and the dissimilarities between isoforms in the same species, such as $\alpha_2$ [26] and $\alpha_3$ [18] from human and $\alpha_1$, $\alpha_2$ and $\alpha_3$ from rat [1]. This would indicate that the leader sequence upstream of the first exon is common in the same isoform irrespective of species. There might be specific sequences characteristic of the isoforms, which are significantly related to the expression of the genes in various tissues.

Since the cap site of horse DNA has not been determined, the exact site of transcription initiation cannot be specified. The comparison of 886 bp sequenced in horse and 318 bp of human $\alpha_1$ cDNA [4] in the 5′-region shows the similarity of the sequence of about 230 bp upstream from ATG translation initiation site. This implies that no introns exist at least in the region of 230 bp upstream of ATG triplet. Sequences which correspond to the TATA box [27] or CCAAT box [28] are not discernible. GC-rich clusters, which are common to housekeeping genes [29], are conspicuous in the 5′-noncoding region. The sequence, CCCTCGCTC, occurs twice at −137 to −129 and −115 to −107. These are located in the vicinity of TCC repeats which are sensitive to S1 nuclease [30]. The sequence, GGGGGCGGAG, at −406 to −397 coincides with the Sp1 promoter-binding sequence [31] and its inverted sequence, GAGGCGGGGG, is located at −277 to −268. The sequence GGCGGAGGAGGCGG at −232 to −219 forms an inverted repeat and this might also be related to the Sp1 promoter. The sequence at −29 to −18, CCCGGCGCCGGG, exists as a palindrome. In the 3′-noncoding region the similarity of sequences of $\alpha_1$ cDNAs from various species are characteristic [7]. The polyadenylation signal AATAAA was observed at 300 bp downstream from the stop codon.

The $\alpha_1$ gene of the horse Na,K-ATPase is thought to represent a single gene separate from genes of other isoforms of the horse enzyme. As regards other cation-transporting ATPases, two isoforms of plasma membrane $Ca^{2+}$-ATPase from rat brain are encoded on separate genes [32] like Na,K-ATPases. In contrast, neonatal and adult type isoforms are produced by the alternative splicing of the 3′-region of the gene for rabbit fast twitch $Ca^{2+}$-ATPase [33]. Further extensive analysis of genomic DNA of Na,K-ATPase should facilitate an understanding of the regulatory factors of expression.

