PARTIAL SEQUENCE OF THE SODIUM HYDROGEN EXCHANGER FROM WINTER FLOUNDER RED BLOOD CELLS.

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Regulatory Volume Increase (RVI) in response to osmotic perturbation is a well-documented phenomenon in many different species and cell types. One of the first descriptions of this response was in the winter flounder (Pseudopleuronectes Americanus) red blood cells (RBCs) at the Mount Desert Island Biological Laboratory (Cala, Bull. MDIBL 13:20-25, 1973). In response to hypertonic stress, the winter flounder RBCs were shown to exhibit regulatory volume increase (RVI) following shrinkage by gaining Na, Cl and osmotically obligated water, returning the cell to normal volume (Cala, J Gen. Physiol., 69:537-52 1977). Several membrane proteins have been linked to RVI in different cell types and species. These include the type 1 Na/H exchange (NHE1) and the Na-K-2Cl co-transporter (Cala, In: Chloride Channels and Carries, In Nerve, Muscle and Glial Cells, New York: Plenum Press, 67-81, 1990). Since NHE1 has been detected in other teleost fish RBCs (Borgese, Proc Natl Acad Sci 89(15): 6765-9, 1992), we revisited the winter flounder RBC studies to determine if NHE1 activity is responsible for RVI. Last year we demonstrated that the RBC Na influx is accompanied by obligatory proton efflux consistent with NHE1mediated RVI (Rigor, Bull. MDIBL 40:68-70, 2001). Expression of NHE1 in winter flounder RBCs was confirmed using antibodies specific to NHE1 (Rigor, Bull. MDIBL 40:68-70, 2001). Western blots stained with antibodies to NHE1, but not NHE3, detected a protein running at approximately 110 kD on SDS-PAGE, comparable to the molecular weight of NHE1 from other species. In this study, we also demonstrated that although the transport properties of the winter flounder NHE1 are similarly to NHE1 from other species, the protein is somewhat unique in that it is unaffected by the common NHE1 inhibitors, amiloride, EIPA and HOE694 (Rigor, Bull. MDIBL 40:68-70, 2001). Our lab has previously shown that the NHE1 also differs from human in its sensitivity to the inhibitor HOE694 (McLean, Am. J. Physiol. 276: C1025-37, 1999). To investigate the molecular basis for species-specific NHE1 inhibitor sensitivity, we have begun sequencing the winter flounder NHE1. Since NHE1 is fairly homologous across species, a comparison of the winter flounder sequence to the human and may identify the regions necessary for inhibitor function.

In these experiments, winter flounder blood was taken from the caudal vein by puncture with a heparin containing syringe. RBCs were isolated by centrifugation and washed twice with isotonic saline. Messenger RNA (mRNA) was purified from the RBCs using the PolyTract A system (Promega, Madison, WI). Briefly, cells were lysed in a strong denaturing solution to inhibit RNase activity. Biotinylated oligo dT primers were added to the lysate and annealed to the mRNA. The mRNA complex was then purified using streptavidin coupled magnetic beads. DNA was synthesized from the mRNA using avian myeloblastosis virus reverse transcriptase (Promega) and degenerate primers based on homologous regions of the trout, amphiuma and human NHE1 (forward, ttacagaattcgtgcagggcatgacaattcggc and reverse, cttctcgaggtgtggcccggggtcacttaggc) were used to amplify the C-terminal region of the winter flounder NHE1.

Using this approach, we isolated an approximately 1-kb DNA segment from winter flounder RBCs that corresponds to the carboxy terminal tail of NHE1. We began sequencing from this region since it is fairly homologous between species. We have, so far, sequenced 788 nucleotides of the winter flounder NHE1 carboxyl terminus (Fig. 1), corresponding to amino acids 511 to 790 of the NHE1.

