EVIDENCE FOR THE PRESENCE OF A NA^+/H^+ EXCHANGER IN THE GILL OF THE LITTLE SKATE, *RAJA ERINACEA*

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Acid-base balance involves the maintenance of an internal pH within a narrow range despite both internal and environmental challenge (Claiborne, *Physiology of Fishes* 177-193, 1998). Because of the aquatic environment that fishes inhabit, acid-base balance can be widely influenced by variations in many different parameters such as P_{O_2}, P_{CO_2}, temperature, salinity, and pH (Claiborne et al., *J. Exp. Zool.* 279:509-520, 1997). One of the transport proteins thought to be involved in acid-base regulation in the gills of marine fish is the sodium hydrogen exchanger (NHE) (Yun et al., *Am. J. Physiol.* 269:G1-G11, 1995). NHEs were initially detected in intestine, urinary bladder, and gall bladder (Yun ibid.) and have been found in all eukaryotes including mammals, yeast (Noel et al., *J. Cell Science* 109:929-939, 1996), crustaceans (Towle et al., *J. Exp. Zool* 200:1003-1014, 1997), and some prokaryotes (Tse et al., *J. Membrane Biology* 135:93-108, 1993). To date, seven isoforms have been cloned, NHE 1-6 and β-NHE and all are structurally similar (Yun ibid.). Recently our laboratory has detected the presence of NHE in the gill tissue of two marine teleosts, the long-horned sculpin *Myoxocephalus octodecimspinosus*, (Blackston et al., *Bull MDIBL* 37:33-35, 1998) and the killifish, *Fundulus heteroclitus* (MacKenzie et al., *Bull MDIBL* 37:36-37, 1998). Choe et al. (*Bull MDIBL,* this volume) showed that antibodies for NHE-1 could detect proteins in the gills of the little skate, *Raja erinacea*, but to our knowledge no other specific NHE mRNA has been reported in any elasmobranch, and only one partial sequence has been obtained from the dogfish shark, *Squalus acanthias* (Morrison, MacKenzie, unpublished). The goal of this study was to determine if NHEs are present in the gill tissue of the little skate, *Raja erinacea.*

Skates were exposed for 48 hours to water equilibrated with 1% CO_2 following a protocol from Claiborne and Evans (Claiborne and Evans, *J. Exp. Zool.* 261:9-17, 1992). Following this procedure, total RNA was extracted by removal and homogenization of a gill arch from the skate using the TRI reagent protocol (Molecular Research Center, Inc.). Reverse-transcriptase polymerase chain reaction (RT-PCR) was used with oligo dT to reverse transcribe mRNA to cDNA. Primers were developed from a previously obtained sequence of the dogfish shark, *Squalus acanthias,* (Morrison and MacKenzie, unpublished) and used in PCR to amplify specific sequences. The products from these reactions were run on a 1% Agarose (Gibco BRL) gel stained with Ethidium Bromide (Sigma) against a 1 kb standard (Gibco BRL). The PCR product obtained, which was approximately 500bp in size, was ligated into a pCR II TOPO vector and the plasmids transformed using INVα competent cells (TOPO TA Cloning Kit, Invitrogen). The bacteria containing the plasmids were grown on ampicillin-treated LB Agar plates containing X-gal (Sigma) for blue-white screening. White colonies were chosen and grown overnight in LB Broth (Gibco BRL) with shaking at 37 degrees Celsius. An alkaline lysis preparation was performed to obtain the isolated plasmid (Birnboim and Doly, *Nucleic Acid Res* 7:1513, 1979). An EcoRI restriction enzyme was used with gel electrophoresis to determine the size of the insert. Inserts of an expected size were sequenced at the Medical College of Georgia as well as the Mount Desert Island Biological Laboratory Sequencing Facility. The sequence generated was submitted to the NIH Blast web server for identification and found to have 85% homology

and 71% identity to the NHE human isoform 2 and a high degree of similarity to the shark sequence with 97% identity (Figure1).

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                                                    30
SKATE                                 L x p I I V G I I Y G F V A A F T T
SHARK                       H F F V V G I G G I I V G I I Y G F V A A F T T
HUMAN V F A G I A N F F V V G I G G V L I G I f l G F I A A F T T
                            F F V V G I G G I I V G I I Y G F V A A F T T

          60                        90                       120
SKATE R F T Q N I R I I E P q F V F L Y S Y L C Y L T A E M F R L
SHARK R F T Q N I R I I E P L F V F L Y S Y L C Y L T A E M F R L
HUMAN R F T H N I R V I E P L F V F L Y S Y L S Y I T A E M F H L
      R F T Q N I R I I E P L F V F L Y S Y L C Y L T A E M F R L

          150                       180                      210
SKATE S G I V S L I A C S I S M K H Y V E E N V S M K S S T T I K
SHARK S G I M S L I A C S I S M K R Y V g E N V S M K S S T T I K
HUMAN S G I M A I t A C A M T M n K Y V E E N V S q K S y T T I K
      S G I M S L I A C S I S M K . Y V E E N V S M K S S T T I K

          240                       270                      300
SKATE Y F L K M W S S V S E T p I F I F L G Y S T I T E A H E W N
SHARK Y F L K M W S S V S E T L I F I F L G Y S T I T E A H E W N
HUMAN Y F M K M l S S V S E T L I F I F M G V S T V g k n H E W N
      Y F L E M W S S V S E T L I F I F L G V S T I T E A H E W N

          330                       360                      390
SKATE W A F V C F T L L F C L V W R A L G V I V L T Q I I N R F R
SHARK W A F V C F T L L F C L V W R A L G V I V
HUMAN W A F V C F T L a F C L M W R A L G V f V L T Q I I N R F R
      W A F V C F T L L F C L V W R A L G V I V L T Q . I N R F R
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Figure 1. The skate sequence was found to have 85% homology and 71% identity to the NHE2 human isoform (gil5305405) at the amino acid level. To bring the sequence into full alignment, an N was inserted at base 197 of the nucleotide sequence. High similarity was found between the skate and the shark sequence (97% identity). Lower case letters indicate areas of non-homologous amino acids. Bold characters show the consensus of identical matches for at least two of the three sequences.

Studies to date have shown evidence for the presence of NHE in the long-horned sculpin, *Myoxocephalus octodecimspinosus,* the killifish, *Fundulus heteroclitus,* and the dogfish shark, *Squalus acanthias.* This research suggests that this same transport protein may be present within the gills of the little skate. Further studies are needed to determine the full sequence, as well as the functional role of this antiporter and other isoforms which may be found in *Raja erinacea*, thus providing further evidence for the role that NHE isoforms play in acid-base regulation in the gills of fish. This research was funded by the REU NSF DBI-9820400 and the NSF IBM-9808141 to JBC and AIMS.