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Decrease in glucose transport activity of Friend erythroleukemia cell caused by dimethylsulfoxide, a differentiation-inducing reagent

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A transport system for D-glucose was found in a Friend erythroleukemia cell line, T-3-C1-2-O and was characterized as a facilitated diffusion system. D-Glucose transport activity showed a half-saturation concentration of 2.2 mM and was inhibited by mercuric ions, cytochalasin B, phloretin, and stilbestrol, but was not strongly inhibited by phloridzin. Transport of 3-*O*-methyl-D-glucose was faster than D-glucose and the intracellular concentration of the sugar was found to reach the concentration in the assay medium. The treatment of cells with a differentiation-inducing reagent, dimethylsulfoxide(Me_2SO), for 24 h caused a marked decrease in glucose transport activity due to a decrease in V_{max}. In an induction-insensitive Friend cell line, T-3-K-1, D-glucose transport activity was low in untreated cells and Me_2SO treatment did not cause a significant decrease in transport activity. The results obtained in this study indicate that the decrease in glucose transport activity is not due to the direct effect of Me_2SO on transport activity, but is associated with the induction of differentiation. By immunoblotting cell lysates of T-3-C1-2-O cells using antibody to human erythrocyte glucose transporter, a single major band having a molecular weight of 52000 was detected, which may be a glucose transporter in Friend cells.

Introduction

Changes of glucose transport activity in animal cells occur in several cases, such as treatment with a hormone [1–3], cellular transformation [4–6], glucose starvation [7,8] and differentiation [9–11]. Insulin treatment of fat cells [2,3,12,13] or muscle cells [14] causes rapid translocation of the glucose transporter from a microsome fraction to plasma membranes. Translocation does not require protein synthesis [12] and contributes to an increase in glucose transport activity. A variety of transformed cell show higher glucose transport activity, due to an increased amount of transporter [6]. In other cases, including cell differentiation, the detailed mechanism of the regulation is still obscure.

The change in glucose transport activity associated with cell differentiation has been studied in the process of erythropoiesis. Glucose transport activity is high in fetal or neonatal erythrocytes [9,11]. Transport activity decreases when early reticulocytes induced by the administration of phenylhydrazine mature to erythrocytes [10].

Murine erythroleukemia cell lines transformed with Friend virus complex have been used to study erythroid cell differentiation [15–17]. These

cells show many properties associated with the later stages of erythropoiesis when treated with a variety of including reagents; several plasma membrane related phenomena have been described, including an increase in plasma membrane proteins [18–20] and a decrease of the major histocompatibility antigen, H-2 [21]. These changes occur relatively late after the addition of differentiation-inducing reagents. Early changes in plasma membrane functions include a decrease in Na^+/K^+-pump activity [22,23], amino acid transport [23] and cell surface charge [24], an increase in Ca uptake [25] and a change in lectin agglutinability [26]. Although changes in membrane functions are implicated in the processes of differentiation [15,16], biochemical analyses of membrane-related functions are still limited.

In this report we describe a glucose transport system in Friend erythroleukemia cells which shows the typical characteristics of a facilitated diffusion system. The transport activity declined when the cells were treated with Me_2SO for 24 h. A 52 kDa protein was tentatively identified as a glucose transporter.

Experimental procedures

Materials. D-[^{14}C]Glucose (CFB.96), L-[^{14}C]glucose (CFA.328), 2-deoxy-D-[^{14}C]glucose (CFB.181), 3-*O*-[^{3}H]methyl-D-glucose (TRK.372), and ^{125}I-labeled protein A(IM.112) were purchased from Amersham and [^{14}C]inulin (NEC-164P) and $^{3}H_2O$(NET-001B) from New England Nuclear. Two types of silicone oil, TSF440 and TSF451-50, were gifts from Toshiba Silicone, Tokyo. Other reagents used were of analytical grade.

Analytical methods. Protein was determined by the method of Lowry et al. [27]. SDS-gel electrophoresis was performed with 10% polyacrylamide gels [28]. Molecular weight standards used were bovine serum albumin (69 000), ovalbumin (45 000), chymotrypsinogen (25 000) and human erythrocyte membrane proteins: band 1 (240 000), band 2 (220 000), band 3 (98 000), band 4.1 (78 000), band 4.2 (72 000), band 5 (43 000), band 6 (35 000) and band 7 (29 000). Benzidine-positive cells were counted after staining with 3,3′-diaminobenzidine following the method of Orkin et al. [29].

