CLONING AND SEQUENCING OF THE RENAL SODIUM-D-GLUCOSE COTRANSPORTER FROM MARINE ANIMALS

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The sodium-D-glucose cotransport system is a member of a family of proteins important in the kidney and intestine for the uptake of glucose and sodium across the membrane. This system functions primarily due to the sodium gradient that exists across the membrane. Under normal conditions the cell contains a low amount of sodium while in the urine or digestive fluids the sodium content is high. Sodium moves across the membrane into the cell following its concentration gradient, and is known to take a variety of solutes, such as glucose and amino acids with it. The cDNA and amino acid sequence for the sodium-D-glucose cotransporter in rabbit renal tissue is known (Morrison et al., Biochim. Biophys. Acta, 1089:121, 1991). This information has been used to determine the presence of the protein, its functional aspects and sequence differences among species through evolution and development (Morrison-Shetlar, J. Exp. Zool., 265:373, 1993). Seventy eight percent of the dogfish (Squalus acanthias) sequence is known from previous studies but two regions have not been isolated and sequenced, the 5' end including the start codon and a region of 400 bases close to the middle of the sequence. In this study cDNA libraries were made using mRNA from dogfish renal tissue and screened using existing cDNA probes. Additionally, the cDNA probes were used to isolate the genomic DNA coding for this transporter in an attempt to understand the regulatory mechanisms of this gene thorough evolution.

Total RNA was isolated from dogfish (Squalus acanthias), winter flounder (Pseudopleuronectes americanus) and skate (Raja erinacea) renal tissue by homogenization in guanidinium isothiocyanate and extraction with ethanol. mRNA was isolated by oligo dT affinity chromatography and used for cDNA library synthesis following the procedure of the BRL Superscript cDNA synthesis kit. The cDNA produced was size selected, Sal I and Not I adapters ligated to the cDNA and only the largest cDNA's were cloned directionally into pSport plasmid. The resulting library was screened using the radiolabelled cDNA clone. From the 5×10^7 colonies screened 27 positives were obtained and rescreened. Six

strong positive colonies are currently being sequenced to obtain data that will complete the dogfish DNA sequence and allow further experimentation to be carried out. The complete sequence will be compared to the existing rabbit sequence and protein modeling experiments carried out to determine the topography of the protein in the membrane. A new model for this protein has been suggested (Morrison-Shetlar et al, submitted) which gives some insight into the possible topography of the protein in the membrane.

Genomic DNA was isolated from dogfish, scallop, flounder and skate using the method of Blin and Stafford (Nucl. Acid Res. 3:2303, 1976). The DNA was then digested using the restriction enzyme Hind III, the fragments separated depending on their size by gel electrophoresis and blotted onto nylon membrane. The dogfish cDNA clone was radiolabelled and used to detect homologous regions of the genomic DNA. Hybridization was carried out in phosphate buffer containing SDS at 50^{o}C over night and washed in phosphate buffer at 50^{o}C until unbound radioactivity was removed. As can be seen in Fig 1. the probe bound to several distinct bands in dogfish, winter flounder and skate but not to the scallop (Placopecten magellanicus) which does not express the sodium-D-glucose cotransporter (Morrison-Shetlar, unpublished data). The homologous bands range in size from 1950 base pairs to 8200 base pairs in size. The temperature at which hybridization was carried out may reflect a difference in homology between the dogfish probe and the sequences present in the genomic DNA. The amount of homology will be determined by the DNA sequence obtained. The bands have been excised from the gel matrix, ligated into a vector and are currently being sequenced. These data will allow the gene to be studied at the DNA level and allow comparison to the human genomic sequence that has recently become available (Turk et al., J. Biol. Chem., 269:15204, 1994). Regions of importance such as the promoter and regulatory binding sites will be determined.

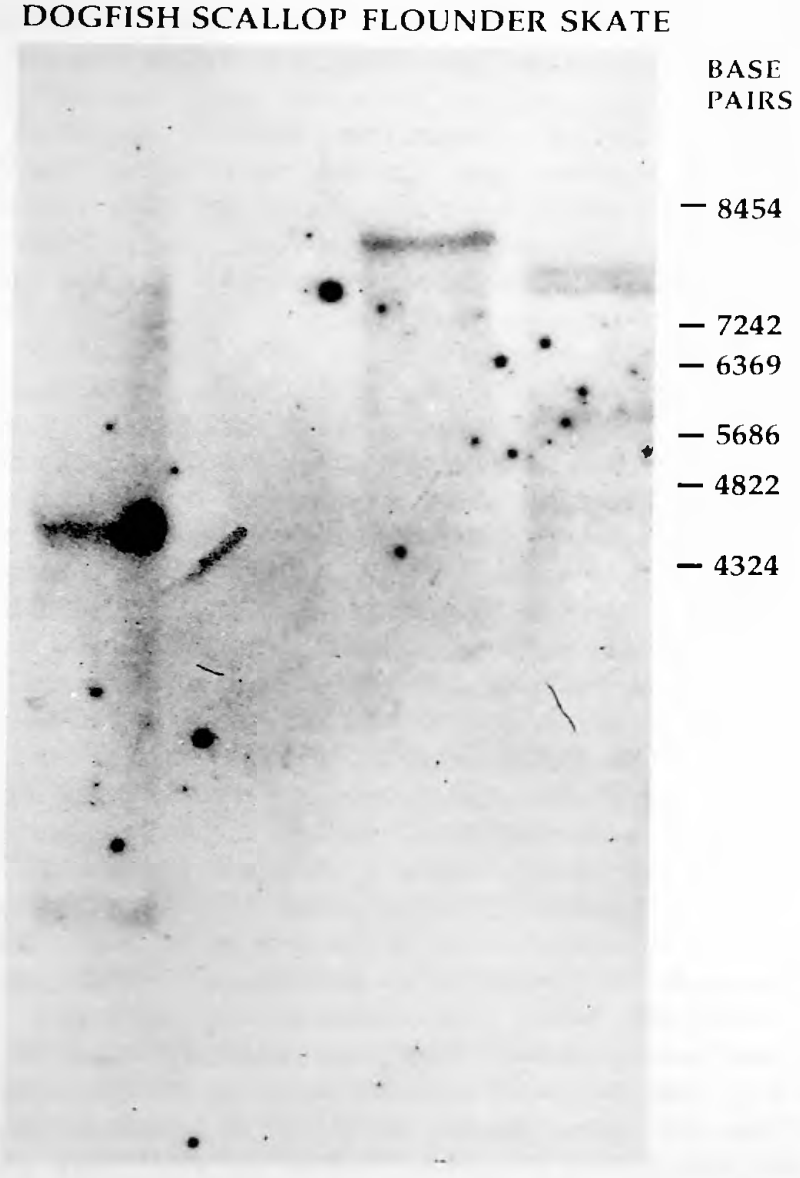

Figure 1. Southern blot analysis of Dogfish, scallop, flounder and skate genomic DNA hybridized with a radioactively labeled dogfish cDNA clone coding for part of the sodium-D-glucose cotransport system. Three bands of 7800, 7400 and 5700 base pairs (bp) were observed, a band at 8200 bp in the flounder genomic DNA and two bands at 4400 and 1959 bp were obtained from dogfish DNA. 10μg of genomic DNA loaded per lane. Positions of standard markers are indicated (bars). A 48 hour exposure is shown.

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