### 3.3. *Comparison of amino acid sequences*

Although the amino acid sequence of the horse

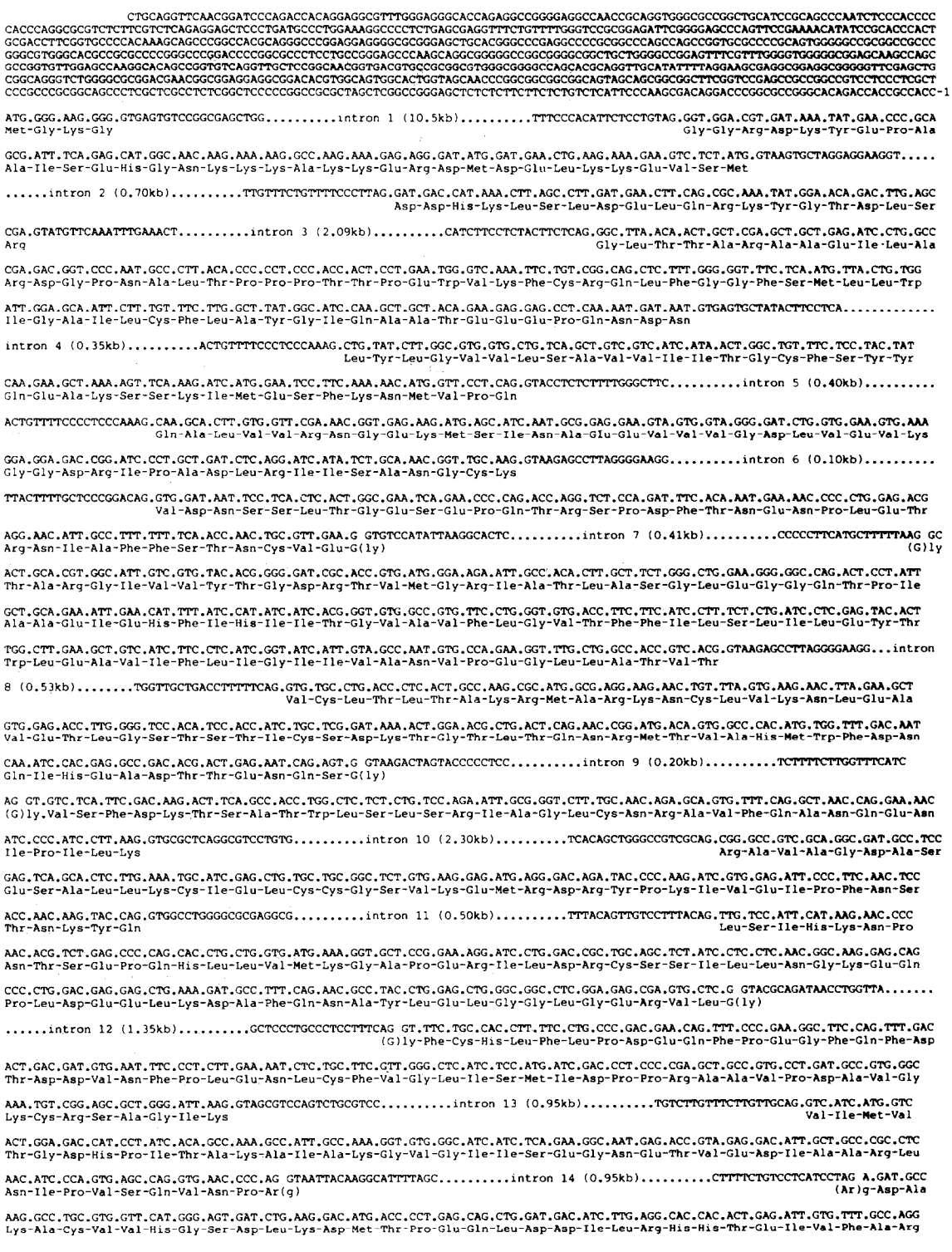


Fig.2. Nucleotide sequence of Na,K-ATPase $\alpha_1$ subunit gene. Lengths of introns are shown in parentheses.

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2071-2118  ACC.TCC.CCT.CAG.CAG.AAG.CTT.ATC.ATC.GTG.GAA.GGC.TGC.CAG.AGG.CAG.GTTCCAAGGTGGCCCTCTCA.....intron 15 (0.61kb).....CATGTCA
           Thr-Ser-Pro-Gln-Gln-Lys-Leu-Ile-Ile-Val-Glu-Gly-Cys-Gln-Arg-Gln

2119-2205  ATGTTTGTGGTAG.GGT.GCC.ATT.GTG.GCT.GTA.ACT.GGC.GAT.GGT.GTC.AAT.GAC.TCT.CCA.GCT.TTG.AAG.AAG.GCG.GAC.ATT.GGG.GTT.GCT.ATG.GGG.ATA.GCT
                     Gly-Ala-Ile-Val-Ala-Val-Thr-Gly-Asp-Gly-Val-Asn-Asp-Ser-Pro-Ala-Leu-Lys-Lys-Ala-Asp-Ile-Gly-Val-Ala-Met-Gly-Ile-Ala

2206-2287  GGC.TCA.GAC.GTG.TCT.AAA.CAA.GCT.GCT.GAC.ATG.ATT.CTT.TTG.GAC.GAC.AAC.TTT.GCC.TCA.ATT.GTG.ACT.GGA.GTA.GAG.GAA.G GTGAGAGCACTACATTT
           Gly-Ser-Asp-Val-Ser-Lys-Gln-Ala-Ala-Asp-Met-Ile-Leu-Leu-Asp-Asp-Asn-Phe-Ala-Ser-Ile-Val-Thr-Gly-Val-Glu-Glu-G(ly)

