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SIDE-SPECIFIC ANALOGUES FOR THE RAT ADIPOCYTE SUGAR TRANSPORT SYSTEM

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(1) 4,6-*O*-Ethylidene-D-glucose is a good inhibitor of adipocyte sugar transport from the external surface. Using radioactively labelled 4,6-*O*-ethylidene-D-glucose we have shown that this compound is not taken up into cells by the hexose transporter but through a route which is insulin insensitive, D-glucose insensitive, temperature sensitive and which is slightly inhibited by phloretin. When efflux of 3-*O*-methyl-D-glucose is studied with 4,6-*O*-ethylidene-D-glucose only present inside the cells then no detectable inhibition is observed indicating that this compound is a good side-specific analogue with a high affinity for only the external site of the hexose transporter. (2) Radioactively labelled alkyl-β-D-glucosides have been prepared. These also penetrate the adipocyte membrane by an insulin and D-glucose insensitive route. The half-times for equilibration with methyl-, *n*-propyl-, and *n*-butyl-β-D-glucosides are 255, 9.45 and 3.3 min, respectively, indicating that the uptake rates are dependent upon the size of the alkyl group. (3) The glucosides show poor inhibition of 3-*O*-methyl-D-glucose transport when added to the external solution only. When cells are preincubated with *n*-propyl-β-D-glucoside and *n*-butyl-β-D-glucoside an increase in the amount of inhibition of 3-*O*-methyl-D-glucose uptake is observed. This increase in inhibition correlates well with the glucoside uptake rates and indicates that availability of the glucosides at the internal surface of the transporter is required for inhibition. This has been confirmed by measuring 3-*O*-methyl-D-glucose exit in the presence of *n*-propyl-β-D-glucoside at the internal surface only. Thus, *n*-propyl-β-D-glucoside is a good side-specific analogue with high affinity only for the internal site of the hexose transporter. (4) *n*-Propyl-β-D-glucoside inhibition of D-allose transport is fully reversible. If cells are washed after a preincubation with the analogue then the inhibition is lost. The *n*-propyl-β-D-glucoside inhibition of D-allose transport is reduced competitively by 3-*O*-methyl-D-glucose. (5) 6-*O*-Propyl-D-galactose has low affinity for both internal and external sites.

Introduction

Sugar transport in adipocytes is stimulated by more than 10-fold by insulin (Whitesell and Gliemann) [1]. For a transported sugar such as 3-*O*-methyl-D-glucose the system behaves symmetrically both in the presence and in the absence of insulin. For 3-*O*-methyl-D-glucose the internal and external affinity constants and V values are approximately equal in zero-*trans* and in infinite-*cis* experiments (Taylor and Holman) [2].

The symmetrical affinity constants measured for a transported sugar do not necessarily indicate an identical site at internal and external surfaces. For a transported sugar without sterically hindering alkyl groups the factors determining affinity will be mainly the positions of the hydrogen bonding groups throughout the transport channel. A sugar passing through the channel will interact with hydrogen bonding groups at both surfaces

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and may well show equal affinity constants for entry and exit. However, if a sugar analogue, because of sterically hindering alkyl groups, cannot fully enter the channel but can only bind at the pore gates then the observed affinity for such an analogue will reflect the spatial requirements of the pore gates which may be asymmetric.

In studying the affinity of alkyl-substituted sugars for the transport system we have found that the external site requires a specific orientation of the sugar such that C-1 faces inwards while C-4 projects into the external solution [3]. This conclusion was based on the observations that trehalose and the methyl-glucosides (with bulky groups at C-1) had low affinity for the external site but maltose and 4,6-*O*-ethylidene-D-glucose (with bulky groups at C-4) had high affinity. We also noted [3] that there was very little space around C-2 but much more around C-3 since 3-*O*-methyl-D-glucose had high affinity but 2,3-di-*O*-methyl-D-glucose had low affinity.

