Expression of the rat GLUT1 glucose transporter in the yeast *Saccharomyces cerevisiae*

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We expressed the rat GLUT1 facilitative glucose transporter in the yeast *Saccharomyces cerevisiae* with the use of a galactose-inducible expression system. Confocal immunofluorescence microscopy indicated that a majority of this protein is retained in an intracellular structure that probably corresponds to endoplasmic reticulum. Yeast cells expressing GLUT1 exhibited little increase in glucose-transport activity. We prepared a crude membrane fraction from these cells and made liposomes with this fraction using the freeze–thaw/sonication method. In this reconstituted system, D-glucose-transport activity was observed with a K_m for D-glucose of 3.4 ± 0.2 mM (mean $\pm$ S.E.M.) and was inhibited by cytochalasin B ($IC_{50} = 0.44 \pm 0.03$ μM), $HgCl_2$ ($IC_{50} = 3.5 \pm 0.5$ μM), phloretin ($IC_{50} = 49 \pm 12$ μM) and phloridzin ($IC_{50} = 355 \pm 67$ μM). To compare these properties with native GLUT1, we made reconstituted liposomes with a membrane fraction prepared from human erythrocytes, in which the K_m of D-glucose transport and ICs of these inhibitors were approximately equal to those obtained with GLUT1 made by yeast. When the relative amounts of GLUT1 in the crude membrane fractions were measured by quantitative immunoblotting, the specific activity of the yeast-made GLUT1 was 110 % of erythrocyte GLUT1, indicating that GLUT1 expressed in yeast is fully active in glucose transport.

INTRODUCTION

The facilitative uptake of glucose in mammalian cells is mediated by a family of at least six glucose transporter isoforms (the GLUT family) [1–3]. A large number of genes encoding related active and facilitative transporters have been identified and sequenced in eubacteria, cyanobacteria, yeasts, green algae, protozoans and green plants [4,5]. In the yeast *Saccharomyces cerevisiae* more than twenty transporters were reported to be homologous to the mammalian GLUT family transporters ([5,6] and database search thereafter), of which Gal2 is a major high-affinity galactose transporter [6,7]. *HXT2* and *SNF*3 seem to be intimately involved in glucose uptake, since disruption of either gene led to a large reduction in high-affinity glucose transport [8].

There are several reports on heterologous expression of GLUT family transporters using *Xenopus* oocytes, tissue-culture cells and insect cells [1–3]. With these heterologous systems, differences in substrate specificities and K_m values are described among GLUT transporters. There are three difficulties in these studies. The first is that the measurement of glucose-transport activity in these systems requires ordinarily lengthy assay time due to insufficient activity, which causes problems in determination of the initial rates and in eliminating the effects of metabolism. The second is the existence of intrinsic glucose transporters in these systems that hindered an unequivocal interpretation [9]. The third is the difficulty in estimating the proportion of transporter contributing to actual transport: the retention of a considerable portion of GLUT1 in intracellular sites [9–11] or production of inactive transporter [12] are described. In order to challenge these problems, we introduced rat *GLUT1* in yeast. Using reconstitution techniques, we were able to determine the initial rate of uptake and various characteristics of the expressed GLUT1 glucose transporter, and these were found to be almost indistinguishable from those of erythrocyte GLUT1.

EXPERIMENTAL

Expression of rat *GLUT1* in *S. cerevisiae*

The construction of plasmids for expression of *GLUT1*, *HXT2* and *GAL2* in yeast was achieved by using a *GAL* expression system in a multicopy plasmid [7]. A DNA fragment containing *GAL2* was cut out by *Eco*RI and *Pma*CI from the plasmid pS25 [13], provided by H. Ronne (Ludwig Institute for Cancer Research, Uppsala, Sweden) and ligated to *Eco*RI and *Sma*I sites in a multicloning site of the multicopy plasmid pTV3 (YEp *TRP1 bla*) [14] provided by J. Nikawa (Kyushu Institute of Technology, Iizuka, Japan). The nucleotide sequence immediately following the termination codon of *GAL2*, TAATGCGTT, was modified to TAATCGATT by using PCR to make a *Cla*I site. Then a single *Eco*RI site in the plasmid was disrupted by blunting with T4 DNA polymerase. To make a cassette to replace the open reading frame (ORF) of *GAL2* with that of *GLUT1* or another gene, a new *Eco*RI site was introduced after the initiation codon of *GAL2*, by using PCR. The nucleotide sequence ATGGCAGTTGAG was modified to ATGGCAGAATTC, which changed the deduced amino acid sequence from Met-Ala-Val-Glu to Met-Ala-Glu-Phe. This plasmid, GAL2-pTV3e, exhibited an unmodified galactose transport activity when it was introduced to LBY416 (*MATα hxt2::LEU2 snf3::HIS3 gal2 lys2 ade2 trp1 his3 leu2 ura3*) [7,8], provided by L. Bisson (University of California, Davis, CA, U.S.A.).

