GLUT8, a Novel Member of the Sugar Transport Facilitator Family with Glucose Transport Activity*

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GLUT8 is a novel glucose transporter-like protein that exhibits significant sequence similarity with the members of the sugar transport facilitator family (29.4% of amino acids identical with GLUT1). Human and mouse sequence (86.2% identical amino acids) comprise 12 putative membrane-spanning helices and several conserved motifs (sugar transporter signatures), which have previously been shown to be essential for transport activity, e.g. GRK in loop 2, PETPR in loop 6, QQLSGVN in helix 7, DRAGRR in loop 8, GWGIPPW in helix 10, and PETKG in the C-terminal tail. An expressed sequence tag (STS A005N15) corresponding with the 3′-untranslated region of GLUT8 has previously been mapped to human chromosome 9. COS-7 cells transfected with GLUT8 cDNA expressed a 42-kDa protein exhibiting specific, glucose-inhibitable cytochalasin B binding ($K_D = 56.6 \pm 18$ nm) and reconstitutable glucose transport activity (8.1 ± 1.4 nmol/(mg protein × 10 s) versus 1.1 ± 0.1 in control transfections). In human tissues, a 2.4-kilobase pair transcript was predominantly found in testis, but not in testicular carcinoma. Lower amounts of the mRNA were detected in most other tissues including skeletal muscle, heart, small intestine, and brain. GLUT8 mRNA was found in testis from adult, but not from prepubertal rats; its expression in human testis was suppressed by estrogen treatment. It is concluded that GLUT8 is a sugar transport facilitator with glucose transport activity and a hormonally regulated testicular function.

Hexose transport into mammalian cells is catalyzed by the members of a small family of 45–55-kDa membrane proteins, GLUT1–GLUT5 (1–4). These hexose transporters belong to the larger family of transport facilitators, which comprises yeast hexose transporters, plant hoxose-proton symporters, bacterial sugar-proton symporters (5, 6), and organic anion as well as organic cation transporters (7, 8). Defining characteristics in the family of hexose transporters are the presence of 12 membrane-spanning helices and a number of conserved residues and motifs (see Fig. 3). These sugar transporter signatures have been characterized by sequence comparisons as well as by mutagenesis. Substitutions, e.g. of the conserved arginine and glutamate residues on the cytoplasmic surface (9), of tryptophan residues 388 and 412 in helix 10 and 11 (10, 11), tyrosines 146 and 292/293 in helix 4 and 7 (12, 13), glutamine 161 in helix 5 (14), and glutamine 282 (15), have been shown to markedly affect transporter function. In addition, mutagenesis experiments have implicated a motif (QLS) in helix 7 in determining the sugar recognition of GLUT1–GLUT5 (16).

The known glucose transporter (GLUT) isoforms differ in their expression in different tissues, in their kinetic characteristics, i.e. $K_m$ values (2), and in their substrate specificity. GLUT1 mediates glucose transport into erythrocytes and through the blood-brain barrier, and appears to provide a basal supply of glucose for most cells. GLUT2 catalyzes glucose uptake into the liver (17), and is an essential component of the glucose sensing mechanism of the pancreatic β cell (18). GLUT3 is predominantly expressed in neuronal cells (19), whereas GLUT4 is exclusively found in muscle and adipose tissue (20, 21); its subcellular localization is controlled by insulin (22, 23). GLUT5 mediates transport of fructose, but probably not glucose, in intestine and spermatozoa (24).

The diverse tissue distribution and the specific functions of GLUT1–GLUT5 appear to indicate that these genes are sufficient to control glucose uptake in all mammalian tissues. However, two arguments may be raised that suggest the possibility that additional sugar transport facilitators exist. First, in some tissues, only low levels of mRNA of the known isoforms were detected (25). Second, GLUT4 knockout mice exhibited an almost normal glucose transport in muscle, although no compensatory increase of the GLUT1 or GLUT3 gene expression was detected (26). Therefore, in order to identify additional hexose transporters, we conducted a search of the EST data bases taking advantage of the conserved "sugar transporter signatures." This search led to the identification of several novel GLUT-like genes. Here we describe the identification and characterization of GLUT8, a novel sugar transporter with unusual structural features and tissue-specific gene expression.