In the present study we have examined whether 4,6-*O*-ethylidene-D-glucose is transported by the glucose transporter and whether it also has a high affinity for the internal site. We also examine whether *n*-propyl-β-D-glucoside and *n*-butyl-β-D-glucoside are inhibitors of transport, whether they are transported, and whether they have selective affinity for the internal site only. The results indicate that the internal site has different spatial requirements to the external site. Analogues with a bulky C-1 group (*n*-propyl-β-D-glucoside and *n*-butyl-β-D-glucoside) are good inhibitors at the internal but not at the external site while 4,6-*O*-ethylidene-D-glucose is a poor internal but a good external inhibitor. Similar results have been previously observed in the human erythrocyte (Barnett et al. [4,5]).

We have previously shown [3] that in adipocytes the specificity requirements for basal and insulin-treated cells are indistinguishable and the present investigation thus only uses insulin-treated cells.

Materials and Methods

Crude collegenase (Type 1) was from Worthington Enzymes, bovine serum albumin (fraction V) was from Sigma. Porcine monocomponent insulin was a gift from Novo Laboratories. Silicone oil was from Hopkins and Williams and phloretin was from K and K Laboratories. 4,6-*O*-Ethylidene-D-glucose was from Koch-Light and 3-*O*-methyl-D-glucose and D-glucose were from Sigma. 3-*O*-[^{14}C]Methyl-D-glucose was prepared from [^{14}C]methyl iodide (The Radiochemical Centre, Amersham) as previously described [3], D-[3-^{3}H]allose was prepared as previously described [6].

Preparation of 4,6-O-ethylidene-D-[U-^{14}C]glucose

The method involved slight modification of the method of Barker and MacDonald [7].

18 mg of D-glucose and 100 μCi D-[U-^{14}C]glucose (The Radiochemical Centre, Amersham) were dried and then shaken for 30 min with 13.5 μl of paraldehyde and 0.1 μl of concentrated sulphuric acid. The solid mixture was left to stand for 3 days. The product was dissolved in ethanol, neutralised with ethanolic potassium hydroxide and then deionised with Amberlite (MB1) mixed bed ion-exchange resin. The product was then purified by preparative paper chromatography yield 16 mg, 80 μCi.

Preparation of glucosides

Methyl-β-D-[6-^{3}H]glucoside, *n*-propyl-β-D-[6-^{3}H]glucoside, *n*-butyl-β-D-[6-^{3}H]glucoside, *n*-propyl-β-D-glucoside and *n*-butyl-β-D-glucoside were all prepared by similar methods. The method is described for *n*-propyl-β-D-[6-^{3}H]glucoside. 2 g of methyl(2,3,4-tri-*O*-acetyl)-α-D-glucouronide bromide (Koch-Light) were dissolved in 15 ml of dry *n*-propanol and stirred with 2 g silver oxide overnight at 40°C. Silver salts were removed and the product was extracted into chloroform. The chloroform extract was washed with sodium thiosulphate solution and then dried over anhydrous sodium sulphate. The solvents were then removed and the product, methyl(2,3,4-tri-*O*-acetyl)-*n*-propyl-β-D-glucouronide was recrystallised from ethanol. Yield 2 g chromatographically pure. This product was used for the synthesis of radioactively labelled and nonlabelled *n*-propyl-β-D-glucoside. For the radioactively labelled preparation 33 mg of methyl(2,3,4-tri-*O*-acetyl)-*n*-propyl-β-D-glucouronide were dissolved in 2 ml of ethanol at 0°C. 8 mCi of sodium borotritide (The Radiochemical Centre, Amersham) were then added

slowly with continuous magnetic stirring. After 1 h 37 mg of sodium borohydride in 0.2 ml of water were added at 0°C. After a further hour the solution was allowed to warm to room temperature and was left overnight. After the addition of 2 ml of water the solution was neutralised with Dowex 50 H^+ resin and then deionised with Amberlite MB1 mixed bed resin. After removal of the ion exchange resins and solvents the product (*n*-propyl-β-D-glucoside) was purified by preparative paper chromatography. Yield 2 mCi (> 10 mCi/mmol). In the preparations of nonlabelled glucosides the sodium borotritide addition was omitted and 10-fold larger quantities were used. The reactions were followed by thin-layer chromatography and ^{1}H-NMR spectroscopy was used to confirm the introduction of the aglycone and the reduction and deacetylation by sodium borohydride.