The nucleotide sequence immediately following the termination codon of *HXT2* in a plasmid pAK5a, provided by L. Bisson, was modified from TAAGAGATT to TAATCGATT to make a *Cla*I site. Since *HXT2* has an *Eco*RI site extending from the 7th to the 12th nucleotides of the coding sequence, *HXT2* is replaced with *GAL2* in GAL2-pTV3e as a cassette to make HXT2-pTV3e.

GLUT1 was cloned from a rat kidney cDNA library (RL1007b;

Abbreviations used: Ab, antibody; ORF, open reading frame.

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Clontech). To make an *Eco*RI site, the nucleotide sequence following the initiation codon of *GLUT1*, ATGGAGCCCAGC, was modified to ATGTCTGAATTC, which changed the deduced amino acid sequence from Met-Glu-Pro-Ser to Met-Ser-Glu-Phe. To make a *Cla*I site, the nucleotide sequence following the termination codon of *GLUT1*, TGAGGAGCC, was modified to TAATCGATC. The plasmid GLUT1-pTV3e was made by replacing *GAL2* with *GLUT1* as a cassette. The nucleotide sequences of ORFs of modified *GAL2*, *HXT2* and *GLUT1* were verified by sequencing the two strands.

These plasmids, GAL2-pTV3e, HXT2-pTV3e, GLUT1-pTV3e and pTV3e, were introduced to LBY416: the cells are called GAL2, HXT2, GLUT1 and control cells respectively. All cells were grown at 30 °C in a synthetic medium [15] with 2% galactose as a carbon source supplemented with uracil, adenine and amino acids except for tryptophan. To evaluate the effects of proteolytic digestion, GLUT1-pTV3e was also introduced in BJ3505 (*MATa pep4::HIS3 prb1-Δ1.6R his3 lys2-208 trp1-Δ101 ura3-52 gal2 can1*), provided by Y. Osumi (University of Tokyo, Tokyo, Japan).

Preparation of crude membrane fraction of yeast

Cells of strain LBY416 possessing one of these plasmids were cultured to an early log phase (D_{650} 0.2–0.4), washed twice with H_2O and suspended with 10 mM Tris/HCl, pH 7.5, containing 0.1 mM EDTA. Cells were disrupted by glass beads (Mini-Beadbeater; Biospec Products) and spun for 1 min at 6200 ***g***. The supernatant was spun for 20 min at 247000 ***g*** to recover the pellet as a crude membrane fraction.

Preparation of human erythrocyte glucose transporter

Erythrocyte GLUT1 glucose transporter was prepared from human red blood cells as described previously [16]. In most experiments, alkali-washed membrane was used as the source for GLUT1.

Reconstitution of glucose transport

The modified freeze–thaw/sonication method was used to reconstitute glucose transporter from the crude membrane fraction of yeast [17]. Briefly, crude soybean phospholipid treated with acetone was suspended in 10 mM Tris/HCl, pH 7.5, flushed with nitrogen gas and sonicated in a bath-type sonicator. A portion of the membrane fraction (25–83 μl) was added to the sonicated liposomes (7.5 mg of phospholipid) in a final volume of 250 μl, flushed with nitrogen gas and sonicated for 10 s. The mixture was quickly frozen in a test tube using ethanol at −70 °C, and thawed at room temperature. The turbid mixture was supplemented with 250 μl of 10 mM Tris/HCl, pH 7.5, sonicated for 10–15 s and diluted to produce a solution of 10 mM Tris/HCl/2 mM $MgSO_4$/8 mg/ml phospholipid. GLUT1 from human erythrocytes was reconstituted in exactly the same way except for the use of alkali-washed erythrocyte membrane.