2288-2337  AAA.....intron 16 (0.16kb).....AAAATATTTTGCCTTCCTAG GT.CGT.CTG.ATC.TTC.GAT.AAC.TTG.AAG.AAA.TCC.ATT.GCC.TAC.ACC.CTG.ACC
                                                                (G)ly-Arg-Leu-Ile-Phe-Asp-Asn-Leu-Lys-Lys-Ser-Ile-Ala-Tyr-Thr-Leu-Thr

2338-2433  AGT.AAC.ATT.CCA.GAG.ATC.ACC.CCC.TTC.CTG.ATA.TTT.ATT.ATT.GCA.AAC.ATT.CCA.CTG.CCC.CTG.GGG.ACT.GTC.ACC.ATC.CTC.TGC.ATT.GAC.TTG.GGC
           Ser-Asn-Ile-Pro-Glu-Ile-Thr-Pro-Phe-Leu-Ile-Phe-Ile-Ile-Ala-Asn-Ile-Pro-Leu-Pro-Leu-Gly-Thr-Val-Thr-Ile-Leu-Cys-Ile-Asp-Leu-Gly

2434-2469  ACA.GAC.ATG.GTAATGATGTCGAGCTTCCA.....intron 17 (0.36kb).....CTCGCCGCCCCTACCCCCAG.GTC.CCC.GCC.ATC.TCC.CTG.GCT.TAT.GAG
           Thr-Asp-Met                                                                                  Val-Pro-Ala-Ile-Ser-Leu-Ala-Tyr-Glu

2470-2565  CAA.GCT.GAG.AGC.GAC.ATC.ATG.AAG.AGA.CAG.CCC.AGA.AAC.CCC.CAG.ACG.GAC.AAA.CTT.GTG.AAT.GAG.CGG.CTG.ATC.AGC.ATG.GCC.TAC.GGA.CAA.ATT
           Gln-Ala-Glu-Ser-Asp-Ile-Met-Lys-Arg-Gln-Pro-Arg-Asn-Pro-Gln-Thr-Asp-Lys-Leu-Val-Asn-Glu-Arg-Leu-Ile-Ser-Met-Ala-Tyr-Gly-Gln-Ile

2566-2601  G GTGAGCTGCCACATGGCGTC.....intron 18 (1.30kb).....TTACTTTTCTAACTTCTTAG GT.ATG.ATC.CAG.GCC.CTA.GGG.GGC.TTC.TTC.ACC.TAC
           G(ly)                                                                (G)ly-Met-Ile-Gln-Ala-Leu-Gly-Gly-Phe-Phe-Thr-Tyr

2602-2697  TTT.GTG.ATT.CTG.GCT.GAG.AAT.GGC.TTC.CTC.CCA.ATT.CAC.CTG.CTG.GGA.CTC.CGC.GTG.GAC.TGG.GAT.GAC.CGC.TGG.GTC.AAC.GAC.GTG.GAG.GAC.AGC
           Phe-Val-Ile-Leu-Ala-Glu-Asn-Gly-Phe-Leu-Pro-Ile-His-Leu-Leu-Gly-Leu-Arg-Val-Asp-Trp-Asp-Asp-Arg-Trp-Val-Asn-Asp-Val-Glu-Asp-Ser

2698-2733  TAC.GGG.CAG.CAG.TGG.GTGAGTGAGCTCCTCAGTTC.....intron 19 (0.09kb).....TTTTGCCTTTTGCGTTTCAG.ACT.TAC.GAA.CAG.AGG.AAA.ATC
           Tyr-Gly-Gln-Gln-Trp                                                                  Thr-Tyr-Glu-Gln-Arg-Lys-Ile

