COMPARATIVE STUDIES OF THE SODIUM-D-GLUCOSE COTRANSPORT SYSTEM IN RABBITS AND DOGFISH (SQUALUS ACANTHIAS)

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The renal brush border membrane plays an important role in the absorption of molecules and ions across the membrane into the cell. The membrane requires specific transport proteins to be present in a functional state to take up these solutes and one such protein is the sodium-D-glucose cotransporter which is involved in the secondary active process of taking up sodium and glucose into the cell. The functional activity of this protein has been investigated by several authors but there is still little known about the glucose and sodium binding sites and which segments of the sequence are important for function. We have been able to clone and sequence the sodium-D-glucose cotransporter system from rabbit renal cortex [Morrison et al., BBA, 1089:121, 1991] and have shown that the sequence does induce radioactive glucose uptake when expressed in Xenopus laevis oocytes [Morrison-Shetlar et al., in preparation]. Antibodies raised against the sequence have been used successfully to determine the presence of this protein in winter flounder (Pseudopleuronectes americanus), little skate (Raja erinacea) and dogfish (Squalus acanthias) but not in toadfish (Opsanus tau) [Shetlar et al., Bull. MDIBL 30:35, 1991]. These results correspond well with transport studies carried out on brush border membrane (BBM) vesicles isolated from the renal tissue of these fish. Glucose uptake has been measured in vesicles from flounder, skate and dogfish [Bevan et al., Bull. MDIBL 28:60, 1989] but not in toadfish [Wolff et al., J. Comp. Physiol. 157:573, 1987] suggesting that the toadfish lacks significant amounts of this cotransporter.

To continue this investigation we looked at two aspects of the expression of the sodium-D-glucose cotransporter in shark. Using the polymerase chain reaction (PCR) we isolated fragments of the mRNA-cDNA sequence coding for the cotransporter from renal tissue of the dogfish and sequenced part of the resulting amplified product.

RNA was isolated from freshly caught dogfish renal tissue by the method of Stallcup and Washington, [J. Biol. Chem., 258:2802, 1983]. The dogfish RNA was transcribed into cDNA and the PCR reaction was carried out using the GeneAmp RNA PCR kit from Perkin Elmer Cetus and a Perkin Elmer PCR 480 Cycler. PCR products were separated by weight on agarose gels, blotted by the method of Southern [J. Mol. Biol 98:503, 1975], hybridized with biotin-labelled-dUTP conjugated cDNA coding for the complete sodium-D-glucose cotransporter in rabbit, washed under differing stringency conditions of salt and temperature and detected with the Photogene non-radioactive detection method (BRL Life technologies).

A PCR product located at the 5'-end of the sequence was isolated and subcloned into a vector containing an T overhang (TA cloning kit, Invitrogen). The PCR product was sequenced by the method of Sanger et al. [Mol. Biol. 143:161, 1980] and the resulting sequence indicated approximately 65% homology to that of the rabbit sequence at a DNA level but 86% (identical or well conserved) at the amino acid level.

This sequence has subsequently been verified by screening a cDNA library from dogfish. The clone and the cDNA clone are currently being used to screen a cDNA library for more clones to obtain the full length cDNA sequence for the shark.

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