Cell and cell culture. A Friend erythroleukemia cell line, T-3-C1-2-O (abbr. 2-O) derived from T-3-C1-2 [30] was grown in Ham F-12 medium supplemented with 10% calf serum as previously described [30]. Another Friend cell line, T-3-K-1 (abbr. K-1), which is also a subclone of T-3-C1-2 but does not respond to Me_2SO [30], was used and grown similarly.

Me_2SO treatment. Friend cells were inoculated into 50 ml culture medium ($3 \cdot 10^4$ cells/ml) in a 250 ml plastic flask (5375, Lux) and grown at 37°C for 24 h. Me_2SO was then added at 1.5% and the incubation was continued. At appropriate times (usually 24 h) cells were harvested by centrifugation for 7 min at 1500 rpm and washed twice with phosphate-buffered saline (136.9 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4).

Uptake assay. Friend cells were suspended in 180 μl of phosphate-buffered saline supplemented with 1 mM $MgCl_2$ and preincubated for 2–5 min at 37°C. Transport assay at 37°C was started by the addition of 20 μl of 2.0 mM radioactive sugar in phosphate-buffered saline. After an appropriate time (15 s, if not stated otherwise), the assay was stopped by the addition of 20 μl cold phosphate-buffered saline supplemented with 1 mM $MgCl_2$ and 4.25 mM $HgCl_2$. The mixture was subjected to three-layer silicone centrifugation [31]. The silicone layer (100 μl) consisted of a mixture of TSF440 and TSF451-50 (5.2 : 1). The lowest layer (50 μl) contained a mixture of 5% trichloroacetic acid and 8.5% (w/v) glycerol. Immediately after the addition of cold stopping solution, a 190 μl portion was removed, layered above the silicone layer and spun at 8000 rpm for 30 s using a table top centrifuge (MC-15A, Tomy). The tube was cut in the middle of silicone layer by a razor blade. Radioactivity contained in the lower portion of the tube was counted in scintillation fluid (ACS II, Amersham) using a scintillation counter. The amount of cells was limited to 0.1 to 0.7 mg protein/assay for the sake of accuracy and linearity with protein. The breakage of cells during the assay, estimated by the measurement of protein in supernatants, was less than 4% of total protein in control or Me_2SO-treated cells. Systematic assays (e.g. time-course or kinetic studies) were performed mainly with single samples and other as-

says were performed in triplicate. All experiments reported in this study were repeated more than three times. For the determination of cell volume, ^3H_2O (2 μCi) and [^{14}C]inulin (77 μg, 0.18 μCi) were used and cell-associated radioactivity was usually separated 1 min after the addition of radioisotope by the silicone centrifugation method.

Immunoblotting. Washed cells were suspended in a small volume of phosphate-buffered saline (2–10 mg protein/ml). An equal volume of a lysing solution consisting of 4% SDS and 4 mM phenylmethylsulfonyl fluoride was added and the mixture was immediately immersed in a boiling water bath for 7 min. The cell lysate was then centrifuged at 100000 × *g* for 30 min and a portion of the supernatant was subjected to SDS-gel electrophoresis. Immunoblotting was performed [32] with modifications. After electrophoresis, the SDS-gels were soaked in a transfer buffer (0.192 M glycine/25 mM Tris/20% methanol/0.02% SDS (pH 7.5)) for 15 min at room temperature. Proteins were electrophoretically transferred from the gels to nitrocellulose sheets (0.3 μm, Toyo, Japan) in transfer buffer with a d.c. current of 0.5 A for 4–5 h. The transfer of proteins was almost complete as judged by the staining of gels after transfer with Coomassie blue R and of nitrocellulose sheets with 0.1% Amido black. Nitrocellulose sheets were rinsed in a rinse buffer (10 mM Tris/0.15 M NaCl/1 mM EDTA/0.1% Triton X-100, pH 7.5 adjusted with HCl) for 15 min at room temperature and incubated in rise buffer supplemented with 30 mg/ml bovine serum albumin for 2 h at room temperature. The sheets were then incubated with 10 μl of antibody to human erythrocyte glucose transporter [33] in 10–15 ml of rinse buffer supplemented with 3 mg/ml bovine serum albumin overnight at 4°C. The sheets were washed three times with rinse buffer and incubated with 1 μCi ^{125}I-protein A in rinse buffer supplemented with 3 mg/ml bovine serum albumin for 5–6 h at 4°C. Sheets were washed three times with rinse buffer containing 1 M NaCl. After briefly washing with H_2O, the sheets were dried and subjected to autoradiography with XAR film (Kodak) using an intensifying screen (lightning plus, Du Pont) for 1–3 days at −70°C. For quantitation, appropriate portions of nitrocellulose sheet were cut out and radioactivity was counted in a gamma counter. Molecular weights of bands in autoradiographic films were determined by comparing films with the corresponding nitrocellulose sheets stained with Amido black.

