Biochimica et Biophysica Acta 812 (1985) 98–106
Elsevier

BBA 72415

A kinetic analysis of hexose transport in cultured human lymphocytes (IM-9)

William D. Rees * and Jørgen Gliemann **

Institute of Physiology, University of Aarhus, DK-8000 Aarhus C (Denmark)

(Received August 14th, 1984)

Key words: Hexose transport; Methylglucose; Kinetic asymmetry; (Human lymphocyte)

3-*O*-Methyl-D-glucose transport across the plasma membrane of cultured human lymphocytes of the IM-9 line was followed for net entry into sugar-free cells (zero trans entry), net exit into sugar-free medium (zero trans exit) and for equilibration of labelled sugar in cells with the same sugar concentration in the intracellular water as in the medium (equilibrium exchange). The measurements were performed at 37°C (pH 7.4). Equilibrium exchange of 1 mM 3-*O*-methylglucose ($t_{1/2}$ about 7 s) was exponential, suggesting a homogeneous cell suspension. Initial rates of transport showed a Michaelis-Menten dependency on the sugar concentration. The transport system was found to be asymmetric with the following kinetic parameters. Zero trans entry: K_m = 2.8 mM, V_{max} = 10.7 mM · min^{-1}. Zero trans exit: K_m = 9.5 mM, V_{max} = 37.9 mM · min^{-1}. Equilibrium exchange: K_m = 9.9 mM, V_{max} = 44.0 mM · min^{-1}. Finally, the affinity constant for the internal site was measured as approx. 1.2 mM using the infinite cis protocol.

Introduction

The transport of hexoses by mammalian cells is mediated by a facilitated diffusion process. The kinetic parameters of the transport system have been studied in a number of cell types (for review, Ref. 1). Rat adipocytes [2,3] and rat hepatocytes [4] have been reported to show symmetrical transport parameters at 37°C, i.e., the K_m and V_{max} is the same regardless of the direction of the sugar transport or the sugar concentration on the opposite side of the membrane. The transport system of human adipocytes also appears to exhibit kinetic symmetry [5].

The human erythrocyte has been studied extensively, mainly at room temperature, and the transport system is kinetically asymmetric under these conditions. The zero trans entry, i.e., transport into initially sugar-free cells, is characterized by a low K_m and low V_{max} [6–8] whereas the zero trans exit is characterized by a high K_m and high V_{max} [9,10]. When the affinity of the inside site is measured in the presence of sugar at a high concentration on the outside of the cell (infinite cis entry), a low K_m site becomes apparent on the inside [10–13]. Equilibrium exchange of hexose is characterized by K_m and V_{max} values even higher than those obtained in zero trans exit experiments [14]. These results have led to the conclusion that a simple carrier model is unable to explain the kinetics of human erythrocyte hexose transport, and several kinetic models have been proposed. However, it has recently been reported that changes in the properties of the human erythrocyte transport system occur on storage [15] or upon ATP depletion [16]. In addition, Brahm [17] studied the erythrocyte transport system at 38°C using techniques suited to measure rapid efflux. The K_m values for zero trans exit and equilibrium exchange were essentially equal at that temperature.

* Present address: Department of Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.
** To whom correspondence should be addressed.

At 25°C K_m for equilibrium exchange was slightly higher than that for zero trans efflux, but both constants were markedly lower than previously reported values [17].

These findings raised the question of whether or not kinetic asymmetry is expressed at 37°C in some cell types and, if so, in which way. The half-time for transport of glucose at a tracer concentration is about 0.4 s in human erythrocytes at 37°C [17]. This makes it technically difficult to apply a full kinetic analysis, which requires both exit and entry protocols, to glucose transport in human erythrocytes. We have attempted to answer the question by studying transport of the non-metabolizable glucose analogue 3-*O*-methylglucose in human cultured lymphocytes of the IM-9 line, which turned out to be technically suitable for this purpose.

Materials and Methods

3-*O*-[^{14}C]Methyl-D-glucose (60 Ci/mol), [U-^{14}C]sucrose and ^3H_2O were from Amersham International. 3-*O*-Methyl-D-glucose was from Sigma. Salts (analytical grade) were from Merck. Dibutylphthalate (D 1.04) was from British Drug Houses. Phloretin was from K&K laboratories and was dissolved in ethanol/dimethyl sulphoxide (7 : 3, v/v) to a final concentration of 82.5 mg/ml (0.3 M). Stopping solution was prepared by slowly adding 1 vol. of phloretin stock solution to 1000 vol. 0.1 μM $HgCl_2$ in the Hepes buffer (20°C) described below.