**EXPERIMENTAL PROCEDURES**

RNA Preparation and PCR Cloning—Tissues were homogenized in 4 mM guanidine thiocyanate, and total RNA was isolated by centrifugation on a cesium chloride cushion (5.88 M) at 33,000 rpm (rotor SW40) for 22 h. 5′ RACE (rapid amplification of cDNA ends) was performed with a kit from Life Technologies, Inc., Eggenstein, Germany, according to the instructions of the manufacturer. Primers for cDNA synthesis and the first amplifications were derived from the sequence of the IMAGE clone 46121. DNA fragments were isolated and subcloned into pUC18 with the SureClone kit (Amersham Pharmacia Biotech, Freiburg, Ger-

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‡ The abbreviations used are: GLUT, glucose transporter; EST, expressed sequence tag; RACE, rapid amplification of cDNA ends; PCRpolymerase chain reaction.
many). Since the first RACE amplification yielded fragments still lacking the 5' end of the cDNA, a second and third amplification was performed on the basis of the sequence information obtained in the previous RACE procedures. All cDNA clones and PCR products were sequenced in both directions by the method of Sanger (ThermoSequenase fluorescent labeled primer cycle sequencing kit; Amersham Pharmacia Biotech, Braunschweig, Germany). Blots generated with RNA from different human tissues were purchased from CLONTECH (Palo Alto, CA). Probes were generated with the Klenow fragment of DNA polymerase I and (α-32P)dCTP by random oligonucleotide priming (27). The nylon membranes were hybridized at 42 °C and washed two times at 55 °C with 0.12 x NaCl, 0.012 x sodium citrate, 0.1% SDS.

Expression of GLUT8 in COS-7 Cells—A fragment of the GLUT8 cDNA comprising the 5'-untranslated region and the full reading frame was amplified by PCR and was subcloned into the mammalian expression vector pCMV, which harbors an SV40 origin, a cytomegalovirus promoter, and a polyadenylation site. GLUT4 cDNA (21) was subcloned into the same expression vector as described (28). COS-7 cells were transfected with calcium phosphate/DNA co-precipitates as described in detail previously (29), and were harvested 64 h after transfection.

Preparation of Membrane Fractions from Transfected Cells—Cells transfected with glucose transporter cDNA were homogenized and fractionated as described previously (28) with a modification of a protocol employed in 3T3-L1 cells (30). For detection of GLUT8 in the membrane fractions (plasma membranes, 13,000 × g; high density microsomes, 45,000 × g; and low density microsomes, 200,000 × g), antiserum against a C-terminal peptide (sequence in single-letter code: KGRTLEQVTAHLG) was used.

Assay of Cytochalasin B Binding—Equilibrium cytochalasin B binding in plasma membranes from transfected cells was assayed by a method established with fat cell membranes (31) with modifications performed on the basis of the sequence information obtained in the previous RACE-PCR.

Isolation of Human and Mouse GLUT8 cDNA—In order to identify unknown glucose transporter-like sequences, we performed a search of the EST data bases with the protein sequences of the known GLUT isoforms (thlaspt program). A total of approximately 200 EST sequences found in this search were further analyzed by individual comparisons. Among these, several human and murine EST sequences exhibited significant similarity with the GLUT family but differed from the known GLUT isoforms. By sequencing a clone obtained from the IMAGE consortium (clone no. 46121, EST HS414155) we generated a partial cDNA sequence of a glucose transporter-like protein; this sequence exhibited significant similarity with a portion of the GLUT1 comprising membrane-spanning helices 5–12. Screening of several cDNA libraries failed to isolate longer clones. Thus, the sequence information of the missing 5’ portion was obtained by three sequential 5’-RACE amplifications with cDNA from human testis. Similarly, the mouse cDNA sequence was obtained by sequencing of a partial IMAGE clone (clone 1178770, EST AA734465) and subsequent RACE-PCR.

Genomic Localization of the GLUT8 Gene—Additional data base searches with the GLUT8 cDNA led to the identification of a human EST (STS A005N15/HSG20347) for which genomic localization has been determined by radiation hybrid mapping. This sequence tag is identical with the 3’ end of 12 independent GLUT8 cDNA clones. Thus, it can be concluded that the sequence tag maps the genomic localization of GLUT8 to chromosome 9.

