mRNA for the NHE2 exchanger is expressed in a variety of epithelial tissues in the spiny dogfish, *Squalus acanthias*

Abraham Freiji and James B. Claiborne
Department of Biology, Georgia Southern University, Statesboro, GA 30460

Ion transporters are thought to be responsible for acid excretion and ion regulation in marine fish[2]. Recently the Na^+/H^+ exchanger 2 isoform[1] (NHE2) in the gill tissue of the spiny dogfish was located and sequenced[3]. The objective of the following study was to determine whether the mRNA for NHE2 was transcribed in other epithelial tissues in spiny dogfish, specifically: stomach, kidney, intestine, rectal gland, liver, or white muscle.

Tri Reagent (MRC, Inc.) was used to prepare total RNA from different tissues (gill, stomach, kidney, liver, white muscle, intestine, and rectal gland). Oligo-dT primers were used to carry out reverse transcription. The sequence of the NHE2 in gill was used to design the specific primers (5'-GGT GTC ATC ATC TGC TTC CCT G-3' and 5'- TGG ATT CCT ATT GTT CTC CCTTCG-3'). Actin primers were used as a control in paired PCR reactions. PCR was performed in a thermocycler using a 50 μl reaction. PCR cycling parameters were: initial denaturation temperature of 95 °C, 1 m; followed by 35 cycles of 95 °C for 1 m; 57.5 °C for 1 m; 72 °C for 1 m; and a final extension 10 m at 72 °C. As predicted, the cDNA obtained from the dogfish gill, yielded a band of about 500 bp. The PCR product was sequenced at the DNA Sequencing Facility at MDIBL to confirm that band was indeed NHE2.

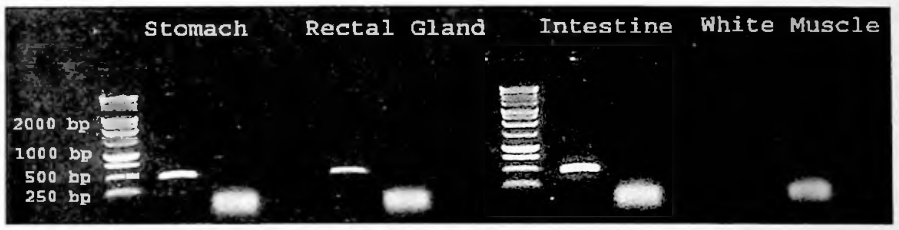

Figure 1. PCR products separated on a 1% agarose gel. For each tissue the first well is the NHE2 band, the second is the actin positive control, and the third is the negative control (does not contain cDNA).

As shown in Figure 1, PCR results confirmed that NHE2 mRNA is highly detectable in the intestine, stomach, and rectal gland. In contrast, only low quantities of product were found in the white muscle. Additional experiments revealed NHE2 message is also found in the kidney, while no NHE2 signal was identified in liver tissue. Furthermore, preliminary immunohistochemical evidence using specific antibodies against dogfish NHE2 suggests that the protein is expressed in the dogfish rectal gland (Edwards et al. unpublished). Future work will employ RT-PCR to better quantify relative levels of NHE2 mRNA across the different epithelial tissues and monitor potential changes in message levels when the dogfish is subjected to acid-base disturbance. Funding was provided by NSF IBN-0111073 to J.B.C.

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