The IM-9 lymphocytes were maintained in culture at 37°C by splitting 1 : 3 every 3rd day with RPMI-1640 supplemented with 10% newborn calf serum (GIBCO), 20 mM Hepes, 2 mM L-glutamine, $2.5 \cdot 10^4$ U/l mycostatin and 50 mg/l neomycin. The cells were used in the late exponential or early stationary phase. They were harvested by centrifugation and washed three times in a buffer composed of 140 mM Na^+, 4.7 mM K^+, 2.5 mM Ca^{2+}, 1.25 mM Mg^{2+}, 142 mM Cl^-, 2.5 mM $H_2PO_4^-/HPO_4^{2-}$, 1.25 mM SO_4^{2-} and 10 mM Hepes. The total osmolarity (π) was thus 304 mM. All transport experiments were carried out in this buffer at 37°C, pH 7.4. Packed cell volume was measured by sucking a concentrated cell suspension into haematocrit tubes followed by centrifugation at 10000 × *g* for 5 min. The diameter was measured on 200 cells using an ocular micrometer.

Entry experiments were performed by squirting 50 μl of a suspension containing $(2–5) \cdot 10^7$ cells/ml on to 15 μl of buffer containing 30 nCi ^{14}C-labelled methylglucose and unlabelled methylglucose as required. The incubations were carried out in 3.5 ml plastic roundbottom mini scintillation vials (Hansac Plastic, Hasselager, Denmark) at 37°C. They were terminated by the addition of 3 ml stopping solution. The timing was assisted by the use of a metronome for incubations up to 10 s. The cells were pelleted by centrifugation at full speed on a bench centrifuge (about 4000 × *g*) for 1 min. The supernatant was aspirated and the cells were resuspended in 3 ml stopping solution and centrifuged again. The supernatant was aspirated, 3 ml of scintillation fluid (Quickzint, Zinsser Analytic, Frankfurt) was added to the tube and the radioactivity was determined by scintillation counting. The methylglucose equilibrium space was determined by 15–30-min incubations, and blank values were determined by adding cells and stopping solution simultaneously to the isotope. In some experiments, aliquots of cell suspension were incubated for 30 min with [^{14}C]methylglucose, [^{14}C]sucrose or ^3H_2O and added without dilution to 500 μl microfuge tubes containing 100 μl dibutylphthalate followed by centrifugation [18]. The intracellular waterspace was determined as the ^3H_2O space minus the [^{14}C]sucrose space; the intracellular distribution space for methylglucose was determined as the [^{14}C]methylglucose space minus the [^{14}C]sucrose space.

Exit experiments were performed by loading cells $(4 \cdot 10^7$/ml) with labelled and unlabelled methylglucose for 15 min at 37°C. 15 μl of the suspension was then placed in a 3.5 ml mini scintillation vial containing a small piece of wire. The vials were placed in a water-bath at 37°C over a magnetic stirrer. Exit of sugar from the cells was initiated by the addition of 1.5 ml Hepes buffer and terminated by further addition of 1.5 ml of buffer containing 0.6 mM phloretin and 0.2 μM $HgCl_2$ (2 × stopping solution). The wire was removed and the cells were pelleted, washed with standard stopping solution and assayed for radioactivity as described for the entry experiments.

The equilibrium distribution spaces were determined by adding stopping solution directly to the loaded cells. Blank values were determined by allowing exit to continue for 30 min in the 1.5 ml buffer. By that time the content of radioactivity in the cells should have decreased almost by the dilution factor of 1 : 100. The measured values at 30 min showed a decrease in radioactivity to 1/80 of the value obtained at equilibrium.

Results

The mean lymphocyte diameter was 12 μm, with 90% of the cells ranging from 10 to 14 μm. If the lymphocytes are regarded as perfect spheres, the mean cell surface area will be about 450 μm^2 and the mean cell volume about 900 μm^3. In reasonable agreement with the latter number, 10^7 cells constituted a packed cell volume of 11–13 μl. The intracellular water space, determined as the ^3H_2O space minus the [^{14}C]sucrose space, was 7.1 ± 0.5 μl per 10^7 lymphocytes (mean value of four experiments $\pm$ 1 S.D.). The intracellular distribution space for [^{14}C]methylglucose was determined in parallel tubes as 7.0 ± 0.6 μl. Thus the sugar analogue was distributed in the entire intracellular water space. In these experiments the [^{14}C]sucrose space accounted for 20–25% of the [^{14}C]methylglucose space in the cell pellet. The intracellular distribution space for [^{14}C]methylglucose was also measured after incubation for 30 min using the methodology described for the transport experiments; that is, after dilution with the stopping solution. The value was 6.9 ± 0.3 μl per 10^7 cells (1 S.D., $n = 4$). This shows that the stopping solution was effective. Incubation for 5 min or 60 min did not change the calculated distribution space, indicating lack of conversion of the tracer to metabolites. In these experiments the [^{14}C]sucrose space accounted only for about 2% of the [^{14}C]methylglucose space in the pellet. Thus, the stopping solution served both to define the timing and to decrease the blank value arising from the extracellularly trapped isotope.

