Primary structure of the β-subunit of *Torpedo californica* ($Na^+ + K^+$)-ATPase deduced from the cDNA sequence

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DNA complementary to the *Torpedo californica* electroplax mRNA coding for the β-subunit of ($Na^+ + K^+$)-ATPase has been cloned by screening a cDNA library with an oligodeoxyribonucleotide probe. Nucleotide sequence analysis of the cloned cDNA has revealed that this polypeptide consists of 305 amino acid residues (including the initiating methionine). The transmembrane topology and the potential *N*-glycosylation sites of this polypeptide are discussed.

($Na^+ + K^+$)-ATPase *cDNA cloning* *Nucleotide sequence* *RNA blot hybridization analysis* *Transmembrane topology* N-*Glycosylation site* *Primary structure*

1. INTRODUCTION

Sodium- and potassium-dependent adenosine triphosphatase [($Na^+ + K^+$)-ATPase] mediates the active transport of Na^+ and K^+ across the plasma membrane and is known to consist of 2 kinds of subunits, α and β [1–4]. The primary structure of the larger α-subunit, which plays the principal catalytic role, has recently been elucidated by cloning and sequencing DNAs complementary to the *Torpedo* electroplax and sheep kidney mRNAs coding for the polypeptide [5,6]. The β-subunit is a glycoprotein with an M_r of 40000–60000. In this study, the complete amino acid sequence of the β-subunit of *Torpedo californica* ($Na^+ + K^+$)-ATPase has been deduced by cloning and sequencing the cDNA.

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Abbreviations: ($Na^+ + K^+$)-ATPase, sodium- and potassium-dependent adenosine triphosphatase; PTH-amino acid, phenylthiohydantoin-amino acid

2. MATERIALS AND METHODS

($Na^+ + K^+$)-ATPase from the electric organ of *T. californica* was purified as in [5]. The purified enzyme was treated with 1% SDS and fractionated by gel filtration on a Biogel A-1.5 m column (2.5 × 100 cm) [7]. The fraction consisting mainly of the β-subunit was further purified by electrophoresis on 0.1% SDS/8% polyacrylamide gels according to [8]; approx. 0.4 mg of the protein was loaded per slab gel (0.2 × 14 × 14 cm). The protein band corresponding to the β-subunit, visualized with 0.25 M KCl and 1 mM dithiothreitol [9], was excised and the gel piece was crushed and subjected to electroelution [10] with an ISCO model 1750 sample concentrator in 40 mM (outer chamber) and 4 mM (inner chamber) Tris-acetate buffer, pH 8.6, containing 0.1% SDS.

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The Okayama-Berg cDNA library [11] screened for $(Na^+ + K^+)$-ATPase β-subunit cDNA was the same as that used for cloning cDNAs encoding the acetylcholine receptor subunits [12]. The transformation and screening procedures were as in [13]. The oligodeoxyribonucleotide probe (see below), synthesized by the triester method [14], was labelled with ^{32}P at the 5′-end and used for hybridization at 37°C. The 118 bp *Hae*III(−35)-*Sau*96I(84) fragment excised from clone pNKAβ1 was labelled with ^{32}P at the 5′-end and used for hybridization at 60°C; the restriction sites are identified by numbers indicating the 5′-terminal nucleotide generated by cleavage. DNA sequencing was carried out according to [15]. Reagents were obtained as in [5].

3. RESULTS AND DISCUSSION

Tryptic peptides from the purified β-subunit of *T. californica* $(Na^+ + K^+)$-ATPase were isolated by reverse-phase HPLC and analysed for amino acid sequence with a gas-phase sequencer (fig.1). Six peptide sequences were thus determined. The synthetic oligodeoxyribonucleotide 5′-AC$\overset{A}{G}$TG$\overset{A}{G}$T-T$\overset{T}{C}$TC$\overset{A}{G}$TA-3′, corresponding to the pentapeptide sequence Tyr-Glu-Asn-His-Val contained in fraction IVa (fig.1), was used as hybridization probe to screen a cDNA library derived from poly(A)$^+$ RNA from an electric organ of *T. californica*. Three hybridization-positive clones were thus isolated from about 1×10^4 transformants. One of them (pNKAβ1), which apparently carried the largest cDNA insert, was subjected to nucleotide sequence analysis. Further screening of about 8×10^4 transformants from the same cDNA library with a hybridization probe derived from the 5′-terminal region of clone pNKAβ1 yielded 24 positive clones. One of them (pNKAβ144), which apparently harboured the largest cDNA insert, was analysed for nucleotide sequence.

