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In vivo regulation of GLUT2 mRNA in sea bass (*Dicentrarchus labrax*) in response to acute and chronic hypoxia

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ABSTRACT

The expression and regulation of sodium-independent glucose transporter (GLUT)-2, in relation to hypoxia has not yet been explored in fish or other vertebrates. In this study, the complete open-reading frame for sea bass GLUT2 was isolated and deposited in the GenBank. The predicted 12 transmembrane domains of the protein (508 amino acids) are presented. A phylogenetic tree was constructed on GLUT2 sequences of sea bass and those of other teleost, amphibian, avian, and mammalian species. We also analyzed acute and chronic hypoxia-induced changes in the expression of hepatic GLUT2 mRNA, using one-tube, two-temperature, real-time RT-PCR with which gene expression can be absolutely quantified by the standard curve method. The number of GLUT2 mRNA copies was significantly increased in response to both acute (1.9 mg/L, dissolved oxygen for 4 h) and chronic (4.3 mg/L, DO for 15 days) hypoxia conditions. The hypoxia-related changes in GLUT2 mRNA copy number support the view that GLUT2 is involved in the adaptation response to hypoxia in sea bass, a marine hypoxia-sensitive species. We realize that the GLUT2 mRNA levels in our study do not measure the physiological effects produced by the protein. Thus, we can only speculate that, under hypoxic conditions, GLUT2 probably functions to allow the glucose produced from liver glycogen to leave the hepatocytes.

1. Introduction

Glucose transporters are membrane proteins active in the transport of hexoses such as glucose, galactose, and fructose across plasma membranes. They are divided into two structurally and functionally distinct families: sodium/glucose cotransporters (SGLTs) and facilitative sodium-independent glucose transporters (GLUTs) (Wood and Trayhurn, 2003). SGLTs reside in the membranes of intestinal and kidney epithelial cells (Burant et al., 1991), whereas GLUTs can be found in most animal tissues.

At least 13 different GLUT isoforms have been thus far identified in different mammalian and avian tissues (Wood and Trayhurn, 2003; Wu and Freeze, 2002), each being the product of a separate gene. These integral membrane glycoproteins are characterized by the presence of 12 putative transmembrane spanning domains with cytosolic amino and carboxyl termini (Pessin and Bell, 1992). They display significant sequence homology but different affinities for glucose and different tissue-specific patterns of distribution and may be subject to differential hormonal regulation (Joost et al., 2002). On the basis of sequence similarities and characteristic signature motifs, GLUT isoforms are categorized into class I (GLUT1–4), class II (GLUT5, 7, 9, and 11), and class III (GLUT6, 8, 10, 12 and the myo-inositol transporter HMIT1) (Joost and Thorens, 2001; Joost et al., 2002; Wood et al., 2007). The class I facilitative transporters have been thoroughly characterized in mammals. Given their high glucose specificity and the predominant expression in high energy demanding tissues, they have been the focus of several metabolic studies examining glucose and energy homeostasis (Katsumata et al., 1999; Joost and Thorens, 2001).

In recent years, the four mammalian GLUT homologues belonging to class I have also been identified in fish: GLUT1 in tilapia, rainbow trout, common carp, and Atlantic cod (Wright et al., 1998; Teerijoki et al., 2000, 2001; Hall et al., 2004, 2006); GLUT2 in rainbow trout and Atlantic cod (Krasnov et al., 2001; Hall et al., 2006); GLUT3 in grass carp and Atlantic cod (Zhang et al., 2003; Hall et al., 2005); and GLUT4 in brown trout and Atlantic cod (Planas et al., 2000; Hall et al., 2006).

The presence of mammalian GLUT homologues in fish has kindled an interest in studying their transcriptional regulation, given not only the importance of glucose uptake in the overall energetics and regulation of carbohydrate metabolism, but also since many fish species face either acute or chronic low oxygen challenges.

Expression of GLUT genes in mammals is known to be hypoxia-sensitive; it is regulated through the transcription factor hypoxia inducible factor-1 (HIF-1), via its binding to the hypoxia-responsive elements present in the GLUT genes (Iyer et al., 1998; Ebert et al., 1995). Increased expression of GLUT genes in mammalian hypoxic tissues has been associated with an enhanced glucose uptake rate to facilitate the supply of metabolic energy (Bunn and Poyton, 1996) and to protect cells from hypoxic injury (Lin et al., 2000). Much less information is available on tissue expression patterns of GLUTs in fish exposed to hypoxia, and the mechanisms involved in the hypoxia response of these genes in fish are almost unknown.