Preparation of adipocytes

Isolated adipocytes were prepared from epididymal fat tissue from male Wistar rats weighing 150—170 g. The method was essentially that of Foley et al. [8]. Tissue, chopped with scissors was digested in Hepes buffer (pH 7.4 at 37°C) (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-}; 10 mM Hepes) containing 3.5% albumin, 0.5 mg/ml collagenase and 0.5 mM D-glucose. The digestion time was approx. 1 h. The digested tissue was filtered through a nylon mesh (mesh size 250 μm) and the isolated cells were carefully washed five times in Hepes 1% albumin buffer.

Transport measurements

Sugar influx measurements were carried out as previously described [2,6]. 50 μl of 40% cytocrit cell suspension, in some cases preincubated with inhibitor and nonlabelled substrate, were added to 15 μl of albumin-free buffer containing radiolabelled substrate and an appropriate concentration of inhibitor. Transport was terminated at the indicated times. In all cases transport was terminated by the addition of 3 ml of Hepes buffer containing 0.3 mM phloretin. The cells were then spun through a 1-ml layer of silicone oil in a bench centrifuge for 1 min at 2500 × *g*. The separated cells were removed from the top of the oil with a pipe cleaner. The trapped radioactivity was estimated by liquid scintillation counting. Tracer uptake rate constants (v/s) were calculated from the equation:

$$v/s = \ln\left(\frac{1}{(1-f)}\right)/t \qquad (1)$$

where f is the fractional filling and t is the time. Inhibition constants (K_i) were calculated from the equation:

$$v_0/v = 1 + (I/K_i)$$

where v is the inhibited rate constant and v_0 is the uninhibited rate constant and I is the inhibitor concentration.

To determine 3-*O*-methyl-D-glucose efflux rates cells were loaded with 40 μM 3-*O*-[^{14}C]methyl-D-glucose (with and without inhibitor) for 45 min. 50 μl of 50% cytocrit cell suspensions were then diluted in 5 ml of albumin free Hepes buffer. After 2 and 3 s 5 ml of 0.6 mM phloretin in Hepes buffer were added. Background radioactivity was estimated from samples in which efflux was allowed to proceed for 5 min. In efflux experiments the radioactivity present in the cells at time zero was estimated from samples incubated with 50 μM cytochalasin B for 30 s before the addition of 5 ml of albumin-free Hepes buffer containing 50 μM cytochalasin B. These samples were incubated for 2 and 3 s before the addition of 5 ml of phloretin solution. The cell associated zero-time radioactivity determined in this way is less than that determined by adding cells directly to 10 ml of phloretin solution. We do not know the reason for this difference but clearly the present procedure has the advantage that noninhibited samples and totally inhibited samples are diluted equally before the addition of phloretin. The difference may be related to a phloretin-dependent sealing of cell membrane invaginations or of a small subpopulation of broken cells that may built up over the lengthy loading time required for an efflux experiment. Thus dilution before phloretin addition allows loss of radioactivity nonspecifically associated with this pool.

To determine the K_m for zero-*trans* exit

(nomenclature according to Eilam and Stein [10]) Eqn. 2 was used. This is derived from the integrated zero-*trans* exit equation previously described [2,10]

$$V/K_m v = 1 + (S_{corr}/K_m)$$

where v is the efflux rate constant determined as in Eqn. 1 except that f is fractional loss. S_{corr} is $S(f/(\ln\ 1/(1-f)))$ where S is the initial 3-*O*-methyl-D-glucose concentration. Substituting the tracer (40 μM) 3-*O*-methyl-D-glucose efflux rate constant (v_0) for V/K_m gives

$$v_0/v = 1 + (S_{corr}/K_m) \qquad (2)$$

Results

Baker and Widdas [9] (using an osmotic swelling method) have shown that 4,6-*O*-ethylidene-D-glucose is not transported by the erythrocyte hexose transport system but enters the cell by an alternative route that is D-glucose insensitive. Fig. 1 shows the uptake of 20 mM 4,6-*O*-ethylidene-D-[^{14}C]glucose by a preparation of isolated adipocytes at 37 and at 16°C in the presence of insulin. At 37°C this compound equilibrates across the adipocyte plasma membrane in less than 15 min (the rate constant is 0.228 min^{-1}). The rate constant for uptake at 16°C is slower and equal to 0.021 min^{-1}. Fig. 1 also shows that uptake of 4,6-*O*-ethylidene-D-glucose is reduced by 0.3 mM phloretin (the rate constant is 0.124 min^{-1}).

