Possible multifunction of glucose transporter

Transport of nicotinamide by reconstituted liposomes

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A kinetic study of the uptake of nicotinamide by reconstituted liposomes containing the human erythrocyte glucose transporter, compared with that of D-glucose, demonstrated that the K_m and V_{max} values were almost the same for each compound, and that the uptake of D-glucose was competitively inhibited by nicotinamide. At 20 mM concentration, 2-deoxy-D-glucose, 3-O-methyl-D-glucose and 4,6-O-ethylidene-D-glucose all caused 50% inhibition of nicotinamide uptake, but L-glucose and nicotinic acid were not inhibitory. Similar results were obtained for the uptake of D-glucose. Cytochalasin B binding to the liposomes was inhibited in a dose-dependent manner by either nicotinamide or D-glucose. Antibody for glucose transporter detected in band 4.5 by SDS/PAGE inhibited the uptake of D-glucose and nicotinamide. A possible uptake of nicotinamide by nucleoside transporter was excluded. In human erythrocytes, cytochalasin B binding was inhibited dose-dependently by either nicotinamide or D-glucose, and cytochalasin B depressed the uptake of both nicotinamide and 2-deoxy-D-glucose. These findings were well reproduced in the reconstituted liposomes. The very close similarities between uptake of nicotinamide and D-glucose suggest that the glucose transporter plays a direct role in transport of nicotinamide, which is structurally quite different from monosaccharides, and thus that the transporter is probably multifunctional.

INTRODUCTION

In most mammalian cells, glucose transport is mediated by a glucose-specific transport protein (glucose transporter) in the cell membrane. This glucose transporter is reported to be also present in pancreatic β -cells (Thorens *et al.*, 1988; Permutt *et al.*, 1989). Recently we suggested that the glucose transporter might be closely related to the uptake of nicotinamide, because the uptake of nicotinamide by rat pancreatic β -cells in monolayer culture was inhibited dose-dependently by 2-deoxy-D-glucose added to the culture medium, and their uptake of 2-deoxy-D-glucose was also inhibited in the same manner by added nicotinamide, the concentrations of the two compounds for 50% inhibition of uptake of the other being almost the same (Sofue *et al.*, 1991). Similar phenomena were observed in human erythrocytes (M. Sofue, Y. Yoshimura, M. Nishida & J. Kawada, unpublished work). However, it is difficult to analyse transport systems in intact cells. Therefore we tried to obtain direct evidence that the glucose transporter mediates uptake of nicotinamide, using liposomes reconstituted with the isolated glucose transporter from human erythrocytes. From the results presented here, we propose a possible multifunction of the glucose transporter.

EXPERIMENTAL

Materials

Soybean phospholipids (type II-S), cytochalasin B, cytochalasin E, L-glucose and *p*-nitrobenzylthioinosine (NBMPR) were purchased from Sigma (St. Louis, MO, U.S.A.). 2-Deoxy-D-glucose and 3-O-methyl-D-glucose were from Nacalai Tesque (Kyoto, Japan). 4,6-O-Ethylidene-D-glucose was synthesized as described previously (Kawada *et al.*, 1986). D-[U- 14 C]glucose (10.6 GBq/mmol), [4- 3 H]cytochalasin B (555.0 GBq/mmol) and Aquasol-2 were from New England Nuclear (Boston, MA, U.S.A.). [*carboxyl*- 14 C]Nicotinamide

(2.04 GBq/mmol) and [2- 3 H]adenosine (888.0 GBq/mmol) were from Amersham International (Amersham, Bucks., U.K.). L-[1- 14 C]Glucose (2.04 GBq/mmol) was from American Radio-labelled Chemicals (St. Louis, MO, U.S.A.). All other reagents used were of analytical grade. Fresh human erythrocytes were obtained from the Red Cross Blood Center (Tokushima, Japan).

Preparation of glucose transporter from human erythrocytes

Erythrocyte ghosts (Steck & Kant, 1974) and alkali-treated vesicles (Baldwin *et al.*, 1980) were prepared as reported in the references. The glucose transporter was solubilized with Triton X-100 and purified by DEAE-cellulose column chromatography (Kasahara & Hinkle, 1977). The purified protein was detected in band 4.5 by SDS/PAGE (Kasahara & Hinkle, 1977).