Fig. 1 shows the time-course for the equilibrium exchange of 1 mM 3-*O*-methyl-D-glucose following the protocol for entry experiments. If the cell population is homogeneous, then the exchange between the extracellular and intracellular compartment is exponential regardless of the kinetics of the transport system. The exponential uptake should follow the equation:

$$\frac{v}{S} = \ln\left(\frac{1}{1-f_i}\right) \cdot t^{-1} \qquad (1)$$

where v is the transport velocity, S is the substrate concentration (v/S is the rate constant of entry), f_i is the fractional filling of the intracellular [^{14}C]methylglucose space and t is the incubation time. As shown in the inset to Fig. 1, the uptake closely approximates an exponential process, suggesting simple two-compartment kinetics. In this connection, it should be noted that rat thymocyte suspensions consist of two distinct cell types. About one-third are 'active' cells which equilibrate methylglucose with a half-time of about 1 min and two-thirds are 'quiescent' with an equilibration half-time of 30–50 min [19,20]. Some compounds, such as arsenate (2 μM) and the Ca^{2+} ionophore A23187 (1.4 μM) convert the 'quiescent' thymocytes to 'active' ones [20]. We found no effect of

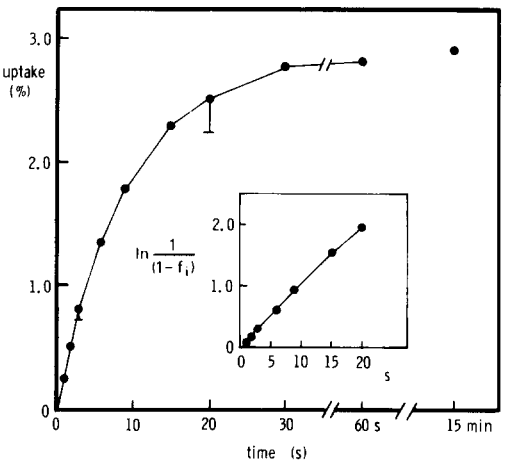

Fig. 1. Time-course of equilibrium exchange of 1 mM 3-*O*-methyl-D-glucose in IM-9 lymphocytes. The cells were preincubated with 1 mM sugar analogue for 30 min and entry of 1 mM 3-*O*-[^{14}C]methyl-D-glucose was measured. The ordinate shows the percentage uptake of the added tracer into $2.6 \cdot 10^6$ cells (final concentration $4 \cdot 10^7$ cells/ml suspension). The data are corrected for trapped isotope at time zero (cpm_0). The inset shows a logarithmic transformation with the fractional filling, f_i, calculated as $(cpm_t - cpm_0)/(cpm_\infty - cpm_0)$ where cpm_0, cpm_t and cpm_∞ indicate the radioactivity in the cell pellets at time zero, time t and 15 min. Each point is average of four replicates $\pm$ 1S.D.

these compounds on the equilibrium exchange of 1 mM methylglucose in human lymphocytes (data not shown). This further supports the idea of a homogeneous lymphocyte population.

The permeability was calculated from the data shown in Fig. 1 and similar experiments, as the rate constant of entry times the intracellular distribution volume for methylglucose in the cell sample divided by the total surface area of the cells. It was $(14 \pm 2.8) \cdot 10^{-6}$ cm · s^{-1} (mean of four experiments ± 1 S.D.). This is close to the maximal permeability, since K_m for transport is much higher than 1 mM. This permeability is of the same magnitude as that of insulin-treated rat adipocytes, as measured under comparable conditions [2] and it is one order of magnitude lower than the maximal permeability of the human erythrocyte [17].

The rate of methylglucose equilibrium exchange at different sugar concentrations was determined using Hanes' transformation of the Michaelis-Menten equation:

$$\frac{S}{v} = \frac{K_m}{V_{max}} + \frac{S}{V_{max}} \quad (2)$$

Fig. 2A shows the data combined from six experiments. The K_m for equilibrium exchange with entry of the labelled sugar analogue from compartment 1 to compartment 2 (K_{11}^{ee}) was calculated as 9.9 mM and V_{12}^{ee} as 44.0 mM · min^{-1}. However, the V_{12}^{ee} values determined on the individual cell batches varied in the range of 30–50 mM · min^{-1} and this complicates the analysis of the kinetic parameters. A similar situation has been reported previously for Novikoff cells [21] and for other cell lines [22]. Table I shows the mean values for K_m and V_{max} calculated from these six individual equilibrium exchange experiments.