Results

Characteristics of glucose transport system of Friend cell

A Friend erythroleukemia cell line, 2-O, exhibited rapid uptake of 2-deoxy-D-glucose, an analog of D-glucose which is not metabolized beyond initial phosphorylation in mammalian cells [4] (Fig. 1A). The uptake activity was inhibited by $HgCl_2$, cytochalasin B and phloretin (Fig. 1B). The inhibitor-insensitive part of uptake may be a background level of 2-deoxy-D-glucose uptake since the amount was the same with all the inhibitors and was comparable to that observed with L-glucose, a non-transportable analog (see Tables I, II and Figs.3, 4, 6). Uptake of D-glucose was faster in initial rate but the extent of uptake was less com-

TABLE I

INHIBITORS OF D-GLUCOSE UPTAKE IN FRIEND CELLS

A Friend cell line, 2-O was cultured for 3 days and harvested in phosphate-buffered saline containing 1 mM $MgCl_2$. The cells were preincubated for 2–5 min with an indicated inhibitor at 37°C. Uptake of D-, or L-glucose was measured at 37°C for 15 s. All inhibitors except for $HgCl_2$ were dissolved in 10 to 40% ethanol and at most 1% ethanol was carried over into the uptake assays, which had no significant effect on D-, or L-glucose uptake. D-Glucose-specific uptake was calculated by subtracting L-glucose uptake from D-glucose uptake. Cyt. B, cytochalasin B.

Addition		Uptake (nmol/mg protein per 15 s)			% Inhibition
		D-Glc	L-Glc	D-Glc-specific	
None		2.83	0.83	2.0	
$HgCl_2$	50 μM	1.32	0.96	0.36	82
	500 μM	1.13	0.98	0.15	93
Cyt.B	100 nM	1.98	0.82	1.2	40
	500 nM	1.35	0.79	0.56	72
Phloretin	80 μM	0.99	0.93	0.06	97
Stilbestrol	80 μM	1.30	0.92	0.38	81
Phloridzin	80 μM	2.44	1.0	1.4	30

