CLONING AND CHARACTERIZATION OF SEQUENCES ENCODING THE MAJOR ISOFORM OF NA,K-ATPase IN SHARK RECTAL GLAND

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We and others (Matsuda, T. et al., J. Biol. Chem. 259:3858-3863, 1984; Sweadner, K.J., et al., Biochim Biophys. Acta 988:185-220, 1989; Orlowski, J., et al., J. Biol. Chem., 263:17817-17821, 1988; Orlowski, J., et al., J. Biol. Chem., 263:10436-10442, 1988; Schneider, J.W., et al., Proc. Natl. Acad. Sci., USA, 85:284-288, 1988) have previously demonstrated that 3 isoforms of the α subunit of the Na,K-ATPase exist in mammalian and avian species. However, only one isoform is present in invertebrates (Varadi, A. et al. FEBS Letters, 258:203, 1989). This isoform most closely resembles the A3, or neural, isoform of mammals and birds (Schneider, J.W., et al., Proc. Natl. Acad. Sci, USA, 85:284-288, 1988; Varadi, A. et al., FEBS Letters, 258:203, 1989), on the basis of nucleic acid and amino acid sequence homology. The Al isoform predominates in epithelial tissues, while A2 is especially abundant in muscle. We have attempted to define the number and types of isoforms of Na,K-ATPase present in the rectal gland of the common dogfish shark, Squalus acanthus. We focused our studies upon the rectal gland for two reasons. First, the behavior the Na,K-ATPase has been extensively studied in this tissue; it would be of considerable interest to know whether the Na,K-ATPase activity of the rectal gland arose from a single isoform, or several. Second, if multiple isoforms exist, it would be of interest to know whether the transport epithelium of the rectal gland expresses the Al isoform, because Al is the only isoform expressed in epithelial cells of mammals and birds. This information would aid efforts to discern a functional basis for the existence of multiple isoforms.

In order to examine the isoforms present in the dogfish rectal gland, we elected to clone their cDNAs. We adapted methods, based upon the polymerase chain reaction (PCR), that we had employed to characterize Na,K-ATPase cDNAs in the common fruitfly, Drosophila melanogaster (Varadi, A. et al., FEBS Letters, 258:203, 1989). Briefly, messenger RNA was isolated from six rectal glands by use of standard commercial RNA extraction kits. The mRNA was reverse transcribed into cDNA with reverse transcriptase. Selective amplification of portions of the cDNA moieties that were complementary to the Na,K-ATPase mRNA was then accomplished by PCR, using oligonucleotide primers complementary to highly conserved regions of Na,K-ATPase mRNA. The primers were chosen by comparing previously cloned cDNA sequences from many species: rat, Torpedo, chicken, sheep, pig, human, and Drosophila (Varadi, A. et al., FEBS Letters, 258:203, 1989). Two extremely conserved regions of homology emerged from this comparison: amino acids within the phosphorylation site (amino acids 368-376) and a stretch of amino acids within the FITC site (amino acids 502-510). The former site was used to design a "sense" strand oligonucleotide primer, 23 bases in length, while the latter site was utilized for design of the "anti-sense" strand primer, also 23 bases in length. The region between these two primers was then selectively amplified by PCR, using temperatures of 95°C for one minute (denaturation), 55° for 2 minutes (annealing), and 72 °C for 2.5 minutes (chain extension). The DNA products from this reaction were then analyzed by agrose

gel electrophoresis. A single DNA band, 429 bases in length, was observed. This result was consistent with selective amplification of cDNA sequences located between the two primer sites, which are 130-145 amino acids apart in the Na,K-ATPase's of many species; it suggested strongly that the band represented a portion of the shark rectal gland Na,K-ATPase cDNA.

The 429 base pair band was recovered by subcloning into a standard bacterial plasmid vector, and subjected to DNA sequencing using standard techniques (Varadi, A., et al., FEBS Letters.258:203, 1989). The sequence analysis revealed that the amplified cDNA segment encoded an Na,K-ATPase isoform highly homologous to the A3 isoform. The amino acid sequence encoded by this cDNA segment exhibited 89% homology to the rat and human A3 isoform; homologies for the A1 and A2 isoforms were significant, but considerably lower: 76 and 73%, respectively. The shark sequence also matched the A3 sequence at several positions where rat and human A3 amino acid sequences diverge from their A1 counterparts. This is most apparent in the regions encoding amino acids 489-498, where A1 and A3 isoforms differ significantly. Within this region, the shark sequence exhibits a 9/10 sequence match with the A3, but a 0/10 match with the A1 sequence. These findings strongly suggest that the shark sequence is most closely related to the A3 isoform of mammals.

The amplified cDNA segment was also utilized as a probe for RNA analysis, employing stringent Northern blotting hybridization techniques that eliminate cross-hybridization among mRNAs encoding different isoforms (Schneider, J.W., et al., Proc. Natl. Acad. Sci, USA, 85:284-288, 1988). Under these conditions, the probe detected a prominent mRNA band in shark rectal gland RNA; the RNA band was 3,800 bases long, consistent with the length of mRNAs encoding Na,K-ATP'ase alpha subunits from all species previously examined (Orlowski, J., et al., J. Biol. Chem., 263:17817-17821, 1988; Orlowski, J., et al., J. Biol. Chem., 263:10436-10442, 1988; Schneider, J.W., et al., Proc. Natl. Acad. Sci, USA, 85:284-288, 1988; Varadi, A., et al., FEBS Letters, 258:203, 1989). The mRNA was present, but less abundant, in other tissues.

Our preliminary results suggest, but do not prove, that the predominant Na,K-ATPase isoform expressed in the dogfish shark is the A3 form, since we found this form rather than A1 in transport epithelium. This pattern is much more typical of that seen in invertebrates, where only one isoform is produced. In mammals, the isoform in transport epithelium is almost always exclusively the A1 isoform. Further studies will be necessary to determine whether other shark isoforms exist and their tissue distributions. This initial result, however, is striking because it identified an A3 isoform in epithelial cells.

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