Measurement of glucose transport in reconstituted liposomes

The initial rate of glucose transport was assessed by the transport of 0.1 mM D-[^{14}C]glucose (CFB96; Amersham) or 0.1 mM L-[^{14}C]glucose (CFA328; Amersham) for 5–15 s at 25 °C with 0.32–0.48 mg of reconstituted liposomes. In the kinetic studies, the transport of 0.5–16 mM D-[^{14}C]glucose was measured. The uptake reaction was started by the addition of 20 μl of the isotope to 180 μl of the liposome suspension in a salt solution containing 10 mM Tris/HCl, pH 7.5, and 2 mM $MgSO_4$ with or without an inhibitor. In the studies of substrate specificity, the reaction was started by the addition of a mixture of 20 μl of 1 mM D-[^{14}C]glucose and 10 μl of 400 mM non-radioactive glucose analogue to 170 μl of the liposome suspension. The reaction was terminated by the addition of 5 ml of cold salt solution containing 0.5 mM $HgCl_2$. The mixture was filtered through a 0.3 μm nitrocellulose membrane filter, followed by a 10 ml wash with the cold salt solution containing $HgCl_2$. The radioactivity retained in the filter was measured with a liquid scintillation counter. Non-specific uptake of D-glucose was estimated from the transport in the presence of 0.5 mM $HgCl_2$ or L-glucose transport.

Glycosidase treatment

Aliquots of 20 μg each of the yeast crude membrane fraction or alkali-washed human erythrocyte membrane were incubated with 0.3 unit of *N*-glycosidase F (EC 3.2.2.18; Boehringer–Mannheim) in a solution containing 20 mM potassium phosphate (pH 7.5), 10 mM EDTA, 0.5% Triton X-100 and 0.5% 2-mercaptoethanol at 37 °C overnight. A portion of the mixture was subjected to SDS/PAGE (10% gel) and immunoblotting.

Immunoblotting

Yeast cells grown to an early log phase were washed with H_2O and disrupted by glass beads. Immunoblotting of the disrupted cells or the crude membrane fraction was performed as described previously [18] with minor modifications. Polyclonal rabbit antibodies (Abs) to erythrocyte glucose transporter or polyclonal rabbit anti-GLUT1 Abs raised against the synthetic peptide corresponding to amino acids 480–492 (13 amino acids of the C-terminus) of the deduced amino acid sequence of human *GLUT1* were used. No appreciable difference in reactivity was observed between these two Abs. The amino acids 480–492 are identical between rat and human GLUT1. Autoradiography of [^{125}I]Protein A (IM144; Amersham) was performed with imaging plates (BAS2000; Fuji Film). The intensity of radioactivity of GLUT1-containing band was measured with BAS2000 and was expressed as PSL (photo-stimulated luminescence unit) instead of c.p.m. Under the present conditions, a linear relation of amounts of protein and radioactivity was observed with at least 0–500 ng of protein (the crude membrane fraction of GLUT1 cells) or 0–20 ng of protein (alkali-washed erythrocyte membrane).

Confocal immunofluorescence microscopy

Yeast cells grown to early log phase were fixed with 5% formaldehyde [19]. After methanol and acetone treatment, cells were treated successively with anti-GLUT1 Abs and goat anti-(rabbit IgG) Abs conjugated with tetramethylrhodamine isothiocyanate (111-026-003; Jackson Immuno Research). The samples were mounted with FluorSave (Calbiochem) and observed with confocal immunofluorescence microscopy (MRC600; Bio-Rad).

Protein assay

Protein content was determined with bicinchoninic acid, according to the manufacturer's instructions (Pierce).

