Characterization of the rabbit intestinal fructose transporter (GLUT5)

Ken-ichi MIYAMOTO,* Sawako TATSUMI, Aki MORIMOTO, Hisanori MINAMI, Hironori YAMAMOTO, Karako SONE, Yutaka TAKETANI, Yukihiko NAKABOU, Tatsuzo OKA and Eiji TAKEDA

Department of Nutrition, School of Medicine, University of Tokushima, Tokushima, Japan

Recent studies suggest that the jejunal/kidney-type facilitative glucose transporter (GLUT5) functions as a high-affinity D-fructose transporter. However, its precise role in the small intestine is not clear. In an attempt to identify the fructose transporter in the small intestine, we measured fructose uptake in *Xenopus* oocytes expressing jejunal mRNA from five species (rat, mouse, rabbit, hamster and guinea-pig). Only jejunal mRNA from the rabbit significantly increased fructose uptake. We also cloned a rabbit GLUT5 cDNA from a jejunal library. The predicted amino acid sequence of the 487-residue rabbit GLUT5 showed 72.3 and 67.1% identity with human and rat GLUT5 respectively. Northern-blot analysis revealed GLUT5 transcripts in rabbit duodenum, jejunum and, to a lesser extent, kidney. After separation of rabbit jejunal mRNA on a sucrose density gradient, the fractions that conferred D-fructose transport activity in oocytes also hybridized with rabbit GLUT5 cDNA. Hybrid depletion of jejunal mRNA with a GLUT5 antisense oligo-

nucleotide markedly inhibited the mRNA-induced fructose uptake in oocytes. Immunoblot analysis indicated that GLUT5 (49 kDa) is located in the brush-border membrane of rabbit intestinal epithelial cells. *Xenopus* oocytes injected with rabbit GLUT5 cRNA exhibited fructose uptake activity with a K_m of 11 mM for D-fructose. D-Fructose transport by GLUT5 was significantly inhibited by D-glucose and D-galactose. D-Fructose uptake in brush-border membrane vesicles shows a K_m similar to that of GLUT5, but was not inhibited by D-glucose or D-galactose. Finally, cytochalasin B photolabelled a 49 kDa protein in rabbit brush-border-membrane preparations that was immunoprecipitated by antibodies to GLUT5. Our results suggest that GLUT5 functions as a fructose transporter in rabbit small intestine. However, biochemical properties of fructose transport in *Xenopus* oocytes injected with GLUT5 cRNA differed from those in rabbit jejunal vesicles.

INTRODUCTION

Fructose is a natural constituent of many fruits, in which it is present as a free monosaccharide and also combined with various sugars. Several studies indicate that fructose feeding leads to the development of hyperinsulinaemia and insulin resistance in normal rats [1,2]. The absorption of fructose in the small intestine is stimulated specifically by adding fructose to the diet [3,4]. However, little attention has been paid to the structure and function of the intestinal fructose transporter. Furthermore, the data on the characteristics of intestinal transport of D-fructose are contradictory [3–11], which is probably a reflection of the differences in the animal species studied and in the techniques applied [4]. Recently, cDNAs encoding the Na⁺-dependent glucose co-transporter (SGLT1) and at least seven facilitative glucose transporters (GLUT1–GLUT7) have been isolated [12,13]. Burant et al. [14] reported that the human jejunal/kidney-type facilitative glucose transporter (GLUT5) transports fructose with high affinity and exhibits a much greater capacity to transport fructose than glucose. This transporter is abundant in the upper small intestine, and antiserum to human GLUT5 revealed that this protein is located in the intestinal epithelial brush-border membrane (BBM) [15]. However, the properties of D-fructose transport in human small intestine are not well characterized. We previously showed that rat GLUT5 mRNA concentration was significantly increased by feeding with fructose, but not with other sugars [16]. We suggested that GLUT5 may be the fructose transporter. Rand et al. [17] recently cloned the rat GLUT5 cDNA, the encoded protein of which showed 81.5% amino acid identity with human GLUT5. Expression of rat GLUT5 cRNA in *Xenopus* oocytes showed that

the rat GLUT5 protein mediated the uptake of D-fructose and, to a lesser extent, D-glucose. Furthermore, D-fructose uptake was significantly inhibited by D-glucose. Rand et al. [17] suggested that uptake of dietary glucose from the lumen of the small intestine in the rat may occur by both facilitated diffusion and active transport. However, transport of glucose from the lumen into the epithelial cells, under normal circumstances, is mediated predominantly by the Na⁺-dependent glucose transporter. The presence of a facilitative glucose transporter in the BBM is therefore not easily explained [18]. Furthermore, Sigrist-Nelson and Hopfer [6] showed that the transport system for D-fructose in the BBM of rat small intestine is distinct from aldohexose transporters (it is not the Na⁺-coupled co-transporter or a facilitative glucose transporter) and is not inhibited by D-glucose or D-galactose. Thus further characterization of the fructose transporter in the small intestine is required; it is also not clear if other fructose transporters exist.

We observed that D-fructose uptake in *Xenopus* oocytes expressing jejunal mRNA from rabbit, but not from other species (rat, mouse, hamster or guinea-pig) was greater than that in oocytes injected with water. Schultz and Strecker [5] also demonstrated the presence of a high-affinity D-fructose transport system in rabbit small intestine. We therefore focused our investigation on the role of GLUT5 as a fructose transporter in rabbit small intestine.

