

Na⁺,K⁺-ATPase: tissue-specific expression of genes coding for α -subunit in diverse human tissues

E.D. Sverdlov, N.S. Akopyanz, K.E. Petrukhin, N.E. Broude, G.S. Monastyrskaya and N.N. Modyanov

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

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The expression of genes coding for α and α III isoforms of Na⁺,K⁺-ATPase α -subunit has been studied in human kidney, brain, thyroid and liver cells. The expression was shown to be subjected to a tissue-specific control and also depended on the developmental stage. The tissue-specific expression of genes coding for different isoforms of the catalytic subunit of Na⁺,K⁺-ATPase perhaps may be attributed to various functions of proteins belonging to this family.

Na⁺,K⁺-ATPase; Catalytic subunit; Isoform; Tissue-specific expression

1. INTRODUCTION

Na⁺,K⁺-ATPase of the plasma membrane of animal cells consists of the α -subunit (110 kDa) forming all known functionally important regions and the glycosylated β -subunit (protein part 35 kDa), its role to be established (for a review see [1]).

Studies on the genomic organization of the genes coding for the α -subunit conducted here [2] and by other authors [3] revealed a family of closely related genes in the human genome, that contains no less than five genes and/or pseudogenes. At least two of them were shown to be transcribed in human brain [4]. The primary structure of one of the transcripts was identical to the cDNA coding for the α -subunit from HeLa cells [5]. Three homologous mRNA species were found in rat brain [6], one of these mRNAs closely related to the mRNAs identified in human brain and HeLa cells (α), the second mRNA represented so called α -form, whereas the third contained an open reading

frame for the protein highly homologous but not identical to the α or α ⁺ isoforms. This hypothetical protein was designated as α III-isoform of the catalytic subunit [6]. The primary structure of the human gene corresponding to unknown protein (α III) was established by us [7].

This paper reports our results in the expression of genes encoding the α -subunit of Na⁺,K⁺-ATPase and α III hypothetical protein in diverse human tissues as well as on different developmental stages of brain and renal tissues. Part of the work was published earlier [8]. (In the same paper we presented data on Southern blots of DNA isolated from various human tissues and hybridized with α - and β -subunit specific probes. The differences in hybridization patterns were observed and probable tissue-specific rearrangements of Na⁺,K⁺-ATPase genes were proposed as one of the explanations of the effect. Later we found the explanation to be incorrect [7]. At the moment the preliminary data appear to allow explanation of the observed differences in hybridization patterns as a result of some peculiarities of the rate of DNA restriction in the locus of α -subunit genes. At present we are investigating the problem more thoroughly.)

Correspondence address: E.D. Sverdlov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

2. MATERIALS AND METHODS

2.1. RNA isolation

Total cellular RNA from normal human tissues was isolated by hot phenol procedure using guanidinisoithiocyanate as RNase inhibitor [9]. To prepare poly(A⁺) RNA the material was chromatographed twice on an oligo(dT) cellulose column [10].

2.2. RNA blot hybridization

10 µg of poly(A⁺) RNA was denatured by heating at 65°C in 50% (v/v) formamide and subjected to electrophoretic separation in 1.5% agarose gel containing 2.2 M formaldehyde [11]. Transfer to a Hybond membrane (Amersham, England) was performed according to the recommendation. Oligonucleotide 5'-termini were radiolabelled, and hybridization was performed as described [12]. Oligonucleotide probes were synthesized by V. Rostapshov, I. Chernov, and T. Ashikina (Shemyakin Institute of Bioorganic Chemistry). Hybridization temperature was calculated by the formula of Meinkoth and Wahl [13]. Blots were stripped twice before used for repeated hybridization. Intensity of hybridization signals was estimated quantitatively by scanning of radioautograms using 2202 laser densitometer (LKB, Sweden).

3. RESULTS

When estimating the expression level of Na⁺,K⁺-ATPase genes coding for various isoforms of the catalytic subunit, we hybridized poly(A⁺) RNA fractionated by agarose gel electrophoresis and immobilized on the membrane with specific radioactive oligonucleotide probes complementary to two forms (α and αIII) of the RNA in regions of the least homology (fig.1).