2734-2829  GTG.GAG.TTC.ACC.TGC.CAC.ACA.GCA.TTC.TTC.GTC.AGT.ATC.GTG.GTG.GTA.CAG.TGG.GCC.GAC.TTG.GTC.ATC.TGC.AAG.ACC.AGG.AGG.AAC.TCA.GTC.TTC
           Val-Glu-Phe-Thr-Cys-His-Thr-Ala-Phe-Phe-Val-Ser-Ile-Val-Val-Val-Gln-Trp-Ala-Asp-Leu-Val-Ile-Cys-Lys-Thr-Arg-Arg-Asn-Ser-Val-Phe

2830-2865  CAG.CAG.GGG.ATG.AA GTGTAAAGGACAGTCAGGTT.....intron 20 (0.35kb).....TTTTTTCCTCCTTGACTTTAG G.AAC.AAG.ATC.CTA.ATA.TTT.GGC
           Gln-Gln-Gly-Met-Ly(s)                                                                (Ly)s-Asn-Lys-Ile-Leu-Ile-Phe-Gly

2866-2945  CTC.TTC.GAA.GAG.ACG.GCC.CTT.GCT.GCC.TTC.CTT.TCC.TAC.TGC.CCT.GGA.ATG.GGT.GTG.GCC.CTG.AGG.ATG.TAT.CCC.CTC.AA GTAAGTCCATCCTCTCCAGC..
           Leu-Phe-Glu-Glu-Thr-Ala-Leu-Ala-Ala-Phe-Leu-Ser-Tyr-Cys-Pro-Gly-Met-Gly-Val-Ala-Leu-Arg-Met-Tyr-Pro-Leu-Ly(s)

2946-3000  .....intron 21 (2.17kb).....GGTACCTGCTTTTTCCAG G.CCT.ACC.TGG.TGG.TTC.TGT.GCC.TTC.CCG.TAC.TCT.CTT.CTC.ATC.TTC.GTG.TAT.GAC
                                                                (Ly)s-Pro-Thr-Trp-Trp-Phe-Cys-Ala-Phe-Pro-Tyr-Ser-Leu-Leu-Ile-Phe-Val-Tyr-Asp

3001-3039  GAA.GTC.AGA.AAA.CTC.ATC.ATC.AGG.CGA.CGC.CCT.GGC.G GTAATTACAGGCATTTTAGC.....intron 22 (0.53kb).....CTGTGTTTGCTGCCGCAG GC
           Glu-Val-Arg-Lys-Leu-Ile-Ile-Arg-Arg-Arg-Pro-Gly-G(ly)                                                                (G)ly

3040-3159  TGG.GTG.GAG.AAG.GAG.ACC.TAC.TAC.TAG.ACGCCATCCTGCAGCCGCGGAATCGCTCACCCTGCACCCCTCACCCACCCCCCTCTGTGTACTTCAGTCCCGGAAGCTCGGAACTCTACCCTG
END (3063) Trp-Val-Glu-Lys-Glu-Thr-Tyr-Tyr-***