MATERIALS AND METHODS

RNA preparation

Total RNA from rat (Sprague–Dawley), rabbit (New Zealand White), mouse (Crj:CD-1), hamster (Syrian) and guinea pig

Abbreviations used: GLUT5, jejunal/kidney-type facilitative glucose transporter; BBM, brush-border membrane; BLM, basolateral membrane.

* To whom correspondence should be addressed.

(Hartley) jejunal mucosa was extracted by the caesium tri-fluoroacetate/guanidinium thiocyanate centrifugation method described previously [19]. mRNA was isolated by oligo(dT)-cellulose chromatography, size-fractionated on a 6–20% (w/w) sucrose gradient in a solution containing 1 mM EDTA and 10 mM Tris (pH 8.0), denatured (100 µg in 100 µl of water) for 3 min at 65 °C, and then rapidly chilled on ice [19].

Oocyte injections

Xenopus laevis females were obtained from Hamamatsu Jitsukenn (Sizuoka, Japan). Small clumps of oocytes were treated twice for 90 min with collagenase (2 mg/ml) (Boehringer Mannheim) in a Ca²⁺-free solution (ORII solution: 82.5 mM NaCl/2 mM KCl/1 mM MgCl₂/10 mM Hepes/Tris, pH 7.5) to remove the follicular layer. After extensive washing first with ORII solution and then with modified Barth's solution [88 mM NaCl/1 mM KCl/0.82 mM MgSO₄/0.4 mM CaCl₂/0.33 mM Ca(NO₃)₂, 2.4 mM NaHCO₃/10 mM Hepes/Tris (pH 7.5)], the oocytes were maintained in modified Barth's solution overnight at 18 °C. Healthy stage-V oocytes were then injected with mRNA (dissolved in water at concentrations from 0.2 to 1 mg/ml) or water with the use of a manual injector (Narishige, Tokyo, Japan).

Uptake measurements in oocytes

The uptake of D-fructose was measured in groups of seven to ten oocytes incubated for 5 min at 30 °C in 0.25 ml of modified Barth's solution in a 6 ml tube. Transport measurements were initiated by the addition of D-[U-¹⁴C]fructose or 2-deoxy-D-[2,6-³H]glucose as indicated in the Figure legends. For assay of L-[¹⁴C]leucine uptake, the assay medium contained 50 µM L-[¹⁴C]leucine, and oocytes were incubated at 20 °C for 60 min. Oocytes were washed three times with 4 ml of ice-cold PBS (150 mM NaCl/10 mM sodium phosphate, pH 7.5) containing 100 mM D-fructose or 0.1 mM phloretin, a potent transport inhibitor. Oocytes were solubilized in 0.5 ml of 1% (w/v) SDS and radioactivity was measured by scintillation spectroscopy.

cDNA cloning

A cDNA library in λgt 10 (5 × 10⁵ independent recombinants) was constructed from rabbit jejunal mRNA by oligo(dT)-primed cDNA synthesis (BRL). Plaques (5 × 10⁶) were screened with ³²P-labelled human GLUT5 cDNA under low-stringency conditions. Two 2.2 kb clones were isolated, subcloned into the *NotI* site of pBluescriptII (Stratagene), and sequenced on both strands by the dideoxy-chain-termination method with a T7 sequencing kit (Pharmacia).

Northern-blot analysis

Scrapings of rabbit gastrointestinal mucosa were obtained from duodenum (between the pyloric sphincter and the ligament of Treitz), jejunum (60 cm distal to the ligament of Treitz), and ileum (60 cm distal to the cecum). Total RNA (20 µg) from rabbit duodenum, jejunum, ileum, kidney, skeletal muscle, liver, brain and adipose tissue was subjected to electrophoresis on a 1.2% agarose gel containing formaldehyde, transferred to a nylon membrane (GeneScreen; du Pont-New England Nuclear), and probed with ³²P-labelled, randomly primed full-length rabbit cDNA as described previously [16].

Hybrid depletion experiments

mRNA (total or size fractions 13–20) was denatured at 65 °C for 5 min in a solution containing 50 mM NaCl and 40 µM of an 18-nucleotide oligomer (5'-AGCTATCAGGGTCCGAAG-3') complementary to the rabbit GLUT5 mRNA sequence starting nucleotide position 56 of the coding region, and was then further incubated at 42 °C for 30 min prior to injection. Oocytes were injected with a 50 nl sample and incubated at 18 °C for 3 days in modified Barth's solution. Uptake of D-fructose and L-leucine was then determined as described above.

In vitro transcription of rabbit GLUT5 cDNA

The 2.2-kb cDNA insert was excised from pBluescriptII with *NotI*, blunt-ended with Klenow enzyme, and ligated into *SmaI*-digested pSP64-poly(A) vector (Promega). Template DNA was linearized by digestion with *EcoRI* and transcribed into rabbit GLUT5 cRNA with SP6 RNA polymerase according to standard methods [20]. Briefly, 1–2 µg of linearized plasmid DNA was incubated for 1 h at 37 °C in 50 µl of 1 × transcription buffer supplied with the enzyme (Promega) containing 0.5 mM ATP, CTP, UTP, and m⁷pppGTP (New England Biolabs), 10 mM dithiothreitol, 0.1 mM GTP, 50 units of RNA-guard (Pharmacia), and SP6 polymerase. DNA was then digested with 10 units of DNase I and 50 units of RNA-guard. RNA was extracted twice with phenol/chloroform (1:1, v/v) and precipitated with ammonium acetate and ethanol. The cRNA was finally suspended in 10 µl of water and assayed by absorbance at 260 nm, and its integrity was checked by electrophoresis on a 1% agarose gel containing formaldehyde.

