Use of a genetic variant to study the hexose transport properties of human skin fibroblasts

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Human skin fibroblasts from 'normal' subjects were found to possess at least two hexose transport systems. One system was responsible for the uptake of 2-deoxy-D-glucose (dGlc), D-glucose and D-galactose, whereas the other was responsible primarily for the uptake of 3-O-methyl-D-glucose (MeGlc). The transport of dGlc was the rate-limiting step in the uptake process; over 97 % of the internalized dGlc was phosphorylated and the specific activity of hexokinase was several times higher than that for dGlc transport. The dGlc transport system was activated by glucose starvation, and was very sensitive to inhibition by cytochalasin B and energy uncouplers. Fibroblasts isolated from a patient with symptoms of hypoglycaemia were found to differ from their normal counterparts in the dGlc transport system. They exhibited a much higher transport affinity for dGlc, D-glucose and D-galactose, with no change in the respective transport capacity. Transport was not the rate-limiting step in dGlc uptake by these cells. Moreover, the patient's dGlc transport system was no longer sensitive to inhibition by cytochalasin B and energy uncouplers. This suggested that the intrinsic properties of the patient's dGlc transport system were altered. It should be noted that the patient's dGlc transport system could still be activated by glucose starvation. Despite the changes in the dGlc transport system, the MeGlc transport system in the patient's fibroblasts remained unaltered. The observed difference in the properties of the two hexose transport systems in the 'normal' and the patient's fibroblasts strongly suggests that the two transport systems may be coded or regulated by different genes. The present finding provides the first genetic evidence from naturally occurring fibroblasts indicating the presence of two different hexose transport systems.

INTRODUCTION

Human skin fibroblasts have been used extensively to investigate metabolic defects in patients with inheritable diseases. The rationale for using these cells was based on the ease of their isolation and the ability to grow them under defined conditions [1]. The uptake of hexose by fibroblasts has been used as a parameter to monitor the metabolic alterations of patients in response to various growth hormones [1,2]. Most eukaryotic hexose transport systems are responsible for the uptake of D-glucose and D-galactose, but not fructose [2–5]. They are also sensitive to inhibition by very low concentrations of the fungal metabolite cytochalasin B [6–8]. Both high- and low-affinity hexose transport systems have been demonstrated in chick embryo fibroblasts, rat and human myoblasts, and rat adipocytes [3,6,7,9–14]. In the case of rat myoblasts, 2-deoxy-D-glucose (dGlc), D-glucose and D-galactose were preferentially taken up by the high-affinity transport system, whereas 3-O-methyl-D-glucose (MeGlc) was taken up predominantly by the low-affinity system [3,6,10]. Both whole cell and membrane vesicle studies indicated that dGlc was taken up by an active transport process; consequently, dGlc uptake was very

sensitive to energy uncouplers such as CCCP or 2,4-dinitrophenol [3,5]. On the other hand, MeGlc uptake was mediated only by facilitated diffusion. The transport capacity of the high-affinity transport system was also found to be elevated by glucose starvation [15].

Transport mutants have proved to be a valuable tool in elucidating transport mechanisms in prokaryotes [16]. To date, relatively little work has been done using genetic variants to examine the molecular mechanism and regulation of various transport processes. Rat myoblast mutants defective in hexose transport have been isolated [10]. They have not only provided valuable information on the number and nature of the transport systems present in the cells, but have also served as a powerful tool in the identification of the hexose transport components [7,10,15]. More recently, a galactokinase-negative Chinese hamster fibroblast cell line has also been used to study the regulation and kinetic properties of galactose transport in the absence of substrate metabolism [17]. Genetic variants can be isolated in the form of somatic cell mutants from permanent cell lines, and can also be obtained from primary cultures isolated from patients or animals. We have recently demonstrated that myoblasts from patients with Duchenne Muscular Dys-

Abbreviations used: dGlc, 2-deoxy-D-glucose; MeGlc, 3-O-methyl-D-glucose; PBS, phosphate-buffered saline; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; FIB, fibroblasts from a patient with symptoms of hypoglycaemia; FIBC, fibroblasts from an age-matched control boy; FIBMC, fibroblasts from another age-matched control boy; FFIBC, fibroblasts from the patient's father; MFIBC, fibroblasts from the patient's mother.

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trophy exhibit different hexose transport properties [18].