The equilibrium exchange was also demonstrated to be approximately exponential when using the exit protocol (data not shown). The initial rate can therefore be determined using the equation:

$$\frac{v}{S} = \ln(1 - f_r) \cdot t^{-1} \quad (3)$$

where f_r is the fraction of sugar remaining in the cells. Fig. 2B shows an (S/v) vs. S plot of the

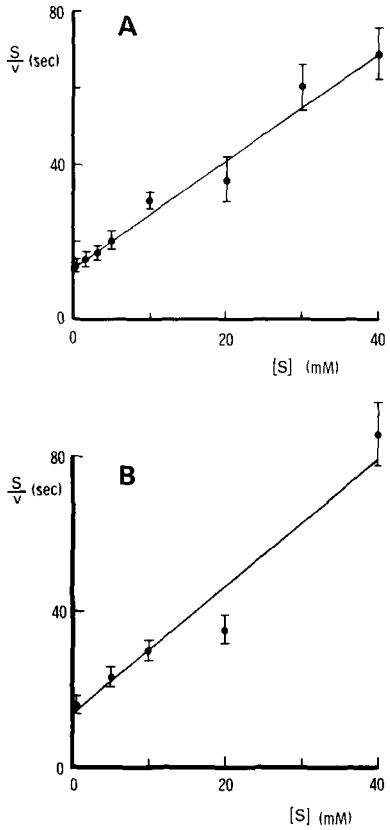

Fig. 2. (A) Concentration dependence of equilibrium exchange entry. The points represent the average of six experiments, each with three observations for every concentration. The incubation time was 1–15 s and the rates of exchange were calculated using Eqn. 1. The concentration dependence is plotted according to Eqn. 2. The error bars represent ± 1 S.E. and the line is drawn by linear regression. K^{ee} is calculated as 9.9 mM and V^{ee} as 44.0 mM · min^{-1}. (B) Concentration dependence of equilibrium exchange exit. Five duplicate observations were made over a time-course in two experiments and the exchange rates were calculated using Eqn. 3. The error bars represent ± S.E. and the line is drawn by linear regression. K^{ee} is calculated as 7.9 mM and V^{ee} as 35.7 mM · min^{-1}.

equilibrium exchange exit. K_{21}^{ee} was 7.9 mM and V_{21}^{ee} was 35.7 mM · min^{-1}. The kinetic constants for equilibrium exchange should theoretically be identical when using entry and exit protocols and the similarity of the measured values further supports the validity of the method.

The following additional experiments (not shown) were carried out using the equilibrium exchange entry protocol. The lymphocytes were split in two lots and incubated for 6 h in Hepes

TABLE I

KINETIC PARAMETERS FOR TRANSPORT OF 3-*O*-METHYL-D-GLUCOSE IN IM-9 LYMPHOCYTES

The values obtained by regression of the combined data for each protocol are summarized from Figs. 2–5. The mean values for n individual experiments are given ± 1 S.D.

Protocol	n	K_m (mM)		V_{max} (mM · min^{-1})	
		combined data	mean of individual experiments	combined data	mean of individual experiments
Equilibrium exchange	6	9.9	11.4 ± 3.7	44.0	41.6 ± 10.6
Zero trans entry	4	2.8	4.5 ± 1.0	10.7	13.2 ± 3.3
Zero trans exit	4	9.5	12.9 ± 3.3	37.9	43.4 ± 8.3
Infinite cis entry	3	1.2	1.5 ± 0.4	51.1	39.4 ± 22.4

buffer supplemented in 1% bovine serum albumin in the presence or absence of 1 mM D-glucose. The cells were then washed and transport measured. The (S/v) vs. S plots remained linear and K^{ee} and V^{ee} remained unchanged in the two situations. Thus, the cells remain stable after their removal from the culture medium. The IM-9 lymphocyte possesses a high concentration of insulin receptors on its surface, although internalization and degradation of insulin is minimal [23,24]. However, neither 1 μM insulin nor 5 μM adrenaline had any effect on methylglucose entry, suggesting that the transport system is not under hormonal control.

Zero trans entry experiments (entry into initially sugar-free cells) were performed in two ways, with the initial rates of uptake being estimated either directly from the initial portion of the progress curve or using the integrated rate procedure for calculation of the initial velocities as described by Eilam and Stein [25,1]. The former method was used when f_i did not exceed about 0.2 and the integrated rate procedure was applied to time-courses with f_i values up to about 0.5. According to this procedure t/C is plotted vs. $-[\ln(1 - C/S_o) + C/S_o]/C$, where t is the time of uptake, C is the internal substrate concentration and S_o is the external substrate concentration. These integrated rate plots were linear (data not shown) and the initial velocity was obtained as the reciprocal of the intercepts on the ordinate [25,1]. Fig. 3 shows that similar kinetic constants were obtained when both approaches were employed, yielding $K_{12}^{zt} = 2.8$ mM and $V_{12}^{zt} = 10.7$ mM · min^{-1}. Table I shows that similar results were obtained as the mean values of the inidvidual experiments. Thus, the values obtained in the zero trans entry experiments were about one-third of those obtained in the equilibrium exchange experiments.