Immunoblotting

Antiserum to a peptide corresponding to the COOH-terminus of rabbit GLUT5 (amino acids 474–487) was prepared by immunized rabbits with the peptide DREELKDFPPDVSE, which was coupled to keyhole-limpet haemocyanin (Sigma) via a cysteine residue added to the N-terminus of the peptide. GLUT5-specific antibodies were affinity purified on a peptide-coupled immunopurification column described previously [21]. Rabbit jejunal BBM and basolateral membrane (BLM) vesicles were prepared in the presence of protease inhibitors as described previously [22] and subjected to SDS/PAGE. Separated proteins were transferred electrophoretically to a nitrocellulose filter as described by Towbin et al. [23], and probed with the antibodies to rabbit GLUT5 (1 µg/ml) and ¹²⁵I-labelled Protein A.

Uptake measurements in rabbit jejunal BBM vesicles

Rabbit jejunal BBM vesicles for uptake assays were prepared by the Ca²⁺-precipitation method described previously [24]. BBM vesicle purity was assessed by enrichment of marker enzymes (alkaline phosphatase and sucrose). The uptake assay medium contained 1 mM Hepes/Tris (pH 7.5)/0.1 mM MgSO₄/100 mM NaSCN/100 mM D-mannitol, and either D-[U-¹⁴C]fructose, D-[U-¹⁴C]glucose or L-[1-³H]glucose at a concentration of 1 mM. L-[1-³H]Glucose uptake was measured as a control for passive diffusion. The effect of Na⁺ removal on fructose transport was determined by the replacement of NaSCN with D-mannitol. Incubations were performed at 20 °C, and uptake was terminated by protein (50–70 µg of protein) of the incubation medium and its rapid dilution 50-fold with ice-cold PBS. The diluted sample was immediately filtered (0.6 µm pore size) as described previously [24].

Photolabelling of GLUT5 with cytochalasin B

Rabbit jejunal BBM vesicles (2.4 $\mu\text{g}/\mu\text{l}$), prepared as described above, were incubated in PBS containing 20 nM [^3H]cytochalasin B, 2 μM cytochalasin E and 0.5 M D- or L-glucose. Samples were irradiated in a Rayonet reactor (450 W) for 60 s, mixed and further irradiated for another 60 s. Photoaffinity-labelled BBM vesicles were then solubilized in a solution containing 1% Triton X-100, 1 mM phenylmethanesulphonyl fluoride, 150 mM NaCl and 50 mM Hepes, pH 7.6, for 30 min at 4 °C, followed by centrifugation at 13000 g for 30 min at 4 °C. The supernatants were incubated with affinity-purified antibodies to rabbit GLUT5 (1 $\mu\text{g}/\text{ml}$) at 4 °C for 20 h. After addition of 20 μl of Protein A-Sepharose (Pharmacia), samples were incubated for an additional 16 h at 4 °C. The samples were then centrifuged at 13000 g for 10 min at 4 °C, and the pellets washed three times with a solution containing 0.1% Triton X-100, 150 mM NaCl and 50 mM Hepes, pH 7.6. The pellets were then suspended in 1% SDS/50 mM dithiothreitol and subjected to SDS/PAGE on a 10% polyacrylamide gel. The gel lanes were sliced, solubilized with Protosol (du Pont-New England Nuclear), and combined with liquid-scintillation mixture for determination of radioactivity [25].

Chemicals

D-[U- ^{14}C]Fructose (230 mCi/mmol), 2-deoxy-D-[2,6- ^3H]glucose (40 Ci/mmol), L-[^{14}C]leucine (300 mCi/mmol) and D-[U- ^{14}C]glucose (230 mCi/mmol) were obtained from the Radiochemical Center, Amersham, Japan. L-[1- ^3H]Glucose (50 mCi/mmol), ^{125}I -labelled Protein A (70 $\mu\text{Ci}/\mu\text{g}$) and [^3H]cytochalasin B (15 Ci/mmol) were obtained from du Pont-New England Nuclear.

RESULTS

Expression of mRNA in *Xenopus* oocytes

D-[U- ^{14}C]fructose uptake was significantly increased in oocytes injected with jejunal mRNA from rabbit, but not from rat, mouse, hamster or guinea-pig (Table 1). The maximum increase in D-fructose uptake in oocytes injected with rabbit jejunal mRNA, relative to oocytes injected with water, was 2.1–2.4-fold between 2 and 3 days after injection. This difference increased further to a plateau after 3 to 4 days (results not shown). The increase in D-fructose transport rate was dependent on the amount of mRNA injected (Table 1). Uptake in the presence of 1 mM D-[U- ^{14}C]fructose increased linearly during the first 15 min of incubation at 30 °C of oocytes injected with 50 ng of rabbit jejunal mRNA (results not shown). Fructose uptake was not inhibited by D-glucose (100-fold molar excess) or phloridzin (0.1 mM), a potent inhibitor of the Na $^+$ -dependent glucose co-transporter (results not shown). In the absence of Na $^+$, D-fructose uptake also was significantly higher in rabbit jejunal mRNA-injected oocytes in water-injected oocytes (Table 1). Schultz and Strecker [5] also described the existence in rabbit jejunal epithelial cells of a distinct D-fructose transport system that was phloridzin-insensitive and Na $^+$ -independent.