Fig.2 shows the 2105 nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the β-subunit of *T. californica* $(Na^+ + K^+)$-ATPase, determined with clones pNKAβ1 and pNKAβ144. All the 6 partial amino acid sequences determined by peptide analysis were found to be encoded by the cDNA sequence in the same reading frame (amino acid residues 16–22, 73–82, 87–106, 152–158, 226–245 and 280–290). This

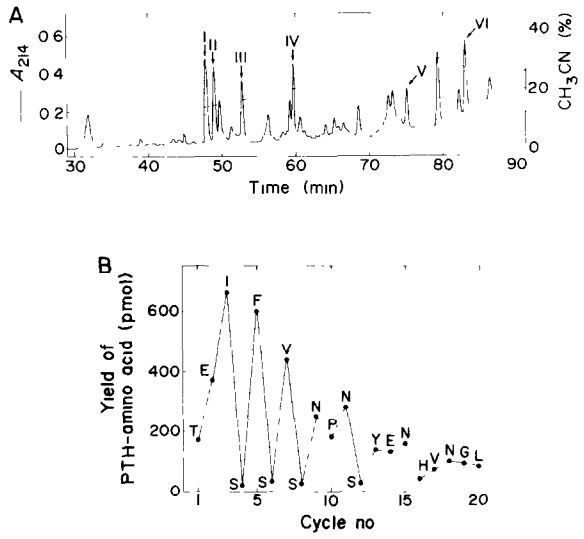

Fig.1. Partial amino acid sequence analysis of the β-subunit of *T. californica* $(Na^+ + K^+)$-ATPase. (A) Reverse-phase HPLC of tryptic peptides. After dialysis to remove SDS, approx. 1 mg of the purified β-subunit was digested by trypsin and then subjected to reverse-phase HPLC. Six fractions (I–VI) corresponding to absorbance peaks were collected and rechromatographed on the same column to yield fractions Ia–VIa, respectively. (B) Sequence analysis of peptides. The yield of PTH-amino acids at each cycle of Edman degradation is shown for fraction IVa; the one-letter amino acid notation is used. The sequences determined for the other fractions (not shown) were as follows: Ia, VFGDNIAYSEK; IIa, FLLQWLK; IIIa, FLWDSEK; Va, VAPPGLSHSP; VIa, IGTIEYFG-MGGVGGFPLQYY. For experimental details, see [5].

reading frame was used to deduce the primary structure of the $(Na^+ + K^+)$-ATPase β-subunit (fig.2). The translational initiation site was assigned to the methionine codon composed of nucleotide residues 1–3 because this is the first ATG triplet that appears downstream of a nonsense codon (TAA at positions −51 to −49) found in frame and because the second ATG triplet encodes amino acid residue 58, which is preceded by one of the peptide sequences determined. The 305th codon specifying serine is followed by 2 successive translational termination codons (TGA and TAA). Thus, it is concluded that the β-subunit of *T. californica* $(Na^+ + K^+)$-ATPase consists of 305 amino acid residues (including the

