CLONING AND SEQUENCING OF COMPLIMENTARY DNA FOUND IN THE GILL TISSUE OF THE LONG-HORNED SCULPIN (*MYOXOCEPHALUS OCTODECIMSPINOSUS)*

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The sodium/hydrogen exchanger (NHE) is a transport protein thought to be involved in ion regulation and acid excretion in marine fishes (Claiborne et al., *J. Exp. Biol.* 279:509-520, 1997). Recently, two partial sequences of NHE, a βNHE and an NHE2 isoform, have been cloned and sequenced from a single RT-PCR product obtained from the gill tissue of the long-horned sculpin using NHE degenerate primers (Claiborne et al., *J. Exp. Biol.* 202:315-324, 1999). We have used the partial product of the NHE2 sequence to screen a sculpin gill cDNA library to 1) obtain the complete or partial sequence of a sodium/hydrogen exchanger; 2) determine which isoform is being expressed in the gill tissue, and; 3) determine if other sculpin cDNA can be found using the NHE2 probe at low stringency.

The partial NHE2 sequence (Claiborne et al., *J. Exp. Biol.* 202:315-324, 1999) was reamplified by PCR incorporating radioactive phosphorus (^{32}P-dCTP) and used as a species-specific probe to screen the sculpin gill complimentary DNA (cDNA) library. Previously made sculpin gill cDNA libraries (Morrison-Shetlar, Claiborne, and McKenzie, unpublished) were plated out onto agar plates containing ampicillin. Colonies of bacteria containing the ampicillin resistant gene were grown and transferred onto nylon membranes. The membranes were sequentially placed, bacterial side up, in solutions 1-4 for five minutes each (solution 1: 0.5mM EDTA/1% SDS, solution 2: 0.5M NaOH/1.5M NaCl, solution 3: 1M Tris pH 8.0/1.5M NaCl, and solution 4: 2x SSC, (sodium citrate and sodium chloride buffer)). The solutions caused disruption of the bacterial cells and denaturation and renaturation of the plasmid DNA. To permanently bind the plasmid DNA to the membranes, an UV cross-linker (Fisher Scientific) was used. Membranes were then soaked in prehybridization solution (for 40ml: 12ml 6x SSC, 4ml 10x Denhardts, 2ml 1% SDS, .2 ml 50μg salmon sperm DNA, 21.8 ml distilled water) for 1 hour at 42°C with shaking. Radioactively labeled NHE2 probe was purified through a sephadex G50 fine column. Membranes were then soaked in hybridization solution (for 40 ml: 20ml 50% formamide, 12ml 6x SSC, 2ml 1% SDS, .2 ml 50 μg, 4 ml 10x Denhardts, and 1.8ml of distilled water) with the radioactive NHE2 probe and incubated overnight at 42°C with shaking. Membranes were then washed to remove any unbound probe and exposed to autoradioagraphy film for 48 hours at -70°C. Positive colonies were cut out of the agar, placed in LB medium and grown for thirty minutes before being replated on agar plates and grown overnight. The screening process was repeated with these new plates through three cycles until a single colony could be isolated. Single positive colonies were isolated from the agar plates and grown up in LB medium. The plasmid DNA was isolated by alkaline lysis and a restriction digest was done to measure the size of the insert. A Southern blot (Maniatis et al., *Molecular Cloning* 2:9.38-9.43,1989) was performed using the same radioactive NHE2 probe and hybridizing solution, for confirmation that positive colonies were isolated properly. Positive bands obtained from the Southern blot procedure reconfirmed the colonies selected from the screening process, and the plasmid DNA was sequenced at the Medical College of Georgia.

Two different sculpin gill cDNA libraries were screened, a library made from the gills of fish adapted to 20% seawater and a library from gills of seawater fish. A total of 15 plates were screened from each library and the screening process was repeated three times. Of the 30 plates screened, 19 positive colonies were isolated from the 20% seawater library and ten positive colonies isolated from the seawater library. Out of 29 positive colonies only eight were submitted for sequencing. From the sculpin gill seawater library a partial sequence with high homology to a zinc finger protein was detected. A probable full-length sequence of glutathione s-transferase (GST) was obtained from the sculpin gill 20% seawater library. The sculpin GST sequence was found to be 85% homologous at the amino acid level to the flounder, *Pleuronectes platessa* (Figure 1).

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Sculpin:     118 MAKDMTLLWGSGSPPCWRVMIALEEKSLQGYNEKLLSFEKGEHKSKEVMA 267
                 ||||||||||||||||||||||+||||+||||| |||||||||||| |||
Flounder:      1 MAKDMTLLWGSGSPPCWRVMIVLEEKNLQGYNSKLLSFEKGEHKSAEVMS 50

Sculpin:     268 MNPRGQLPAFKHKDYVLNESYGACMYLANQFKSQGTKLIPDCPAEQAMMY 417
                 ||||||||+|||   |||||| |||||  ||||||+|||||||||||||
Flounder:     51 MNPRGQLPSFKHGSKVLNESYAACMYLESQFKSQGNKLIPDCPAEQAMMY 100

Sculpin:     418 QRIFESLTLNQKMADVIYYTWKVPEGERHDSAVKRNKKTLTTEVKLWEGY 567
                 ||+||+|||+|||||||||+||||| |||||||||||| +||||+|||| |
Flounder:    101 QRMFEGLTLTQKMADVIYYSWKVPEAERHDSAVKRNKENLTTELKLWEEY 150

Sculpin:     568 LNKGSGSXLAGKNFSLADVVVYPSIAYLFHFQLCEERYPKLAXYYXXFKD 717
                 |+| ||| +||| |||||| |+| +||||+| |+|||||||+ ||   |+
Flounder:    151 LQKTSGSFVAGKSFSLADVSVFPGVAYLFRFGLSEERYPKLTAYYNSLKE 200

Sculpin:     718 RASIKAFWXSTWL- 759
                 | |||| |   ||
Flounder:    201 RPSIKASWPPTWL- 214
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Figure 1: Sculpin amino acid sequence as compared to the published flounder Glutathione s-Transferase sequence. The two sequences are 85% homologous and 79% identical. Vertical lines represent identical matches and (+) represent homology between the two sequences.

Interestingly, glutathione s-transferase is thought to be involved in detoxification of reactive electrophiles and free radical scavenging. It may also protect the cell from the harmful effects of oxidative stress (Deleve, *Seminars in Liver Disease*. 18:403-413, 1998 and Sram, *Environmental Health Perspect.* 106:231-237, 1998). To our knowledge, this is the first time that the cDNA of GST has been found in the gill tissue of the long-horned sculpin. It is likely that the low stringency used in the library screening process, allowed non-specific binding of the NHE2 probe.

While no positive colonies containing NHE sequences have been detected to date, we are screening the sculpin gill cDNA libraries at a higher stringency, to obtain the full-length sequence of a sodium/hydrogen exchanger. Total sculpin gill RNA samples acquired this summer are also being used in 5'/3' RACE (rapid amplification of cDNA ends) reactions in order to obtain the full-length message of one or more NHE isoforms being expressed in the gill tissue of the sculpin. This research is supported by NSF IBM 9808141 to J.B.C and A.I.M.S.