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# An Explanation of the Asymmetric Binding of Sugars to the Human Erythrocyte Sugar-Transport Systems

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6-*O*-Alkyl-D-galactoses competitively inhibit the erythrocyte sugar-transport system when added to the outside of the cells, but not to the inside. *n*-Propyl β-D-glucopyranoside competitively inhibits the system on the inside of the cells, but not on the outside. A model for sugar transport is proposed.

In recent years it has been shown that the apparent affinity of D-glucose for the human erythrocyte sugar-transport system varies with the method of measurement (Miller, 1968; Karlish *et al.*, 1972; Lieb & Stein, 1970).

Several models have been proposed to explain these observations, which do not fit a simple symmetrical carrier model. These include a tetramer with high-affinity and low-affinity sites (Lieb & Stein, 1970), models in which two (Le Fevre, 1973) or more (Naftalin, 1970) fixed sites transfer sugars within the membrane, and an asymmetric carrier system (Geck, 1971).

Baker & Widdas (1973*a*) have shown that the competitive inhibitor of sugar transport 4,6-*O*-ethylidene-D-glucose is not transported by the erythrocyte transport system, but penetrates the membrane by an alternative route. It has a high affinity for the transport system on the outside of the cell, but a weak or negligible affinity on the inside. In the present communication we report an extension of these observations on competitive, but non-transported, inhibitors that leads to a possible explanation of the asymmetry of the carrier system at the molecular level.

Using the inhibition of L-sorbose entry (Levine *et al.*, 1971), we had observed that 6-*O*-propyl-D-galactose, 6-*O*-pentyl-D-galactose and 6-*O*-benzyl-D-galactose are inhibitors of the sugar-transport system (J. E. G. Barnett, G. D. Holman & K. A. Munday, unpublished work). We have now established that they also fall into the class of non-transported competitive inhibitors that slowly penetrate the membrane by an alternative route to the sugar-transport system.

The L-sorbose penetration method of Levine *et al.* (1971) does not distinguish competitive from non-competitive inhibition, and we have adapted the 'net entry' method of Levine & Stein (1966) to measure the extent and nature of the inhibition. The rate of entry of D-[1-$^3$H]glucose was measured in the concentration range 2–20mM in the presence and in the absence of the inhibitor at 15°C. From Lineweaver–Burk reciprocal plots the D-galactose derivatives were shown to be competitive inhibitors, and the values of $K_i$ obtained are shown in Table 1.

Rates of penetration of 6-*O*-alkyl-D-[6-$^3$H]galactoses were measured in the presence and in the absence of 50mM-D-glucose at 25°C. The rates of penetration were low (Table 1), and uninhibited by glucose, showing that penetration was not by the sugar-transport system.

Because the rate of penetration of the 6-*O*-alkyl sugars is low relative to the time taken to measure the transport of glucose, it is possible, as with 4,6-*O*-ethylidene-D-glucose (Baker & Widdas, 1973*b*), to measure independently their ability to inhibit both entry and exit of sugars with the inhibitor effectively on either the inside or outside of the membrane.

The ability of the 6-*O*-alkylgalactoses to inhibit sugar entry was measured by the inhibition of either L-sorbose entry at 25°C (Levine *et al.*, 1971) or D-glucose entry at 15°C (Levine & Stein, 1966) (the $K_m$ for glucose entry was 3mM). For measurements with the inhibitors outside the cells, cells were added to a mixture of the penetrating sugar and inhibitor. For measurement with the inhibitor inside the cell, the cells were pre-equilibrated with the inhibitor for 2h at 25°C, the inhibitor outside the cells was removed by washing or dilution, and the preloaded cells were added to the penetrating sugar. Measurement of the inhibition of glucose exit was by an extension of the method of Karlish *et al.* (1972). Cells were preloaded with D-[1-$^3$H]glucose and inhibitor for 2h at 25°C or overnight at 0°C, and added to a large volume of exit medium containing buffered saline [290mM-NaCl and 20mM-$Na_2HPO_4$ adjusted to pH 7.4 with HCl, to which sufficient inositol (40mM) was added to maintain isosmolarity, when inhibitor or malonamide was inside the cell]; apparatus and solutions were maintained at 18°C. Malonamide, which has a similar penetration rate, was used in the controls to allow for the osmolarity of the inhibitors. Measurements of the rate of exit of D-glucose at 10s intervals over 70s were fitted to an integrated rate equation. At least four determinations were made for each