We have previously reported [4,5] that *n*-propyl-β-D-glucoside also penetrates the erythrocyte plasma membrane independently of the hexose transport system. Fig. 2 shows the uptake of 5 mM *n*-butyl-β-D-[6-^{3}H]glucoside, 5 mM *n*-propyl-β-D-[6-^{3}H]glucoside and 5 mM methyl-β-D-[6-^{3}H]glucoside by adipocytes at 37°C. The 4,6-*O*-ethylidene-D-glucose, *n*-propyl-β-D-glucoside and *n*-butyl-β-D-glucoside space at equilibrium was identical to the space occupied by 3-*O*-methyl-D-glucose at equilibrium and equal to 1.8 μl/100 μl of packed cells. This value remained constant for at least 90 min for all four sugars. The methyl-β-D-glucoside did not reach equilibrium over the time course studied and the rate constant is calculated for equilibration with a hypothetical distribution space equal to that found for 3-*O*-methyl-D-glucose in the same experiment.

Table I summarises the rate constants for the uptake of 4,6-*O*-ethylidene-D-glucose and the alkyl

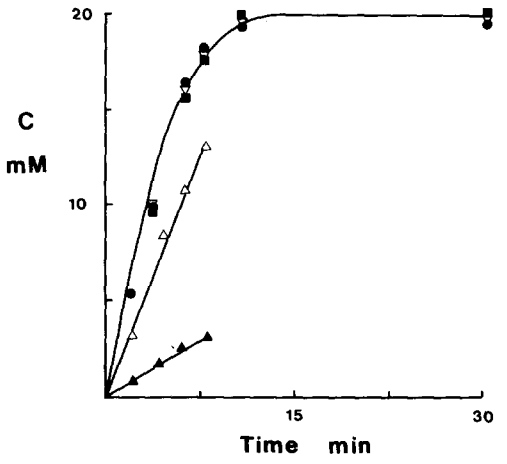


Fig. 1. The uptake of 4,6-*O*-ethylidene-[U-^{14}C]-D-glucose at 37°C in basal (▽) and adipocytes treated with 10 nM insulin (●) and insulin-treated cells in the presence of glucose (■). Also shown is 4,6-*O*-ethylidene-D-glucose uptake in the presence of 0.3 mM phloretin (△) and uptake at 16°C (▲). Two experiments with duplicate observations except phloretin and low temperature treatment which were single experiments in duplicate.

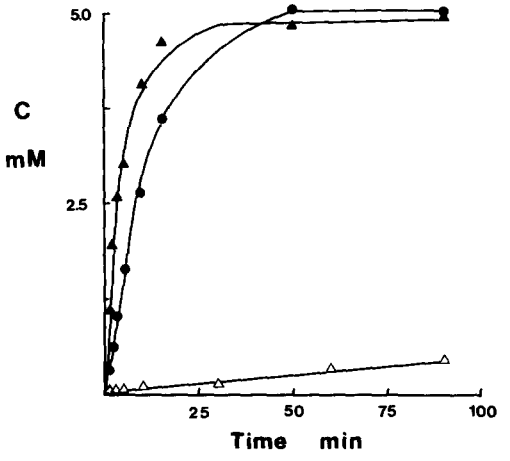


Fig. 2. The uptake of methyl-β-D-glucoside (△), *n*-propyl-β-D-glucoside (●) and *n*-butyl-β-D-glucoside (▲) in insulin-treated cells at 37°C. Single experiments with triplicate observations in each case.

