

Cloning of aquaporin1e gene homologues in the dogfish shark (*Squalus acanthias*) and hagfish (*Myxine glutinosa*)

Christopher P Cutler

Department of Biology, Georgia Southern University, Statesboro, GA30460

Information concerning the first aquaporin water channel in elasmobranchs was recently published¹. However, despite this original report and other reports on aquaporin water channels in teleost fish², their presence and role in elasmobranchs and other evolutionarily more ancient fish lineages, such as agnathans, remains virtually un-investigated. This study set out to begin the process of remedying this situation.

The nucleotide sequence of bullshark aquaporin1e¹ (AQP1e in *Carcharhinus leucas*) was utilized to redesign oligonucleotide primers that were targeted at amplifying AQP1e homologues in the dogfish and hagfish. The primers designed used the nucleotide analogue, inosine, at positions of uncertainty in the sequence and were made using regions of the amino acid sequence that are the most conserved in aquaporins from a variety of species. Initial PCR amplifications resulted in products of approximately the correct size, as estimated in agarose gel electrophoresis. Successful amplification was achieved in cDNA samples made from total RNA extracted from dogfish rectal gland and hagfish esophagus. Complementary DNA (cDNA) was made from RNA samples (for PCR), using Invitrogen's Superscript III reverse transcriptase. Putative positive DNA bands from agarose gels were purified using a GeneClean DNA purification kit (Bio 101, Irvine, CA) and cloned into a TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). The initial products sequenced for the dogfish and hagfish AQP1e homologues were situated in the center of the coding region of the gene. Amplification of the 5' and 3' ends of both genes was attempted using cDNA made for that purpose, with a Marathon cDNA Cloning kit (Clontech, Mountain View, CA). The 5' and 3' ends of the dogfish and the 5' end of the hagfish AQP1e homologues were successfully amplified (using PCR), cloned and sequenced. Overlapping sequences were pieced together using GeneJockey DNA analysis software (Biosoft, Cambridge, UK).

As described previously¹, bullshark AQP1e is roughly equally similar to human AQP's 1, 2 and 5. This property is shared to some extent by the hagfish AQP1e homologue, although this partial cDNA has slightly higher levels of homology to human AQP5. The homologies of all of the genes in Table 1, in comparison to human AQP2, are somewhat lower than might be expected for other mammalian AQP2 genes, due to the presence of a small insert of 12 amino acids that is unique to human AQP2. Interestingly, although the hagfish cDNA appears to be an orthologue of bullshark AQP1e, the low level of homology between these two genes in particular leaves that open to question.

The situation with the cDNA amplified from dogfish rectal gland is entirely different. Unlike the bullshark and hagfish cDNAs, the dogfish cDNA shares significantly higher levels of homology with human AQP1 than with other human AQP's, suggesting that it represents an orthologue of mammalian AQP1. In fact, the level of amino acid homology between the putative dogfish AQP1 and human AQP1 is almost identical to that between eel (teleost) AQP1³ and human AQP1 (61.0%). The amino acid homology between eel AQP1 and the putative dogfish AQP1 is somewhat lower at 56.5% (in the region of the sequence used for the table). The relatively high level of homology of the putative dogfish AQP1 compared to human AQP2 or AQP5 leaves open the possibility that it may represent a contamination artifact from another species. Confirmation of its status as a dogfish AQP1 orthologue

will await Northern blotting experiments that will determine if there is significant and consistent expression of the gene in dogfish tissues.

Table 1. Comparison of bullshark AQP1e, dogfish and hagfish AQP1e homologues, and human AQP1, AQP2 and AQP5, partial derived amino acid sequences. Only the portions of sequences corresponding to the partial hagfish AQP1e homologue amino acid sequence were used in the comparison. Data represent the percentage of identical amino acids in the alignment between any two sequences.

	Hagfish	Dogfish	Bullshark	Human AQP1	Human AQP2	Human AQP5
Hagfish	-	46.1	34.4	42.2	41.1	46.4
Dogfish	46.1	-	47.3	61.1	48.7	53.0
Bullshark	34.4	47.3	-	42.8	42.2	43.6
Human AQP1	42.2	61.1	42.8	-	43.4	46.7
Human AQP2	41.1	48.7	42.2	43.4	-	68.8
Human AQP5	46.4	53.0	43.6	46.7	68.8	-

The presence or absence of aquaporin genes in agnathan or elasmobranch genomes of course, tells us nothing of their physiological role. Experiments were begun to assess the physiological role of these genes (and/or potential osmoregulatory role), using manipulation of the external environmental salinity to influence any mechanisms controlling ion and water balance in these fish. Tissue samples obtained from dogfish and hagfish held in different environmental salinities will be used for total RNA extraction to assess any changes in the expression of the new aquaporin genes.

This work and the PI were supported by a New Investigator Award Fellowship from MDIBL. I would also like to acknowledge the help of students funded by MDIBL: Gina Luchini (from Ellsworth High School, on a Hancock County Scholars award with support from the Betterment Fund) and Makesha Foster (from Georgia Southern University, on an NSF REU fellowship (DBI-0453391)).

1. **Cutler, C. P., L. Meischke, and G. Cramb.** Evolutionary and comparative analysis of aquaporin water channel genes in fish. *Bull. Mt. Des. Isl. Biol. Lab.* 44: 55, 2005.
2. **Cutler, C. P., and G. Cramb.** Molecular physiology of osmoregulation in eels and other teleosts: The role of transporter isoforms and gene duplication. *Comp. Physiol. Biochem.* 130: 551-564, 2001.
3. **Martinez, A-S., C. P. Cutler, G. Wilson, C. Phillips, N. Hazon and G. Cramb.** Cloning and expression of three aquaporin homologues from the European eel (*Anguilla anguilla*): effects of seawater acclimation and cortisol treatment on renal expression. *Biol. Cell.* 97: 615-627, 2005.