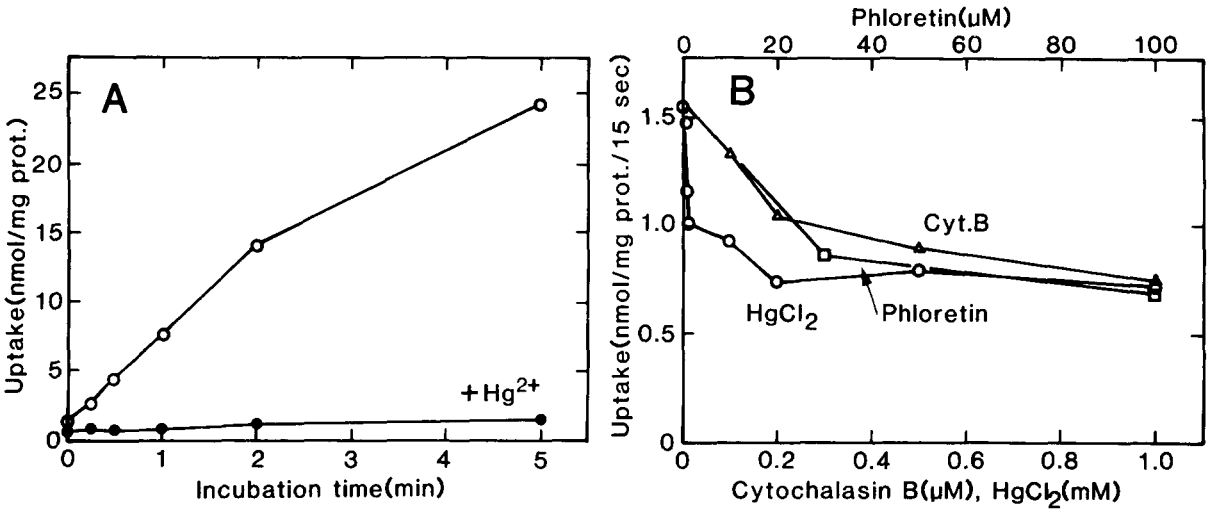

Fig. 1. 2-Deoxy-D-glucose uptake in Friend cell. A Friend cell line, 2-O, was cultured in 250 ml plastic bottles for 2 days. Uptake of 0.2 mM 2-deoxy-D-glucose (10 mCi/mmol) in 2-O cells (0.29 mg protein in (A) and 0.15 mg protein in (B)) was measured at 37°C in phosphate-buffered saline containing 1 mM $MgCl_2$ or in phosphate-buffered saline supplemented with 1 mm $MgCl_2$ and an indicated inhibitor. At indicated times, a portion (190 μl) of the assay mixture was subjected to silicone layer centrifugation. Other conditions are described in Experimental Procedures. (A) Uptake assay was performed in the presence of 1 mM $HgCl_2$ (●) or in its absence (○). (B) Uptake was measured for 15 s with the indicated amounts of the following inhibitors; $HgCl_2$ (○), cytochalasin B (△) and phloretin (□).

pared to 2-deoxy-D-glucose uptake (see Fig. 4). 3-*O*-Methyl-D-glucose, another non-metabolizable analog of D-glucose [4] equilibrated in cells with a half time of less than 30 s (see Fig. 5). Uptake of D-glucose was inhibited by mercuric ions, cytochalasin B, phloretin, and stilbestrol but was not strongly inhibited by phloridzin, a preferential

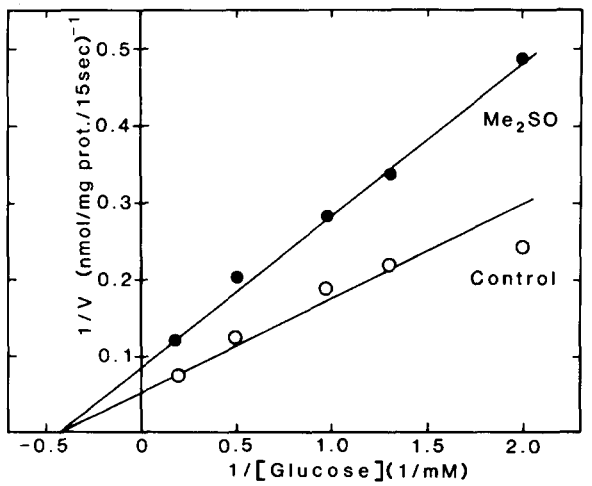

Fig. 2. Kinetic analysis of D-glucose-specific uptake in control (○) and Me_2SO-treated cells (●). 2-O cells were cultured for one day with or without 1.5% Me_2SO. Uptake of D-, or L-glucose for 15 s in control (0.32 mg) or Me_2SO-treated (0.29 mg) cells was measured, with varying concentrations of radioactive sugars. The difference in D-glucose uptake and L-glucose uptake was taken as the D-glucose-specific uptake.

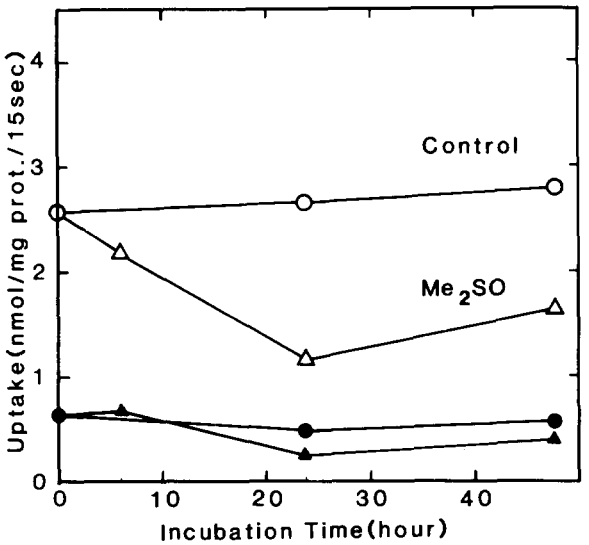

Fig. 3. Decrease in glucose uptake in 2-O cells with Me_2SO treatment. 2-O cells were cultured with or without 1.5% Me_2SO for the indicated times and harvested for uptake assay. Uptake for 15 s of 0.2 mM D-glucose (13.3 mCi/mmol) in control (○) and Me_2SO-treated (△) cells and of 0.2 mM L-glucose (13.3 mCi/mmol) in control (●) and Me_2SO-treated (▲) cells was measured as described in Experimental Procedures.