The present investigation reports on the kinetic properties of hexose transport in human skin fibroblasts. Whereas a significant difference in the apparent K_m values of dGlc and MeGlc transport could not be observed in normal fibroblasts, the dGlc transport system was found to be altered in a genetic variant isolated from a patient with suspected hypoglycaemia. Since the MeGlc transport system remained unaltered in the genetic variant, it might be surmised that the transport processes for dGlc and MeGlc are coded or regulated by different genes. Thus as in other eukaryotic cells, at least two hexose transport processes are present in human skin fibroblasts.

MATERIALS AND METHODS

Cell lines and culture media

Human fibroblast cultures were established from skin biopsies from a patient (M.V.) with symptoms of hypoglycaemia, the patient's mother and father, and two age-matched control boys, with the subjects' consent. Cells were grown and maintained in F-10 medium (Gibco) supplemented with 20% fetal calf serum, 50 μ g of gentamicin/ml, 200 units of penicillin G/ml and 100 units of streptomycin sulphate/ml. Transfers were made every week, using 0.1% trypsin to detach cells from plates. The glucose starvation medium (referred to as the fructose medium) was composed of essentially the same ingredients, except that glucose was replaced with 0.1% fructose, and the fetal calf serum was first dialysed extensively against phosphate-buffered saline (PBS; 137 mM-NaCl, 2.7 mM-KCl, 8.1 mM- Na_2HPO_4 , 1.5 mM- KH_2PO_4 , 0.9 mM- CaCl_2 , 2H₂O, 0.5 mM- MgCl_2) [15].

Transport studies

Transport studies were carried out as described previously [3]. In order to examine the properties of the hexose transport systems independent of the cell density inhibition effect, sub-confluent monolayers were used in these studies. Our approach also differed from those of other workers [2,19] in that a wide range of substrate concentrations (20–300 μ M) was used in determining the transport kinetics. The same amount of radioactive sugar was added to different concentrations of the unlabelled sugar. Using cells treated with 20 μ M-cytochalasin B or with 10 mM-mercuric chloride, the amount of sugar that entered the cells by passive diffusion at these concentrations was found to be negligible (results not shown). Cells were grown for 3 days in 6-well Costar plates (35 mm $\times$ 15 mm) to yield a final cell density of around 3×10^4 cells/well. Medium was aspirated and each well was washed with 8 ml of PBS. A 900 μ l portion of uptake buffer (PBS containing 1 mg of bovine serum albumin/ml) was added to each well. Transport studies were carried out at 23 °C, and were initiated by the addition of 100 μ l of radioactive substrate to give the desired final concentration. At appropriate times, uptake was terminated by washing the cells rapidly (for less than 15 s) twice with ice-cold PBS or PBS supplemented with 10 mM-mercuric chloride. In all cases, 1 min uptake assays were performed and samples were taken at 15, 30, 45 and 60 s after the addition of the radioactive substrate. Cells were solubilized with 1 ml of 0.1% Triton X-100; 0.8 ml aliquots were counted for radioactivity in 10 ml of scintillation

fluid. Two wells on each plate were treated with trypsin and used to determine the average cell number/well. Zero-time controls and background counts were subtracted from the raw data. All transport studies were carried out in triplicate, and each experiment was repeated at least twice; Figures illustrate the results of representative experiments. Results were found to be very consistent in all cases.

Fate of dGlc after uptake

Transport studies were carried out as described above. Uptake was terminated 1 min after the addition of radioactive dGlc (60 μ M), by washing the cells twice with 8 ml of ice-cold PBS containing non-radioactive dGlc (1 mM). Washing with non-radioactive dGlc was essential for displacing the radioactive dGlc bound to the cell surface. The internalized dGlc was then released by treatment with 800 μ l of ethanol. The ethanol-soluble material from six wells was pooled and centrifuged to remove any particulate material. Phosphorylated and non-phosphorylated dGlc were then separated by h.p.l.c. using a Hewlett-Packard 1084B instrument equipped with a refractive index detector and a Partisil-10 SAX column (Whatman Inc., Clifton, NJ, U.S.A.). The ethanol extracts were evaporated under N_2 at room temperature and dissolved in 75 μ l of water containing unlabelled sugar analogues (100 μ mol). The compounds were eluted with 35 mM- KH_2PO_4 (pH 4.6) at a flow rate of 1.2 ml/min at 40 °C. Carrier sugars were detected by the refractive index detector, and column fractions (360 μ l) were collected for liquid scintillation counting. Under these conditions, the phosphorylated and non-phosphorylated sugars were well separated.