Reconstitution of glucose transporter into liposomes

Liposomes were prepared by the freeze-thaw method (Kasahara & Hinkle, 1977; Sase *et al.*, 1982). Reconstituted liposomes were separated from unreconstituted protein by centrifuging 3 ml of liposomal solution layered on 5 ml of 0.4 M-sucrose at 39000 *g* for 30 min in a Hitachi RP65 rotor (Wheeler & Hinkle, 1981). The cloudy layer was collected, and sucrose was removed on a Sephadex G-50 column (1 cm $\times$ 25 cm). Samples (185 μ l) of the cloudy eluate were used for all experiments described below. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Transport assays

Experiments were generally carried out as follows. The reconstituted liposomes were incubated with D-[14 C]glucose (15 kBq, final concn. 0.2 mM) or with [14 C]nicotinamide (7.5 kBq, final concn. 0.2 mM) at 25 $^{\circ}$ C in various conditions as described in the Results section. Then the uptake reaction was stopped by adding 5 ml of cold stopping solution, containing

Abbreviation used: NBMPR, *p*-nitrobenzylthioinosine.

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150 mM-KCl, 1 mM-HgCl₂, 2 mM-MgSO₄ and 10 mM-Tris/HCl (pH 7.4). The mixture was filtered on a 0.2 μ m-pore membrane filter (Advantec) and washed with 2 $\times$ 5 ml of the same solution. The washed filters were solubilized in Bray's (1960) solution, and radioactivity was determined in a liquid-scintillation counter (Aloka). All determinations were done in triplicate. The amounts of glucose and nicotinamide transported into liposomes were calculated from the specific radioactivity of each radiotracer.

Effects of various compounds on uptake of D-glucose and nicotinamide. The effects of D-glucose, L-glucose, 2-deoxy-D-glucose, 3-O-methyl-D-glucose, 4,6-O-ethylidene-D-glucose (ethylideneglucose), nicotinamide and nicotinic acid at 20 mM concentration on the uptake of D-[¹⁴C]glucose and [¹⁴C]-nicotinamide in 15 s were examined.

Effects of D-glucose and nicotinamide on cytochalasin B binding. The reconstituted liposomes were incubated with [³H]-cytochalasin B (20 kBq, final concn. 4 μ M) and cytochalasin E (4 μ M) for 5 min in the presence of 0–50 mM unlabelled D-glucose and nicotinamide. The reaction was stopped by adding 5 ml of cold stopping solution. The mixture was filtered on the membrane filter and was washed with 3 $\times$ 5 ml of the same solution. This number of washings was determined in our preliminary experiments, comparing the changes in binding of cytochalasin B produced by various washing times. It was enough to remove the unbound tracer, and the amounts of bound cytochalasin B were not substantially decreased by further washing. The washed mixture was treated in the same way as described above for counting. Cytochalasin E was used to decrease the non-specific binding of cytochalasin B (Dick *et al.*, 1984).

Effect of antibody on the uptake of D-glucose and nicotinamide by reconstituted liposomes

The liposomes were incubated for 10 min with 0–200 μ g/ml concentrations of antibody against isolated glucose transporter (IgG fractionated from the rabbit anti-glucose-transporter serum, kindly prepared by Dr. M. Kurata, Faculty of Pharmaceutical Sciences, Josai University, Sakato, Japan). Then D-[¹⁴C]glucose (15 kBq, final concn. 0.2 mM) or [¹⁴C]nicotinamide (7.5 kBq, final concn. 0.2 mM) was added and the uptake of these radiolabelled compounds for 15 s at 25 °C was determined.

Effect of NBMPR on the uptake of D-glucose and nicotinamide by reconstituted liposomes

The liposomes were preincubated with 10 nM- and 50 nM-NBMPR for 5 min, and then the uptake of [¹⁴C]glucose (15 kBq, final concn. 0.2 mM) and [¹⁴C]nicotinamide (7.5 kBq, final concn. 0.2 mM) for 20 s at 25 °C was determined. To check the inhibitory effect of NBMPR on the transport of adenosine, [³H]adenosine (7.5 kBq, final concn. 0.2 mM) was used in the same conditions. The effect of cytochalasin B at 1 μ M concentration was also examined at the same time.