TABLE I

UPTAKE OF GLUCOSE ANALOGUES

	Rate constant (min^{-1})		
	Basal cells	+ 10 nM insulin	+ 10 nM insulin + 50mM D-glucose
4,6-*O*-Ethylidene-D-glucose	0.221 ± 0.027 (n = 3)	0.222 ± 0.038 (n = 4)	0.202 ± 0.035 (n = 3)
Methyl-β-D-glucoside	–	0.0035 ± 0.0004 (n = 10)	–
n-Propyl-β-D-glucoside	0.061 ± 0.002 (n = 6)	0.076 ± 0.003 (n = 15)	0.061 ± 0.004 (n = 6)
n-Butyl-β-D-glucoside	0.202 ± 0.015 (n = 6)	0.238 ± 0.015 (n = 12)	0.197 ± 0.007 (n = 6)

-β-D-glucosides measured in the presence and absence of insulin and shows that uptake of these compounds is insensitive to insulin. Table I also shows that the uptake of these compounds is not inhibited by 50 mM D-glucose. Thus the alkyl sugars used here are transported by an insulin and D-glucose insensitive route. The uptake rates are considerably increased by increasing the size of the alkyl group and this is consistent with penetration through lipid regions of the membrane. The uptake rates increase (Table I) in the order methyl < propyl < butyl < ethylidene.

4,6-*O*-Ethylidene-D-glucose added to the external solution of a rat adipocyte suspension inhibits the exchange of D-allose with a $K_i = 6.11$ mM [3]. The K_i for the inhibition of 40 μM 3-*O*-methyl-D-glucose exchange by 4,6-*O*-ethylidene-D-glucose added to the external solution is 6.54 ± 0.56 mM ($n = 9$) and is thus similar to our previous estimate using D-allose as the substrate. The exchange uptake estimates for 3-*O*-methyl-D-glucose can be made rapidly ($t_{1/2} = 3.14$ s and uptake is measured at up to 3 s) so that any additional inhibition by 4,6-*O*-ethylidene-D-glucose as it reaches the internal solution of the adipocyte would be expected to reduce the K_i for 3-*O*-methyl-D-glucose exchange. Fig. 3 shows $1/K_i$ for 4,6-*O*-ethylidene-D-glucose remains constant for up to 90 min indicating that there is no increase in affinity for this compound as it reaches the inside site of the sugar transport system.

Also shown in Fig. 3 is $1/K_i$ for 6-*O*-propyl-D-galactose plotted against increasing incubation time. The K_i for 6-*O*-propyl-D-galactose is high and within experimental error is approximately constant over the course of the experiment.

Fig. 4 shows the inhibition of 40 μM 3-*O*-methyl-D-glucose exchange by *n*-propyl-β-D-glucoside and by *n*-butyl-β-D-glucoside with increasing preincubation time. As the glucosides enter the intracellular space the K_i for *n*-butyl-β-D-glucoside changes from 63.25 ± 8.85 mM ($n = 12$) to 10.98 ± 1.23 mM ($n = 6$) while the K_i for *n*-propyl-β-D-glucoside changes from 138.3 ± 47.5 mM ($n = 12$) to 13.4 ± 2.15 mM ($n = 6$) in the

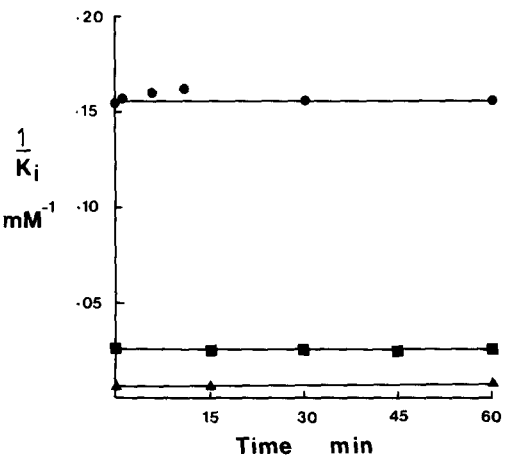


Fig. 3. A plot of $1/K_i$ against time of incubation with 20 mM inhibitor. ●, 4,6-*O*-ethylidene-D-glucose (■: 6-*O*-propyl-D-galactose; ▲, methyl-β-D-glucoside. Two or three experiments with triplicate observations in each case. Cells were insulin treated and at 37°C.