inhibitor for Na^+-dependent glucose transport systems [34,35] (Table I). Kinetic analysis showed a half maximum D-glucose concentration (K_t) of 2.2 mM and a V_{max} of 19 nmol D-glucose/mg protein per 15 s (Fig. 2). Based on results of five experiments, a K_t of 2.0 ± 0.3 (means ± S.D.) and a V_{max} of 19 ± 7.5 nmol/mg protein per 15 s were obtained. With 2-deoxy-D-glucose as the substrate, a K_t of 2.0 mM and a V_{max} of 11 n mol 2-deoxy-D-glucose/mg protein per 15 s were observed when the background level was estimated by the addition of 0.5 mM $HgCl_2$ (data not shown). The rapid uptake of D-glucose and its analogs, and inhibitor specificity indicate that the uptake activity of Friend cells represents transport by a facilitated diffusion system. Further evidence which supports the notion of facilitated diffusion of D-glucose is given in later sections.

Effect of Me_2SO treatment on glucose transport activity of Friend cells

The change in glucose transport activity following culture in the presence of Me_2SO was measured. The initial rate of D-glucose transport decreased to half with 24 h culture in 1.5% Me_2SO (Fig. 3). It decreased from 2.3 ± 0.6 nmol/mg protein per 15 s ($n = 12$) to 1.3 ± 0.6 nmol/mg protein per 15 s, corresponding to an average 46 ± 17% decrease in individual experiments. In parallel experiments without Me_2SO, no change in glucose transport activity was found. A decrease in glucose transport was observed 6 h after the addition of Me_2SO; 23 ± 8.5% decrease in Me_2SO-treated cells ($n = 3$, $P < 0.05$). Under the present culture conditions, the induction of hemoglobin synthesis proceeded more slowly. Benzidine-positive cells began to appear 3 days after the addition of Me_2SO and reached a level of 83–97% of cells on day 5. Culture for 2 days with Me_2SO caused no further decrease in transport.

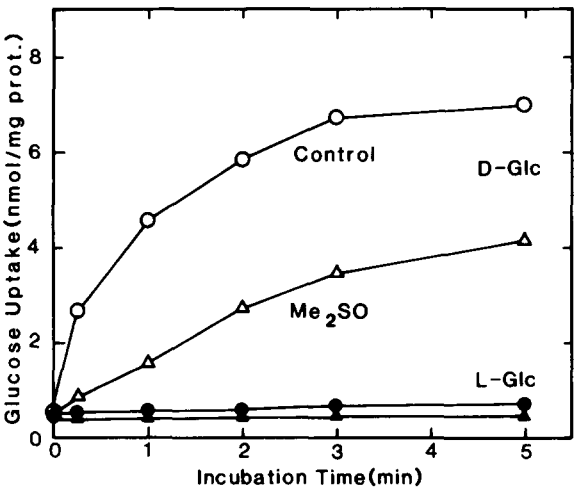

Fig. 4. Time-course of D-glucose uptake in control and Me_2SO-treated 2-O cells. 2-O cells were cultured for one day with or without 1.5% Me_2SO. Uptake of 0.2 mM D- and L-glucose in Me_2SO-treated (0.28 mg) and control (0.41 mg) cells was measured as described in Experimental Procedures. Symbols used are the same as in Fig. 3.