Experiments with human erythrocytes

Effect of D-glucose and nicotinamide on cytochalasin B binding. Human erythrocytes (10⁸ cells/ml) were incubated with [³H]cytochalasin B (20 kBq, final concn. 1 μ M), cytochalasin E (final concn. 1 μ M) and D-glucose or nicotinamide (0–50 mM) for 15 min at 37 °C. The reaction was terminated by addition of 2 ml of cold stopping solution and centrifuged at 1500 *g* for 10 min. The resulting pellet was washed with 3 $\times$ 2 ml of the same solution, and then suspended in 1 ml of stopping solution. A 200 μ l portion of this suspension was mixed with 200 μ l of H₂O₂ and 200 μ l of 2 M-KOH/propan-2-ol to solubilize and decolorize the erythrocytes completely. Acetic acid (10%, v/v; 400 μ l) and

7 ml of Aquasol-2 were then added, and the radioactivity was determined.

Inhibition of uptake of D-glucose and nicotinamide by cytochalasin B. Human erythrocytes (10⁸ cells/ml) were incubated with 10 nM–10 μ M-cytochalasin B and the same concentration of cytochalasin E for each of them for 15 min at 37 °C. Then 2-deoxy-D-[³H]glucose (18.5 kBq, final concn. 1 mM) or [¹⁴C]nicotinamide (18.5 kBq, final concn. 1 mM) was added and the incubation was continued for 5 min. This second incubation time was determined to be appropriate by our preliminary experiments. After the reaction was stopped, each sample was treated as described above for radioactivity counting.

RESULTS

Comparison of the time-dependent uptakes of D-glucose and nicotinamide by reconstituted liposomes showed that two curves were virtually identical after subtracting the high values probably caused by non-specific interaction or adsorption of nicotinamide on the liposomes. Both compounds had the same half-time for equilibration, approx. 15 s. In the presence of 1 μ M-cytochalasin B, the uptake of both compounds was equally inhibited. No significant uptake of L-glucose was observed. No uptake was demonstrated by unreconstituted liposomes (Figs. 1a and 1b).

Concentration-dependence of the uptakes of D-glucose and nicotinamide by reconstituted liposomes is shown in Figs. 2(a) and 2(b). From these data, almost the same K_m and V_{max} values for each compound were obtained by an Eadie-Hofstee plot (v/s versus v): K_m = 6.0 mM, V_{max} = 2.8 μ mol/min per mg of protein for D-glucose; K_m = 7.5 mM, V_{max} = 3.6 μ mol/min per mg of protein for nicotinamide. In the presence of 1 μ M-cytochalasin B, both uptakes were inhibited. Nicotinamide and D-glucose each at 20 mM depressed the uptake of the other.

Fig. 1. Time course of uptake of D-glucose and nicotinamide by reconstituted liposomes

(a) Reconstituted liposomes were incubated with D-[¹⁴C]glucose in the absence (○) or the presence (●) of 1 μ M-cytochalasin B for the indicated times at 25 °C. For controls, unreconstituted liposomes were incubated with D-[¹⁴C]glucose (□), but because there was no protein the uptake values are expressed in pmol, where 5 pmol was nearly equivalent to 1 nmol/min per mg of protein. Reconstituted liposomes were also incubated with L-[¹⁴C]glucose (▲) under the same conditions. Values are means $\pm$ S.E.M. for three samples. The S.E.M. was omitted when it was less than 5% of the mean. (b) Reconstituted liposomes were incubated with [¹⁴C]nicotinamide in the absence (○) or the presence (●) of 1 μ M-cytochalasin B. Unreconstituted liposomes were also incubated with the same radiotracer (□), but the uptakes are expressed in pmol, where 6 pmol was nearly equal to 1 nmol/min per mg of protein in this case.

Fig. 3. Kinetics of the inhibition of D-glucose uptake by nicotinamide (Dixon plot)

The D-glucose uptakes at concentrations of 0.25 mM (○) and 0.5 mM (●) in the presence of various concentrations of nicotinamide were determined. The S.E.M. of each original value was less than 5 % of the mean (three samples) and was omitted.

Table 1. Effects of various compounds on the uptake of D-glucose and nicotinamide by reconstituted liposomes

The concentration of each compound was 20 mM. Values are means ± S.E.M. for three samples: **P* < 0.001 versus control (Student's *t* test).