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Table 1
Primers used

Primer	Sequence 5′–3′	Purpose
Glut2-sense1	CATCAATGCACCACAGAAG	RT-PCR
Glut2-antisense1	CTCAGCCAGTCTATGCCACA	RT-PCR
Glut2-sense2	ATGAGGAGAGAGAAAGAGGAAGC	RT-PCR
Glut2-antisense2	AAGTCGGCACAGAGCCCTAA	RT-PCR
Glut2- 5′GSP1	GGATGCCAATGACAATAGCC	5′ RACE
Glut2- 5′GSP2	GCAAAGACCCATCAGCAGTC	5′ RACE
Glut2-5′GSP3	GATTGCCACCGACAATGACC	5′ RACE
Glut2-3′GSP1	GGTCCCATTCCATGGTTCAT	3′ RACE
Glut2-3′GSP2	CTGGACCAGCAACTTCATCA	3′ RACE
GLUT2 - ESO1s	GAGGAAGCGGACAGGCA	Real-time
GLUT2 - ESO1as	CTTTGATCCGCTCATCTGTGTACA	Real-time
GLUT2 - ESO1FAM	CAGGGTCTCCATCCTTT	Real-time
Glut2-T3sense	*caattaaccctcactaaaggg*GAGCCCACGGTACCTTTACA	Standard curve
Glut2-antisense1	CTCAGCCAGTCTATGCCACA	Standard curve

We have recently reported in sea bass, a marine hypoxia-sensitive teleost species (Terova et al., 2008), that hypoxia (either acute or chronic) enhances the expression of the hypoxia inducible factor-1α (HIF-1α), which is a central regulator of hypoxia response. Once it has been activated, HIF-1 stimulates the transcription of a series of genes for adaptation to hypoxia, including glucose transporters.

GLUT-2, which is the subject of this study, is the major glucose transporter in the hepatocytes and in β-cells of pancreatic islets in mammals. It is unique among the GLUTs by virtue of its high half-maximal saturation constant (Km ~30 mM compared with 1–10 mM for the other GLUT isoforms) and its ability to transport both D-glucose and D-fructose (reviewed in Burant et al., 1991; Baldwin, 1993;

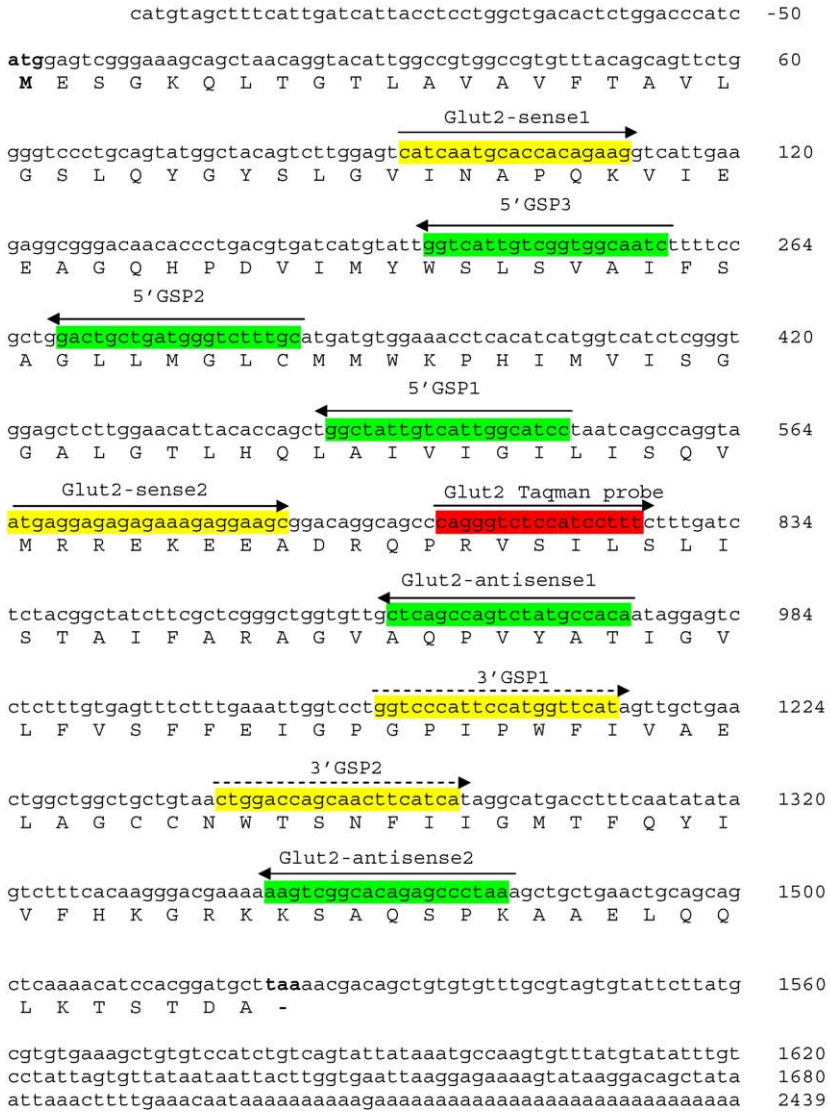

Fig. 1. The nucleotide sequence of sea bass (*Dicentrarchus labrax*) GLUT 2 (accession no. EF014277), with the deduced amino acids shown below the sequence in single-letter code. Nucleotides are numbered to the left. The locations of the primers used in PCR of the full-length transcript and in the 5′ or 3′ RACE are also indicated by solid and broken horizontal arrows, respectively.