Fig. 4A shows the time-courses for the zero trans exit at varying methylglucose concentrations. These data were analyzed by the integrated rate equation of Karlish et al. [9]:

$$\frac{-\ln\frac{S_t}{S_0}}{S_0 - S_t} = \frac{V_{21}^{zt}}{K_{21}^{zt}} \cdot \frac{t}{S_0 - S_t} - \frac{1}{K_{21}^{zt}} \qquad (4)$$

where S_o is the intracellular sugar concentration at time zero and S_t is the intracellular concentration at time t. Note that Eqn. 4 is equivalent to a form

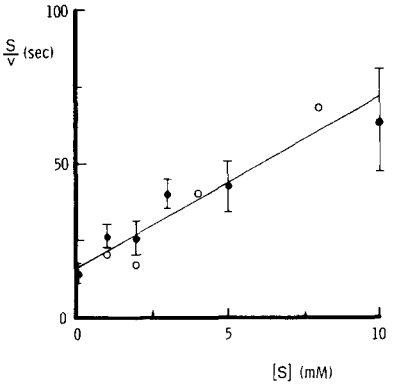

Fig. 3. Concentration dependence of zero trans entry. The initial rates were either derived from early uptake points with $f_i < 0.2$ (●, averages of two experiments, six observations at each concentration, error bars indicate ± 1 S.D.) or determined from 45-s time-courses as described in the text (○). The line is drawn by linear regression through all points. K_{12}^{zt} is calculated as 2.8 mM and V_{12}^{zt} as 10.7 mM · min^{-1}.

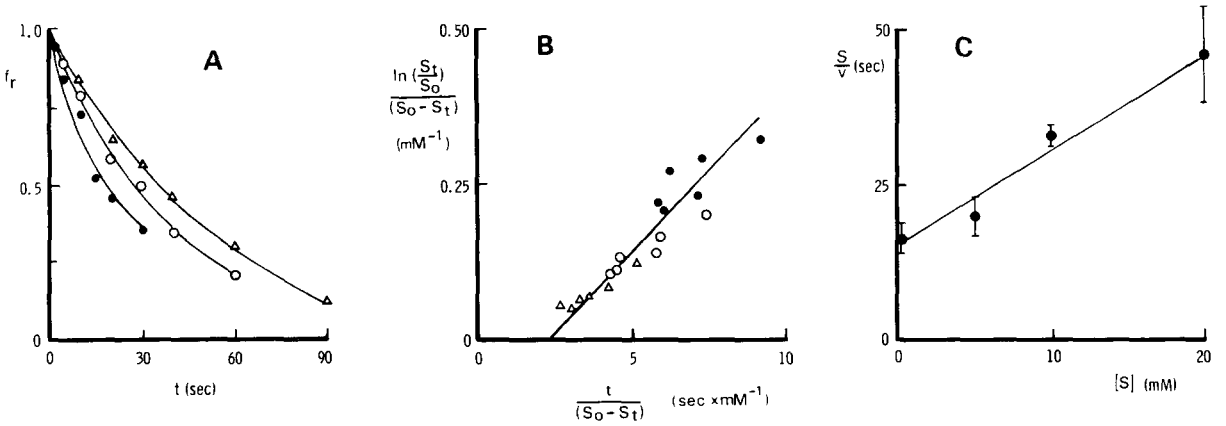

Fig. 4. (A) Time courses for the zero trans efflux of 5 mM (●), 10 mM (○) and 20 mM (△) 3-*O*-methyl-D-glucose. f_r was calculated as $(cpm_t - cmp_\infty)/(cpm_0 - cpm_\infty)$. The points represent the average of four experiments, each with duplicate observations at each time point. (B) Integrated rate replot of the same data using Eqn. 4. K_{21}^{zt} is calculated as 8.3 mM and V_{21}^{zt} as 26.2 mM·min^{-1}. (C) S/v vs. S plot of the concentration dependence of zero trans efflux. The initial rates were determined using Eqn. 4 as described in the text. The rates were determined for each of the separate experiments and then averaged. The error bars represent ± S.D. K_{21}^{zt} is calculated as 9.5 mM and V_{21}^{zt} as 37.9 mM.

of the Lineweaver-Burk transformation of the Michaelis-Menten equation:

$$\frac{1}{S} = \frac{V\max}{K_m} \cdot \frac{1}{v} - \frac{1}{K_m} \qquad (4a)$$

where v is the initial transport velocity. It can be seen from Fig. 4B that the points obtained from time-courses of different starting concentrations form a family of curves which tend to fall away from a straight line with increasing deviation at longer times. This may be due in part to the differences in the V_{max} between different cell batches. As differences in V_{max} are seen as a change in the slope of this plot, averaged progress curves can lead to a distorsion.