	Uptake (nmol/15 s per mg of protein)	
	D-Glucose	Nicotinamide
Control	7.7 ± 0.16	13.6 ± 0.20
D-Glucose	3.1 ± 0.19*	5.6 ± 0.22*
L-Glucose	8.1 ± 0.74	13.3 ± 0.09
2-Deoxy-D-glucose	3.3 ± 0.05*	6.2 ± 0.53*
3-O-Methyl-D-glucose	2.8 ± 0.08*	6.0 ± 0.13
Ethylideneglucose	3.3 ± 0.22*	5.8 ± 0.52*
Nicotinamide	3.2 ± 0.12*	5.4 ± 0.28*
Nicotinic acid	8.2 ± 0.31	12.9 ± 0.79

Similar results were obtained on the uptake of nicotinamide. L-Glucose and nicotinic acid were again not inhibitory, but other glucose analogues had similar inhibitory effects to those on D-glucose uptake.

The specific binding of cytochalasin B to reconstituted liposomes was inhibited in a dose-dependent manner by either D-glucose or nicotinamide, the inhibition curves for the two being practically identical (Fig. 4).

The antibody against the glucose transporter isolated from human erythrocytes caused dose-dependent inhibition on the uptake of D-glucose and nicotinamide by reconstituted liposomes. The inhibition curves were parallel, and the concentration for

Fig. 2. Concentration-dependence of uptake of D-glucose and nicotinamide by reconstituted liposomes

(a) Reconstituted liposomes were incubated with D-[¹⁴C]glucose in the presence of various concentrations of unlabelled D-glucose without (○) or with 1 μM-cytochalasin B (●) and with 20 mM unlabelled nicotinamide (▲). Unreconstituted liposomes were also incubated with D-[¹⁴C]glucose in the presence of various concentrations of unlabelled D-glucose (□). The values are means ± S.E.M. for three samples. The S.E.M. was omitted when it was less than 5 % of the mean. (b) Reconstituted liposomes were incubated with [¹⁴C]nicotinamide in the presence of various concentrations of unlabelled nicotinamide without (○) or with 1 μM-cytochalasin B (●) and with 20 mM unlabelled D-glucose (▲). Unreconstituted liposomes were also incubated with the same radiotracer in the presence of various concentrations of unlabelled nicotinamide (□).

Next, we examined whether the inhibitory effects of D-glucose and nicotinamide on the uptake of the other was competitive or not. We determined D-glucose uptake at concentrations of 0.25 mM- and 0.5 mM-glucose in the presence of 5–25 mM-nicotinamide and analysed the data by Dixon (1953) plot (1/*v* versus [*I*]). The result demonstrated that two straight lines intersected on the left (1/*v*) ordinate, showing the inhibition of D-glucose uptake by nicotinamide was competitive and the estimated *K_i* value was approx. 13 mM (Fig. 3).

The effects of various related compounds on the uptake of D-glucose and nicotinamide were examined at 20 mM concentration of each (Table 1). L-Glucose had no effect on D-glucose uptake, indicating that it was not recognized by the glucose transporter as shown above in Fig. 1(a) and also as previously reported (Kasahara & Hinkle, 1977). The glucose analogues 2-deoxy-D-glucose and 3-O-methyl-D-glucose caused about 50 % inhibition. Ethylideneglucose, an inhibitor of glucose transporter (Baker & Widdas, 1972; Holman & Rees, 1982), caused similar inhibition of the uptake. Nicotinic acid, unlike nicotinamide, had no effect.

Fig. 4. Effects of D-glucose and nicotinamide on cytochalasin B binding to reconstituted liposomes

The liposomes were incubated with [3 H]cytochalasin B and cytochalasin E for 5 min in the presence of 0–50 mM unlabelled D-glucose (○) or nicotinamide (●) at 25 °C. Values are means $\pm$ S.E.M. for three samples. S.E.M. values are omitted when less than 5% of the mean.

Fig. 5. Inhibition by antibody of uptakes of D-glucose and nicotinamide by reconstituted liposomes

The liposomes were incubated for 10 min at 25 °C with antibody to erythrocyte glucose transporter at 0–200 μg/ml, and then D-[14 C]glucose (○) or [14 C]nicotinamide (●) was added. The uptakes of these radiotracers in 15 s were determined. Values are means $\pm$ S.E.M. for three samples. S.E.M. values are omitted when less than 5% of the mean.