RESULTS

GLUT1 was cloned from a rat kidney cDNA library. The cDNA was modified to create an *Eco*RI site at the very beginning of the ORF and a *Cla*I site just after the stop codon, the ORF of which was cut out and used to replace that of *GAL2* in the multicopy

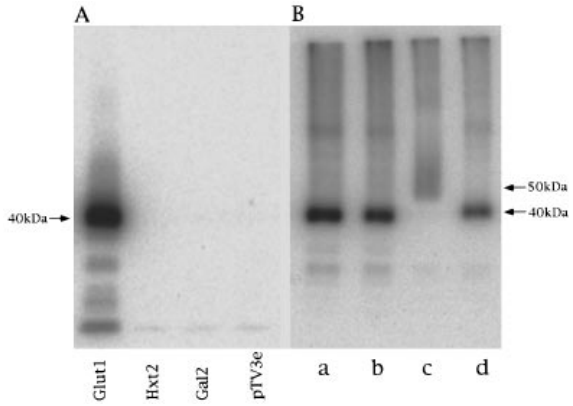

Figure 1 Immunoblotting of GLUT1 expressed in yeast

(**A**) GLUT1, HXT2, GAL2 and control cells were cultured to an early log phase and disrupted by glass beads. Each homogenate (10 μg) was subjected to immunoblotting with anti-erythrocyte glucose transporter Ab as described in the Experimental section. To ascertain that the Ab used in this study is specific to GLUT1, proteins were intentionally overloaded. (**B**) Aliquots of 20 μg each of the crude membrane fraction prepared from GLUT1 cells or alkali-washed human erythrocyte membrane were incubated with *N*-glucosidase F at 37 °C overnight. Aliquots of 100 ng of the glycosidase-treated GLUT1 membrane (lane b) or 5 ng of the glycosidase-treated erythrocyte membrane (lane d), together with the equivalent amounts of the mock-treated GLUT1 membrane (lane a) or erythrocyte membrane (lane c) were subjected to immunoblotting as in (**A**). Note that the overnight incubation caused aggregation of GLUT1, which shows as smears in high-molecular-mass regions.

plasmid GAL2-pTV3e to make GLUT1-pTV3e. GLUT1-pTV3e was introduced in LBY416 cells, in which two major glucose-transport-related genes, *HXT2* and *SNF*3, are disrupted. LBY416 cells possessing GLUT1-pTV3e (GLUT1 cells) exhibited little glucose-transport activity (results not shown). Similarly, the ORF of *HXT2* was cut out at an *Eco*RI site and a *Cla*I site (introduced after the stop codon) and was used to replace that of *GAL2* in GAL2-pTV3e to make HXT2-pTV3e. LBY416 cells possessing HXT2-pTV3e (HXT2 cells) exhibited rates of glucose transport about 10-fold higher than those possessing the vector pTV3e only (control cells) (results not shown). These results suggest that there is difficulty in expressing functional GLUT1 in plasma membrane of yeast.

The immunoblotting of GLUT1 cells with anti-GLUT1 Ab is shown in Figure 1. A single band corresponding to 40 kDa was observed with these cells, whereas no detectable band was seen with LBY416 possessing GAL2-pTV3e (GAL2 cells), HXT2 cells or control cells (Figure 1A). The apparent molecular mass of 40 kDa is about 10 kDa smaller than the calculated molecular mass of 54 kDa. Similarly, the apparently smaller molecular mass detected with immunoblotting has been observed in Gal2 and Hxt2 [7]. The apparent molecular mass of GLUT1 in animal cells varied from 44 to 55 kDa depending on tissues and cell types [20]. To examine whether glycosylation of GLUT1 contributes to the difference in molecular mass, glycosidase treatment was used (Figure 1B). After *N*-glycosidase F treatment, the molecular mass of human erythrocyte GLUT1 changed from 50 to 40 kDa, while that of GLUT1 expressed in yeast showed the same