3.1. Tissue specific expression of genes encoding two isoforms of α-subunit in normal human tissues

Fig.2 and table 1 present the results of blot hybridization analysis of poly(A⁺) RNA isolated

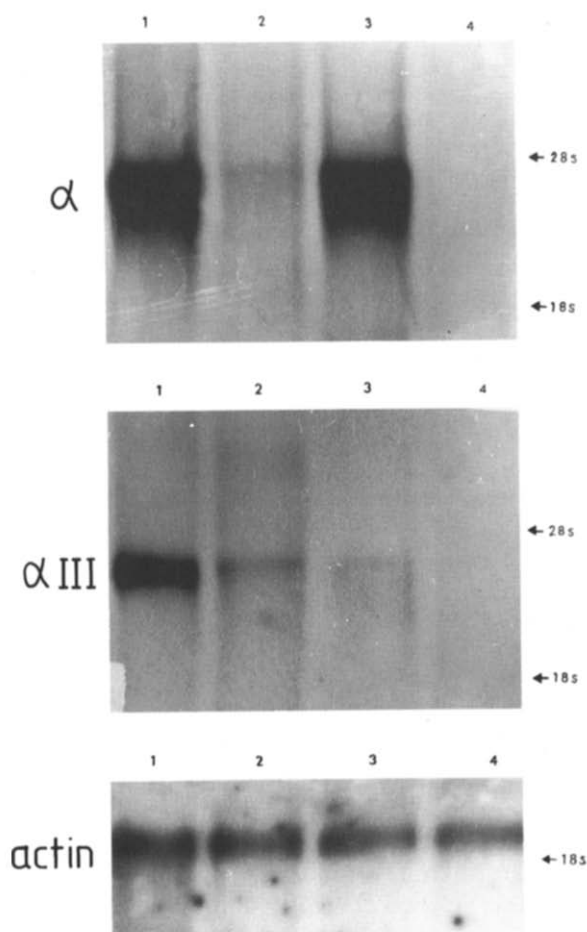


Fig.2. Expression of genes coding for the α- and αIII-isoforms of Na⁺,K⁺-ATPase catalytic subunit in different human tissues. RNA was isolated from adult kidney (1), brain (2), thyroid (3), and liver (4). Here and in fig.3 hybridization of the same filter with actin cDNA shows that approximately the same quantities of RNA are present in all samples analyzed. Arrows mark position of 28 S and 18 S ribosomal RNAs.

peptide	Lys-Gly-Val-Gly-Arg-Asp-Lys-Tyr-G(lu)
α-mRNA	5'-AAG GGG GUU GGA CGU GAU AAG UAU G-3'
oligonucleotide probe	3'-TTC CCC CAA CCT GCA CTA TTC ATA C-5'
peptide	Asp-Lys-Lys-Asp-Asp-Lys-Asp-Ser-P(ro)
αIII-mRNA	5'-GAC AAG AAA GAU GAC AAG GAC UCA C-3'
oligonucleotide probe	3'-CTG TTC TTT CTA CTG TTC CTG AGT G-5'

Fig.1. Structure of oligonucleotide probes specific for two isoforms (α and αIII) of Na⁺,K⁺-ATPase catalytic subunit. Both probes correspond to mRNAs regions which code for peptides, analogous to peptide 3-11 of pig kidney α-subunit [14].

Table I
Scanning of radioautographs by laser densitometer

Tissue	Relative amount of α -mRNA as compared with the amount in adult kidney	Relative amount of α III-mRNA as compared with the amount in adult kidney
Adult kidney	100	100
Adult brain	10	33
Adult thyroid	90	11
Adult liver	0	0
Embryo kidney	5	3
Child kidney	95	90
Embryo brain	1,5	3
Child brain	10	30

Average data from 3 experiments are shown

from adult human kidney, brain, thyroid, and liver with oligonucleotide probes corresponding to two isoforms of Na^+, K^+ -ATPase α -subunit. Hybridization was performed sequentially using the same filter after stripping the previous radioactive probe. With α - and α III-isoforms, hybridization points to the presence of only one band of size 4.0 kb. The amount of both mRNAs is highest in kidney. The level of α -mRNA in thyroid gland is comparable with the RNA level in kidney, whereas in brain this mRNA equals about 10% of its amount in kidney.

When estimating α III-mRNA level in various human tissues we must state that this mRNA is most abundant in kidney, present in lower levels in the brain (see comment in parentheses at end of section 1) and still lower levels in thyroid. No specific hybridization bands being hybridized with probes corresponding to both mRNA forms (α and α III) were found in human liver.

3.2. Expression of two genes coding for α - and α III-isoforms of the Na^+, K^+ -ATPase α -subunit in ontogeny

Poly(A⁺) RNA isolated from embryo (12–13 weeks), child and adult kidney, as well as from embryo, child and adult brain was hybridized with the probe specific for the α III-isoform. After radioautography the filter was washed free of radioactivity and then hybridized repeatedly under similar conditions with the probe specific for the α -isoform. The results are shown in fig.3 and table 1.