50% inhibition was estimated to be 50 μg/ml (Fig. 5). Rabbit IgG was not inhibitory in the same concentration range (results not shown). However, in fact the antibody used was that for band 4.5 proteins, detected by SDS/PAGE, and the results described above were not decisive to prove the transport of nicotinamide through the glucose transporter itself. Among proteins in band 4.5, the nucleoside transporter is the sole protein with a transport function except the glucose transporter, but these two are not separable at present (Jarvis & Young, 1981; Wu *et al.*, 1983). Consequently, the reconstituted liposomes contain the nucleoside transporter. We examined a possible role of this transporter for the uptake of nicotinamide. Its specific inhibitor NBMPR showed no effect on the uptake of nicotinamide and D-glucose at concentrations of either 10 nM or 50 nM, in spite of a marked decrease (> 60%) in the uptake of adenosine (Table 2). Therefore, at least, the participation of the nucleoside transporter in nicotinamide transport was excluded.

Table 2. Effects of NBMPR and cytochalasin B on the uptake of D-glucose, nicotinamide and adenosine by reconstituted liposomes

Values are means $\pm$ S.E.M. for three samples: * P < 0.001 versus control (Student's t test).

	Uptake (pmol/20 s)		
	D-Glucose	Nicotinamide	Adenosine
Control	56 $\pm$ 2	101 $\pm$ 10	139 $\pm$ 10
NBMPR			
10 nM	55 $\pm$ 2	98 $\pm$ 5	55 $\pm$ 6*
50 nM	52 $\pm$ 2	99 $\pm$ 6	46 $\pm$ 5*
Cytochalasin B			
1 μM	30 $\pm$ 2*	51 $\pm$ 3*	136 $\pm$ 8

Fig. 6. Effects of D-glucose and nicotinamide on cytochalasin B binding to human erythrocytes

Human erythrocytes (10^8 cells/ml) were incubated with [3 H]cytochalasin B, cytochalasin E, and D-glucose (○) or nicotinamide (●) at 0–50 mM for 15 min at 37 °C. Values are means $\pm$ S.E.M. for three samples. S.E.M. values are omitted when less than 5% of the mean.

Fig. 7. Inhibition of the uptakes of 2-deoxy-D-glucose and nicotinamide by cytochalasin B in human erythrocytes

Human erythrocytes (10^8 cells/ml) were incubated with 10 nM–10 μ M-cytochalasin B for 15 min at 37 °C. Then 2-deoxy-D- $[^3\text{H}]$ glucose (○) or $[^{14}\text{C}]$ nicotinamide (●) was added and further incubated for 5 min. Values are means $\pm$ S.E.M. for three samples. S.E.M. values are omitted when less than 5% of the mean.

To see whether or not the results obtained by using reconstituted liposomes are artifacts, we examined the effects of D-glucose and nicotinamide on the binding of cytochalasin B, and those of cytochalasin B on the uptake of 2-deoxy-D-glucose and nicotinamide by human erythrocytes. The binding of cytochalasin B to human erythrocytes was inhibited by either D-glucose or nicotinamide in a dose-dependent manner, and the two curves were almost identical (Fig. 6). On the other hand, the uptake of nicotinamide and 2-deoxy-D-glucose was depressed dose-dependently in the presence of cytochalasin B between concentrations of 10 nM and 10 μ M. The inhibitory effect of cytochalasin B was also similar for both compounds (Fig. 7). Control uptakes in the absence of cytochalasin B were not significantly different from those with 10 nM-cytochalasin B. These results suggest that the functions of glucose transporter in native cell membranes are well reproduced in the reconstituted liposomes used in our experiments.

DISCUSSION

The volume of reconstituted liposomes, estimated with $[^3\text{H}]$ inulin (results not shown), was calculated to be 4.2 $\mu\text{l}/\text{mg}$ of lipid (mean of ten samples), which was approximately twice the volume previously reported (Sase *et al.*, 1982). This discrepancy may be attributable to the different method for estimation from ours. This volume is equivalent to about 600 $\mu\text{l}/\text{mg}$ of protein reconstituted, because usually approx. 5 μg of glucose transporter protein and 0.75 mg of lipid were incorporated into liposomes in our experimental conditions.