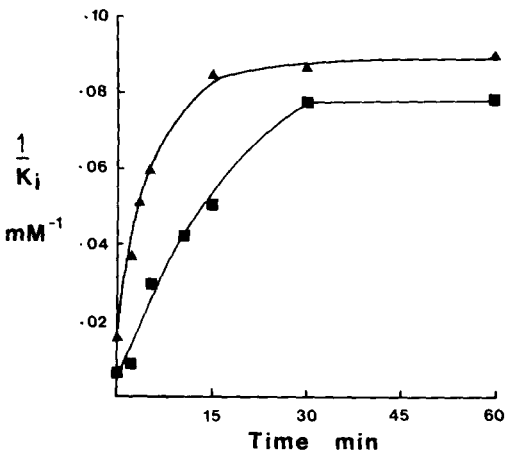


Fig. 4. A plot of $1/K_i$ against time of incubation with 20 mM inhibitor. ■, *n*-propyl-β-D-glucoside and ▲, *n*-butyl-β-D-glucoside. Two experiments with triplicate observations in each case. Cells were treated with insulin and at 37°C.

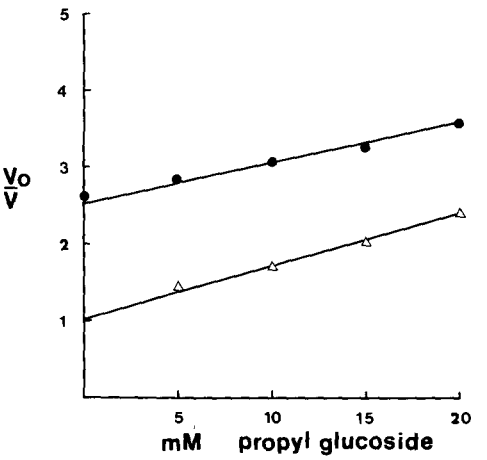


Fig. 5. Inhibition of 1.3 mM D-allose uptake in insulin-treated cells at 37°C by: △, *n*-propyl-β-D-glucoside ($K_i = 17.89 \pm 1.46$ ($n = 30$)) and ●, *n*-propyl-β-D-glucoside in the presence of 5 mM 3-*O*-methyl-D-glucose ($K_{i,app} = 50.68 \pm 6.26$ ($n = 15$)).

same period. There is no change in these final inhibition constants for longer preincubation times up to 90 min. Methyl-β-D-glucoside shows no change in K_i over a 90-min preincubation time which is consistent with the very slow pentration rate for this compound. Thus in each case the K_i closely follows the penetration rates for the glucosides indicating that C-1 alkyl-glucosides can bind to the inside site of the transport system. The rapid penetration of *n*-butyl-β-D-glucoside may account for the apparent external inhibition by this compound. When *n*-butyl-β-D-glucoside and radiolabelled 3-*O*-methyl-D-glucose are added together to the external solution sufficient inhibitor may have entered the cell during the assay to give detectable inhibition.

Fig. 5 shows the inhibition of 1.3 mM D-allose exchange by *n*-propyl-β-D-glucoside after a 30-min preincubation. This time is sufficient to equilibrate the inhibitor and substrate to equal concentrations on both sides of the membrane. The K_i for *n*-propyl-β-D-glucoside inhibition of D-allose exchange is 17.89 ± 1.46 mM ($n = 30$). In the same experiments cells were equilibrated with 20 mM *n*-propyl-β-D-glucoside for 30 min then washed twice over period of 30 min in 1% albumin-Hepes buffer at 37°C before the transport assay was performed. These cells showed no detectable inhibition of D-allose exchange indicating that *n*-propyl-β-D-glucoside inhibition of hexose transport is reversible. The inhibition of D-allose exchange by 20 mM ethanol and by 20 mM propan-1-ol after a 60-min preincubation is slight with the ethanol $K_i > 100$ mM and the propan-1-ol $K_i > 150$ mM. Also shown in Fig. 5 is the inhibition of D-allose exchange by *n*-propyl-β-D-glucoside in the presence of 5 mM 3-*O*-methyl-D-glucose. Both sugars were equilibrated across the cell membrane. 3-*O*-Methyl-D-glucose increases the K_i for *n*-propyl-β-D-glucose indicating that the glucoside is displaced from its binding site by 3-*O*-methyl-D-glucose.