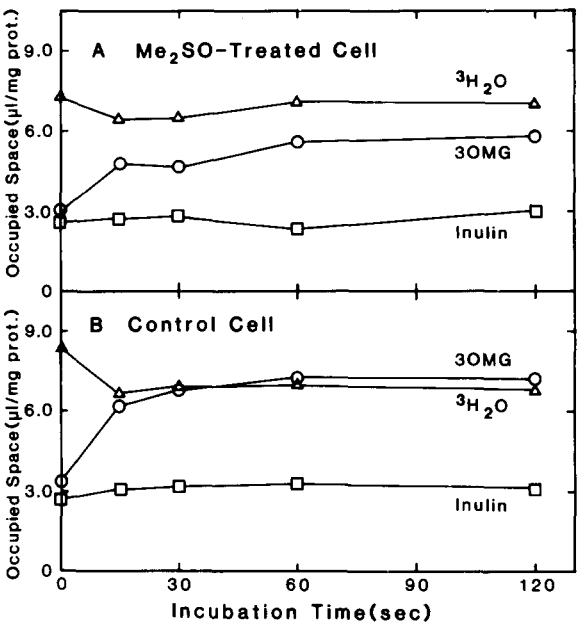

Fig. 5. 3-*O*-Methyl-D-glucose transport and cell volume of Me_2SO-treated (A) and control (B) 2-O cells. 2-O cells were cultured for one day with or without 1.5% Me_2SO. Transport of 0.2 mM 3-*O*-methyl-D-glucose (20 mCi/mmol) (○) in Me_2SO-treated (0.38 mg) and control (0.38 mg) cells was measured. Other conditions for the transport assay are described in Experimental Procedures. Uptake of ^3H_2O (△) or inulin (□) was measured in the same manner as 3-*O*-methyl-D-glucose transport. After centrifugation, 10 μl of the supernatant was removed and the radioactivity was counted after the addition of glycerol/trichloroacetic acid and silicone in order to get the same quenching conditions as in the lower layer. The uptake of radioactive substances is expressed as the occupied space in μl, which was calculated from the ratio of the cell-associated radioactivity and radioactivity in 1 μl of the corresponding supernatant. The difference between H_2O space and inulin space was interpreted as cell volume.

The time-course of D-glucose transport was measured with cells treated with Me_2SO for 24 h (Fig. 4). The extent as well as the initial rate of D-glucose-specific transport declined considerably with Me_2SO treatment. Kinetic studies showed that the change in transport activity was due to a decrease in V_{max} (Fig. 2); control cells showed a V_{max} of 19 ± 8 nmol/mg protein per 15 s ($n = 5$) and a decrease of $37 \pm 10\%$ was observed with Me_2SO treatment. No significant change was observed in the K_t of transport (2.0 ± 0.33 mM in control cells and 2.3 ± 0.59 mM in Me_2SO-treated cells). During culture of 2-O cells for 3 days with or without the addition of Me_2SO, the protein content of cells was 0.26 ng/cell and did not change significantly. No significant change in cell volume was observed in cells treated with Me_2SO for 24 h (Fig. 5). When cell volume was measured as the difference in H_2O space and inulin space, it was found to be 5.8 ± 1.6 μl/mg protein ($n = 6$) in control cells and 4.6 ± 0.7 μl/mg protein in Me_2SO-treated cells. In control cells, the steady-state level of 3-*O*-methyl-D-glucose transport reached an amount almost equivalent to the ^3H_2O space ($103 \pm 6\%$, $n = 6$), indicating equilibration of the sugar inside and outside the cells. This result supports the notion of sugar transport by facilitated diffusion. The transport of 3-*O*-methyl-D-glucose in Me_2SO-treated cells leveled off at about 80% of cell volume ($82 \pm 19\%$, $P < 0.05$), suggesting that about 20% of cells had little glucose transport activity. Since the initial rate of D-glucose decreased to only 46% and about 10% of the cells may not be committed to differentiation, as observed by the benzidine staining, it is conceivable that cells are heterogeneously sensitive to Me_2SO and that individual cells may exhibit different levels of glucose transport activity.

In both control and Me_2SO-treated cells, the addition of Me_2SO (0.2–1.5%) to the transport assay did not change the D-glucose transport activity nor the background level as measured by L-glucose uptake (Fig. 6). Another Friend cell line, K-1, which shows no induced differentiation with Me_2SO treatment [30], was cultured under the same conditions and subjected to transport assays. The K-1 cells exhibited a lower glucose transport activity and Me_2SO treatment did not change the transport activity, which was comparable to Me_2SO-treated 2-O cells (Table II); 0.70 ± 0.54 nmol/mg protein per 15 s ($n = 3$) in control K-1