Assay for hexokinase activity

Hexokinase activity was assayed by a coupled enzyme assay using glucose-6-phosphate dehydrogenase and glucose [3,20]. To measure the kinetic properties of the type II isoenzyme of hexokinase, eight different concentrations (ranging from 0.1 mM to 5 mM) of D-glucose were used.

Materials

2-Deoxy-D-[G-³H]glucose (8.3 Ci/mmol), 3-O-[methyl-³H]-D-glucose (60–90 Ci/mmol), D-[6-³H]glucose (30.2 Ci/mmol) and D-[4,5-³H(n)]-galactose were purchased from New England Nuclear (Boston, MA, U.S.A.) and Amersham Canada Ltd. (Oakville, Ontario, Canada). Tissue culture media and antibiotics were from Gibco. All other chemicals were from Sigma Chemical Co. All chemicals were of the highest available purity from commercial sources.

RESULTS

Uptake of hexoses by human skin fibroblasts

dGlc has been used as the non-metabolizable hexose analogue in studying the kinetic properties of hexose transport systems in eukaryotic cells. In order to determine the general hexose transport properties in human skin fibroblasts, the initial rates of dGlc uptake by fibroblasts from different individuals were determined (Fig. 1). The skin fibroblasts used were isolated from a 'normal' human subject, a patient with symptoms of hypoglycaemia (FIB) and the patient's parents. The normal human subject used was an age- and sex-matched control for the patient. When the external dGlc con-

Fig. 1. Uptake of dGlc by normal and patient's fibroblasts

Uptake studies were performed as described in the Materials and methods section. The concentration of dGlc used was 0.06 mM, and the specific radioactivity of dGlc was 35.8 pmol/1000 d.p.m. $\square$, $\diamond$ and $\triangle$ denote the uptake by control fibroblasts (FIB) and fibroblasts from the patient's mother (MFIBC) and father (FIBC); $\bullet$ denotes uptake by fibroblasts (FIB) isolated from the patient.

centration was 60 μ M, both paternal (FFIBC) and maternal (MFIBC) fibroblasts took up dGlc at a rate of 19 pmol/min per 10^5 cells; this was similar to the rate by fibroblasts (FIBC) from an age-matched control boy (Fig. 1). Thus regardless of the age and sex of the subjects from which they were isolated, human skin fibroblasts exhibited similar dGlc transport activities. However, under identical growth and uptake conditions, the rate of dGlc uptake by the hypoglycaemic patient's fibroblasts was significantly higher; dGlc was taken up at a rate of 45 pmol/min per 10^5 cells. There was no detectable difference in the size and morphology of these cells (results not shown). The protein contents for both types of fibroblasts were also very similar, having a value of around 14.6 μ g of protein/ 10^5 cells.

In order to verify that the observed alteration was indeed associated with the hexose transport system, transport studies were carried out with 60 μ M-D-glucose and 60 μ M-D-galactose, the natural substrate of the hexose transport system. As shown in Fig. 2, D-glucose was taken up by FIB and FIBC at rates of 55 and 27 pmol/min per 10^5 cells respectively. D-Galactose has been shown to be an excellent natural substrate for studying hexose transport, as the phosphorylation of this sugar by galactokinase is not the rate-limiting step in the uptake process [4]. Whereas both FIBC and MFIBC exhibited similar rates of uptake for D-galactose (26 pmol/min per 10^5 cells), FIB transported this sugar at a much faster rate (51 pmol/min per 10^5 cells) (Fig. 2). Thus the above observations suggest that 'normal' human skin fibroblasts, regardless of the age and sex of the subjects from which they were isolated, exhibit similar rates of hexose transport. This study also reveals that fibroblasts from a patient with symptoms of hypoglycaemia show changes in the transport system responsible for the uptake of dGlc, D-glucose and D-galactose.