In order to confirm that the alkyl-β-D-glucosides are inhibitors at the inner face of the transport system zero-*trans* exit experiments were performed (nomenclature according to Eilam and Stein [10]). Zero-*trans* exit was performed so that the inhibitors are only present at the inner face. Thus inhibitors acting at the outside binding site would not be expected to inhibit 3-*O*-methyl-D-glucose exit. Table II shows that K_i values calculated for inhibition of 40 μM 3-*O*-methyl-D-glucose exit at 37°C, 20 mM *n*-propyl-β-D-glucoside is an effective inhibitor whereas 20 mM 4,6-*O*-ethylidene-D-glucose and 6-*O*-propyl-D-galactose are not. The determined K_i values are subject to a large error due to technical difficulties involved in measuring exit but clearly show that the inner site accepts propyl-

TABLE II

INHIBITION OF 3-*O*-METHYL-D-GLUCOSE TRACER EXIT BY ALKYL SUGAR DERIVATIVES INSIDE THE CELL

	K_i (mM)	n
20 mM 3-*O*-methyl-D-glucose	6.42 ± 2.34	6
(corrected for efflux using Eqn. 2)	5.65 ± 2.05	6
20 mM 4,6-*O*-ethylidene-D-glucose	143.2 ± 48.9	12
20 mM *n*-propyl-β-D-glucoside	8.88 ± 1.64	11
20 mM 6-*O*-propyl-D-galactose	433.9 ± 87.9	12
Average tracer efflux ($t_{1/2}$ in s)	3.48 ± 0.42	12

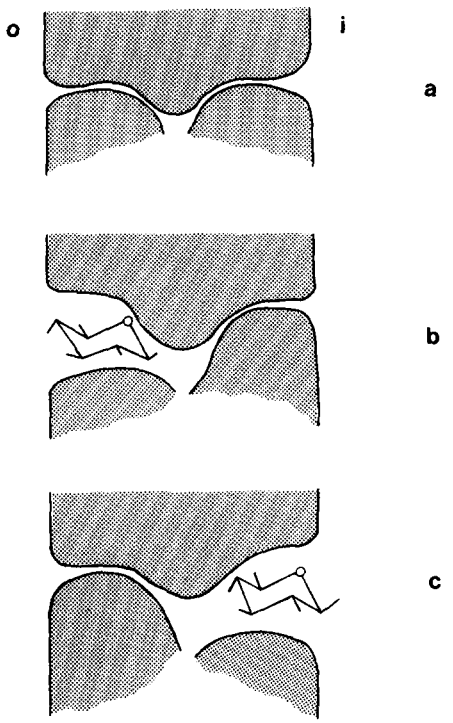
β-D-glucoside but not 4,6-*O*-ethylidene-D-glucose. The lack of detectable inhibtion in the zero-*trans* exit experiment by 6-*O*-propyl-D-galactose indicates that C-6 alkylated sugars may not bind well to the inside site. Taken with the 3-*O*-methyl-glucose exchange results this result is consistent with 6-*O*-propyl-D-galactose being a better external inhibitor than internal inhibitor but the K_i values for both sites are high and no great confidence in the K_i estimates for this compound can be claimed. 6-*O*-Propyl-D-galactose will thus have limited use as a side specific analogue but 4,6-*O*-ethylidene-D-glucose and *n*-propyl-β-D-glucoside are very side specific for external and for internal sites of the transporter, respectively.