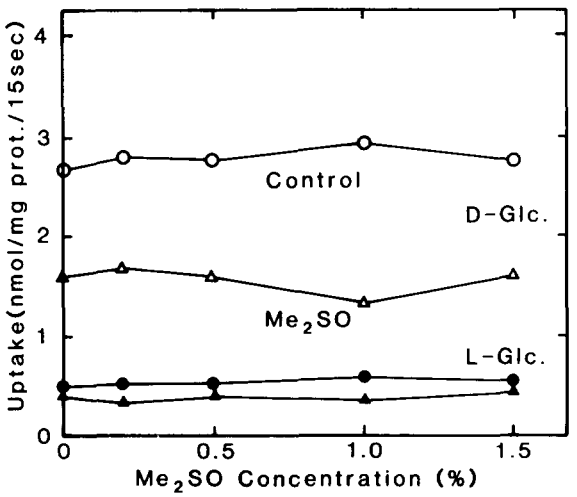

Fig. 6. Effect of Me_2SO on D-glucose uptake in control and Me_2SO-treated Friend cells. 2-O cells were cultured for one day with or without 1.5% Me_2SO. Cells were washed and preincubated with the indicated concentration of Me_2SO in uptake medium for 5–10 min at 37°C. Uptake of D-, or L-glucose for 15 s in control (0.3 mg) or Me_2SO-treated cells was measured. The symbols used are the same as in Fig. 3.

TABLE II

EFFECT OF Me_2SO TREATMENT ON GLUCOSE UPTAKE IN 2-O AND K-1 CELLS

An induction-insensitive cell line, K-1 and the 2-O cell line which exhibits induced differentiation by Me_2SO were cultured for one day with or without 1.5% Me_2SO. Uptake for 15 s of D- and L-glucose in control (0.27 mg) and Me_2SO-treated (0.26 mg) 2-O cells and in control (0.22 mg) and Me_2SO-treated (0.10 mg) K-1 cells was measured as described in Experimental Procedures. Data are expressed as the mean ± S.D. of three determinations from a typical experiment. The Me_2SO-dependent decrease of the D-glucose-specific transport in 2-O cells is significant ($P < 0.01$).

Cell line	Me_2SO treatment	Uptake (nmol/mg protein per 15 s)			% Activity
		D-Glc	L-Glc	D-Glc-specific	
2-O	–	1.93 ± 0.18	0.75 ± 0.02	1.18	100
	+	1.25 ± 0.11	0.60 ± 0.04	0.65	55
K-1	–	1.15 ± 0.03	0.56 ± 0.07	0.59	50
	+	1.18 ± 0.06	0.56 ± 0.05	0.63	53

cells and 0.66 ± 0.41 nmol/mg protein per 15 s in Me_2SO-treated K-1 cells. These results indicate that the decrease in glucose transport in Me_2SO-treated 2-O cells is not due to the direct effect of Me_2SO on the plasma membrane transport system, but to the effect of Me_2SO on cell differentiation. The low glucose transport activities of control and Me_2SO-treated K-1 cells were similar to Me_2SO-treated 2-O cells, which might be interpreted as indicating that the K-1 cells are at a similar erythropoietic stage to Me_2SO-treated 2-O cells.

