Biochimica et Biophysica Acta, 1149 (1993) 339–342

BBAMEM 70726

Short Sequence-Paper

Sequence analysis of DNA encoding an avian Na^+,K^+-ATPase $\beta 2$-subunit

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(Received 2 April 1993)

Key words: ATPase, Na^+/K^+-; Alpha subunit; Beta subunit; Isoform; cDNA sequence

The DNA encoding a chicken Na^+,K^+-ATPase $\beta 2$-subunit was cloned and sequenced. The deduced amino acid sequence has structural features common to all known Na^+,K^+-ATPase β-subunits. It is proposed to belong to the $\beta 2$-isoform family, though the amino acid sequence has significantly diverged from mammalian $\beta 2$-subunit sequences. Similar to other Na^+,K^+-ATPase $\beta 2$-isoforms, the chicken $\beta 2$-isoform mRNA is predominantly expressed in brain tissue.

The Na^+,K^+-ATPase is an electrogenic ion pump responsible for maintaining the high internal potassium and low internal sodium levels characteristic of most animal cells. The enzyme consists of two subunits. The α-subunit contains the ouabain binding site and the catalytic site for ATP-hydrolysis. The β-subunit is a glycoprotein that associates non-covalently with the α-subunit to form an active complex [1,2]. The β-subunit appears to be involved with the folding and maturation of the α-subunit [3] and subsequent transport to the plasma membrane [1,4,5].

Three isoforms of the α-subunit ($\alpha 1$, $\alpha 2$, and $\alpha 3$) and three isoforms of the β-subunit ($\beta 1$, $\beta 2$, and $\beta 3$) are known and have been reviewed [6,7]. The $\beta 2$-isoforms have been identified only from mammals [8,9]. A $\beta 3$-isoform, but no $\beta 2$-isoform, has been isolated only from *Xenopus* [10]. We earlier reported the cloning of the chicken putative $\beta 2$-isoform and compared portions of it to other published β-isoforms [11]. In this report, we present the entire nucleotide sequence of the encoding cDNA of the chicken $\beta 2$-isoform and its mRNA distribution in tissue.

Alignment of amino acid sequences from the previously known β-isoforms (see Review [7]) revealed a sequence (FTLTMWVMLQT) within the proposed transmembrane domain that is conserved in the $\beta 2$-isoforms and the *Xenopus* $\beta 3$-isoform but is not found in $\beta 1$-isoforms. These alignments also revealed an amino acid sequence [YYPY(Y/F)GK] towards the carboxyl-terminus that is conserved in all the known β-isoforms. Thus, degenerate oligonucleotides, [5′TT(T/C)ACI (T/C)TIACIATGTGGGTIATG(T/C)TICA(A/G)-AC-3′] and [5′TTICC(A/G)TA(A/G)TAIGG(A/G) (T/A)A(A/G)TA-3′], that encode these two conserved regions, were used for polymerase chain reaction (PCR) with a chicken brain cDNA library [12] as template according to methods provided by Perkin-Elmer Cetus. Primers for PCR and sequencing were synthesized on a PCR-Mate (391 DNA Synthesizer, Applied Biosystems) and purified with Oligonucleotide Purification Cartridges (Applied Biosystems). The PCR-generated DNA fragment (~ 500 nucleotides) was random-primer ^{32}P-labeled and used to screen the recombinant phages from the same brain cDNA library. A clone (1.965 kb) encoding the putative $\beta 2$-subunit, including the coding sequence and poly-A tail, was recovered. DNA sequencing was performed on double-stranded and on single-stranded plasmid DNA by the dideoxy method [13], with deoxyadenosine 5′-(α-[^{35}S]thio)triphosphate and Sequenase™ (United States Biochemical). Several chicken brain libraries were thoroughly screened by PCR with isoform-specific oligonucleotides. The oligonucleotides used in the PCR should have amplified any $\beta 3$-isoforms present in the brain cDNA library. However, the only DNA fragment amplified was that encoding the $\beta 2$-subunit presented here.