The zero-*trans* exit K_m for 3-*O*-methyl-D-glucose is 5.65 ± 1.05 mM ($n = 6$) which is similar to our previously determined estimate using a slightly different procedure. Our previous estimate (K_m = 2.66 mM) was probably low because of an overestimate of the zero-efflux radioactivity. In our previous experiments we measured S (the internal concentration at $t = 0$) radioactivity by adding cells loaded with 3-*O*-methyl-D-glucose directly to the stopping solution. This does not allow loss by dilution of radioactive label nonspecifically associated with the cells. In the present experiments we have avoided this difficulty by measuring S in the presence of 50 μM cytochalasin B (Methods). The present estimate for the internal 3-*O*-methyl-D-glucose K_m confirms that the system interacts symmetrically with this transported analogue and that asymmetry is more evident when bulky substitutions are made in the sugar. This difference in asymmetry between transported and nontransported sugars is probably because the latter compounds only interact at the surface of the transport system and do not pass through it.

Discussion

The alkyl-substituted D-glucoses have been shown to inhibit the monosaccharide transport system of rat adipocytes but are not transported by this system. They are taken up into adipocytes at rates which are dependent upon the size of the lipophilic substitution. Thus the methyl β-D-glucoside uptake $t_{1/2} = 255$ min which is 27-times slower than the uptake $t_{1/2}$ of *n'*-propyl-β-D-glucoside ($t_{1/2} = 9.45$ min). The slow penetration rate of methyl-β-D-glucoside probably represents the lowest limit for a nonmediated uptake rate [11]. The uptake of the alkyl sugars is through a route which is insulin insensitive and which is noninhibitable by 50 mM D-glucose. The uptake for 4,6-*O*-ethylidene-D-glucose has been shown to be very temperature sensitive and to be slightly inhibited by 0.3 mM phloretin. The effect of phloretin may be related to a generalised reduction in membrane fluidity [13]. These results all suggest that the alkyl sugars enter the adipocyte via a nonmediated route through the membrane lipid.

The asymmetric inhibition shown by the alkyl sugars for the inner and outer sites of the adipocyte sugar transport system indicate that the binding sites in the transport protein are organised such that the orientation of the sugar at the external site is conserved at the inner site. Thus at both sites C-1 faces inwards while C-4 faces the external surface. (Scheme I). This means that the positions



Scheme I. The proposed structure of the transporter. (a) In the absence of the substrate the system is closed.(b) Binding to the external site destabilises the interface between subunits. Sufficient space is available to accomodate a bulky group at C-4. (c) Binding to the internal site opens the internal subunit interface. Sufficient space is available to accommodate a bulky group at C-1.

of least steric constraint to binding are C-4 at the outer site and C-1 at the inner site and these are the positions where a bulky substitution into the sugar can be tolerated. The poor inhibition by 6-*O*-propyl-D-galactose outside was unexpected as the human erythrocyte system has high affinity outside for this analogue [4,5]. This probably means that the rat adipocyte and human erythrocyte sugar transport systems are structurally slightly different at the C-6 bonding positions. Other minor differences between the two systems have also been attributed to a difference in the C-6 bonding position. The C-4 and C-6 positions seem to have less crucial hydrogen bonding requirements in the adipocyte system [6]. On the whole, the spatial requirements for the erythrocyte and adipocyte systems are very similar and both systems show the same side-specificity for *n*-propyl-β-D-glucoside and for 4,6-*O*-ethylidene-D-glucose.

Other sugar analogues have been tested for side-specificity in the erythrocyte system. 1,2-Isopropylidene-α-D-glucofuranose is not side specific [14,15,19] and this is probably because the compound only exists as a furanose ring while the site requires a pyranose ring [6,16,17]. Similar considerations also apply to the highly substituted (dialkyl) sugars used by Novack and LeFevre [18]. Introducing too many alkyl groups into the sugar may allow some nonspecific detergent-like interactions with the transporter.

All our evidence on the effects of the selectively substituted alkyl sugars suggests that they are side-specific, competitive, reversible inhibitors that may (because of their side specificity) be useful in further studies on mechanisms of sugar transport and for testing transporter orientation in membrane disrupted systems and golgi membrane fractions. If insulin action involves recruitment to the plasma membrane of golgi vesicles containing sugar transporter (Cushman and Wardzala [20] andSuzuki and Kono [21]) then it might be expected that the transporter orientation in the golgi vesicles would be such that the 4,6-*O*-ethylidene-D-glucose binding site is inside and the *n*-propyl-β-D-glucoside binding site is on the outer surface of the vesicles.

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