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                                5'-----ACCTTCCTCGAAACTCCTCACACCTGGAGCTTCTACTCAGTCCTCCCTTTCACCCCTAGCCCCCCCCCCCTTTTCCTACCCAACTGCCT  -241
CTTTTTTTAAAAAAAACCCACCTACCTGGCAGCTTCAAGCAGCCGCCACCACCCCCGCGGGAGGCGACGTCTCGAGTGAGTGCCTTCCAACCGTCGCTCTCTTTTTTTAAAAGGTAACCG  -121
ACGCGGGCGAGGGCGCGCGGCCGATCGGCTCCGCGAGCAAGGCTCGAAGGGGGCACCTCGCCTCCAGCGTAACAGGGCGCCTTGGCCTCTCCGCCAACTGCCACCGCAAAGTCAGCGAAG   -1
  1                                10                                20                                30
Met Ala Arg Glu Lys Ser Thr Asp Asp Gly Gly Gly Trp Lys Lys Phe Leu Trp Asp Ser Glu Lys Lys Gln Val Leu Gly Arg Thr Gly
ATG GCA CGA GAA AAG TCC ACG GAT GAT GGT GGA GGA TGG AAG AAA TTC CTC TGG GAT TCC GAG AAG AAA CAG GTT TTG GGC AGG ACC GGC    90
                                40                                50                                60
Thr Ser Trp Phe Lys Ile Phe Val Phe Tyr Leu Ile Phe Tyr Gly Cys Leu Ala Gly Ile Phe Ile Gly Thr Ile Gln Val Met Leu Leu
ACG AGC TGG TTC AAG ATA TTT GTA TTC TAC TTG ATC TTT TAC GGT TGC CTC GCT GGA ATC TTC ATC GGT ACC ATC CAA GTA ATG TTA CTG   180
                                70                                80                                90
Thr Ile Ser Asp Phe Glu Pro Lys Tyr Gln Asp Arg Val Ala Pro Pro Gly Leu Ser His Ser Pro Tyr Ala Val Lys Thr Glu Ile Ser
ACC ATA AGT GAT TTC GAA CCA AAA TAC CAG GAC AGA GTT GCA CCT CCA GGT CTA TCG CAT AGT CCA TAT GCT GTA AAA ACT GAG ATA AGC   270
                                100                               110                               120
Phe Ser Val Ser Asn Pro Asn Ser Tyr Glu Asn His Val Asn Gly Leu Lys Glu Leu Leu Lys Asn Tyr Asn Glu Ser Lys Gln Asp Gly
TTT AGT GTT TCA AAC CCA AAC TCC TAT GAA AAT CAT GTG AAT GGT CTG AAG GAA CTG TTG AAG AAT TAC AAT GAA TCA AAG CAA GAT GGC   360
                                130                               140                               150
Asn Thr Pro Phe Glu Asp Cys Gly Val Ile Pro Ala Asp Tyr Ile Thr Arg Gly Pro Ile Glu Glu Ser Gln Gly Gln Lys Arg Val Cys
AAC ACT CCT TTT GAG GAT TGT GGC GTA ATT CCT GCA GAC TAC ATA ACT AGA GGT CCA ATA GAG GAA TCG CAG GGA CAG AAG AGA GTA TGC   450
                                160                               170                               180
Arg Phe Leu Leu Gln Trp Leu Lys Asn Cys Ser Gly Ile Asp Asp Pro Ser Tyr Gly Tyr Ser Glu Gly Lys Pro Cys Ile Ile Ala Lys
AGA TTT TTG CTT CAG TGG CTT AAA AAT TGC TCT GGA ATT GAT GAT CCT AGT TAT GGC TAT TCT GAA GGA AAA CCC TGC ATC ATT GCC AAG   540
                                190                               200                               210
Leu Asn Arg Ile Leu Gly Phe Tyr Pro Lys Pro Pro Lys Asn Gly Thr Asp Leu Pro Glu Ala Leu Gln Ala Asn Tyr Asn Gln Tyr Val
CTC AAC AGG ATC CTT GGT TTC TAT CCT AAG CCT CCG AAA AAT GGC ACT GAT CTT CCT GAA GCA TTG CAA GCA AAT TAT AAT CAG TAT GTC   630
                                220                               230                               240
Leu Pro Ile His Cys Gln Ala Lys Lys Glu Glu Asp Lys Val Arg Ile Gly Thr Ile Glu Tyr Phe Gly Met Gly Gly Val Gly Gly Phe
CTT CCT ATT CAT TGT CAA GCA AAG AAA GAA GAA GAC AAA GTC AGA ATT GGA ACC ATC GAA TAT TTT GGT ATG GGT GGA GTT GGT GGC TTT   720
                                250                               260                               270
Pro Leu Gln Tyr Tyr Pro Tyr Tyr Gly Lys Arg Leu Gln Lys Asn Tyr Leu Gln Pro Leu Val Gly Ile Gln Phe Thr Asn Leu Thr His
CCC TTG CAG TAC TAT CCA TAC TAT GGA AAG CGC CTG CAG AAA AAT TAC CTT CAA CCC TTG GTT GGT ATT CAG TTC ACC AAC CTC ACA CAC   810
                                280                               290                               300
Asn Val Glu Leu Arg Val Glu Cys Lys Val Phe Gly Asp Asn Ile Ala Tyr Ser Glu Lys Asp Arg Ser Leu Gly Arg Phe Glu Val Lys
AAT GTG GAG CTG CGT GTT GAG TGT AAA GTG TTT GGT GAC AAT ATT GCA TAT AGT GAA AAA GAT CGC TCA CTG GGG CGC TTT GAA GTA AAA   900