Although there are many reports using reconstituted liposomes for studying the mechanism of transport, it is generally difficult

to distinguish real transport of substrate from its binding to a transporter protein in this model system. There is no direct evidence showing the presence of a translocated substrate, including glucose, inside liposomes. However, although it is an indirect proof, the following calculation is possible in our experiments, provided that (i) proteins used for reconstitution are all the glucose transporter, (ii) the molecular mass of glucose transporter is 50 kDa, (iii) all glucose transporters are arranged in the right direction, as in intact cells, in the liposomal membranes, and (iv) the molar binding ratio of nicotinamide and the transporter is unity. For example, we estimated that the amount of nicotinamide in reconstituted liposomes as a whole 20 s after starting of uptake was 50 times that expected from only binding of nicotinamide to the transporter when 32 mM-nicotinamide was present in the medium (Fig. 2b). This value is probably underestimated, therefore such a high value might suggest a possible translocation of nicotinamide into liposomes rather than its binding to transporter proteins.

The configurational requirements of the glucose transporter for monosaccharide transport are very strict (Holman *et al.*, 1981). Consequently, it seems unlikely that the transporter plays a direct role in the transport of compounds other than monosaccharides. A mechanism for active transport of nicotinamide has been found in the K-562 human leukaemic cell line (Pero *et al.*, 1989). However, our model system does not contain any high-energy source such as ATP and its related enzyme(s). Our series of experiments using liposomes reconstituted with the human erythrocyte glucose transporter strongly suggest the direct role of the glucose transporter in the uptake of nicotinamide, which has no structural similarities to monosaccharides.

For examination of the properties of reconstituted liposomes, it is necessary, as far as possible, to remove unreconstituted transporter protein from the liposomes, especially for the cytochalasin-B-binding assay. However, the widely used method of separation by filtration with membrane filters seems to be insufficient, considering the insolubility of transporter proteins. In our experiments, liposomes were separated from unreconstituted proteins by centrifugation, and about 40% of the protein used was actually recovered as a distinct pellet. Therefore the results obtained here were thought to be those for reconstituted liposomes slightly contaminated with free transporter proteins.

The nucleoside transporter protein of erythrocyte membrane is co-purified with the glucose transporter by the same purification method, and both transporters are detected in band 4.5 in SDS/PAGE (Jarvis & Young, 1981; Young & Jarvis, 1983; Wu *et al.*, 1983). Therefore the reconstituted liposomes with the erythrocyte glucose transporter necessarily include the nucleoside transporter. The transport of nicotinamide through this transporter has never been examined. However, our results using NBMPR, a potent inhibitor of the nucleoside transporter (Paterson & Simpson, 1965; Paterson & Oliver, 1971; Jarvis *et al.*, 1982; Jarvis & Young, 1982), clearly demonstrated that nicotinamide and D-glucose transport was not affected by this inhibitor, although they were commonly inhibited by cytochalasin B. On the other hand, cytochalasin B did not change the transport of adenosine, as shown in our study and also as reported by Tse *et al.* (1985). It is concluded from these facts that the nucleoside transporter is not involved in the transport of nicotinamide.

We observed similarity between the uptake of D-glucose and nicotinamide by pancreatic β -cells (Sofue *et al.*, 1991), but the glucose transporter of β -cells is reported to be different from that of human erythrocytes (Type 1) (Thorens *et al.*, 1988). However, similar phenomena were observed in different systems, i.e. liposomes reconstituted with erythrocyte glucose transporter as

described here, pancreatic β -cells (Sofue *et al.*, 1991), and 3T3-L1 fat-cells, whose uptake of 2-deoxy-D-glucose was inhibited by nicotinamide, but not by nicotinic acid (Grunfeld & Shigenaga, 1984). These findings demonstrate a general function of glucose transporters for nicotinamide uptake, regardless of their different types.

There may be some criteria for recognition of compounds including nicotinamide by glucose transporters, other than that the chemical and configurational properties of these compounds may allow the transport. These criteria are unknown at present, but other substances besides nicotinamide satisfying these criteria may exist, and such compounds may help the understanding of the transport mechanism in molecular level. Irrespective of the mechanism involved, the present finding that, in reconstituted liposomes the features of transport of nicotinamide and D-glucose are very similar, provides evidence of multifunction of the glucose transporter.

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