Kinetic properties of the human skin fibroblast hexose transport system

In order to define the properties of the human skin fibroblast hexose transport system, a detailed kinetic

Fig. 2. Uptake of D-glucose and D-galactose by normal and patient's fibroblasts

Uptake studies were performed as described in Fig. 1. The concentration of hexose used was 0.06 mM, and the specific radioactivities of D-glucose and D-galactose used were 34.2 and 36.5 pmol/1000 d.p.m., respectively. $\bullet$ and $\blacksquare$ denote uptake of D-glucose by FIB and FIBC respectively; $\circ$, $\square$ and $\triangle$ denote uptake of D-galactose by FIB, FIBC and MFIBC respectively.

Fig. 3. Kinetics of dGlc uptake by glucose-grown and glucose-starved fibroblasts

Transport studies were carried out as described in the text. The amounts of dGlc taken up at 23 $^{\circ}$ C in 15, 30, 45 and 60 s were determined, and the rates of uptake were then calculated. The velocity (v) is expressed as nmol of dGlc taken up/min per 10^5 cells. S refers to the concentrations of dGlc (in mmol/litre) used. An equal number of counts was added to each dGlc concentration. $\blacktriangle$, $\blacksquare$ and $\bullet$ denote the uptake by glucose-grown control fibroblasts (FIBMC and FIBC) and the patient's fibroblasts (FIB) respectively; $\circ$ and $\square$ denote the uptake by fructose-grown (glucose-starved) FIB and FIBC cells respectively.

analysis was carried out. The sugar concentrations used ranged from 0.02 mM to 0.3 mM, and the initial rates of uptake were determined. Fibroblasts from two different age-matched control boys (FIBC and FIBMC) were found to have similar K_m and V_{max} values (280 ± 30 μ M and 65.5 ± 2 pmol/min per 10^5 cells respectively) for dGlc uptake (Fig. 3). It is interesting to note that this is significantly different from values measured in human myoblasts [18]. Kinetic analysis of dGlc uptake by the patient's fibroblasts (FIB) revealed that the K_m value was around 135 ± 10 μ M, whereas the V_{max} value was

65.5 ± 2 pmol/min per 10^5 cells (or 4.42 ± 0.06 nmol/min per mg of protein) (Fig. 3). Thus the observed increase in dGlc transport activity in FIB was due to an alteration in transport affinity.

D-Galactose transport kinetics were also examined using various fibroblasts (Table 1). The apparent K_m values were 510 ± 50 and 250 ± 20 μ M for FIBC and FIB respectively; furthermore, both FIBC and FIB had a similar transport capacity (116 ± 16.5 pmol/min per 10^5 cells or 7.98 ± 1.13 nmol/min per mg of protein) for D-galactose. The above observation indicates that FIB are altered in their transport affinities for dGlc and D-galactose. It is interesting to note that the dGlc transport systems in rat myoblasts [3], hamster fibroblasts [4] and Chinese hamster fibroblasts [17] are also responsible for the uptake of D-galactose. Although competitive inhibition studies have to be done before one can conclude that these two sugars are transported by the same transporter, the above observation does suggest this possibility. It may be surmised from this study that the intrinsic activity of this transport system is altered in FIB.

Kinetic properties of MeGlc uptake

Another hexose analogue that has been used frequently in hexose transport studies is MeGlc. Studies with different cell types have suggested that this sugar analogue might be taken up preferentially by a hexose transport system distinct from the one primarily responsible for the uptake of dGlc and D-glucose [3,9,21]. The K_m and V_{max} values for MeGlc uptake by FIBC were found to be 250 ± 16 μ M and 148 ± 5 pmol/min per 10^5 cells respectively (Table 1). Thus the MeGlc transport capacity of 'normal' fibroblasts is significantly different

from that for dGlc uptake, whereas the transport affinities for dGlc and MeGlc are quite similar.

A more definitive indication of the difference between the dGlc and MeGlc transport systems comes from analysis of FIB. The FIB K_m and V_{max} values for MeGlc were found to be identical with those of FIBC (Table 1). Thus, unlike the dGlc transport system, the MeGlc transport system in FIB was not altered, indicating that MeGlc is not taken up by the altered dGlc transport system found in FIB. It is interesting to note that the MeGlc transport properties are also not altered in rat myoblast mutants defective only in the dGlc transport system [10]. This difference in dGlc and MeGlc transport properties in FIBC and FIB may be taken as an indication that two different hexose transport systems are present in human fibroblasts.