Ile Glu Val Lys Ser
ATT GAA GTT AAA AGC TGA TAACTGTAATAGAATTTACCCCACATTACAAATTAGTCTTGAACAAACTGTCATACATATGGGACCTACACTTAATCTGTATGCTTTACACTAGCT  1014
TTCTGCATCTAGGTATGTAAAATAAAAGCAATAGTAGTACATATTTATTCTACTGTAAATGATACTACTTGAGCCATGGGTTTCCCCATAACTGTAAATTATAATTCCACTAATGTGTAG  1134
CAGTGTTTCTGTCCTTTGGGTATTGCTGCCTTGTGCCTCATGTGGGTTTGTGTACAGTACTGTAGTGCATTCACTGGTCTTTCAAGCCATGTTGCAGTTTTTAAAGCAAACTTTGCTTTC  1254
CAAGTTGTAAATACTCTGAAAATTGCGATGGTACTTCATTTTCTGTTATGCCCATGTGTATGAACACACTGCACTCTTTCTTTTTGTCAGGAGGGTGGGTGGGTAGGAGAATATGCTTTC  1374
TTTTGGCAACATTAGTGCAGGGTGATGGGTTTTATTCCACTTAGGATGGAGGCTGAAACACAAACTATGCTGAGATTTGGGTTTGGTTGTTAAGCTACTTTGATTAGTTAAAGGTTGTTT  1494
GCTGGAGCCACCATGTCATGTCTAAATGCTTTAACTAACTATGGTGAAAGATTTTATATATGCAGTTGTACAATTTTTCTGCAAAGTGTAATGTATAAAAGATACCAAAGTCACTGGTAA  1614
AATCTTTATTTGAAGTAAGCCAATGCCATTGGATGTTCATGTTAAAATGCAAATGCAATGTAACATCTGTAAACTTGTTTGGAGAACAGTGCTCCTCACCTGATTTAAAGCATCGGAAAA  1734
ATAAAACTTAAGAACCCATTCTTGTGGGACTTCTCTGGAAATT-----3'
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Fig.2. Nucleotide sequence of the cDNA encoding the β-subunit of *T. californica* $(Na^+ + K^+)$-ATPase. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5′-side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each line is given. The deduced amino acid sequence of the $(Na^+ + K^+)$-ATPase β-subunit is shown above the nucleotide sequence, and amino acid residues are numbered beginning with the initiating methionine. The 5′-terminal sequence presented does not extend to the 5′-end of the mRNA. The 3′-terminal sequence of the cDNA insert of clone pNKAβ1 (carrying nucleotides −67 to 1777) as well as clone pNKAβ144 (carrying nucleotides −328 to 1751) is followed by a poly(dA) tract connected with the vector DNA sequence [11]. The 3′-noncoding region contains 2 copies of the polyadenylation signal AATAAA [16] (nucleotides 1035–1040 and 1734–1739). No nucleotide difference was found between the 2 clones.

initiating methionine) and has a calculated M_r of 34671, which agrees with the reported M_r of the protein moiety of this subunit (32000–38000) [4,17–20]. The sequence of amino acid residues 2–10 or 2–35 is homologous with the reported amino-terminal sequence of the lamb [21] or dog kidney $(Na^+ + K^+)$-ATPase β-subunit [22]; in the latter case, residue 12 or 13 in the *Torpedo* sequence is assumed to be an insertion. Furthermore, the amino-terminal residue has been shown to be

alanine for all the mammalian, avian and fish β-subunits hitherto examined [23–25]. These findings suggest that the alanine residue following the initiating methionine represents the amino terminus of the β-subunit.

The blot hybridization analysis of *T. californica* electroplax RNA with $(Na^+ + K^+)$-ATPase β-subunit cDNA as probe exhibited a single hybridizable RNA species with an estimated size of approx. 2600 nucleotides (fig.3). This implies that a 5′-terminal sequence of about 400 nucleotide is missing from the cDNA sequence presented in fig.2.