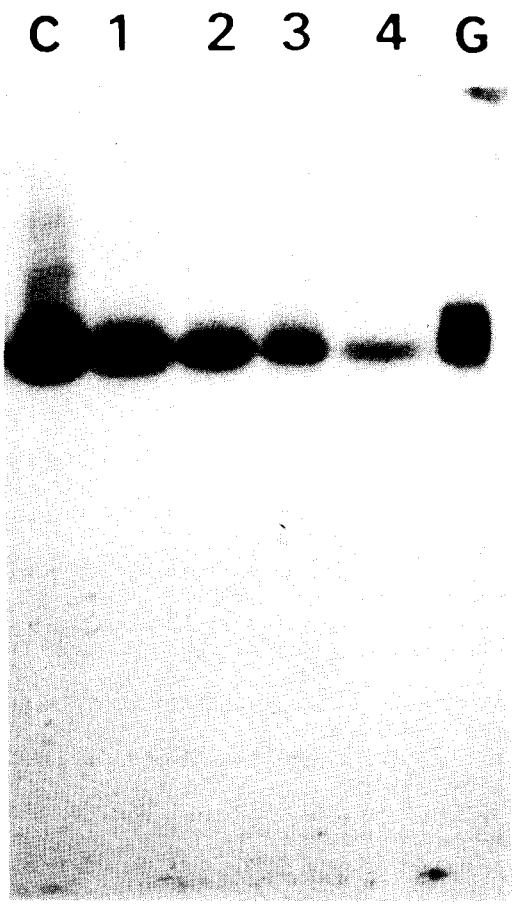

Fig. 7. Immunological detection of the glucose transporter of Friend cells with antibody to human erythrocyte glucose transporter. A Friend cell line, 2-O was cultured in the presence of 1.5% Me_2SO and harvested on the indicated day (1–4 day). Control cells without Me_2SO (C) were cultured for 3 days. SDS-gel electrophoresis was performed with 200 μg 2-O cell protein or 0.1 μg of human erythrocyte ghost (G) prepared as described [37]. Immunoblotting was performed as described in Experimental Procedures. Autoradiography was performed for one day at −70°C.

Detection of glucose transporter of Friend cells by immunoblotting

Antibody to the human erythrocyte glucose transporter is known to crossreact with the glucose transporters of several animal cells [6,12,36]. A single major band having a molecular weight of 52 000 was detected by immunoblotting lysates of 2-O cells using this antibody (Fig. 7). No clear corresponding band was found with Coomassie blue staining, indicating that the transporter is a minor protein. In Fig. 7, the amount of 52 kDa protein decreased to 70% of the control 1 day after Me_2SO addition and the half-time for this decrease was about 24 h, which is significantly slower than the decrease in transport activity (see Fig. 3). The time-course of the decrease varied in individual experiments but always appeared to be slower than the decrease in D-glucose transport. This raises the possibility that the rapid decrease in D-glucose transport activity is not entirely due to a decrease in the amount of glucose transporter in cells, but in some part due to the availability of active transporter in plasma membranes or to a lower activity of the transporter in plasma membranes. At present, however, the possibility of inaccurate quantitation using the present immunoblotting procedures is not entirely excluded.

Discussion

The results obtained in this study clearly indicate that the glucose transport system of a Friend cell line, 2-O, is a typical facilitated diffusion system in that (1) D-glucose and its analogs are good substrates of the system (Figs. 1, 4, 5), (2) the inhibitor specificity (Table I) is of the facilitated diffusion type [38,39] rather than the Na^+-dependent type [34,35], and (3) the steady-state level of 3-*O*-methyl-D-glucose transport indicates the equilibration of sugar inside tand outside cells (Fig. 5B). Moreover, antibody to the transporter of the human erythrocyte glucose transport system, a typical facilitated diffusion system [40], crossreacted with a protein having an apparent molecular weight of 52 000, which is similar to that of the human erythrocyte transporter (55 000) [41]. It should be noted that the reticulocytes of several animals in their early stages exhibit a

glucose transport system of the facilitated diffusion type [9,11,42].

Maturation of erythroid cells is accompanied by changes in various metabolic activities including glucose transport. In fetal and new born animals [9,11,42] or in phenylhydrazine-treated animals [10], the glucose transport activity of erythroid cells markedly declines on maturation. We observed a similar change in the Friend cell line, 2-O, when differentiation was induced with Me_2SO. The onset of the decrease was less than 6 h and it was earlier than the reported onset of commitment of cells to differentiation: a lag of 10 h was observed and less than 10% of the cells were committed to differentiation by 24 h [43,44]. The decrease in D-glucose transport was completed within 24 h and is one of the earliest responses to induction, raising the possibility that decrease in transport is closely connected with the induction of differentiation. This possibility is supported by the fact that glucose transport activity of the induction-insensitive cell line, K-1, was low and did not decrease markedly on Me_2SO treatment. In addition, we have observed that the addition of 5–10 μM cytochalasin B in place of Me_2SO caused differentiation in 10–30% of 2-O cells, which was not observed with the addition of cytochalasin A or E (Amanuma, H. and Kasahara, M., unpublished observation (1985)). On the other hand, a haemopoietic growth factor which promotes the differentiation of early haematopoietic cells was shown to increase glucose transport [45]. Of several early responses studied [22–26,44,46], a change in intracellular Ca^{2+} concentration was recently implicated as the cause of differentiation [25,44], but the claim was not supported by subsequent work [47]. It is thus important to clarify the relation between these early events and differentiation, especially between glucose metabolism and the commitment of cells to differentiation.

Acknowledgements

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