DNA and deduced amino acid sequences of rabbit GLUT5

The GLUT5 cDNA clones rbG5-2 and rbG5-11 were isolated from a rabbit jejunal cDNA library by low-stringency hybridization with ^{32}P -labelled human GLUT5 cDNA [26]. The se-

Table 1 D-Fructose transport in *Xenopus* oocytes injected with jejunal mRNA

Oocytes were injected with 50 ng of mRNA isolated from jejunal mucosa of rat, mouse, rabbit, guinea-pig or hamster (Expt. 1), or with 10, 25 or 50 ng of rabbit jejunal mRNA (Expt. 2). Control oocytes were injected with water. After 3 days, D-fructose uptake was measured by incubating oocytes for 5 min at 30 °C in medium containing 1 mM D-[U- ^{14}C]fructose (230 $\mu\text{Ci}/\text{mmol}$) and either 100 mM NaCl (+Na $^+$) or choline chloride (–Na $^+$) as described in the Materials and methods section (Expt. 2). Values are means $\pm$ S.E.M. for seven to ten oocytes per group of a representative experiment. ** $P < 0.05$ versus water-injected oocytes.

(a) Expt. 1		
Species	D-Fructose uptake (pmol/min per oocyte)	
Water	5.06 ± 1.45	
Mouse	6.92 ± 1.33	
Rat	4.58 ± 2.03	
Rabbit	12.8 ± 3.64**	
Guinea-pig	7.04 ± 2.98	
Hamster	6.33 ± 1.95	
(b) Expt. 2		
Amount of mRNA injected (ng)	D-Fructose uptake (pmol/min per oocyte)	
	+ Na ⁺	— Na ⁺
0 (water)	4.46 ± 0.98	3.32 ± 1.21
10	5.98 ± 3.65	4.43 ± 1.92
25	12.8 ± 4.65	7.54 ± 3.33
50	13.6 ± 3.44	12.4 ± 3.87

quence of the inserts in these clones indicated that they included the entire protein-coding region of the mRNA (Figure 1). The predicted amino acid sequence of the 487-residue rabbit GLUT5 was 72.3 and 67.1% identical with human [26] and rat [17] GLUT5 respectively; rat GLUT5 is 81.5% identical with human GLUT5. Many of the differences between the three proteins occur in the loops connecting the 12 predicted membrane-spanning regions, as well as in the intracellular N- and C-termini, as discussed by Rand et al. [17] for the rat and human proteins. There is less conservation of amino acids in the membrane-spanning regions, especially M10, M11 and M12, between rabbit and the other two species than between rat and human. Furthermore, antibodies prepared to the 14C-terminal amino acids of rabbit GLUT5 did not cross-react with the human, rat, mouse or hamster protein (results not shown). These observations indicate that GLUT5 has been less well conserved during evolution compared with other glucose transporters [12].

Tissue distribution of rabbit GLUT5 mRNA

Northern-blot analysis with a full-length rabbit GLUT5 cDNA probe revealed the presence of a 2.8 kb mRNA in rabbit jejunum, duodenum, and ileum, in decreasing order of abundance (Figure 2). The relative abundance of transcripts in the kidney, however, was much lower than that observed for human [26] or rat [17]. GLUT5 transcripts were also detected in brain, adipose tissue, and skeletal muscle, but not liver.

Size fractionation of mRNA

Rabbit jejunal mRNA was resolved on a linear 6–20% (w/w) sucrose density gradient into 28 fractions. D-Fructose uptake was

Figure 1 Comparison of the predicted amino acid sequences of rabbit, human and rat GLUT5

The 12 predicted transmembrane regions (M1–M12) are indicated above the sequence; both N- and C-termini are predicted to be intracellular. Gaps in the sequence to optimize alignment are represented by dashes. Residues on the human and rat protein identical with the corresponding rabbit residue are indicated by dots; residues identical in all three species are indicated below the sequences with an asterisk. Numbers on the left indicate residue numbers.

greatest (3.8 times that observed with total mRNA) in oocytes injected with fractions 17–20, which contained mRNA species with an average size of 2.2–3.0 kb (Table 2). Similar results were obtained with two independent mRNA preparations. Fractions (0.1–0.3 µg of mRNA/sample) were also analysed by Northern blotting with rabbit GLUT5 cDNA as probe (Figure 3). The fractions that conferred the highest fructose transport activity were those that showed the greatest hybridization with rabbit GLUT5 cDNA. The liver-type glucose transporter (GLUT2) expressed in *Xenopus* oocytes has been shown to mediate D-fructose transport [18]. This transporter isoform is also expressed in the small intestine in rats and humans [18,27,28]. However, fractionated rabbit jejunal mRNA showed no detectable hybridization with a rabbit GLUT2 cDNA probe (results not shown).

Hybrid depletion experiments

Rabbit jejunal mRNA (total or fractions 13–20 from the sucrose gradient) was subjected to hybrid depletion with a GLUT5 antisense oligonucleotide before injection into oocytes and assay

of D-[U-¹⁴C]fructose uptake. Hybrid depletion of GLUT5 mRNA resulted in almost complete inhibition of fructose transport induced by total mRNA or fractions 13–20 (residual uptake, 18.7 and 23.1 % respectively) (Table 3). As a control, we measured uptake of L-[¹⁴C]leucine. The induction of L-leucine uptake into oocytes by total jejunal mRNA or fractions 13–20 was unaffected by hybrid depletion of GLUT5 mRNA (Table 3).