3160-3288  GTAGGAAGGCACCGAAGCATGTGGGGGAAGCCAGACGTCCCGGAATGAAGCATGTAGCTATATGGGGGGAGGGGGGAGGCTGCCTGAAAACCATCCATCTGTGGGAATGACAGCGGGGAAGGTTTTTAT
3289-3417  GTGCCTTTTTGTTTTTGTAAAAAGGATAACTCGGAAAAGACTGAAAGAATACATTTTATATCTGGATTTTTACAAATAAAGATGGCTATTATAATGGAATTTGCCTCATGTCCTTCATCACTTCTGTGG
3418-3546  TCCAGATGTCTGTGTGTTGTGTGAGGATCTGAGCTCACGGGGGCTGCTGCACAGTCCCCAGCCTGGAGACAGGTGCCTGGGCAGCCTCTGTGGGAGGGCTAAGCTTTCCTGACGACCAGCTCGTGCAGT
3547-3569  ATGTGAGCCAAACAGGTGGTCCC
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enzyme protein has not been determined, the N-terminal 5 amino acids might be cleaved off post-translationally as observed in other Na,K-ATPases. The mature protein would then consist of 1016 amino acids and its $M_r$ would be 112264. The amino acid sequence of horse $\alpha_1$ deduced from the exons has the homology values of rat $\alpha_1$ [2] 95.8%, human $\alpha_1$ [4] 97.5%, sheep $\alpha_1$ [5] 97.0%, pig $\alpha_1$ [6] 97.5%, chicken $\alpha_1$ [7] 93.8%, *Torpedo* $\alpha_1$ [8] 86.2%, rat $\alpha_2$ [1] 87.2% and rat $\alpha_3$ [1] 85.6% (fig.3). The horse gene obtained here was proved to correspond to the $\alpha_1$ subunit gene on the basis of the extensive homology of nucleotide and amino acid sequences with those of $\alpha_1$ from other species, but not those of $\alpha_2$, $\alpha_3$ or any other isoforms. Eight transmembrane segments have been predicted [5] from hydrophobicity. Transmembrane segments M1, M2, M3, M5, M7 and M8 [5] were located within the exons 4, 5, 8, 17, 20 and 21, respectively, but M4 and M6 were interrupted by intron 8 and intron 18, respectively (fig.1 and fig.2). Extensive similarities were observed in exons containing the site of phosphorylation [34] in exon 9, the FITC-binding site [35] in exon 12, the FSBA-binding site [36] in exon 16, and in addition, the transmembrane segments and extracellular domains [5] show high homology. These essential segments with important physiological functions and the organization of membrane folding are common in most species. Variable sequences occur rather non-randomly in exons 10, 12, and 13 and exon 19, all of which are on the cytoplasmic side. Variability of the N-terminus is distinct. The amino acid sequence of horse $\alpha_1$ is the most homologous to human $\alpha_1$ and among the 26 different amino acids, 11 are located in the N-terminal intracellular portion.

113Arg and 124Asp at the M1-M2 junction of rat $\alpha_1$ are uniquely different from those of the ouabain-sensitive enzyme species. These differences were suggested to be responsible for the ouabain insensitivity [37]. The horse kidney en-