In order to overcome this difficulty, the initial rates of exit were calculated for each individual time-course. It can be seen from Eqns. 4 and 4a that when $[\ln(S_0/S_t)]/(S_0 - S_t)$ equals $1/S_0$ then $t/(S_0 - S_t)$ equals $1/v$ where v is the initial rate [10]. The values obtained for individual experiments were averaged and used to construct a Hanes plot of the zero trans exit data (Fig. 4C). The values for the 'tracer' concentration of methylglucose used in this plot were calculated using Eqn. 3, since at low concentrations, when S_i is well below K_m, the time-course for exit approximates to a simple exponential. This procedure yielded K_{21}^{zt} = 9.5 mM and $V_{21}^{vt} = 37.9$ mM · min^{-1} which is close to the values obtained from the equilibrium exchange experiments. Table I shows the mean values of the four individual experiments.

The infinite cis procedure provides an additional means of measuring the affinity constant for the internal site by following the concentration dependence of backflux during the uptake of a single high concentration of sugar. We have chosen a concentration of 40 mM as being well above the K_m observed for the zero trans entry experiment and Fig. 5A shows the time-course of uptake. The integrated rate equation of Eilam and Stein [25] can be applied to the infinite cis protocol so that a plot of t/C vs. $-[\ln(1 - C/S_0) - C/S_0]/C$ yields a straight line giving $1/V_{max}$ as the intercept on the ordinate. The intercept on the abscissa is equal to $-K_m/S_0^2(1 + S_0/\pi)$ where π is the effective osmotic concentration of the medium. Fig. 5B shows an infinite cis replot of the progress curve of Fig. 5A yielding $K_{12}^{ic} = 1.2$ mM and $V_{12}^{ic} = 51.1$ mM · min^{-1}. Table I shows the means of the values from the individual experiments. It is clear that the K_m of the inside site measured by this method is considerably lower than that measured in the zero trans exit experiments. However, the variations in V_{max} between different cell batches make it difficult to draw detailed conclusions from

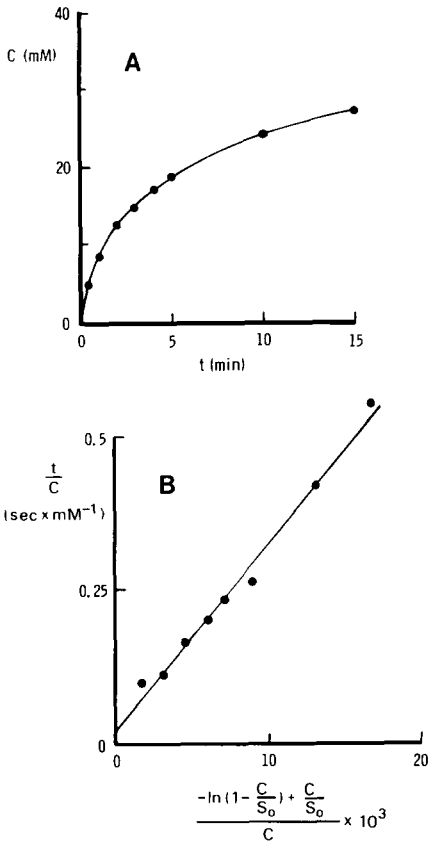

Fig. 5. Time-course of uptake of 40 mM 3-*O*-methyl-D-glucose (infinite cis experiment). The points represent the averages from three experiments with triplicate observations for each experiment. (A) Intracellular concentrations as a function of time. (B) Integrated rate replot; the line is drawn by linear regression yielding $K^{ic} = 1.2$ mM and $V^{ic} = 51.1$ mM·min^{-1}.

this type of experiment and it remains uncertain whether K_{12}^{ic} is the same as or lower than K_{12}^{zt}.

Discussion

This study shows that the hexose transport system of the cultured human lymphocyte is kinetically asymmetric at 37°C. The main features are as follows. The K_m and V_{max} values are equal for equilibrium exchange and zero trans exit of 3-*O*-methyl-D-glucose, about 10 mM and 40 mM·min^{-1}, respectively. The zero trans entry values are lower, about 3 mM and 12 mM·min^{-1}, Finally, K_m for the internal site (K_{12}^{ic}) is 1–2 mM. In contrast, the transport systems of rat adipocytes [2,3] and rat hepatocytes [4] have been reported to show symmetrical transport parameters at 37°C, with the K_m and V_{max} values being similar for all the protocols used [3,4]. However, it was recently reported [26] that the rat adipocyte is also asymmetric with respect to the values for equilibrium exchange and zero trans exit. This asymmetry is different to that reported for the human erythrocyte [17] and the data presented here for the human IM-9 lymphocyte in that both these cell types show apparent equality of the parameters for exchange and zero trans exit [27].