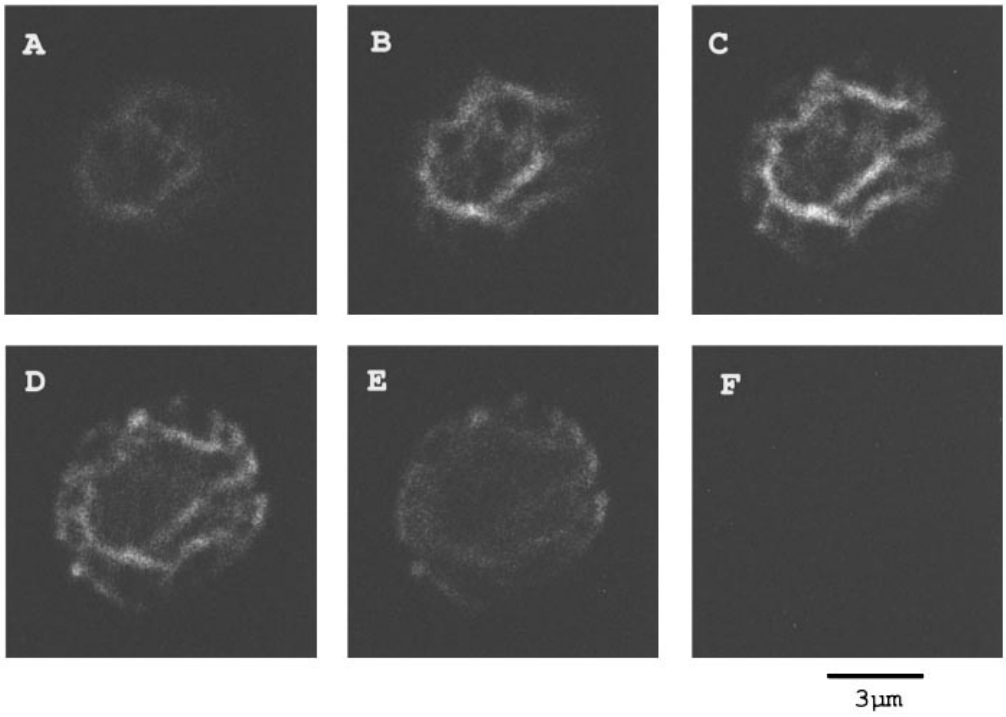

Figure 2 Confocal immunofluorescence microscopy of GLUT1 cells

GLUT1 cells were cultured to an early log phase and fixed with 5% formaldehyde [19]. The fixed cells on a glass slide were treated with anti-(human erythrocyte glucose transporter) Ab and stained with goat anti-(rabbit IgG) Ab coupled with tetramethylrhodamine isothiocyanate. Confocal immunofluorescence microscopy was performed with a $\times 100$ objective attached to a microscope (BH-2; Olympus). A series of 2 μm optical sections is shown (**A**–**E**), together with a section of a control cell possessing the vector, pTV3e (**F**).

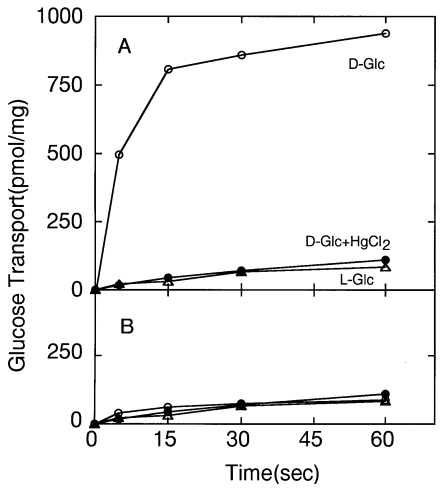

Figure 3 Time course of reconstituted glucose transport

(**A**) The crude membrane fraction of GLUT1 cells was reconstituted in liposomes with the freeze–thaw/sonication method. An aliquot of 50 μg of protein in 0.32 mg of liposomes was used for D-glucose (Glc) transport (○), D-glucose transport in the presence of 0.5 mM $HgCl_2$ (●) or L-glucose transport (△) at 25 °C as described in the Experimental section. When $HgCl_2$ was used, it was preincubated for more than 5 min at 25 °C. (**B**) The crude membrane fraction prepared from control cells was used for reconstitution. D-Glucose transport (○), D-glucose transport in the presence of 0.5 mM $HgCl_2$ (●) or L-glucose transport (△) was measured with 45 μg of protein in 0.32 mg of liposomes.

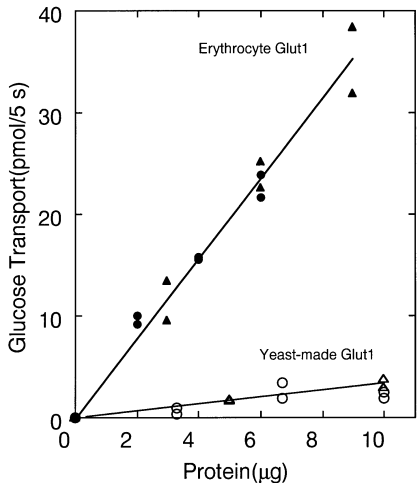

Figure 4 Linear dependence of reconstituted glucose transport on protein content

The crude membrane fraction of GLUT1 cells (○, △) or alkali-washed human erythrocyte membrane (●, ▲) was reconstituted in liposomes with varying amounts of protein. The initial rates of glucose transport in 0.32 mg (○, ●) or 0.48 mg (△, ▲) of liposomes containing 2 to 10 μg of protein were measured. The background was estimated by D-glucose transport in the presence of 0.5 mM $HgCl_2$ and subtracted.

