

**CLONING AND CHARACTERIZATION OF GLUCOSE
TRANSPORTERS FROM COD (*Gadhus morhua*) HEART**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

We are currently studying the effects of temperature and hypoxia on growth rates and survival of cod (*Gadhus morhua*). Of particular interest is cardiac performance under these conditions. In cod heart, glucose is an essential metabolic fuel for the production of ATP and the maintenance of contractile function under both aerobic and particularly anaerobic conditions.

Glucose transport across the membranes of animal cells is mediated by facilitative glucose transporters (GLUTs). Thirteen of these transporters have been identified in humans and are expressed in a tissue specific manner. Of particular interest to the heart are GLUT-1 and GLUT-4. GLUT-1 is the basal glucose transporter and is induced in other species under hypoxic conditions. GLUT-4 is the insulin sensitive glucose transporter. GLUT-1 has recently been cloned from carp (*Cyprinus carpio*) (Teerijoki *et al.*, 2001) and rainbow trout (*Oncorhynchus mykiss*) (Teerijoki *et al.*, 2000). A GLUT molecule with high sequence similarity to GLUT-4 has also recently been cloned from brown trout (*Salmo trutta*) (Planas *et al.*, 2000).

We have identified two glucose transporters in cod heart by reverse transcription - polymerase chain reaction (RT-PCR). Primers were designed based upon

consensus sequences in conserved areas from other species. A phylogenetic tree was generated using AlignX (Informax Inc.) and indicates that the first is most likely GLUT-1 and the second, GLUT-4. Of the sequence data generated thus far, cod GLUT-1 has approximately 82%, 79% and 76% nucleotide identity to rainbow trout, carp and human GLUT-1, respectively. Cod GLUT-4 has approximately 73%, 68% and 70% nucleotide identity to human GLUT-1 and human and brown trout GLUT-4, respectively. Cod GLUT-1 and GLUT-4 have approximately 73% nucleotide identity.

Northern blot analysis indicates that the mRNA transcript for cod GLUT-1 is approximately 6 kB in length and is highly expressed in brain, gill, heart and kidney. The 5' untranslated region (UTR) has been cloned by 5' Rapid Amplification of cDNA Ends (RACE) and is 215 bp. The open reading frame (ORF) is estimated to be 1500 bp based on the published sequences of GLUT-1s from other species. Approximately 1300 bp of ORF sequence has been identified thus far. A cod heart cDNA library has been constructed and is being screened with a probe generated from the known cod GLUT-1 ORF sequence to obtain the remaining ORF sequence and that of the 3'UTR.

The ORF of cod GLUT-4 is also estimated to be about 1500 bp. Approximately 1200 bp of ORF sequence has been generated. The cod heart cDNA library will also be screened to obtain the remaining GLUT-4 sequence data.

References

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