Response to glucose starvation

There is ample evidence that glucose starvation results in elevated hexose transport activities [4,6,9,15,19]. This increase is thought to be due to decreased degradation of the transporter or to increased levels of specific mRNA during glucose starvation [22,23]. In order to determine the glucose-starvation effect, cells were grown in the presence of fructose instead of glucose. It should be noted that dialysed fetal calf serum was used in the glucose-free medium. The dGlc transport systems in both FIBC and FIB were found to respond similarly to glucose starvation by increasing the transport capacity to around 171 ± 4 pmol/min per 10^5 cells (Fig. 3 and Table 1), which was about 2.7 times higher than that of the glucose-grown cells. Thus as in other cell types, glucose starvation resulted in an increase in the dGlc transport capacity in human skin fibroblasts, and this alteration was not accompanied by any alteration of transport affinity [10,15]. The above study also shows that FIB is not altered in the glucose-starvation-mediated regulatory mechanism of dGlc transport.

Response to specific hexose transport inhibitors

If the dGlc transport is indeed altered in its intrinsic property, then it may respond differently to various inhibitors. Cytochalasin B has been demonstrated to bind specifically and tightly to the hexose transporter [6,7,24–26]. It is thought to inhibit hexose transport by an allosteric mechanism [7]. Table 2 shows the effect of cytochalasin B on dGlc transport by fibroblasts. As in other cell types, dGlc uptake in both FIBC and MFIBC was significantly inhibited by 1 μ M-cytochalasin B (Table 2). On the other hand, dGlc uptake by FIB was actually activated by the same concentration of cytochalasin B; instead of the 50 % inhibition observed with the parental fibroblasts, a 30 % activation was observed. This activation by cytochalasin B could be explained by changes in the intrinsic properties of the FIB dGlc transporter, such that a cytochalasin B-mediated allosteric change resulted in activation, instead of inhibition, of the transporter. Alternatively, this increased transport activity may be due to inhibition of efflux by unphosphorylated dGlc (as will be shown later on) from the patient's fibroblasts. If this were the case, then cytochalasin B is not likely to have much effect on the influx process. Again, this implies that the FIB dGlc transporter is altered in its response to cytochalasin B.

Table 1. Kinetic properties of hexose transport systems in fibroblasts from normal controls and a hypoglycaemic patient

The initial rates of dGlc, D-galactose and MeGlc transport were determined as described in the legend to Fig. 3. The K_m and V_{max} values of these transport processes were determined by Lineweaver–Burk plots as illustrated in Fig. 3. The results are expressed as the means $\pm$ S.E.M. of at least two trials.

Substrate and cells	K_m (μ M)	V_{max} (pmol/min per 10^5 cells)
1. dGlc		
Glucose-grown cells:		
FIBC	280 ± 30	66 ± 2
FIBMC	280 ± 30	66 ± 2
FIB	135 ± 10	66 ± 2
Glucose-starved cells:		
FIBC	280 ± 30	171 ± 4
FIB	135 ± 10	171 ± 4
2. D-Galactose		
Glucose-grown cells:		
FIBC	510 ± 50	116 ± 17
FIB	250 ± 20	116 ± 17
3. MeGlc		
Glucose-grown cells:		
FIBC	250 ± 16	148 ± 5
FIB	250 ± 16	148 ± 5

Table 2. Effect of cytochalasin B on the uptake of dGlc

Transport studies were carried out as described in the text. The concentration of dGlc used was 0.06 mM, and the specific radioactivity was 35.8 pmol/1000 d.p.m. The control fibroblasts were those isolated from the father (FFIBC) and the mother (MFIBC) of the patient. FIB denotes the fibroblasts isolated from this patient. The results are expressed as the means $\pm$ S.E.M. of at least two trials.

Fibroblasts	dGlc uptake			
	Control		+ Cytochalasin B (1 μ M)	
	(pmol/min per 10 ⁵ cells)	(%)	(pmol/min per 10 ⁵ cells)	(%)
Father (FFIBC)	19.15 $\pm$ 0.45	100	11.0 $\pm$ 1.0	57 $\pm$ 4
Mother (MFIBC)	17.55 $\pm$ 0.85	89	7.4 $\pm$ 0.6	42 $\pm$ 1
Patient (FIB)	33.25 $\pm$ 1.25	184	43.5 $\pm$ 1.5	228 $\pm$ 3