molecular mass of 40 kDa, indicating yeast-made GLUT1 is little N-glycosylated, so that the apparent molecular mass is lower than those observed in animal cells. The possibility of proteolytic degradation was checked using BJ3505 which lacks two potent proteases. With BJ3505 cells, a band having the same molecular mass of 40 kDa was observed (results not shown), suggesting no significant effects of proteolytic degradation.

Confocal immunofluorescence microscopy showed that a majority of GLUT1 was retained in an intracellular organelle, the structure of which resembles endoplasmic reticulum, and little was found on the cell surface (Figure 2). These results together with those obtained with immunoblotting are consistent with the idea that GLUT1 is expressed but retained in an intracellular organelle so that glucose transport in yeast cells is not exhibited.

A crude membrane fraction was prepared from GLUT1 cells. Using the freeze–thaw/sonication method, we reconstituted GLUT1 in liposomes. Significant D-glucose-specific transport was observed (Figure 3A). A rapid D-glucose transport was drastically inhibited by $HgCl_2$ and reduced to the level of L-glucose transport, thus enabling us to use $HgCl_2$ as a stopping reagent. When liposomes are made with the crude membrane fraction prepared from control cells, no appreciable D-glucose transport was observed (Figure 3B).

Kinetic parameters were determined by the 5 s uptake. The K_m for D-glucose transport was 3.4 ± 0.2 mM ($n = 3$) with yeast-made GLUT1 and 3.8 ± 0.0 mM ($n = 3$) with human erythrocyte GLUT1, which agreed well with values obtained with human erythrocytes under zero *trans* uptake conditions [1,21,22]. To compare the V_{max} values, we measured with immunoblotting the relative amounts of GLUT1 in the crude membrane fraction of yeast and in the alkali-washed human erythrocyte membrane (Table 1). The initial rates of D-glucose transport with both GLUT1 were linearly dependent on the amount of protein in liposomes (Figure 4). The specific activities were calculated from

Table 1 Specific activity of reconstituted GLUT1

The crude membrane fraction of GLUT1 cells or alkali-washed human erythrocyte membrane was used for reconstitution in liposomes as described in the Experimental section. The initial rates of glucose transport in liposomes were measured as shown in Figure 4. The relative content of GLUT1 in each fraction was measured with immunoblotting and expressed as PSL/mg of protein. Note that the GLUT1 contents can only be compared in the same experiment (Exp.), since PSL indicates relative radioactivity and is proportional to exposure time.

	Human erythrocyte GLUT1			Yeast-made GLUT1			
	Content (10^{-6} PSL/mg)	Transport activity (nmol/s per mg of protein)	Specific activity (pmol/s per 10^{-6} PSL)	(10^{-6} PSL/mg)	Transport activity (nmol/s per mg of protein)	Specific activity (pmol/s per 10^{-6} PSL)	Percentage of erythrocyte GLUT1
Exp. 1	896	0.58	0.64	60.6	0.044	0.73	114
Exp. 2	962	0.78	0.81	53.9	0.048	0.89	110
Exp. 3	1018	0.81	0.80	71.4	0.061	0.85	106
Average		0.72			0.051		110

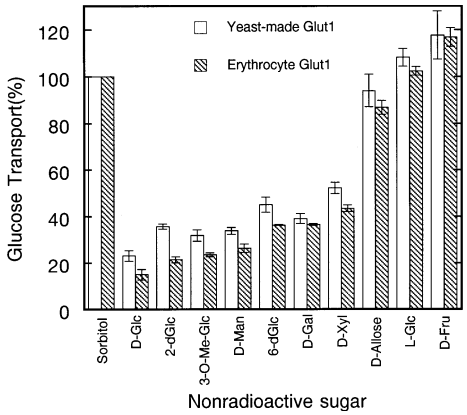

Figure 5 Substrate specificity of reconstituted glucose transport mediated by yeast-made GLUT1 and human erythrocyte GLUT1