Plagemann et al. [22] have previously analyzed the 3-*O*-methyl-D-glucose in lymphocyte cell lines and other cultured cell types. These authors reported equal K_m and V_{max} values for zero trans entry and zero trans exit. On the other hand, K^{ee} and V^{ee} were about 6-times higher than the equivalent zero trans values. These data were interpreted in terms of a simple carrier with directional symmetry but with differing mobilities depending on whether or not the transporter is loaded with substrate. Such model is not compatible with our results. However, some differences in methodology should be noted. First, their studies were carried out at 25°C and ours at 37°C. Secondly, their characterization of the hexose transport relied upon the rapid centrifugation of the cells through an oil layer without employing a stopping solution to trap the sugar analogue within the cell [22]. Originally, we found that about 30 s are required to obtain a stable value of radioactivity in the cell pellet [18,2]. When using adipocytes, the amount of water taken into the oil layer is initially quite large, and this is gradually replaced by oil as the centrifugation proceeds [28]. Therefore, transport cannot be considered as terminated when centrifugation is started.

Whitesell, Regen and co-workers have studied the hexose transport system of rat thymocytes at 35°C. These cells are different from the present cultured human lymphocytes in that they comprise two distinct populations. About one-third of the cells are 'active' ($t_{1/2}$ for equilibration of 3-*O*-[^{14}C]methylglucose about 1 min) and two-thirds are 'quiescent' ($t_{1/2}$, 30–50 min). The Ca^{2+} ionophore A23187 and arsenate can, among several other compounds, convert 'quiescent' thymocytes to 'active' ones [20], but they had no effect on the

cultured human lymphocytes. The 'active' thymocyte shows a 3-times higher K_m for equilibrium exchange than for zero trans entry [29] and its hexose transport system may therefore be quite similar to that of the IM-9 lymphocyte.

The hexose transport system of the human erythrocyte has been studied intensively and asymmetric transport kinetics have been reported (for review, see Refs. 14, 27). Most experiments have been carried out at room temperature, probably because tracer glucose flux is very rapid at 37°C, with a half-time of about 0.4 s [17]. Different authors have reported markedly different kinetic constants even at 25°C, and particularly for zero trans efflux and equilibrium exchange. This is in part caused by previous technical difficulties in measuring initial velocities (for discussion, see Ref. 17), and in part to changes in the kinetic properties upon storage or ATP depletion of human red blood cells [15,16]. However, it still appears almost certain that the human erythrocyte is kinetically asymmetric at 25°C, since the K_m for zero trans exit (5.8 mM according to Ref. 17) is at least 3-times higher than the K_m for zero trans entry [30], since the K_m for equilibrium exchange (8.1 mM according to Ref. 17) is slightly but significantly higher than the K_m for zero trans exit, and since the K_m for infinite cis entry [11] is much lower than the K_m for equilibrium exchange.

It also seems likely that kinetic asymmetry is expressed in human erythrocytes at body temperature. Brahm [17] found similar K_m values for zero trans exit (8.2 mM) and equilibrium exchange (6.7 mM) at 37°C. On the other hand, Sen and Widdas [31] reported a K_m value of 4.0 mM for infinite cis exit. Other kinetic experiments have to our knowledge not been carried out in human erythrocytes at 37°C and particularly not zero trans entry experiments. Nevertheless, the present results with IM-9 lymphocytes and those previously obtained with human erythrocytes are compatible with the idea that the kinetics of hexose transport are similar in the two cell types.

A number of models have been proposed to account for the kinetics of hexose transport in the human erythrocyte [14,27]. The inequality of the kinetic parameters for zero trans entry and exit together with the infinite cis entry experiments are inconsistent with the hypothesis of a simple carrier with directional symmetry but differential mobilities of loaded and unloaded carrier [30,32]. The variation in V_{max} seen with all experimental protocols unfortunately prevents detailed parameter fitting to multiple site models which have been proposed [33–40].

In conclusion, we have demonstrated kinetic asymmetry of the sugar transport system in a human lymphocyte cell line at 37°C. This may be of the same kind as that demonstrated in human erythrocytes although technical difficulties have in part obscured the evaluation of the glucose transport system in these cells. It may be proposed, as a simplifying hypothesis, that the hexose transport system exists in two forms, at least at 37°C, one kinetically asymmetric as in human erythrocytes, rat thymocytes and perhaps several cultured cell lines, and one symmetrical as in rat adipocytes and rat hepatocytes.

Acknowledgements

Dr. O. Sonne is thanked for providing us with the IM-9 lymphocytes. This study was supported by the Nordic Insulin Foundation.