Localization of GLUT5 in intestinal epithelial cells

The purity of BBM and BLM membranes isolated from rabbit jejunum was assessed by assay of marker enzymes. The specific activity of alkaline phosphatase in the BBM preparation was 20 times that of the crude extract, and the specific activity of the Na⁺- and K⁺-dependent ATPase in the BLM preparation was 16 times that of the crude extract [22]. Antibodies to GLUT5 reacted with a protein of 49 kDa in the BBM preparation, but not in the BLM preparation (Figure 4a). Sucrase was present in the BBM preparation, but was not detected in the BLM preparation (Figure 4b).

Functional expression of rabbit GLUT5 in *Xenopus* oocytes

Rabbit GLUT5 cRNA was synthesized *in vitro* and expressed in *Xenopus* oocytes. The rate of uptake of [¹⁴C]fructose in oocytes injected with GLUT5 cRNA was linear up to 15 min. At 5 min the amount of [¹⁴C]fructose accumulated by cRNA-injected oocytes was up to 10 times that accumulated by water-injected oocytes (results not shown). Lineweaver–Burk analysis of cRNA-injected oocytes revealed an apparent K_m of 11 mM for D-fructose transport (results not shown). At all concentrations of

Figure 2 Northern-blot analysis of various rabbit tissues for GLUT5 mRNA

Total RNA (20 µg) from each tissue was resolved on a 1.2%-agarose gel containing formaldehyde, transferred to a nylon membrane, and hybridized with ³²P-labelled rabbit GLUT5 cDNA. Lanes: 1, duodenum; 2, jejunum; 3, kidney; 4, ileum; 5, liver; 6, adipose tissue; 7, brain; 8, skeletal muscle. The positions of 28 S and 18 S rRNA are indicated.

D-fructose used in the kinetic analysis, the concentration of the sugar inside the oocyte was < 8 % of that in the incubation medium. These results demonstrated that the rate of accumulation of fructose in the oocytes expressing GLUT5 is a true reflection of the transport rate; the phosphorylation of the sugar by hexokinase is not rate-limiting under our experimental condi-

Table 2 D-Fructose uptake in *Xenopus* oocytes injected with rabbit jejunal mRNA fractions separated by size on a sucrose gradient

Uptake was assayed at 30 °C for 5 min in the presence of 1 mM D-[U-¹⁴C]fructose 3 days after injection. Values represent means $\pm$ S.E.M. obtained from seven to ten oocytes per group in a representative experiment.

Fraction no.	D-Fructose uptake (pmol/min per oocyte)
1–4	2.26 $\pm$ 1.13
5–8	3.61 $\pm$ 2.12
9–12	9.65 $\pm$ 3.45
13–16	10.8 $\pm$ 2.14
17–20	18.6 $\pm$ 4.32
21–24	8.81 $\pm$ 2.54
25–28	7.72 $\pm$ 4.39

Figure 3 Northern-blot analysis with a ³²P-labelled rabbit GLUT5 cDNA probe of size-fractionated rabbit jejunal mRNA

The odd-numbered fractions of each group (0.1–0.3 μ g per sample) were analysed in the autoradiogram shown. The even-numbered fractions of the same gradient were analysed on a second gel, with similar results.

tions [29]. Oocytes expressing rabbit GLUT5 also transported the glucose analogue 2-deoxy-D-[2,6-³H]glucose (Table 4). D-Fructose uptake was inhibited by D-glucose, D-galactose and L-sorbose at a concentration of 50 mM (Table 4). Cytochalasin B (50 μ M) did not inhibit uptake of D-fructose. The removal of Na⁺ from the transport assay medium did not affect the inhibition of D-fructose uptake by various sugars (results not shown). Transport of 2-deoxy-D-glucose by GLUT5 also exhibited kinetics characteristic of a carrier-mediated system, but was inhibited by cytochalasin B (Table 4). 2-Deoxy-D-glucose transport by GLUT5 also was significantly inhibited by 50 mM D-fructose. These observations are consistent with results obtained with rat GLUT5 [17].

D-Fructose transport in rabbit jejunal BBM vesicles

Schultz and Strecker [5] demonstrated D-fructose transport in rabbit jejunal sheet, with an apparent K_m of 18 mM, that was Na⁺-independent and inhibited by L-sorbose. We investigated in more detail the kinetic properties and substrate specificity of D-fructose transport activity in rabbit jejunal BBM vesicles. D-Fructose uptake was linear for up to 1 min, before reaching an

Table 3 Effect of hybrid depletion of GLUT5 mRNA on D-[U-¹⁴C]fructose and L-[¹⁴C]leucine uptake reduced by rabbit jejunal total mRNA and size-fractionated mRNA in *Xenopus* oocytes

Oocytes were injected with water or with 6–12 ng of rabbit, jejunal total mRNA or size-fractionated mRNA (fractions 13–20). Before injection, mRNA was hybridized with 2.5 ng of a GLUT5 sense (5'-CTTCGGACCCCTGATAGCT-3') or antisense (5'-AGCTATCAGGGTCCGAAG-3') oligonucleotide. Three days after injection, D-[U-¹⁴C]fructose and L-[¹⁴C]leucine uptake were determined as described in the Materials and methods section. Values represent means $\pm$ S.E.M. ($n = 4$) from seven to ten oocytes per incubation from a representative experiment. ** $P < 0.005$.

