EXPRESSION OF A NA^+/H^+ EXCHANGER ISOFORM IN THE GILL TISSUE OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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Maintenance of proper physiological pH and ionic balance is a vital challenge to all organisms, in particular fishes. Due to their aquatic existence, the acid-base balance of fish can be widely influenced by variations in ambient PCO_2, P_{O_2}, pH, temperature and salinity (Claiborne, Acid-Base Regulation. In: *The Physiology of Fishes* . ed.:D. H. Evans, Boca Raton, CRC Press, 179-200, 1997). One proposed mechanism for intracellular pH regulation in fish is the exchange of an internal H^+ for an external Na^+ across the gill epithelia *via* a Na^+/H^+ antiporter (NHE). Multiple NHE isoforms have been identified, and are distinguished on the basis of their sensitivity to amiloride, their specificity to kinases and their kinetic properties (Noel and Pouysseguer, *Am. J. Physiol.* 268: C283-C296, 1995). The excretion of H^+ for the uptake of Na^+ in freshwater fish would function not only to maintain pH during acidosis, but also for ion regulation. In saltwater fish, NHE may aid in pH regulation; however, it would counteract efforts of the fish to ion regulate in its hyperionic environment. Despite this obvious conflict, a number of studies have indicated that a Na^+/H^+ exchange exists in marine fish, possibly emphasizing the significance of acid-base regulation to the fish (for review see: Claiborne, Acid-Base Regulation. In: *The Physiology of Fishes* . ed.: D.H. Evans, Boca Raton, CRC Press, 179-200, 1997). When exposed to an internal experimental acidosis, fish compensate for the acid load over a time period of minutes to a few hours (Claiborne, et al. *J. Exp. Zool.* 279:509-520, 1997). Due to their apical location in epithelial cells, we hypothesize that NHE2 and/or NHE3, along with possibly other isoforms, may be upregulated in response to ɔsis and hence responsible for the rapid compensation after an acid load (Blackston et al., this volume). The killifish, *Fundulus heteroclitus*, is a euryhaline fish capable of surviving in salinities ranging from sea water to fresh water. This characteristic makes it a good model to examine the regulation of NHE in response to varying external environments. The goal of this study was to determine if NHE isoforms are present in the gill tissue of the killifish.

Seawater fish were acid loaded with 0.1N HCl (2 mM/kg) or injected with the same volume of water for a control, and allowed to adjust for five hours. Total RNA was extracted from the gill tissue homogenate of each fish using a TRI reagent and BCP extraction protocol (Molecular Research Center, Inc.). In multiple reverse transcription-polymerase chain reactions, RT-PCRs (Perkin-Elmer Gene Amp RNA PCR Kit), oligo dT primers were used against the different total RNA samples to selectively transcribe mRNA. A reverse degenerate primer, 3F, made against conserved regions of NHE isoforms (Towle et al., *J. Exp. Biol.* 200:1003-1014, 1997) and a forward degenerate primer, NHER, generated against a sculpin ßNHE sequence (Blackston et al., *Bull MDIBL* 36:22, 1997), were used to synthesize specific NHE cDNA from the transcribed mRNA. PCR products were ligated into either Promega pGEM-T Easy vectors or Invitrogen pCR 2.1 vectors and transformed into Promega JM109 or Invitrogen INVαF1 competent cells. The transformations were plated on ampicillin plates containing X-gal (and IPTG for pGEM-T Easy Vectors), and blue-white screening was used to identify colonies containing plasmids with inserts. Selected colonies were grown overnight and plasmids were isolated by a mini-prep procedure (Birnboim and Doly, *Nucleic Acids Res.* 7:1513, 1979). To verify the presence and size of inserts, the isolated plasmids were cut with *Eco* RI in a restriction digest and visualized on a 1% agarose gel containing ethidium bromide. S^{35} labeled dATP and dideoxynucleotides were used in the Sanger sequencing method to sequence the ligated PCR products (Amersham Life Sciences Sequenase Version 2.0 DNA Sequencing Kit). The sequences were entered into the NIH Blast Server program for comparison to all other sequences.

As seen in Figure 1, a PCR product of the correct size, approximately 590 bases, was generated from both seawater control and seawater acid loaded fishes. From the PCR products, about 150 bases were sequenced from the 3F primer end. The sequences from the control and acid loaded fish were identical to each other and found to be 88% homologous at the amino acid level and 68% homologous at the nucleotide level to the ßNHE isoform in trout red blood cells.

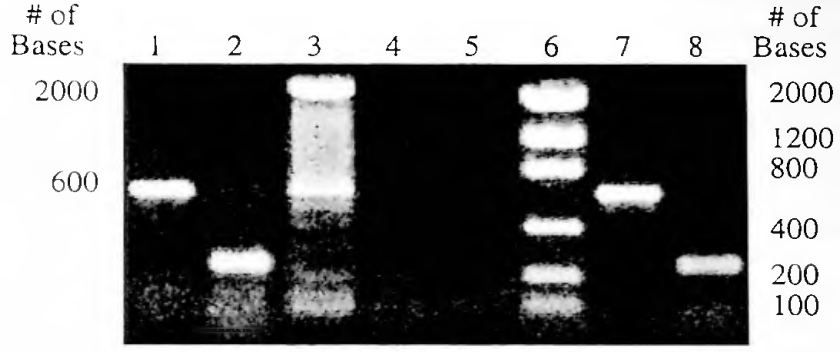

Figure 1. NHE RT-PCR PRODUCTS. RT-PCR products are seen here on a 1% agarose gel containing ethidium bromide. Lane 1 and 7 are the NHE products, ~590 bases, using NHER and 3F primers against the cDNA from a saltwater control fish in lane one and from a saltwater acid loaded fish in lane 7. Lanes 2 and 8 are the control lanes, showing PCR products using actin primers against saltwater control and acid loaded fishes, respectively. The middle two lanes, 4 and 5, show the negative controls (no RNA with primers). The numbers to the left of the gel indicate the size of the top and middle bands in the molecular ladder in lane 3, and the numbers to the right of the gel are the respective sizes of the ladder bands seen in lane 6.

Studies on the ßNHE isoform found in trout red blood cells suggest that it is not involved in physiological pH regulation (Malapert et al., *J. Exp. Biol.* 200:353-360, 1997). Instead, ßNHE appears to be activated in fish red blood cells when they are in hypoxic water to create an alkaline environment; thereby, enhancing the binding of oxygen to hemoglobin. The function of ßNHE in gill epithelial cells *in vivo* is not known. Our laboratory has detected the presence of ßNHE and NHE2 in the gill tissue of another teleost, *Myoxocephalus octodecimspinosus*, (Blackston et al., this volume). In addition, Western blot analysis has shown that an NHE 1- like protein may be physiologically regulated in the sculpin in response to internal acidosis (Choe et al., this volume). The presence of multiple NHE isoforms in the gill tissue of these teleosts suggests that NHE does play an important role in acid-base regulation across the gills of fish. The search for other NHE isoform sequences and the completion of the ßNHE sequence in the killifish are currently underway. Future research will focus on quantifying the relative expression of the NHE isoform(s) in response to varying internal and external conditions by Northern blot analysis. This research was funded by the NSF RUI-94-19849 to J.B.C.