Table 1. *Inhibition of* D-*glucose or* L-*sorbose penetration of the human erythrocyte membrane*

| Sugar | 4,6-*O*-Ethylidene-glucose | 6-*O*-Propyl-galactose | 6-*O*-Pentyl-galactose | 6-*O*-Benzyl-galactose | Propyl β-D-glucopyranoside |
|---|---|---|---|---|---|
| Rate of penetration (10mM) (mmol·litre$^{-1}$·min$^{-1}$) | 0.41* | 0.11 | 0.66 | 0.40 | 0.11 |
| Rate of penetration with 50mM-glucose (mmol·litre$^{-1}$·min$^{-1}$) | 0.41* | 0.11 | 0.66 | 0.40 | 0.11 |
| $K_i$ for inhibition of sorbose entry (mM): | | | | | |
| Outside cell | 7 | 33 | 3 | 2.5 | ∞ |
| Inside cell | — | >200 | — | 11 | 18 |
| $K_i$ for inhibition of glucose entry (mM): | | | | | |
| Outside cell | — | 48 | 10 | 6 | ∞ |
| Inside cell | — | — | — | — | Inhibited† |
| $K_i$ for inhibition of glucose exit (mM): | | | | | |
| Outside cell | 25* | — | — | — | — |
| Inside cell | >200* | >200 | — | — | 20 |

* Data from Baker & Widdas (1973*a*,*b*). The penetration rate for 4,6-*O*-ethylidene-D-glucose is in units of min$^{-1}$. Values given for inhibition of glucose exit are those concentrations of 4,6-*O*-ethylidene-D-glucose that decrease the exchange of 20mM-glucose by 50%.

† The sugar inhibited significantly, but a meaningful $K_i$ cannot be calculated.

inhibitor and the means of the points were used for calculation of the inhibition constants. The $K_m$ for glucose exit was 28mM, both in the presence and in the absence of malonamide. Measurement of glucose exit into solutions containing the inhibitor outside were impracticable because of the large quantities of the inhibitors that would be required.

The results (Table 1) show that the 6-*O*-alkyl sugars are good inhibitors on the outside of the membrane, but poor or undetectable inhibitors on the inside of the membrane. Where some inhibition on the inside of the membrane is observed, as with 6-*O*-benzyl-D-galactose, this could be due to the presence of inhibitor at the outside surface, or there is some evidence that the sugar may interact with the sugar-transport system by a non-specific mechanism involving the benzyl group. It would appear that substitution by an alkyl group at C-6 or by a 4,6-*O*-ethylidene group allows binding to the transport system on the outside of the membrane, but not transport or binding on the inside of the membrane. We therefore decided to investigate the effect of an alkyl group on the C-1 position by the same methods.

It is well known that although 1-deoxy-D-glucose penetrates the membrane by the transport system and inhibits sorbose transport, methyl α- or β-glucopyranosides do not (see Barnett *et al.*, 1973). The methyl glucosides penetrate the membrane very slowly and are effectively present only on the outside of the cells. To test whether a compound of this type can inhibit the sugar-transport system on the inside of the cell we synthesized *n*-propyl β-D-[1-$^3$H]glucopyranoside. This penetrated the membrane by an alternative route to the sugar-transport system, and at the same rate as 6-*O*-propyl-D-galactose (Table 1). *n*-Propyl β-D-glucopyranoside was not an inhibitor of either sorbose or glucose entry when present on the outside of the cells. On the inside, however, it inhibited both sorbose and D-glucose entry, and also glucose exit. The experimental procedure does not allow a distinction between competitive and non-competitive inhibition of entry, because the inhibitor is on the inside of the cell and the substrate on the outside. However, inhibition of exit appeared to be competitive. The $K_m$ for exit was increased to 85mM in the presence of 40mM-propyl D-glucoside.

In summary, therefore, 6-*O*-propyl-D-galactose and the related derivatives are good competitive inhibitors of the sugar-transport system on the outside of the cell, but poor or negligible inhibitors on the inside, whereas *n*-propyl β-D-glucopyranoside is a competitive inhibitor on the inside of the cell, but not on the outside. This confirms the asymmetry of the transport system, and, as pointed out by Baker & Widdas (1973*b*), is inconsistent with the tetramer theory of Lieb & Stein (1970).