Material injected	D-[U- ¹⁴ C]Fructose uptake (pmol/min per oocyte)	L-[¹⁴ C]Leucine uptake (pmol/min per oocyte)
Water	3.61 $\pm$ 1.24	0.36 $\pm$ 0.04
Total mRNA	9.48 $\pm$ 2.32**	2.65 $\pm$ 0.19**
Water	4.68 $\pm$ 0.81	0.44 $\pm$ 0.11
Total mRNA + antisense	5.64 $\pm$ 2.46	2.11 $\pm$ 0.31**
Water	5.45 $\pm$ 2.29	0.52 $\pm$ 0.06
Total mRNA + sense	9.83 $\pm$ 2.82**	3.11 $\pm$ 0.62**
Water	3.11 $\pm$ 1.04	0.33 $\pm$ 0.07
Fraction 13–20	16.92 $\pm$ 4.84**	8.41 $\pm$ 1.27**
Water	3.89 $\pm$ 1.95	0.76 $\pm$ 0.13
Fraction 13–20 + antisense	5.74 $\pm$ 2.88	6.27 $\pm$ 2.25**
Water	5.78 $\pm$ 2.26	0.57 $\pm$ 0.18
Fraction 13–20 + sense	10.66 $\pm$ 3.41**	5.84 $\pm$ 1.26**

Figure 4 Immunoblot analysis of rabbit jejunal BBM and BLM preparations

Rabbit jejunal BBM and BLM vesicles (50 μ g of protein) were subjected to immunoblot analysis with antibodies to GLUT5 (a) or sucrose (marker for BBM) (b). Antibody for sucrose reacted with 120 and 140 kDa protein as described in previous paper [22]. The positions of molecular-size markers are shown in (a).

equilibrium (Figure 5). The rate of D-fructose uptake was less than that of D-glucose, but greater than that of L-glucose (Figure 5). The apparent K_m for D-fructose was 9 mM (results not shown), similar to the value obtained for oocytes expressing GLUT5 cRNA (11 mM). D-Fructose transport was not affected by Na⁺ removal, whereas D-glucose transport was inhibited by $\sim 75\%$ by Na⁺ removal (results not shown). The effects of various sugars on fructose transport in Na⁺-free medium were also examined in isolated BBM vesicles. Of the sugars examined, only L-sorbose inhibited the uptake of D-fructose (Table 5). Cytochalasin B (50 μ M) and phloridzin (0.1 mM) also did not affect D-fructose transport. These observations are consistent with those of Schultz and Strecker [5]. Furthermore, we could not

Table 4 D-[U-¹⁴C]Fructose and 2-deoxy-D-[2,6-³H]glucose uptake in *Xenopus* oocytes expressing rabbit GLUT5

Oocytes were injected with 50 nl of water or 50 nl of water containing 50 ng of rabbit GLUT5 cRNA. After 3 days, D-[U-¹⁴C]fructose and 2-deoxy-D-[2,6-³H]glucose uptake was assayed at a concentration of 1 mM for 5 min at 30 °C in the presence of 50 mM of various unlabelled sugars, 0.1 mM phloridzin or 50 μM cytochalasin B. Values represent means ± S.E.M. (*n* = 5) from seven to ten oocytes per incubation from a representative experiment *P* < 0.05* or 0.005** versus non-treatment of test molecule.

Test molecule	D-[U- ¹⁴ C]Fructose uptake (pmol/min per oocyte)	2-Deoxy-D-[2,6- ³ H]glucose uptake (pmol/min per oocyte)
None	44.7 ± 11.2	28.7 ± 8.9
D-Fructose	5.4 ± 1.8**	14.6 ± 3.9*
D-Glucose	11.7 ± 3.4**	6.4 ± 2.1**
D-Galactose	14.2 ± 2.6**	5.9 ± 1.5**
D-Sorbose	20.6 ± 4.8*	22.5 ± 4.6
D-Tagatose	39.6 ± 6.8	24.1 ± 5.6
D-Mannose	40.5 ± 6.1	19.8 ± 7.4
D-Xylose	56.9 ± 5.8	20.6 ± 4.3
Phloridzin	40.8 ± 9.6	17.4 ± 4.1
Cytochalasin B	36.9 ± 5.3	5.2 ± 1.8**
Control (water-injected oocytes)	6.7 ± 1.4	8.9 ± 2.7

Figure 5 Time course of D-fructose uptake in rabbit jejunal BBM vesicles

The uptake of D-[U-¹⁴C]fructose (●), D-[U-¹⁴C]glucose (○) and L-[1-³H]glucose (□) at a concentration of 1 mM and 20 °C was assayed in purified rabbit jejunal BBM vesicles as described in the Materials and methods section. The values represent the mean ± half the range of measurements on two animals.

detect any facilitative glucose transport activity in the isolated rabbit jejunal BBM vesicles.

Photolabelling of jejunal BBM vesicles with cytochalasin B

To determine whether the protein in rabbit jejunal BBM vesicles detected by the antibodies to GLUT5 binds cytochalasin B, a potent inhibitor of facilitative D-glucose uptake, we photolabelled isolated vesicles with cytochalasin B in the presence of either L-glucose or D-glucose. A labelled 49 kDa protein was immunoprecipitated with antibodies to GLUT5 from the BBM vesicles incubated with L-glucose, but not from those incubated with D-

Table 5 Effects of various sugars on D-[U-¹⁴C]fructose transport in isolated rabbit jejunal BBM vesicles

Vesicles were incubated for 1 min at 20 °C in Na⁺-free medium (containing 200 mM D-mannitol) with 1 mM D-[U-¹⁴C]fructose and either various sugars (100 mM), phloridzin (0.1 mM) or cytochalasin B (50 μM). Values are means ± S.E.M. (*n* = 6). *P* < 0.005**.

