NHE2 and Na^+/K^+-ATPase immunoreactivity in *Myoxocephalus octodecimspinosus*

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Na^+/H^+-exchangers (NHE) were thought to play a role in freshwater (FW) salt uptake and acid excretion in the earliest models of ion regulation in FW fish[6]. While this scenario has since been shown to be thermodynamically unlikely in FW fish, it may explain transepithelial ion movement in seawater fish[7]. Claiborne et al[2] have shown that longhorn sculpin (*Myoxocephalus octodecimspinosus*) acid excretion rates decrease as external salinity decreases. Acid excretion rates also decrease in the presence of amiloride and 5-(N,N-hexamethylene)-amiloride, known NHE inhibitors[1]. Both studies suggest an apical NHE in the gill epithelium that would be consistent with NHE2 localization at the apical membrane of TALH cells in the mammalian kidney[8]. The first full-length gill NHE2 was cloned using 3'/5' RACE-PCR with sculpin mRNA[3] and has now been used to make a fish-specific NHE2 antibody. Our study describes immunoreactivity results using this antibody.

Longhorn sculpin gills were fixed for 4 h in 3% paraformaldehyde, 0.05% glutaraldehyde, and 4% picric acid in 10 mmol l^{-1} phosphate buffered saline (PBS, pH 7.3) at 4 °C. Gill filaments were dehydrated in a CitriSolv-ethanol series and embedded in paraffin wax. Five micron serial sections were cut through the long axis of the gill filament and placed on positively-charged slides (Fisher Scientific). Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in H_2O for 30 min. Tissue sections were blocked in 2.5% normal horse sera (NHS) in H_2O for 30 min. A 5 min wash in PBS followed each step. Sections were incubated in anti-NHE2 (A94-APS; 1/7500 with 2.5% NHS) or anti-Na^+/K^+-ATPase (α5, 1/5000 with 2.5% NHS, Developmental Studies Hybridoma Bank) overnight at 4 °C in a humidified chamber. After a 5 min PBS wash, 20 µl of biotinylated horse anti-rabbit were applied to each tissue section, followed by another PBS wash and incubation in 20 µl of ABC reagent (Vectastain Elite ABC kit, Vector Labs). After 5 min incubation in Vector VIP (purple) or Vector SG (blue/grey, Vector Labs), slides were washed in running water, dehydrated, and permanently mounted by coverslip (Permount, Fisher Scientific). Individual sections were labeled first with anti-NHE2 and then with anti-Na^+/K^+-ATPase for co-localization. A94-APS is a polyclonal rabbit antibody made against AA 694-708 (Ac-CVDNEHGSADNFRDGH-amide) of the longhorn sculpin NHE2 sequence (BioSource International). This peptide is from the variable, carboxyl terminal end of the NHE2 sequence and is not homologous to mammalian NHE1 or NHE3 isoforms or other fish NHE2 isoforms known to date.

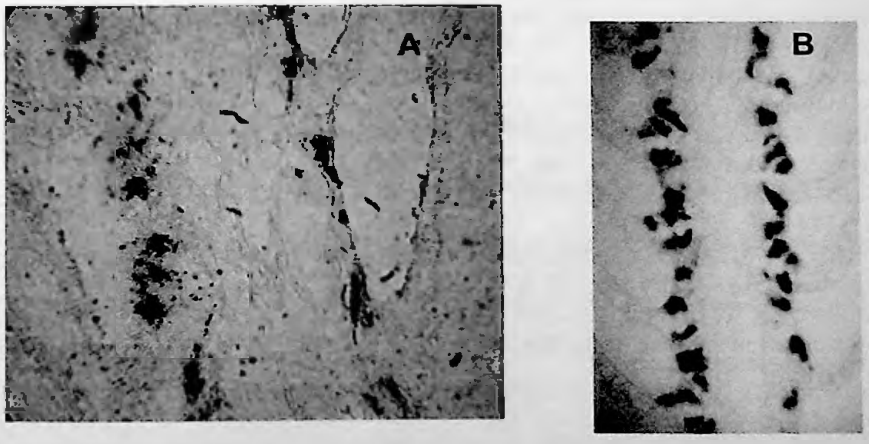

Fig. 1. Representative micrographs of A) anti-NHE2 staining in longhorn sculpin gill (A94-APS: 1/7500, ~1000X magnification) and B) anti-Na^+/K^+-ATPase staining (α5: 1/5000, ~500X magnification). Filaments are vertically oriented with staining in the interlamellar region.

Figure 1A shows the granular staining of NHE2 in the epithelial layer of the interlamellar region. Na^+/K^+-ATPase was used as a cellular marker to show mitochondria-rich cells in the sculpin gill (Fig. 1B)[5]. Anti-Na^+/K^+-ATPase staining was diffuse throughout the cell except in the nucleus and appears to be consistent with basolateral staining. Double-labeled gill sections showed many cells which stained for NHE2 and Na^+/K^+-ATPase, while some cells only stained for NHE2 (Fig. 2).

Fig. 2. Representative micrograph of gill section double-labelled with anti-NHE2 (A94-APS: 1/7500) and anti-Na^+/K^+-ATPase (α5: 1/5000). Gill filament is horizontally oriented with staining in the interlameller region. Magnification ~500X. Closed arrow indicates cell with NHE2 only; open arrow indicates cell with NHE2 and Na^+/K^+-ATPase.

This immunohistochemical study demonstrates staining consistent with an apically located Na^+/H^+-exchanger in the interlamellar region. The basolaterally located Na^+/K^+-ATPase stains diffusely throughout the chloride cells. Conversely, NHE2 shows a granular staining pattern that could indicate staining of NHE2 located in sub-apical vesicles. As noted above, acidosis induces a significant increase in H^+ transfer to the water across the gills. This might be explained by the movement of vesicles towards the apical membrane during an acidosis. Increased mRNA expression levels for NHE2 has been shown to follow an intraperitoneal acid perfusion.[4] Current work seeks to determine if NHE2 protein levels also increase during acidotic events. An increase in NHE2 protein expression would support the role of NHE2 in acid-base regulation. Funding was provided by a NSF IBN-0111073 grant to JBC and a GSU Professional Development Grant to JSC.

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