Analysis of branchial longhorn sculpin (*Myoxocephalus octodecimspinosus*) Na^+/H^+ exchanger isoform 3 using Northern hybridization

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We have previously reported the preliminary PCR detection and partial 1.0 kb cDNA sequence of longhorn sculpin (*Myoxocephalus octodecimspinosus*) gill Na^+/H^+ exchanger isoform 3 (NHE3)[3]. Expression of NHE3 mRNA has been shown in the Osorezan dace *Tribolodon hakonensis*, a cyrinid teleost, which lives in extremely acidic freshwater lakes (pH 3.4 to 3.8)[2]. To date NHE3 mRNA expression is not known in a marine teleost. In this study, Northern hybridization analysis using a chemiluminescent probe was used to further characterize gill NHE3 mRNA in the sculpin.

Gill RNA was isolated from seawater adapted sculpin (n=3) using the TRI Reagent method (Molecular Research Center, Inc.) and samples where loaded on 1 % formaldehyde agarose denaturing gel. Total RNA was transferred to a positively charged nylon membrane (Millipore Corporation) using capillary transfer with 10X SSC[4]. Total RNA was then fixed to the membrane according to manufacturer's protocol using an UV cross-linker (254 nm) at 5,000 microjoules/cm^2 followed by drying at 80°C for 15 minutes. Sculpin NHE3 anti-sense DNA probe was synthesized using a PCR digoxigenin (dig) probe labeling kit (Roche Applied Science). Hybridization of the NHE3 dig labeled probe was performed using ULTRAhyb Hybridization buffer (Ambion). The membrane was transferred through a series of stringency washes for removal of nonspecifically bound DNA probes. The membrane was then blocked (Roche Applied Science) and bound to an alkaline phosphate (AP) conjugated anti-dig antibody. Immuno-Star AP substrate solution (Bio-Rad Laboratories) was then applied to the membrane and visualized on a x-ray film.

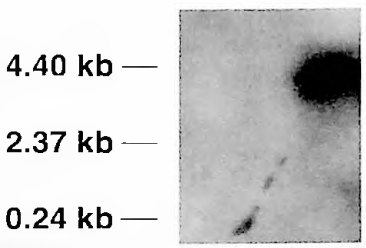

Figure 1: Northern blot of sculpin gill total RNA (10 µg). A transcript at approximately 4.3 kb is visible in RNA from gill tissue. Positions of RNA molecular weight markers are marked (bars). A 5 min. exposure is shown.

Northern detection of sculpin gill homogenates shows a transcript at approximately 4.3 kb. These Northern analysis results are similar in size to the dace (4.0 kb)[3] and rat (5.2 kb)[1] NHE3 mRNA. The data suggest presence of longhorn sculpin gill NHE3 mRNA very similar in size and homology to the dace NHE3, but a complete transcript sequence and investigation of gene expression in the sculpin gill is needed to elucidate NHE3 function. Further studies include complete sequencing of sculpin NHE3 cDNA and northern analysis investigating changes in NHE3 expression in acid infused longhorn sculpin. Funding was provided by a NSF IBN-0111073 to J.B.C.

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