The nucleotide sequence of the DNA encoding the chicken $\beta 2$-subunit isoform is shown in Figure 1, along

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The nucleotide sequence reported appears in the GenBank, EMBL, and DDBJ Data Bases under the accession number L13208.

with the deduced amino acid sequence. Hydrophobicity analysis of the amino acid sequence (data not shown) predicts a single membrane spanning domain near the amino-terminus. Also present are structural features shared by all the related β-subunit isoforms including six conserved cysteine residues [14] and several potential glycosylation sites (see Fig. 1). Although the mammalian β2-isoforms have eight to nine potential glycosylation sites [8,9], the chicken β2-isoform has only four, one more than the three known glycosylation sites in β1-isoforms [15]. The *Xenopus* β3-subunit has the same number of potential glycosylation sites [10] as the chicken β2-subunit, but not all of these sites are located at the same positions.

Percent identities between the amino acid sequences of chicken β2-subunit and other known β-isoforms, including the rat H^+,K^+-ATPase β-subunit [16], were compiled. The chicken β2-isoform has approx. 50% amino acid identity with the mammalian β2-isoforms (Fig. 2). The avian and mammalian β2-isoforms are similarly divergent from the β1-isoforms (approx. 40% amino acid identity). All the known Na^+,K^+-ATPase β-isoforms have less than 37% amino acid identity with the rat H^+,K^+-ATPase β-subunit. To-

Fig. 2. Pairwise comparison of amino acid sequences of several different Na^+,K^+-ATPase β-subunits with chick β1- and β2-subunit. Percent identity among the isoforms was calculated with the GCG-software package program ALIGN.

Subunit	Percent identity	
	chicken β1	chicken β2
Chicken β1	100%	41%
Rat β1	70%	42%
Human β2	41%	50%
Rat β2	41%	50%
Mouse β2	40%	50%
Xenopus β3	43%	65%
Rat H^+,K^+-ATPase β-subunit	31%	35%