Response to energy uncouplers

Both plasma-membrane-vesicle and whole-cell studies have indicated that dGlc is taken up by an active transport process into rat myoblasts [3,5,27]. In order to determine whether this is also the case with human skin fibroblasts, cells were treated with the energy uncoupler CCCP (Fig. 4). Significant inhibition of dGlc transport by FIBC was observed at CCCP concentrations above 10 μ M. The extent of inhibition was similar to that observed with rat myoblasts [3]. Although more work is required, this preliminary finding nevertheless suggests that the uptake of dGlc by normal human fibroblasts may be dependent on membrane potential. In contrast with the above observation, the initial rates of uptake of dGlc by FIB were not affected by CCCP. Thus regardless of the mode of action of CCCP, the above observation again points to altered dGlc transport properties in the patient's fibroblasts.

Fate of internalized dGlc

The uptake of dGlc into cells is the result of both

Fig. 4. Effect of energy uncoupler on dGlc uptake by normal and patient's fibroblasts

Experiments were carried out as described in the legend to Fig. 1, except that cells were treated with different concentrations of CCCP. The concentration of dGlc was 0.06 mM, and the specific radioactivity of dGlc was 35.8 pmol/1000 d.p.m. 100% of dGlc uptake by FIB (●) and FIBC (■) fibroblasts was 38 and 17.9 pmol/min per 10⁵ cells respectively.

transport and phosphorylation by hexokinase. If substrate transport across the membrane is the rate-limiting step, the specific activity of hexokinase should exceed that of transport, and all the internalized sugar should be in the phosphorylated form; the negative charge of the sugar phosphate would prevent its passage across the membrane [3]. If phosphorylation is the rate-limiting step, then a significant amount of unphosphorylated sugar should be present intracellularly; the efflux of the unphosphorylated sugar should result in a reduced amount of internalized sugar upon prolonged incubation.

In the present investigation, eight different concentrations (ranging from 0.1 mM to 5 mM) of D-glucose and various normal fibroblasts were used to determine the kinetic properties of hexokinase in these cells. The K_m and V_{max} values of hexokinase were found to be $96 \pm 5 \mu$ M and 25.8 ± 2.8 nmol/min per mg of protein. Thus the specific radioactivity of hexokinase was significantly higher than that of the transport process. This suggests that transport is the rate-limiting step in the uptake process by the normal fibroblasts. An examination of the fate of dGlc by h.p.l.c. revealed that over 97% of the internalized dGlc in FIBC was in the phosphorylated form. These findings indicate that transport is the rate-limiting step in the uptake of dGlc in normal human skin fibroblasts.

The observed increase in dGlc transport affinity in the patient's fibroblasts raises the question of whether transport is still the rate-limiting step in the uptake process. The K_m and V_{max} values of hexokinase in FIB were found to be $143 \pm 8 \mu$ M and 52.1 ± 9.5 nmol/min per mg of protein respectively. The differences between patient and control K_m and V_{max} were significant ($P < 0.01$ and $P < 0.001$ respectively). Determination of the fate of the internalized dGlc in FIB revealed that only 58% of the internalized dGlc was phosphorylated in these fibroblasts. This suggests that the rate of influx of dGlc is faster than the rate of phosphorylation by hexokinase. It is plausible that the internalized dGlc may not be readily accessible to the hexokinase; compartmentation of the newly taken up D-glucose has been proposed [28].

In view of the finding that around 42% of the internalized dGlc in FIB remained unphosphorylated after 1 min of uptake, it is conceivable that efflux of the internalized dGlc may occur once the internal unphosphorylated dGlc concentration exceeds that of the external concentration. Since Fig. 4 suggests that the

patient's dGlc transport system may be energy-independent, one would predict that the amount of dGlc accumulated upon prolonged incubation should be lower in FIB. Indeed, this was found to be the case. The net amount of dGlc accumulated in FIB after 24 min of uptake was 40% lower than that in FIBC. The amounts present in FIBC and FIB at 24 min were 225 pmol/ 10^5 cells and 137 pmol/ 10^5 cells respectively. This is significantly different from their respective initial rates of uptake (the amount taken up within the first 60 s). This finding is in agreement with the notion that not all of the internalized dGlc is phosphorylated in the patient's fibroblasts.