Sequence Characteristics of GLUT8—The cDNAs of both mouse and human GLUT8 contain open reading frames encoding a sequence of 477 amino acids (Fig. 1). Within the coding region, 85.2% of the nucleotides and 86.2% of the amino acids are identical. The deduced amino acid sequence of human GLUT8 is 29.4% identical with that of the GLUT1 (Fig. 1). Furthermore, 73 (55%) of the 132 residues that are identical in all mammalian GLUT isoforms (marked by asterisks in Fig. 1) are conserved in GLUT8. Analysis of the sequence with the HELIXMEM program suggested the presence of 12 putative membrane-spanning helices, consistent with the presumed tertiary structure of a transport facilitator (Fig. 2). The sequence contains all motifs (sugar transporter signatures) that are characteristic for the family of sugar transporters, in particular two motifs similar to the PESPR/PETKGR motifs following helices 6 and 12 (Fig. 2). Additionally, motifs corresponding with the GRR motifs in loops 2 and 8, and glutamate and arginine residues in the intracellular loops 4 and 10 are present in GLUT8. Furthermore, tryptophan residues corresponding with Trp388 and Trp412 in GLUT1 are present; Trp388 has been implicated in the binding of the transport inhibitor cytochalasin B. There are striking differences from GLUT1. Loop 1 is much shorter than that of GLUT1 and lacks a glycosylation site; instead, the glycosylation site appears to be located in the larger loop 9. Furthermore, the conserved STS motif in loop 7 is replaced by AET.

Fig. 3 illustrates a dendrogram of an alignment of GLUT8 with its closest relatives. The protein with the highest similarity is another novel transport facilitator (GLUT9, 43.6% identical amino acids), which was recently cloned by our group. The next relatives are the mammalian glucose transporters (GLUT1; 29.4% identical residues), the Saccharomyces pombe inositol transporter (30.2% identical residues), and the Escherichia coli arabinose and xylose transporters (32.8% and 29.1% identical amino acids, respectively). Individual alignments of these sequences (PALIGN program) also indicated that the similarity of GLUT8 with the arabinose transporter (155 identical amino acids) is somewhat higher than that of the arabinose transporter with the GLUT1 (140 amino acids). Thus, GLUT8 may be more closely related with a common evolutionary precursor of the sugar transporter family than the GLUT isoforms.

Cytochalasin B Binding Activity of GLUT8—The mouse GLUT8 cDNA was subcloned into an expression vector driven by the cytomegalovirus promoter, and COS-7 cells were transfected with this construct. Plasma membranes from transfected cells were isolated by differential centrifugation and were incubated with 3Hcytochalasin B and different concentrations of unlabeled ligand. As is illustrated in Fig. 4, overexpression of GLUT8 protein caused a marked increase in specific binding of cytochalasin B. The mean KD derived from Scatchard plots of the binding curves (data not shown) was 56.6 ± 18 nM (three independent transfections) and is well within the range of KD values assayed for binding of cytochalasin B to members of the GLUT family (22, 36). Cytochalasin B binding to GLUT8 is fully inhibitable by glucose with an IC50 of approximately 50 mM.

Glucose Transport Activity of GLUT8—Plasma membranes from cells transfected with GLUT8 cDNA were solubilized, and proteins were reconstituted into lecithin liposomes for assay of their glucose transport activity. As is illustrated in Fig. 5

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transfection with GLUT8 cDNA produced an 8-fold increase in D-glucose transport activity as compared with membranes from cells transfected with blank vector. Transfection with GLUT4 cDNA produced a somewhat lower increase. Note, however, that this difference appeared to reflect a lower abundance of GLUT4 in the reconstituted membranes, since normalization of transport rates for cytochalasin B bound (tracer only) indicated a somewhat lower activity of GLUT8 (392 ± 113 pmol of glucose/pmol of cytochalasin B versus 810 ± 228 in membranes with GLUT4).

In order to obtain an additional comparison of the glucose transport activities of GLUT4 and GLUT8, membranes were prepared from transfected cells on a larger scale allowing a full Scatchard analysis of the number of cytochalasin B binding sites. In this experiment, GLUT8 transported 4.1 mol of glucose/mol of cytochalasin B, as compared with 5.2 transported by GLUT4.

In order to ascertain the expression of GLUT8 with a second, independent method, Western blots of the membrane fractions were analyzed with antiserum against a C-terminal peptide. As is illustrated in the lower panel of Fig. 5, cells transfected with the GLUT8 cDNA indeed expressed a protein with a somewhat lower apparent molecular mass (42 kDa) than that of the GLUT4 (45 kDa); no immunoreactivity was found in cells transfected with bland vector. A second specific band was detected at approximately 75 kDa. Since glucose transporters tend to aggregate even under denaturing conditions, this band might represent a homodimer of GLUT8.