Sugars bind to the transport system as a whole by hydrogen bonds at C-1, C-3 and C-4 (Kahlenberg & Dolansky, 1972; Barnett *et al.*, 1973). When the sugar enters the system from the outside of the membrane,

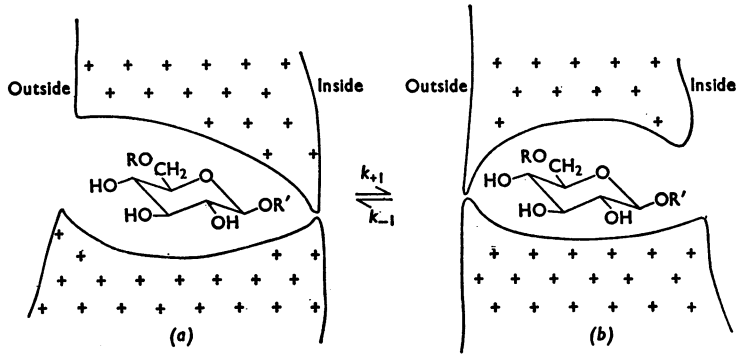


Fig. 1. *Model for glucose transport in the human erythrocyte*

If R = R′ = H, both conformations of the membrane are possible and transport occurs. If R = propyl and R′ = H, binding can occur only with conformation (*a*), on the outside, and transport is impossible. If R = H and R′ = propyl, binding can occur only with conformation (*b*), on the inside, and transport is again impossible. If the affinity of conformation (*b*) for the sugar is less than that of conformation (*a*), then $k_{-1}$ must be greater than $k_{+1}$ to maintain equilibrium with the same concentration of sugar on each side of the membrane.

it appears to do so with C-4 and C-6 towards the solution, so that bulky groups in these positions do not interfere with binding. However, when combining with the transport system from the inside of the membrane the converse is true and C-1 appears to be in contact with the solution.

A model of sugar transport that would explain these observations and the non-transportation of sugars with bulky groups in positions C-4, C-6 or C-1 is shown in Fig. 1. When entering the cell the sugar first binds to a site on the outside of the membrane. Then part of the membrane protein rearranges to a second stable configuration around the binding site, exposing the sugar to the inner solution. Rearrangement is prevented by a bulky group. This model would explain the observed apparent asymmetry of D-glucose binding (Baker & Widdas, 1973*b*) because the hydrogen bonds between sugar and protein may well be different in the two stable configurations, as might other non-bonding interactions. It resembles the allosteric model of Vidaver (1966) in its postulate of a conformational alteration of the transport barrier. However, the Vidaver (1966) model was considered in symmetrical terms and does not suggest a specific orientation of the substrate in the transport site.

Independent evidence for conformational changes in the membrane during transport is to be found in the complex 1-fluoro-2,4-dinitrobenzene inhibition of sugar transport (Krupka, 1971), one of the features of which is substrate-stimulated enhancement of the inactivation (Bowyer & Widdas, 1958). Edwards (1973) has now shown that glucose on the inside surface protects against 1-fluoro-2,4-dinitrobenzene inactivation, whereas glucose on the outside surface enhances it, and he has proposed that this can best be interpreted by changes between two conformational states of the transport system.

Although Fig. 1 shows a single membrane site, the results would be consistent with the multiple-site mechanisms, provided that the sites preserve the polarity and that at the external surface sugars combine with the sites in such a way that C-4 and C-6 are in contact with the outside solution whereas at the internal surface C-1 is in contact with the inside solution.

Baker, G. F. & Widdas, W. F. (1973*a*) *J. Physiol.* (*London*) **231**, 129–142
Baker, G. F. & Widdas, W. F. (1973*b*) *J. Physiol.* (*London*) **231**, 143–165
Barnett, J. E. G., Holman, G. D. & Munday, K. A. (1973) *Biochem. J.* **131**, 211–221
Bowyer, F. & Widdas, W. F. (1958) *J. Physiol.* (*London*) **141**, 219–232
Edwards, P. A. W. (1973) *Biochim. Biophys. Acta* **307**, 415–418
Geck, P. (1971) *Biochim. Biophys. Acta* **242**, 462–472
Kahlenberg, A. & Dolansky, D. (1972) *Can. J. Biochem.* **50**, 638–643
Karlish, S. D. J., Lieb, W. R., Ram, D. & Stein, W. D. (1972) *Biochim. Biophys. Acta* **255**, 126–132
Krupka, R. M. (1971) *Biochemistry* **10**, 1143–1148
Le Fevre, P. G. (1973) *J. Membrane Biol.* **11**, 1–19
Levine, M. & Stein, W. D. (1966) *Biochim. Biophys. Acta* **127**, 179–193
Levine, M., Levine, S. & Jones, M. N. (1971) *Biochim. Biophys. Acta* **225**, 291–300
Lieb, W. R. & Stein, W. D. (1970) *Biophys. J.* **10**, 585–609
Miller, D. M. (1968) *Biophys. J.* **8**, 1339–1352
Naftalin, R. J. (1970) *Biochim. Biophys. Acta* **211**, 65–78
Vidaver, G. A. (1966) *J. Theor. Biol.* **10**, 301–306