DISCUSSION

The present investigation reveals several interesting properties of the hexose transport systems in human skin fibroblasts. Regardless of the age and sex of the subjects from which they were isolated, normal human skin fibroblasts exhibited similar rates of hexose transport. As with human and rat myoblasts [3,18], the transport of dGlc into normal human skin fibroblasts was the rate-limiting step in the uptake process; almost all of the internalized dGlc was phosphorylated immediately by hexokinase, and the specific activity of hexokinase was significantly higher than that of the transport process. Using dGlc concentrations ranging from 20 μ M to 300 μ M, the K_m and V_{max} values of dGlc transport in normal fibroblasts were 280 ± 30 μ M and 65.5 ± 2 pmol/min per 10^5 cells (or 4.42 ± 0.06 nmol/min per mg of protein) respectively (Fig. 3). These values differ significantly from those of human and rat myoblasts [3,18]. As with other cell types, glucose starvation was found to result in a 2.7-fold elevation in hexose transport capacity, with no apparent change in transport affinity. Furthermore, the dGlc transport system in normal fibroblasts was also very sensitive to the fungal metabolite cytochalasin B (Table 2). A very interesting and potentially important finding from the present study was that dGlc uptake in normal fibroblasts could be inhibited by the energy uncoupler CCCP. A similar observation was also made with the rat myoblast dGlc transport system [3]. Although more work is required to substantiate this conclusion, the above observation suggests that dGlc uptake in normal fibroblasts may be dependent on membrane potentials.

More information on the intrinsic properties of the fibroblast hexose transport system was obtained by comparing the hexose transport properties of the normal and the patient's fibroblasts. Data presented in this paper have indicated that the patient's fibroblasts (FIB) differed from their normal counterparts in their uptake rates for dGlc, D-glucose and D-galactose, and that these increased rates were due to changes in the transport affinity. Since similar changes are observed in the uptake of these three sugars, it seems likely that they may be taken up by the same hexose transport system. If this were the case, then the substrate specificity of this fibroblast hexose transport system is similar to that of rat myoblasts [3].

Changes in the intrinsic activity of the transporter are usually reflected by alterations in transport affinity, substrate specificity and/or the response to various reagents. There is increasing evidence that the intrinsic properties of the hexose transporter can be modulated in an allosteric manner [7,29–33]. In view of the changes in

transport affinity, and its altered response to cytochalasin B and CCCP, it seems likely that the FIB dGlc transporter may be altered in its intrinsic activity. Changes in transport activity can also be brought about by altering the number of functional transporters [34]. The findings that the FIB dGlc transport system has a similar transport capacity and response to glucose starvation suggest that the number of dGlc transporters and the rate of translocation across the membrane are not likely to be affected in these cells. It may therefore be surmised that the genetic alteration in the patient's fibroblasts may affect, directly or indirectly, the intrinsic properties of the dGlc transporter. Fig. 1 shows that the hexose transport properties of fibroblasts from the patient's mother and father are similar to those of normal subjects. Thus even though the genetic alteration in this patient has not been identified, the gene responsible for the altered dGlc transport properties is likely to be recessive, as it is not expressed in either parent.

Table 1 shows that the kinetics of MeGlc uptake are essentially identical in both the normal and the patient's fibroblasts. This is quite different from results with the dGlc transport system. It is therefore conceivable that the dGlc and MeGlc transport systems are coded or regulated by different genes, and that the one responsible for dGlc uptake is altered in the patient's fibroblasts. It is interesting to note that dGlc and MeGlc have also been demonstrated to be taken up by different transport systems into rat myoblasts [3], hamster fibroblasts [4], chick embryo fibroblasts [9] and human fibroblasts [21]. The presence of two hexose transport systems in rat myoblasts was substantiated by the isolation of mutants altered only in the dGlc transport system, but not in the MeGlc transport system [10].

In conclusion, the present investigation is unique in that both normal fibroblasts and a naturally occurring genetic variant (as opposed to mutants isolated through mutagenesis) are used in the characterization of the hexose transport systems in human skin fibroblasts. Studies on the transport kinetics and the regulation of the hexose transport systems present in these two types of fibroblasts revealed similar, though not identical, hexose transport properties to those found in other eukaryotic cells. The genetic variant is found to be essential in defining the number of transport systems present, and in our understanding of the mechanism and regulation of the transport process.

This investigation was supported by operating grants from the Medical Research Council of Canada to T.C.Y.L. Technical assistance by A. N. LeCouteur was also appreciated.

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Received 5 June 1989/15 September 1989; accepted 21 September 1989