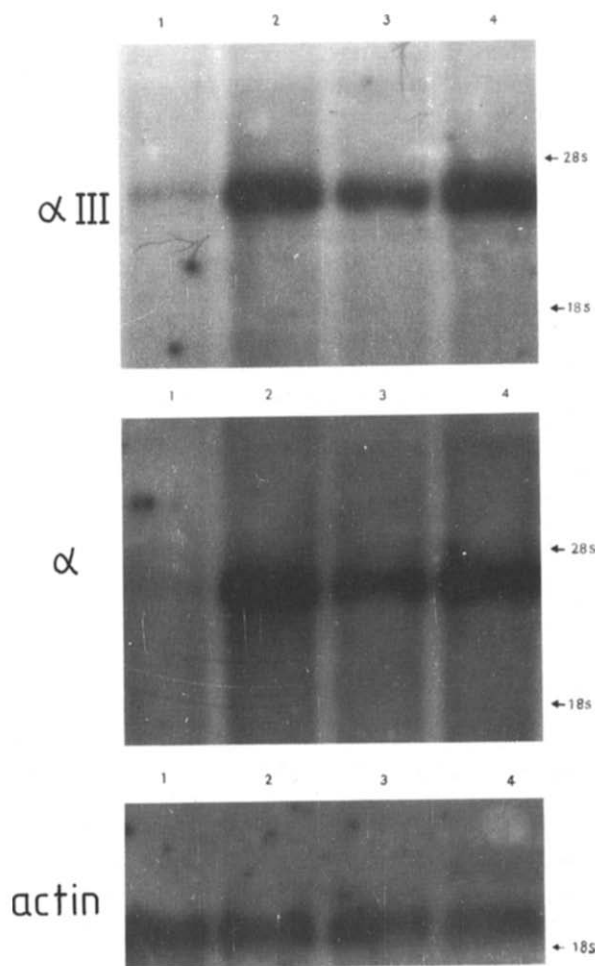


Fig.3. Expression of genes encoding the α - and α III-isoforms of the catalytic subunit of Na^+, K^+ -ATPase at different developmental stages of brain tissue. Poly(A⁺) RNA was isolated from embryo brain (12–13 weeks) (1), child brain (2,3), and adult brain (4). Arrows mark position of 28 S and 18 S ribosomal RNAs.

As seen from these data, α and α III expression during renal tissue development is practically identical in child and adult kidney and much lower in embryo. For brain tissues the picture was similar.

4. DISCUSSION

This paper demonstrates that the expression of genes coding for α and α III isoforms of the catalytic subunit displays a tissue-specific regulation. α and α III mRNA level is the highest in kidney. A

high level of α -mRNA in thyroid is probably associated with intense transport of iodine in the gland cells. The accumulation of iodine in the thyroid gland is known to be associated with ATP hydrolysis, K^+ transport into the cells and Na^+ efflux out of the cell, suggesting coupling of iodine transport with Na^+, K^+ -exchange, i.e. the activity of Na^+, K^+ -ATPase [15]. Estimation of α III-mRNA amount in various human tissues indicates that this mRNA is most abundant in kidney, and, as a rule (at the moment we have found one case when the expression of α III-isoform was higher in human brain as compared with kidney), present in lower levels in brain and thyroid. These results contradict those of α III-isoform expression in rat tissue, where the highest expression was found in brain [16,17]. This can be explained by differences in the expression of this isoform in various species. Also we cannot inter-individual differences in the expression of this isoform in human tissues caused by probable drug treatment.

The disclosure of a tissue in which the level of α III mRNA is particularly high allowed us to start studying isolation of this form of Na^+, K^+ -ATPase catalytic subunit by the protein chemistry technique.

The absence of specific hybridization with probes corresponding to both forms of mRNA in liver tissue appears to be explained by a low level of α - and α III-mRNA in liver, since liver cells are known to contain only 1% of Na^+, K^+ -ATPase molecules as compared to kidney cells [18]. This information agrees with data obtained with rat liver mRNA [16,17].

Results of the present work as well as of similar studies performed with rat tissue [16,17] indicate that the distribution of isoforms is tissue-specific. This appears to be primarily related to a different rate of transcription of various genes in the studied genes. However, other causes of tissue-specific expression of these genes cannot be excluded. They may include diversity of maturation rate of various mRNA species or their different stability.

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