Liposomes were made with the crude membrane fraction of GLUT1 cells or alkali-washed human erythrocyte membrane. Aliquots of 66–95 μg of protein (yeast-made GLUT1) or 5.9 μg of protein (erythrocyte GLUT1) in 0.32–0.48 mg of liposomes was used for the 15 s transport assays. The background was estimated by D-glucose (Glc) transport with 0.5 mM $HgCl_2$ and subtracted. Since it was observed that high concentrations of sugars inhibited glucose transport, the transport activities are expressed as the percentage of the values with 20 mM sorbitol addition, which were 6.2–11.6 pmol/15 s (yeast-made GLUT1) and 19.2–22.1 pmol/15 s (erythrocyte GLUT1). The vertical bars indicate S.E.M. Abbreviations used: Me, methyl; d, deoxy.

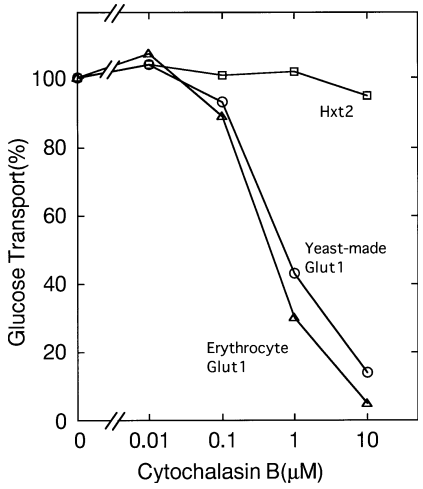

Figure 6 Inhibition of reconstituted glucose transport by cytochalasin B

The crude membrane fraction of GLUT1 cells (○) or of HXT2 cells (□), or purified GLUT1 from human erythrocytes [16] (△), was reconstituted in liposomes. A portion of 54 μg (○), 5 μg (△) or 53 μg (□) of protein in 0.32 mg of liposomes was used for 5 s transport. After the subtraction of the background estimated by L-glucose transport, D-glucose-specific transport activities are expressed as the percentage of those without cytochalasin B, which was 2.8 pmol/5 s (○), 42.6 pmol/5 s (△) or 10.4 pmol/5 s (□).

the initial rates and the amounts of the protein in the two fractions. In three experiments, we observed that the specific activity of rat GLUT1 expressed in yeast is 110% of that obtained with human erythrocyte GLUT1 (Table 1).

Substrate specificity of GLUT1 was assessed by the simultaneous addition of a 200-fold excess of non-radioactive D-glucose analogue (Figure 5). The D-glucose transport in liposomes reconstituted with yeast-made GLUT1 was inhibited by the D-glucose analogues, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, 6-deoxy-D-glucose, and by other known competitors, D-mannose, D-galactose and D-xylose, but not by L-glucose or D-fructose. The results with human erythrocyte GLUT1 were almost indistinguishable from those with yeast-made GLUT1. These results correspond well to those obtained with intact human erythrocytes [23].

Table 2 Inhibitory effects of several reagents on reconstituted glucose transport

The crude membrane fraction of GLUT1 cells or alkali-washed human erythrocyte membrane was reconstituted as described in the Experimental section. Aliquots of 30–105 μg of protein (yeast-made GLUT1) or 5.9 μg of protein (human erythrocyte GLUT1) were used for the 15 s transport assays. Each inhibitor was preincubated for more than 5 min at 25 °C. Inhibition doses were obtained with 4–7 assays using varying concentrations of inhibitors. When cytochalasin B or a diphenolic reagent (phloretin or phloridzin) was used as an inhibitor, dimethyl sulphoxide or ethanol, respectively, was carried over to the assay solution; this amounted to less than 0.5% and caused no apparent effect on glucose transport. Results are expressed as means ± S.E.M.; *n* is the number of experiments. Abbreviations used: PCMB, *p*-chloromercuribenzoate; PCMBS, *p*-chloromercuribenzene sulphonate.

