SEQUENCE COMPARISON OF THE SODIUM-D-GLUCOSE COTRANSPORT SYSTEM IN A VARIETY OF AQUATIC ORGANISMS.

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The sodium-D-glucose cotransporter is an important constituent in the transfer of glucose across the brush border membranes of renal and intestinal epithelia. We have isolated, from rabbit cortex, the full cDNA sequence coding for this transporter [Morrison et al, BBA, 1089:121-123, 1991] and used this information to probe DNA and RNA isolated from a variety of marine organisms in an attempt to find sequence homology between species.

RNA and DNA were isolated from Atlantic hagfish (Myxine glutinosa), bloodworm (Glycera dibranchiata), little skate (Raja erinacea), spiny dogfish (Squalus acanthias), winter flounder (Peseudopleuronectes americanus), toadfish (Opsanus tau), goosefish (Lophius americanus) and crab (Carcinus maenas)

RNA was isolated from kidney cells as described by Stallcup and Washington, [J.Biol Chem. 258:2802, 1983]. The RNA was transcribed into cDNA by the method of Okayama and Berg, [Mol.Cell.Biol, 2:161, 1982] using the GeneAmp RNA PCR Kit (Perkin Elmer Cetus). DNA was isolated from muscle tissue by the method of Gross-Bellard, Oudet and Chambon, [Eur.J,Biochem, 36:32, 1973]. The Polymerase Chain Reaction (PCR) was carried out in the Perkin Elmer PCR 480 Cycler using the GeneAmp PCR System (Perkin Elmer Cetus). Sections of the cDNA/DNA were amplified using selected oligonucleotide primers designed according to the rabbit cortical sequence. Three pairs of primers (Fig 1, segments 1, 2 and 3) were used to obtain the complete sequence from each DNA or RNA pool.

The PCR products were separated on agarose gels and southern blotted [Southern, J.Mol.Biol 98:503, 1975] onto Nytran (Schleicher and Schuell). Sodium-D-glucose cotransport cDNA was labelled by nick translation using biotin labelled-dUTP and Southern blots were screened using the Photogene non-radioactive detection system (BRL Life technologies). The results from gel analysis and southern blots are shown in Table 1.

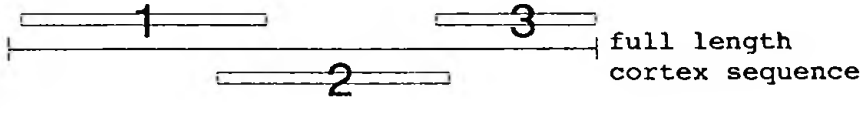

Fig 1. Diagrammatic representation of the full length sequence coding for the rabbit renal sodium-D-glucose cotransporter. Sections 1,2 and 3 indicate the overlapping PCR products synthesized using specific primers.

The results indicate that most animals tested have the sodium-D-glucose cotransport gene present in the DNA and that it is transcribed into

mRNA as indicated by the mRNA-cDNA results (Table 1). The results correspond in general well to studies carried out on the transport system at a functional level. It is known for example that the bloodworm has no sodium-D-glucose cotransport system and was also found to be negative in this study. Of particular interest are the results obtained from the toadfish. It is evident that the DNA contains the sequence for the cotransporter and that it is present in the renal mRNA, however part of the sequence would appear to differ (Table 1, see segment 1) and it is known from transport studies that the toadfish kidney has an extremely low sodium-D-glucose uptake capacity. The goosefish is closely related of the toadfish and the same results were obtained.

Animal	DNA			Renal mRNA			Na^+-D-glucose cotransport
	1	2	3	1	2	3	
hagfish	+	+	+	+	+	+	+
bloodworm	-	-	-	-	-	-	-
skate	+	+	+	+	+	+	+
dogfish	+	+	+	+	+	+	+
flounder	+	+	+	+	+	+	+
toadfish	+	+	+	-	+	+	-
goosefish	+	+	+	-	+	+	-
crab	+	-	+	nd	nd	nd	nd
rabbit	+	+	+	+	+	+	+
mouse	+	+	+	+	+	+	+

Table 1 Results of PCR product analysis by gel electrophoresis and Southern blot analysis. + = PCR product obtained and positive by Southern blot analysis and transport. - = no product or transport obtained. nd = not determined.

The PCR products are at present being sequenced to determine if there are indeed differences in base sequence. Additionally, cDNA libraries are being produced from each of the animals to screen for the complete sodium-D-glucose cotransporter sequence and to determine the presence of regulatory sequences.

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