PARTIAL CLONING AND SEQUENCING OF THE RENAL SODIUM-D-GLUCOSE COTRANSPORTER FROM DOGFISH (SQUALUS ACANTHIAS)

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The proximal tubule of vertebrate kidneys re-absorbs glucose from the renal filtrate through a sodium dependent glucose cotransport system located on the apical surface of kidney epithelial cells. Low intracellular sodium concentration maintained by the Na.K-ATPase leads to the formation of a sodium gradient across the apical membrane which in turn drives the uptake of sodium into the cell. The sodium-D-glucose cotransport (SGLT) protein moves the sodium across the cell membrane, down its concentration gradient and at the same time moves glucose across the membrane against its concentration gradient. The mammalian kidney is known, through functional studies, to express a low affinity, high capacity, and a high affinity, low capacity glucose cotransporter in different locations within the renal tissue, but the difference in stoichiometry at the molecular level is not fully understood. It is the goal of this project to determine the stoichiometry for each of the transport systems, and define the glucose and sodium binding sites on the protein. We have recently cloned, sequenced and expressed the SGLT from rabbit renal tissue in Xenopus oocytes. Using this information we have proposed a model for the orientation of the protein in the membrane. Antibody studies have verified this model but the sodium and glucose binding sites have not been identified.

To continue this study, the spiny dogfish (Squalus acanthias) was used. This fish has been chosen because the dogfish kidney expresses only the low affinity cotransporter. We have made cDNA libraries from renal tissue of dogfish and have obtained positive clones from the library using isolated radio-labeled cDNA sequences from the known rabbit renal sequence. The 5' end of the sequence is shown in Fig 1 and is compared to that of the known rabbit kidney sequence.

A)

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Rabbit     TGTCGTCGCCGCCGCCACGCCGCCATGGACAGCAGCACTTTGAGCCCCCT
Dogfish    gccgGcCGCtgtCttCctctCtgCATaccgAGCtaaAaTgaagGCatCtg

Rabbit     GACCACCTCCACCGCGGCCCCCCTTGAGTCCTA-TGAGCGCATCCGCAAT
Dogfish    cAtCtCCgaCAtCaaccaCgtCtccatccCaaaCTGtGgcCATCaaCAAT

Rabbit     GCGGCCGACATCTCCGTCATCGTCATCTACTTCTTGGTGGTGATGGCCGT
Dogfish    GCtGCaGAtATCagCGTcATCaTCgTGTACTTcgTttTGGTcATcGCCGT

Rabbit     CGGGCTGTGGGCTATCTTTTCCACCAATCGGGGGACGGTCGGAGGCTTCT
Dogfish    tGGaCTGTGGtCTATgTaTaggACCAAcCGtGcGACcGTcGGtGGcTaCT

Rabbit     TCTTGGCGGGTCGGAGTATGGTGTGGTGGCCGATCGGAGCCTCTCTGTTT
Dogfish    TCTTGGCgGGgaGGgacATGcgaTGGTacaCagTcGGAGCCTCaCTgTTT

Rabbit     GCCAGTAACATTGGAAGTGGCCACTTTGTGGGGCTGGCCGGGACGGGAGC
Dogfish    GCtAGTAACATcGGAAGcGGaCACTTTGTtGGttTGGCcGGcACaGGgGC
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Rabbit      TGCTTCAGGCATTGCCACTGGGGGCTTTGAGTGGAACGCCCTGATCATGG
Dogfish     TGCaaacGGCcTgGCCgtcGGtGGCTTTGAGTGGAACGCCCTGtTtgTtG

Rabbit      TGGTCGTGCTGGGCTGGGTGTTTGTCCCCATTTACATCAGGGCTGGGGTG
Dogfish     TGtTacTcCTGGGtTGGCTCTTTGTCCCagTTTACcTgAcaGCTGGGGTC

Rabbit      GTGACGATGCCAGAGTATCTGCAGAAGCGGTTTGGAGGCAAGAGGATCCA
Dogfish     aTcACGATGCCccAaTActTaatGAAGaGGTTcGGAGGaAAccGaATCag

Rabbit      GATCTACCTTTCCATTCTGTCCCTGTTGCTCTACATTTTTACCAAGATCT
Dogfish     acTCTACCTcTCCcTcaTcTCtCTcTTaCTgTACATaTTTACCAAGATCT

Rabbit      CGGCAGACATCTTTTCCGGAGCCATCTTCATCCAGCTGACCTTGGGCCTG
Dogfish     CGGtgGACATgTTcTCCGGAGCGATCTTCATCCAaCaagCtcTGGGatgG

Rabbit      GATATCTATGTGGCCATCATTATCTTATTGGTCATCACTGGGCTCTACAC
Dogfish     aAcATCTATGTtGCagTaATTgcaTTgcTGaTtATtACTtGtaTCTAtAC

Rabbit      CATCACAGGGGG
Dogfish     tATCACAGaGcG

B)
Rabbit      CRRRRHAAMDSSTLSPLTTSTAAPLESYERIRNAADISVIVIYFLVVMAV
Dogfish     paalflsAyrAKmkASaSPTsTtSpSqtvaInNAADISVIVYFVLVIMAV

            GLWAMFSTNRGTVGGFFLAGRSMVWWPIGASLFASNIGSGHFVGLAGTGAA
Dogfish     GLWSMYRTNRATVGGYFLAGRDMrWYTVGASLFASNIGSGHFVGLAGTGAA

Rabbit      SGIATGGFEWNALIMVVVLGWVFVPIVIRAGVVTMPEYLQKRFGGKRIQI
Dogfish     NGLAVGGFEWNALfVVLLLGWLFVPVYLtAGVITMPQYLmKRFGGNRIRL

Rabbit      VLSILSLLLYIFTKISADIFSGAIFIQLTLGLDIYVAIIILLVITGLYTIT
Dogfish     YLSLISLLLYIFTKISVDMFSGAIFIQqALGwNIYVAVIaLLiITcIYTI
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Fig. 1. Comparison of the A) cDNA and B) amino acid sequence obtained from dogfish kidney cDNA and the known rabbit sequence. Identity shown by capital letters in the dogfish sequence for both the DNA and amino acid sequences and similarity in the amino acid sequence shown by underlining. Non-caps means no identity nor similarity.

The results to date suggest a 56% homology at the DNA level and a 86% homology at the amino acid level.

This study will allow additional experiments to be carried out to delineate further the structure and function of this protein. Site directed mutagenesis will facilitate recognition of important functional sites on the protein. The observed functional changes in affinity will be studied in Xenopus oocytes to determine why and how affinity and stoichiometric changes occur. The immunohistochemical studies will indicate if the difference in function is due to a change in the topography of the protein in the membrane. A change in function may affect the internal environment of the cell (change in Na levels transported) and could be related to volume regulation. Additionally, regulation of this protein within the kidney cell is not fully understood and may be determined in the isolated oocyte system where large amounts of the messenger RNA injected increase expression to observable levels.
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