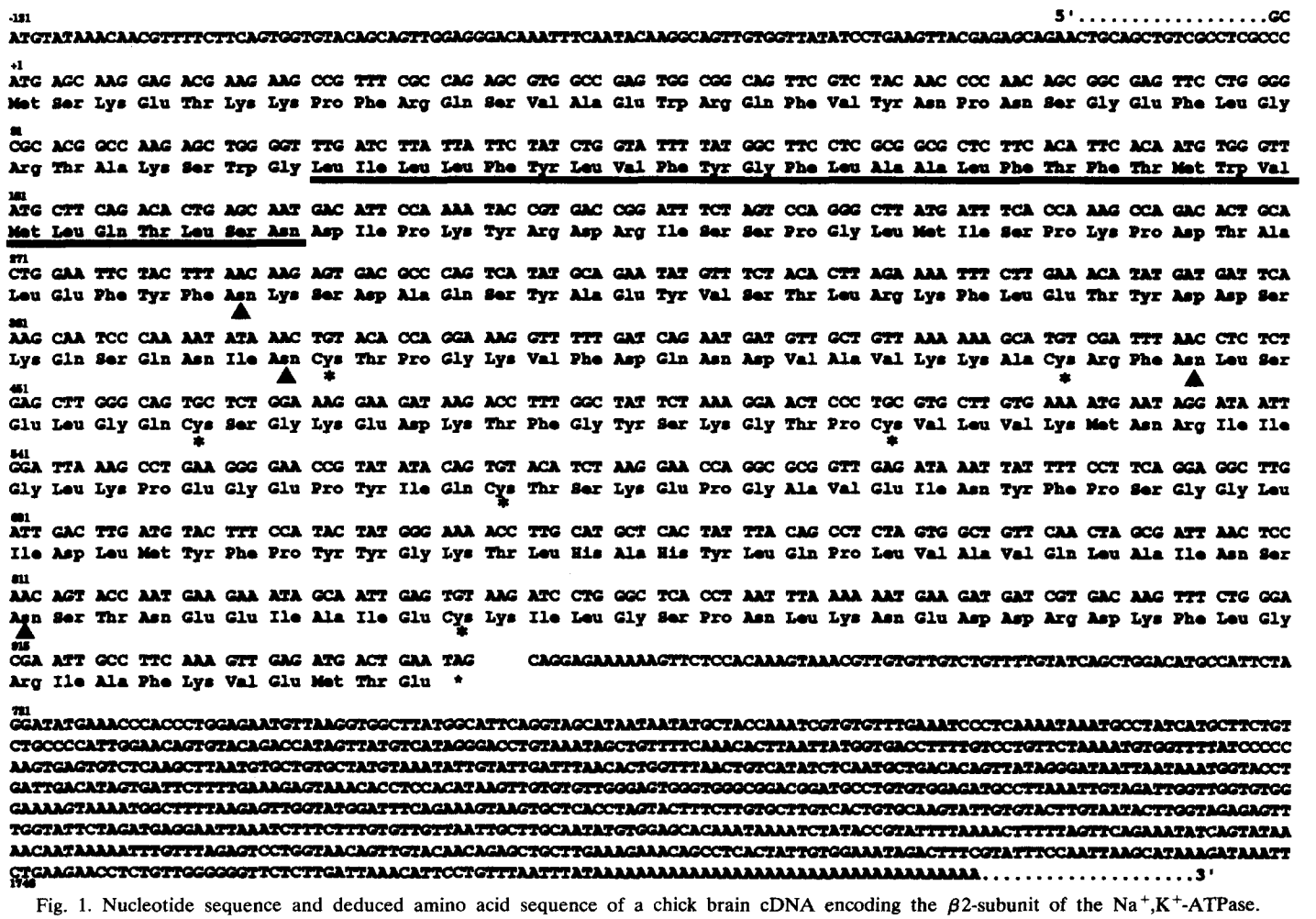

Fig. 1. Nucleotide sequence and deduced amino acid sequence of a chick brain cDNA encoding the β2-subunit of the Na^+,K^+-ATPase. Nucleotide residues are numbered in the 5′ to 3′ direction. The first residue of the translation initiation codon ATG is assigned the number 1. Nucleotides 5′ to this residue are indicated by negative numbers. The four potential glycosylation sites are marked with triangles and the six conserved cysteines are marked with asterisks. The proposed membrane-spanning domain is indicated with a black bar.

gether, these amino acid comparisons suggest that the chicken β-isoform sequence is more closely related to the β2-isoform family than to the β1-isoform family.

Like the mammalian β2-isoforms, chicken β2-subunit is expressed predominantly in neural tissue. RNA blot analysis shows β2-subunit mRNA is primarily in brain tissue (Fig. 3). Some β2-mRNA also appears in heart tissue. Little expression of the message was detected in kidney or liver. A similar mRNA distribution has been observed with other mammalian β2-isoforms [8,9] and the *Xenopus* β3-isoform [10], quite different from mRNA distributions of the β1-isoform. The mouse β2-subunit (also known as adhesion molecule on glia or AMOG) was initially identifed as an adhesion molecule which mediates selective neuron–astrocyte interactions in vitro [17]. Further studies revealed that the mouse β2-subunit may have a functional influence upon the ATPase activity [9]. These data suggest a link between fluctuations in ion concentrations and the dynamic process of cell–cell interactions, critical during nervous system development. It will be interesting to explore the possible role of the Na^+,K^+-ATPase involved in neural–glial interactions.

The chicken β2-isoform shows approx. 16% more identity with the *Xenopus* β3-subunit than with the mammalian β2-isoforms (see Fig. 2). Both the chicken β2- and *Xenopus* β3- subunits resemble the mammalian β2-isoforms more than the β1-isoforms. Several criteria were considered in classifying the *Xenopus* β-subunit as a β3-isoform: the amino acid sequence identity and size (number of amino acids) compared to other β-isoforms, number of potential glycosylation sites, and sequence dissimilarities in the 3′-untranslated region compared to conserved regions in the β1- or β2-isoforms [10]. These characteristics were judged unique enough to justify the *Xenopus* β-subunit as belonging to another isoform family rather than being a diverged form of the β2-isoform family. We consider our chicken β-isoform a member of the β2-isoform family. This is based upon amino acid sequence identity, mRNA distribution in tissue, and no significant 3′-untranslated sequence identity with any of the β-isoforms, including the *Xenopus* β3-isoform (data not shown). Furthermore, PCR amplification of β-subunit encoding DNA fragments from chicken brain cDNA libraries never yielded any fragments other than those encoding the β1- and β2-subunits (see above).