	Yeast-made GLUT1		Human erythrocyte GLUT1	
Reagent	IC_{50} (μM)	*n*	IC_{50} (μM)	*n*
Cytochalasin B	0.44 ± 0.03	3	0.52 ± 0.09	3
Phloretin	49 ± 12	2	61 ± 3	2
Phloridzin	355 ± 67	2	528 ± 51	2
$HgCl_2$	3.5 ± 0.5	3	3.1 ± 0.3	3
PCMB	15 ± 4	3	20 ± 4	3
PCMBS	44 ± 4	3	23 ± 4	3

Effects of several inhibitors of glucose transport that have been described in animal cells [1,23] were studied. A potent inhibitor, cytochalasin B, inhibited glucose transport mediated by yeast-made GLUT1 as well as that by human erythrocyte GLUT1, but did not inhibit Hxt2-mediated glucose transport (Figure 6). The effectiveness of several inhibitors are shown in Table 2, and no notable difference between the two GLUT1s is indicated.

DISCUSSION

The expression of GLUT1 in yeast was observed with immunoblotting with anti-GLUT1 Ab; however, no appreciable glucose transport was observed with GLUT1 cells. Retention of GLUT1 in an intracellular structure as seen by confocal immunofluorescence microscopy is consistent with the notion that mature GLUT1 accumulates intracellularly and does not contribute to glucose transport across plasma membrane. Treatment of GLUT1 with *N*-glycosidase F did not change the size of yeast-made GLUT1, but reduced the size of human erythrocyte GLUT1 to 40 kDa, which is the same as that of yeast-made GLUT1, indicating that the latter is of full length with little N-glycosylation. The reason why GLUT1 is not transferred to yeast plasma membrane is not clear at present. It may well be that the sorting signal for membrane proteins in yeast is different from that of animal cells [24] or that non-glycosylated GLUT1 tends to accumulate intracellularly, as has been observed in CHO cells [25]. In this respect, it is of considerable interest that a plasma membrane H^+-ATPase from *Arabidopsis thaliana* expressed in *S. cerevisiae* was retained at intracellular membranes, but the C-terminal truncated H^+-ATPase was targeted to the plasma membrane and supported the growth of yeast in which an endogeneous H^+-ATPase had been turned off [26].

The glucose-transport activity obtained with yeast-made GLUT1 is almost identical to that with human erythrocyte

GLUT1 in respect to K_m, V_{max}, substrate specificities and inhibitor sensitivities. It may be possible that introduction of GLUT1-pTV3e in LBY416 enhances some intrinsic glucose transporter, the characteristics of which resemble GLUT1. However, when we expressed modified GLUT1 in which Cys^{429} was changed to Ser and reconstituted it in liposomes, glucose transport observed with modified GLUT1 showed a reduced sensitivity to SH inhibitors (T. Kasahara and M. Kasahara, unpublished work). This result indicates that yeast-made GLUT1, but not an unknown yeast glucose transporter(s), is responsible for glucose transport in liposomes. At present, however, we are unable to exclude the possibility that some factor(s) in yeast modulates the properties of glucose transport mediated by GLUT1.

The heterologous expression system of GLUT1 showed a rapid uptake of D-glucose. There was almost no contribution of intrinsic glucose transporter(s) (Figure 3). Yeast-made GLUT1 seems to be retained in endoplasmic reticulum and does not spread over other organelles (Figure 2). The glycosylation pattern is fairly homogeneous (Figures 1A and 1B). The glucose-transport activity obtained with yeast-made GLUT1 is as active as that with human erythrocyte GLUT1. Previously it was shown that GLUT1 expressed in insect cells retained less than 20% of biological activity as detected by cytochalasin B binding [12]. These properties indicate a homogeneous and fully active GLUT1 preparation and have not been described in other heterologous expression systems. However, to further characterize the molecular mechanism of GLUT1, it is necessary to assess the orientation of GLUT1 in reconstituted liposomes. This expression system may be suitable for studies on molecular transport mechanism by using genetically manipulated GLUT family transporters and for the large-scale production of biologically active GLUT1.

We thank T. Fukasawa, Kazusa DNA Research Institute, Chiba, Japan, for discussion, L. Bisson, J. Nikawa, H. Ronne and Y. Osumi for plasmids and yeast cells, and T. Kitagawa, National Institute of Health, Japan, for suggestions on the glycosidase assay. This work was supported by grants from the Ministry of Education, Science and Culture of Japan and Ono Pharmaceutical Co.

Received 11 October 1995; accepted 22 November 1995

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