Test molecule	D-[U- ¹⁴ C]Fructose (pmol/min per mg protein)
None	542 ± 54
D-Fructose	47 ± 11**
D-Glucose	568 ± 72
D-Galactose	522 ± 128
D-Sorbose	386 ± 76
D-Tagatose	589 ± 143
D-Mannose	469 ± 168
D-Xylose	529 ± 166
Phloridzin	488 ± 85
Cytochalasin B	439 ± 67

Figure 6 Photolabelling of rabbit jejunal BBM vesicles with cytochalasin B

Membrane vesicles were photolabelled with cytochalasin B in the presence of cytochalasin E and either L- (●) or D- (○) glucose as described in the Materials and methods section. GLUT5 was immunoprecipitated with specific rabbit antibodies and subjected to SDS/PAGE. The gel was sliced, and the radioactivity extracted with the slices determined by liquid-scintillation spectroscopy. The positions of molecular-size markers are indicated. Values represent the means of two determinations on BBM isolated from one animal.

glucose (Figure 6). Despite numerous attempts, we were unable to measure accurately cytochalasin B binding to or photolabelling of the rabbit GLUT5 transporter expressed in oocytes because of the low level of expression in the oocyte membrane.

DISCUSSION

We have isolated a clone, GLUT5, from a rabbit jejunal cDNA library that encodes a high-affinity D-fructose transporter. Structural features of the D-fructose transporter are similar to those of the other cloned facilitative glucose transporters [12]. After separation of rabbit jejunal total mRNA on a sucrose density gradient, the fractions that conferred D-fructose transport activity on expression in *Xenopus* oocytes were shown to hybridize with rabbit GLUT5 cDNA. Hybrid depletion with a GLUT5 antisense oligonucleotide markedly inhibited the mRNA-induced uptake of D-fructose. A specific antibody prepared to rabbit GLUT5 revealed the protein to be located in the BBM of

intestinal epithelial cells. These observations suggest that GLUT5 functions as a fructose transporter in the rabbit small intestine. However, the properties of GLUT5 expressed in oocytes appeared to differ from a fructose transport system in the BBM with respect to inhibition of D-fructose transport by D-glucose. Transport of D-fructose by rat GLUT5 was shown to be inhibited by D-glucose, but not by cytochalasin B [17]. In contrast, D-fructose transport by human GLUT5 was not inhibited by D-glucose [14]. The reason for this difference in substrate recognition between rat and human GLUT5 is unknown. However, the fructose transport system in BBM vesicles from rat and rabbit small intestine is highly specific for D-fructose [5,6]. Thus rat and rabbit GLUT5 in the BBM may function as a highly specific fructose transporter that does not also mediate glucose transport. A 49 kDa protein that was labelled with [³H]cytochalasin B was immunoprecipitated from rabbit jejunal BBM vesicles by antibodies to GLUT5 in the presence of L-glucose. Labelling was not observed in the presence of D-glucose. These observations suggest that GLUT5 in the intestinal BBM is able to bind cytochalasin B. The cytochalasin B-binding site appears separate from the putative D-fructose binding site, because cytochalasin B did not inhibit D-fructose transport. *Xenopus* oocytes exhibit extremely low basal level of glucose transport and are ideally suited for the expression of heterologous glucose transporters. Many investigators demonstrate that cloned glucose transporters, when expressed in the oocytes plasma membrane, exhibit distinct half-saturation constants under equilibrium exchange flux conditions, consistent with the kinetic properties observed for these proteins in their native cellular environments [18]. One possible explanation is that a second protein associates with GLUT5 in BBM vesicles and thereby reduces the affinity of the transport for D-glucose. This hypothesis is supported by the observation that D-fructose uptake in oocyte expressing rabbit jejunal total mRNA was not inhibited by D-glucose, whereas uptake in oocytes injected with GLUT5 cRNA was. Further studies still need to clarify the differences between the inhibitory patterns of GLUT5 in native BBM vesicles and in oocytes.

GLUT5 is also expressed in brain [12,17], where Mantych et al. [30] suggest it may function as a glucose transporter. A specific fructose-transport system has not been demonstrated in brain. The brain is dependent predominantly on glucose, and uses non-sugar substrates, such as ketones and lactate, in the absence of glucose. Thus fructose does not serve as a substrate for neuronal and glial cell metabolism [30]. Antibodies to GLUT5 recognized a 49 kDa protein in rabbit brain microvascular endothelial cells, but we could not detect facilitative fructose transport activity in these cells (results not shown).

GLUT2 expressed in *Xenopus* oocytes can transport fructose [31]. Cheeseman [32] recently showed that the properties of a fructose transport system in the BLM of rat small intestine were consistent with those of *Xenopus* oocytes expressing rat GLUT2 cRNA. GLUT2 is present in the BLM of both rat and human small intestine [22,31]. GLUT2 mRNA was not detected in rabbit jejunum or ileum. However, fructose transport activity in the rabbit BLM was similar to that in rat jejunum, as reported previously [22]. In a human colonic epithelial cell line (Caco-2 cells), GLUT5 was present predominantly in the apical membrane, but was also detected in the BLM [15,33]. The possibility that GLUT5 in the rabbit BLM may function as both a glucose and fructose transporter, instead of GLUT2, was eliminated, however, because antibodies to GLUT5 did not recognize a

49 kDa protein in the BLM. We previously suggested that a second carrier may contribute to glucose and fructose transport across the BLM in rat jejunum [22]. Cheeseman [32] also suggested the presence of a second carrier in the BLM.