The three chicken α-subunit isoforms can assemble with the chicken β1-subunit [18]. The mouse β2-subunit has been shown to be assembled with mouse α2-subunit (and possibly α3-subunit) in brain tissue extracts [9] and to assemble with the *Torpedo* α1-subunit when coexpressed into frog oocytes [19]. Functional pumps can form when cRNAs encoding the *Xenopus* α1-subunit and β3-subunit are coinjected in *Xenopus* oocytes [20]. The chicken β2-subunit also assembles with the each of the three chicken α-isoforms (manuscript in preparation). We are currently studying the stability of these interactions among the different α/β-isoform combinations.

We would like to thank Dr. Joseph Taormino for the chicken RNA blot, Dr. Peter Good for providing us

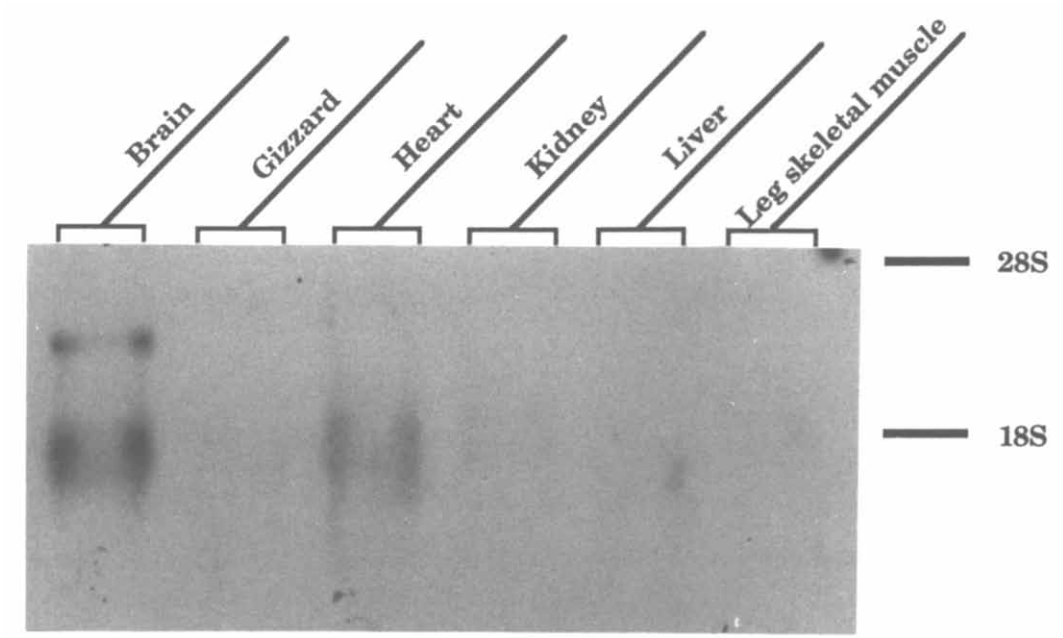

Fig. 3. Distribution of the chicken β2-isoform mRNA in chicken tissues. Total RNA extracted from adult tissues (brain, gizzard, heart, liver, and leg skeletal muscle) was run on a denaturing formaldehyde gel of 1% agarose (20 μg/lane), stained with ethidium bromide, and transferred to nitrocellulose. The RNA blot was hybridized with ^{32}P-labeled cDNA fragment from the β2-subunit clone and exposed to X-ray film for 5 days. The markers indicate positions of the 28S and 18S rRNA bands.

with the amino acid sequence of the *Xenopus* β3-subunit before its publication, Drs. Bernard Rossier and Paul Mathews for their suggestions during the PCR screening of chick cDNA libraries with isoform-specific oligonucleotides, Jason Rome, Christine Hatem, and Delores Somerville for their technical assistance, and Dr. A. Malcolm Campbell, Yuanyi Feng, and Maura Hamrick for their suggestions prior to and during the writing of this manuscript. This work was supported by National Institutes of Health Grants HL-27867 and NS-23241.

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