Fig.4 shows the hydropathy profile [29] and predicted secondary structures [30] of the

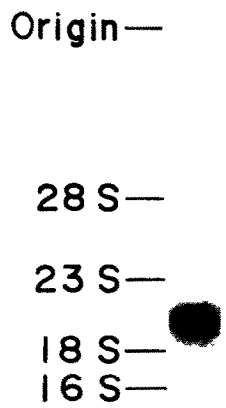

Fig.3. Autoradiogram of the blot hybridization analysis of RNA from *T. californica* electric organ. Total RNA was extracted as in [26], and was analysed by the procedure in [27], using Biodyne membrane (Pall, East Hills, NY); the amount used was 5 μg. The hybridization probe was the *Ban*I(158)-*Fnu*4HI(821) fragment excised from clone pNKAβ1 and labelled by nick-translation [28] with [α-^{32}P]dCTP. The size markers were bovine and *Escherichia coli* rRNA.

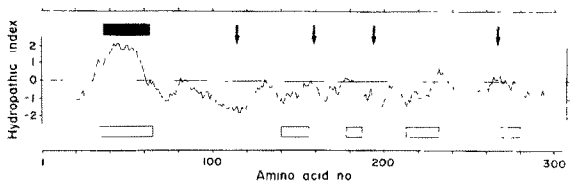

Fig.4. Hydropathy profile and predicted secondary structures of the $(Na^+ + K^+)$-ATPase β-subunit. The averaged hydropathic index of a nonadecapeptide composed of amino acid residues $i-9$ to $i+9$ is plotted against i, where i represents amino acid number; the hydropathy indices of individual amino acids have been taken from [29]. The positions of the predicted structures of α-helix and/or β-sheet [30] that have a length of 10 or more residues are shown by open boxes. The location of the putative transmembrane segment is indicated by a closed box and the potential *N*-glycosylation sites by arrows.

$(Na^+ + K^+)$-ATPase β-subunit. A highly hydrophobic region with predicted secondary structure is observed near the amino terminus. It corresponds to a continuous stretch of 28 uncharged amino acids (residues 36–63) including many nonpolar residues and may represent a transmembrane segment. The $(Na^+ + K^+)$-ATPase β-subunit does not possess a hydrophobic amino-terminal sequence characteristic of the signal peptide [31]. Furthermore, all 4 potential *N*-glycosylation sites [32] present in the β-subunit (asparagine residues 114, 159, 194 and 267) are located on the carboxy-terminal side of the putative transmembrane segment. On the basis of these observations, it is assumed that the large portion of the β-subunit molecule following the putative transmembrane segment is located on the extracellular side of the membrane, whereas the small amino-terminal portion resides on the cytoplasmic side. This transmembrane topology of the β-subunit is consistent with previous findings as follows: (i) immunochemical [33] and chemical labelling studies [34] indicate that the β-subunit spans the membrane; (ii) chemical labelling [35,36] and cell-free translation studies [19] indicate that virtually all of the hydrophilic part of the β-subunit is located on the extracellular side of the membrane; (iii) studies by pulse-chase labelling and endoglycosidase digestion suggest that the β-subunit has 2 or more *N*-linked oligosaccharide chains [20,37]; (iv) the results of papain fragmentation suggest that the carboxy-terminal fragment

of M_r 16000 as well as the preceding fragment of M_r 31000 contains covalently bound carbohydrate, whereas the amino-terminal fragment of M_r 9000 apparently has no carbohydrate [22].

Three additional segments comprising almost exclusively uncharged residues (residues 201–217, 231–249 and 255–272) are present in the carboxy-terminal region of the β-subunit. However, they seem not to be sufficiently hydrophobic to form transmembrane segments (fig.4). It is also to be noted that the sequence Gly-X-Gly-X-X-Gly (X can be any amino acid), which is characteristic of the nucleotide binding region of nucleotide binding proteins [38], is found in the β-subunit (residues 233–238). The functional implication of this sequence is not known because no information is available as to the role of the β-subunit in nucleotide binding. In any event, a second transmembrane segment would be required to locate this sequence on the cytoplasmic side of the membrane.

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