In summary, we have cloned the rabbit GLUT5 cDNA and showed that GLUT5 may function as a fructose transporter. However, biochemical properties of fructose transport in *Xenopus* oocytes injected with GLUT5 cRNA differed from those in rabbit jejunal vesicles. Further studies of this transport should provide further information on the mechanism of substrate recognition.

We thank S. Seino and G. Bell for providing the human GLUT5 cDNA clone.

REFERENCES

- Zavaroni, I., Sander, S., Scott, S. and Reaven, G. M. (1980) *Metabolism* **29**, 970–973
- Sieder, J., Chen, Y.-D. I., Cully, M. D. and Reaven, G. M. (1990) *Metabolism* **29**, 303–305
- Fujisawa, T., Riby, N. and Kretchmer, N. (1991) *Gastroenterology* **101**, 360–367
- Crouzoulon, G. and Korieh, A. (1991) *Comp. Biochem. Physiol.* **100A**, 175–182
- Schultz, S. G. and Strecker, C. K. (1970) *Biochim. Biophys. Acta* **211**, 586–588
- Sigrist-Nelson, K. and Hopfer, U. (1974) *Biochim. Biophys. Acta* **367**, 247–254
- Guy, M. J. and Deren, J. J. (1971) *Am. J. Physiol.* **221**, 1051–1056
- Honegger, P. and Semenza, G. (1973) *Biochim. Biophys. Acta* **318**, 390–395
- Macrae, A. R. and Neudoerffer, T. S. (1972) *Biochim. Biophys. Acta* **288**, 137–144
- Mavrias, D. A. and Mayer, R. J. (1973) *Biochim. Biophys. Acta* **294**, 531–537
- Mavrias, D. A. and Mayer, R. J. (1973) *Biochim. Biophys. Acta* **294**, 538–544
- Bell, G. I., Burant, C. F., Takeda, J. and Gould, G. W. (1993) *J. Biol. Chem.* **268**, 19161–19164
- Wright, E. M. (1993) *Annu. Rev. Physiol.* **55**, 575–589
- Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I. and Davidson, N. O. (1992) *J. Biol. Chem.* **267**, 14523–14526
- Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A., Buse, J. B., Gould, G. W., Burant, C. F. and Bell, G. I. (1992) *Am. J. Physiol.* **262**, C795–C800
- Miyamoto, K., Hase, K., Takagi, T., Fujii, T., Taketani, Y., Minami, H., Oka, T. and Nakabou, Y. (1993) *Biochem. J.* **295**, 211–215
- Rand, E. B., Depauli, A. M. D., Davidson, N. O., Bell, G. I. and Burant, C. F. (1993) *Am. J. Physiol.* **264**, G1169–G1176
- Gould, G. W. and Holman, G. D. (1993) *Biochem. J.* **295**, 329–341
- Miyamoto, K., Oka, T., Fujii, T., Matsubara, T., Minami, H., Nakabou, Y., Natori, Y. and Hagiwara, H. (1992) *Biochim. Biophys. Acta* **1129**, 195–198
- Contreras, R. H., Cheroute, W., Degraeve, W. and Fiers, W. (1983) *Nucleic Acids Res.* **10**, 6353–6356
- Miyamoto, K., Oka, T., Fujii, T., Yamaji, M., Minami, H., Nakabou, Y. and Hagiwara, H. (1989) *J. Biochem. (Tokyo)* **106**, 167–171
- Miyamoto, K., Takagi, T., Fujii, T., Matsubara, T., Hase, K., Taketani, Y., Oka, T., Minami, H. and Nakabou, Y. (1992) *FEBS Lett.* **314**, 466–470
- Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Minami, H., Kim, J. I., Tada, K., Takahashi, F., Miyamoto, K., Nakabou, Y., Sakai, K. and Hagiwara, H. (1993) *Gastroenterology* **105**, 692–697
- Oka, Y., Asano, T., Shibasaki, T., Kasuga, M., Kanazawa, Y. and Takaku, F. (1988) *J. Biol. Chem.* **263**, 13432–13439
- Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan, E. S., Eddy, R. L., Byers, E. L., Shows, T. B., Senio, S. and Bell, G. I. (1990) *J. Biol. Chem.* **265**, 13276–13282
- Miyamoto, K., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagiwara, H. (1991) *Biochem. Biophys. Res. Commun.* **181**, 1110–1117
- Miyamoto, K., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagiwara, H. (1992) *Biochem. Biophys. Res. Commun.* **183**, 626–631
- Thorens, B. (1993) *Annu. Rev. Physiol.* **55**, 591–608
- Mantych, G. J., James, D. E. and Devaskar, S. U. (1993) *Endocrinology (Baltimore)* **132**, 35–40
- Takura, T., Kuentzel, S. L., Raub, T. J., Davies, S. A., Baldwin, S. A. and Borchardt, R. T. (1991) *Biochim. Biophys. Acta* **1070**, 1–10
- Cheeseman, C. I. (1993) *Gastroenterology* **105**, 1050–1056
- Harris, D. S., Slot, J. W., Geuze, H. J. and James, D. E. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7556–7560