Tissue Distribution of GLUT8—By Northern blot analysis (Fig. 6), a 2.4-kilobase pair transcript corresponding with GLUT8 mRNA (size calculated from the sequence: 1.92 kilobase pairs) was predominantly found in testis; lower amounts were detected in most other tissues investigated, e.g. spleen, prostate, small intestine, heart, brain, and skeletal muscle. Because of the predominant expression of the GLUT8 in testis, we investigated its expression in testicular carcinoma and in testicular tissue from patients treated with estrogen. As is illustrated in Fig. 7, GLUT8 mRNA was not detectable in samples from two patients with testicular carcinoma. Furthermore, estrogen treatment fully suppressed the expression of

### Fig. 1.
Comparison of the deduced amino acid sequences of human and mouse GLUT8 and GLUT1. The alignment was performed with the PALIGN program (open gap cost 7, unit gap cost 2). Positions of presumed membrane-spanning helices are underlined. The presumed glycosylation site (Asn<sup>349</sup>) is highlighted by bold italic print. Amino acid residues that are conserved in GLUT1–GLUT5 are marked by asterisks below the alignment. In the mouse sequence, only residues that differ from the human sequence are shown.
GLUT8 in testis.

The results of the Northern blots suggested that GLUT8 is associated with male germ cells, and that its expression is controlled by gonadotropins. Therefore, we studied the mRNA levels in testis from rats of different age. As is illustrated in Fig. 7 (right panel), the 2.4-kilobase pair transcript was found in testis from adult and pubertal, but not in prepubertal rats.

**DISCUSSION**

The novel transporter protein is a close relative of the glucose transport facilitators GLUT1–GLUT4 and shares their ability to catalyze the diffusion of glucose in a system of reconstituted membranes, and their ability to bind the specific ligand cytochalasin B in a glucose-inhibitable manner. Its sequence presents all elements (sugar transporter signatures) that are characteristic for the GLUT family and are required for their function as hexose transporters (9, 29). Thus, the protein is a novel member of the family of sugar transport facilitators and was designated GLUT8. However, its similarity with the GLUT isoforms is not higher than that with the \textit{E. coli} arabinose and xylose transporter and with that of the \textit{S. pombe} inositol transporter. Together with a second novel transport facilitator, it is located on a separate branch within the family of hexose transporters.

On the basis of the sequence comparison between GLUT8 and the GLUT family, we expected the protein to bind cytochalasin B with high affinity in a glucose-inhibitable manner. The findings presented here confirm this assumption and suggest that binding of this ligand requires the presence of few of the sugar transporter signatures, \textit{e.g.} tryptophan 418. In addition to glucose-inhibitable cytochalasin B binding, GLUT8 exhibited a reconstitutable glucose transport activity similar to that of the GLUT4. This finding was unexpected, because we as-
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Assumed that the substrate recognition required a higher similarity with the GLUT1–GLUT5 isoforms. In particular, GLUT8 harbors a motif in the outer loop 7 (AET) that markedly differs from that of the glucose transporters GLUT1–GLUT4 (STS). Previous mutagenesis studies from our group had indicated that these residues are crucial for the conformational alterations during the transport process (29). Furthermore, the striking conservation of this motif among GLUT1–GLUT4 suggested that the residues in this outer loop might define the glucose specificity of these transporters. However, the present data strongly argue against a role of the STS motif in determining the sugar specificity of the GLUT family. Rather, the data are consistent with the hypothesis that it is the QLS motif in helix 7 (residues 283–285 in GLUT8) that determines the glucose specificity of a GLUT isoform (19). However, the possibility cannot be excluded that GLUTS also transports other sugars, and future studies are needed to define the substrate specificity of this sugar transport facilitator.

The tissue distribution of mRNA of GLUT8 suggests that it is widely expressed in different glucose metabolizing tissues such as testis, muscle, brain, liver, and kidney. Thus, it is conceivable that GLUT8 is the unknown glucose transporter that has been postulated to compensate the lack of GLUT4 in GLUT4 knockout mice (26). However, highest mRNA levels were detected in testis, and it appears reasonable to assume that this expression reflects a testis-specific function. Accordingly, GLUT8 was expressed in testis from adult, but not from prepubertal, rats. Furthermore, we observed that the human testicular expression is markedly inhibited by estrogen treatment, which is known to suppress gonadotropin secretion (37). Thus, the results are consistent with a hormonal regulation of the GLUT8 expression by gonadotropins and/or a dependence on spermatogenesis. Thus, GLUT8 might be involved in the provision of glucose required for DNA synthesis in male germ cells.

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Note Added in Proof—After submission of this manuscript, a glucose transporter cDNA (GLUTX1) was described (Ibberson, M., Uldry, M., and Thorens, B. (2000) J. Biol. Chem. 275, 4607–4612), which is essentially identical with that of GLUT8. The cDNA sequences of human GLUT8 and GLUTX1 differ in 5 nucleotides; 2 of these alter the amino acid sequence (S376N, S457F). The cDNA sequences of mouse GLUT8 and human GLUTX1 differ in 4 nucleotides resulting in the alteration of 3 amino acids (S39N, A94S, S429N).

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