References

1 Gliemann, J. and Rees, W.D. (1983) in Current Topics in Membranes and Transport (Martin, R. and Kleinzeller, A., eds.), Vol. 18, pp. 339–379, Academic Press, New York
2 Whitesell, R.R. and Gliemann, J. (1979) J. Biol. Chem. 254, 5276–5283
3 Taylor, L.P. and Holman, G.D. (1981) Biochim. Biophys. Acta 642, 325–335
4 Craik, J.D. and Elliot, K.R.F. (1979) Biochem. J. 182, 503–508
5 Pedersen, O. and Gliemann, J. (1981) Diabetologia 20, 630–635
6 Geck, P. (1971) Biochim. Biophys. Acta 241, 462–472
7 Regen, D.A. and Tarpley, H.L. (1974) Biochim. Biophys. Acta 339, 218–233
8 Baker, G.F., Basketter, D.A. and Widdas, W.F. (1978) J. Physiol. (Lond.) 278, 377–388
9 Karlish, S.J.D., Lieb, W.R., Ravn, D. and Stein, W.D. (1972) Biochim. Biophys. Acta 255, 126–132
10 Baker, G.F. and Naftalin, R.S. (1979) Biochim. Biophys. Acta 550, 474–484
11 Hankin, B.L., Lieb, W.R. and Stein, W.D. (1972) Biochim. Biophys. Acta 288, 114–126
12 Ginsburg, H. and Stein, W.D. (1975) Biochim. Biophys. Acta 382, 353–368
13 Foster, D.M., Jaquez, J.A., Lieb, W.R. and Stein, W.D. (1979) Biochim. Biophys. Acta 555, 349–351

14 Naftalin, R.J. and Holman, G.D. (1977) in Membrane Transport in Red Cells (Ellory, C.J. and Lew, V.L., eds.), pp. 257–259, Academic Press, New York
15 Weisser, M.B., Razin, M. and Stein, W.D. (1983) Biochim. Biophys. Acta 727, 379–388
16 Jaquez, J.A. (1983) Biochim. Biophys. Acta 727, 367–378
17 Brahm, J. (1983) J. Physiol. 339, 339–354
18 Andreasen, P., Schaumburg, B., Østerlind, K., Vinten, J., Gammeltoft, S. and Gliemann, J. (1974) Anal. Biochem. 59, 110–116
19 Reves, J.P. (1977) J. Biol. Chem. 252, 4876–4881
20 Whitesell, R.R., Hoffman, L.H. and Regen, D.M. (1977) J. Biol. Chem. 252, 3533–3537
21 Graff, J.C., Wohlheuter, R.M. and Plagemann, P.G.W. (1978) J. Cell. Physiol. 96, 171–188
22 Plagemann, P.G.W., Wohlheuter, R.M., Graff, J.C., Erbe, J. and Wilkie, P. (1981) J. Biol. Chem. 256, 2835–2842
23 Sonne, O. and Gliemann, J. (1980) J. Biol. Chem. 255, 7449–7454
24 Christensen, E.I., Rennie, O. and Gliemann, J. (1982) Cell Biol. Int. Rep. 6, 757–765
25 Eilam, Y. and Stein, W.D. (1973) Methods Membrane Biol. 2, 283–354
26 Vinten, J. (1984) Biochim. Biophys. Acta 772, 244–250
27 Widdas, W.F. (1980) Curr. Top. Membranes Transp. 14, 165–223
28 Thorsteinsson, B., Gliemann, J. and Vinten, J. (1976) Biochim. Biophys. Acta 428, 223–227
29 Whitesell, R.R., Tarpley, H.L. and Regen, D.M. (1977) Arch. Biochem. Biophys. 181, 596–602
30 Lacko, L., Wittke, B. and Kromphardt, H. (1972) Eur. J. Biochem. 25, 447–454
31 Sen, A.K. and Widdas, W.F. (1962) J. Physiol. (London) 160, 392–402
32 Regen, D.M. and Morgan, H.F. (1964) Biochim. Biophys. Acta 79, 151–166
33 Lieb, W.R. and Stein, W.D. (1972) Biochim. Biophys. Acta 265, 187–207
34 Naftalin, R.J. (1970) Biochim. Biophys. Acta 211, 65–78
35 LeFevre, P.G. (1973) J. Membrane Biol. 11, 1–19
36 Lieb, W.R. and Stein, W.D. (1971) Curr. Top. Membranes Transp. 2, 1–39
37 Bowyer, F. and Widdas, F.W. (1956) Discuss. Faraday Soc. 21, 251–258
38 Foley, J.E., Foley, R. and Gliemann, J. (1980) J. Biol. Chem. 255, 9674–9677
39 Holman, G.D., Buzza, A.L., Pierce, E.J. and Rees, W.D. (1981) Biochim. Biophys. Acta 649, 603–514
40 Holman, G.D. (1980) Biochim. Biophys. Acta 599, 202–213