IMMUNOLOGICAL STUDIES ON THE SODIUM-D-GLUCOSE COTRANSPORTER IN KIDNEY BRUSH BORDER MEMBRANES FROM RABBIT, WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS), DOGFISH (SQUALUS ACANTHIAS), LITTLE SKATE (RAJA ERINACEA) AND TOADFISH (OPSANUS TAU)

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Recently the full amino acid sequence of a renal sodium-D-glucose cotransporter was determined from DNA clones obtained from rabbit renal cortex [Morrison et al., submitted 1990]. The transport protein contains several unique partial amino acid sequences, one of which was used to generate specific monoclonal antibodies against the transporter. The sequence chosen was located on the cytoplasmic side of the plasma membrane. This monoclonal antibody was now utilized to compare the biochemical nature of renal sodium-D-glucose cotransport system of rabbit cortex, rabbit medulla and a variety of fish species. Of particular interest were the following questions:

1. Is there crossreactivity between species?
2. What is the molecular weight of the antigenic protein(s)?
3. Is there evidence for oligomer formation as postulated for renal and intestinal transporters [Lin et al., Biochim.Biophys Acta 777:201-208, 1984. Peerce and Wright, Biophys.J. 47:278a, 1985]?

Brush border membrane vesicles from the kidneys of rabbit (cortex and medulla) were isolated as described by Turner and Moran [Am.J.Physiol. 242:F406-F414, 1982]. Renal brush border membranes from dogfish (Squalus acanthias), winter flounder (Pseudopleuronectes americanus), little skate (Raja erinacea) and toad fish (Opsanus tau) were obtained basically following the protocols published earlier [Eveloff et al., Am.J.Physiol. 237:F291-F298, 1979 and Bevan et al., Toxicol.Appl.Pharmacol. 101:461:469, 1989]. This was followed by transport studies to determine the presence of Na+-D-glucose cotransport [Eveloff et al., J.Comp.Physiol. 135:175-182, 1980]. The isolated membranes were solubilized, separated on 5-15% gradient SDS-polyacrylamide gels (SDS-PAGE) and blotted on to nitrocellulose membranes. The blots were blocked for 2 hrs in 1x TBS (Tris buffered saline) which contained 5% non-fat dry milk followed by incubation for 2 hrs with the monoclonal antibodies mentioned above at different dilutions. The antibody solution was removed and the blots were washed 4 times with 1x TBS+TWEEN (0.05%) followed by 30 min incubation with the second antibody (anti-mouse IgG coupled to alkaline phosphatase in 1x TBS with 5% milk, Promega). The blots were then washed 3 times with 1x TBS+TWEEN and 1 time with 1x TBS. Alkaline phosphatase was detected using the substrates NBT (nitro blue tetrazolium) and BCIP (5- bromo-4-chloro-3-indolyl-phosphate) in alkaline phosphatase buffer (100mM Tris-HCl, 100mM NaCl, 5mM MgCl2, pH 9.5) according to the protocol provided with the second antibody detection kit (Promega).

A positive reaction of the antibody against brush border membrane components was observed for rabbit cortex and medulla, dogfish, winter flounder and little skate but not for toadfish. This parallels with the observation that toadfish brush border membranes lack a significant amount of sodium-dependent glucose transport, and supports the assumption that

the antibody recognizes an epitope specific for the sodium-glucose cotransporter [Kinne et al., Bull. MDIBL 25:120-122, 1985].

Molecular weights of the immunoreactive brush border membrane proteins are given in table 1. It is of interest to note that the minimum size of the cotransporter in all species investigated was between 72 and 91 kD, which is close to the 73 kDa molecular weight calculated from the amino acid sequence of the rabbit cortical sodium-glucose cotransporter [Morrison et al., submitted]. The small differences in the size of the proteins observed between different species may be due to differences in the gels from experiment to experiment or to differences in the carbohydrate chains which may be attached to the cotransporter. It is also interesting that the higher molecular weight bands observed in the various species could easily be considered as multiples of the lowest weight band in a given organism. These bands might therefore represent monomers, dimers or tetramers of the cotransporter protein which may have been incompletely dissociated during pretreatment of the membranes. In rabbit kidney cortex membranes a monomer and a tetramer could be postulated, in rabbit medulla membranes monomers, dimers and tetramers were observed. Membranes from skate kidney gave results which were similar to those observed in rabbit medulla with bands corresponding to a monomer, dimer and tetramer. In the flounder kidney and in the dogfish kidney, however, only one band was observed.

In separate studies it was found that rabbit cortex, flounder kidney and dogfish kidney contain a sodium-D-glucose cotransporter with a 1:1 stoichiometry and this was paralleled by the appearance of only monomer and tetramer structures. Rabbit medulla and skate kidney, on the other hand, were found to possess a cotransporter with a 2:1 glucose stoichiometry which corresponded to the appearance of a dimer in addition to a monomer and a tetramer. Further studies will have to clarify whether there are potentially different association states of monomers which might be related to different transport stoichiometries. It also cannot currently be excluded that there are sequence differences between the various species since antibodies directed against only a small segment of the total cotransport protein were investigated.

Table 1.
Results of experiments with a monoclonal antibody against a peptide from the Na+-D-glucose cotransport protein.

animal	molecular weight in kD		
rabbit cortex	262	---	78
rabbit medulla	341	127	80
skate	354	164	91
flounder	---	---	83
shark	---	---	72
toadfish	---	---	---

1 mg of total membrane protein was typically loaded on to the gels and separation was allowed to proceed overnight at 4° C. The separated proteins were blotted on to nitrocellulose using a wet blotting system (Idea Scientific). Blots were then blocked as described in the text before application of the first antibody.

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