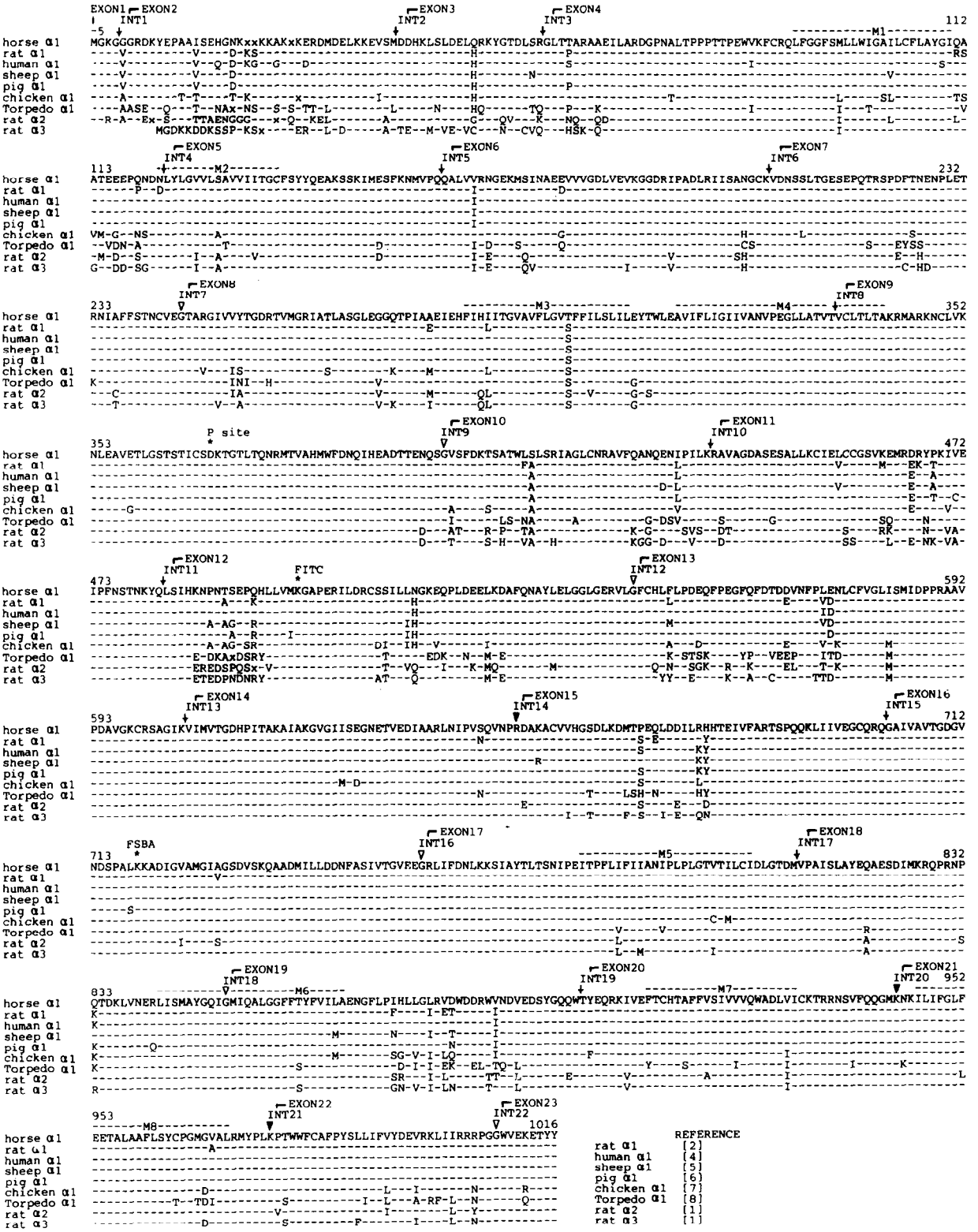
EXON1
EXON2
EXON3
EXON4
INT1
INT2
INT3
M1
EXON5
EXON6
EXON7
INT4
INT5
INT6
M2
EXON8
EXON9
INT7
INT8
M3
M4
EXON10
EXON11
P site
INT9
INT10
FITC
EXON12
EXON13
INT11
INT12
EXON14
EXON15
EXON16
INT13
INT14
INT15
EXON17
EXON18
FSBA
INT16
INT17
M5
EXON19
EXON20
EXON21
INT18
INT19
INT20
M6
M7
M8
EXON22
EXON23
INT21
INT22
horse α1
rat α1
human α1
sheep α1
pig α1
chicken α1
Torpedo α1
rat α2
rat α3
REFERENCE
rat α1 [2]
human α1 [4]
sheep α1 [5]
pig α1 [6]
chicken α1 [7]
Torpedo α1 [8]
rat α2 [1]
rat α3 [1]

Fig.3. Comparison of amino acid sequences of Na,K-ATPase subunits in various species. Positions of introns are indicated by the following symbols: class 0 introns [39] interrupting the reading phase between codons, arrow; class I introns between the first and second nucleotides of the triplet, white arrowhead; and class II introns between the second and third nucleotides, black arrowhead.
The M1-M8 membrane spanning regions [5] are indicated above the sequence. Gaps are introduced to optimize alignment.

zyme is highly sensitive to ouabain [21] and its amino acids, 111Gln and 122Asn (numbers corresponding to 113Arg and 124Asp of rat $\alpha_1$ [2], respectively) are the same as those of other sensitive species. Although the M1-M2 junction is thus related to the ouabain-binding site, it is not clear whether this sequence difference wholly accounts for the insensitivity or not. There is a report that has confirmed the internalization of ouabain [38]. The possibility that the receptor itself is located on the intracellular side and that the M1-M2 hydrophobic domain only works as a modulator for ouabain binding cannot be ruled out.

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