Figure 1: Partial nucleic acid sequence of the winter flounder NHE1 carboxy terminus.

ggtggagcttcttgcagtgaagaagaagaaggagagcaaacgatcaataaacgaggagattcacacacagttcctggatcatctgctcacaggcatcgaagacatctg
tggacattacggacaccatcactggaaagacaagctgaaccgcttcaacaagtcctacgtgaagaagtggctgatcgcgggggaacgctcctccgagcctcagctca
tctccttctacaacaagatggagatgaagcaggccatgatgctggtggagagcgggagcgccgccaagctgccctccatcgtatcctccgtctccatgcagaacattca
gcaaaagggtccgaccagaggacgagcgataccgagcatctccaagagtcgagaggcggagatcagaaagatcctgagagcaaacctgcagaagacaagacag
aggcttcgctcctacagccgacacgacctgatgatcgatcccttcgaggacaatgtgagtgaggttcgcttcaggaagcagagggtggagatggagaggaggatgag
tcactatctcacggtccctgccaatcgccaggaaaccctccagtgaggaaagtctgttttgagccagaacaccaggtttatacgtacgacactgagggcgagagccc
cagaggtcgagcagctcagactcatcccagccctcctgacgccatcggcctgatgaacgaagcgaccccgaggcccaatcagagcagcgcactgcgagatgaagg
tgaaacggagctgagggcgaaagcacccgacgaccaggagg

The winter flounder amino acid sequence was 75% homologous to NHE1 from human and proximal to amino acid 700. After this point, the homology between the winter flounder and other species was lost except for sporadic short segments (Fig. 2).

Figure 2: Amino acid comparison of amphiuma, human, and winter flounder NHE1.

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                          510                                        550
AMPHI                     MTIRPLVELL AVKKKQETKR SINEEIHTQF LDHLLTGIED
HUM                       MTIRPLVDLL AVKKKQETKR SINEEIHTQF LDHLLTGIED
WIN FLOU                        VELL AVKKKKESKR SINEEIHTQF LDHLLTGIED
                  551                                                600
AMPHI     ICGHYGHHHW KDKLNRFNKK YVKKCLIAGE RSTEPQLIAF YHKMELKQAI
HUM       ICGHYGHHHW KDKLNRFNKK YVKKCLIAGE RSKEPQLIAF YHKMEMKQAI
WIN FLOU  ICGHYGHHHW KDKLNRFNKS YVKKWLIAGE RSSEPQLISF YNKMEMKQAM
                  601                                                650
AMPHI     ELVESGGLGR IPSAVSTVSM QNIQPKAKPT DRFIPALSKV KEEEIRKILR
HUM       ELVESGGMGK IPSAVSTVSM QNIHPKSLPS ERILPALSKD KEEEIRKILR
WIN FLOU  MLVESGSAAK LPSIVSSVSM QNIQQKGPTR GRAIPSISKS REAEIRKILR
                  651                                                700
AMPHI     TNLQKTRQRL RSYNRHTLVA DPYEEAWNQM LLRRQKAHQL EQRMNNYLTV
HUMAN     NNLQKTRQRL RSYNRHTLVA DPYEEAWNQM LLRRQKARQL EQKINNYLTV
WIN FLOU  ANLQKTRQRL RSYSRHDLMI DPFEDNVSEV  RFRKQRV.EM ERRMSHYLTV
                  701                                                750
AMPHI     PAHKMDSPTM TRARVGSNPM AYEPKANIRD LPTITIDPA.      ..SPESVDIV
HUM       PAHKLDSPTM SRARIGSDPL  AYEPK.. .ED LPVITIDPAS  PQSPESVDLV
WIN FLOU  PANRQETPPV RKVCFEPEHQ  VYTYDTEGES .PRGRAAQTH PSPPDAIGLM
                  751                                                800
AMPHI     NEEKK..... .     ....      SLPTE KEEEEEEGIV MTAKEPPSPG TDDVFTPGAG
HUM       NEELKGKVLG LSRDPAKVAE  EDEDDDGGIM MRSKETSSPG TDDVFTPAPS
WIN FLOU  .....          A  TPRPNQSSAL RDEGETE...     LRAK.....      .....APDDQE
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We are continuing to sequence the winter flounder NHE1 and hope to have the complete gene shortly. We are also developing chimeras of human and winter flounder NHE1, to help isolate the regions of NHE1 that are responsible for inhibitor sensitivity. In the process, we will also determine those regions required for ion translocation. This work was funded by NIH grant HL21179 and The Salisbury Cove Research Fund.