Biochimica et Biophysica Acta, 946 (1988) 431–438
Elsevier

BBA 70589

BBA Report

Pre-steady-state uptake of D-glucose by the human erythrocyte is inconsistent with a circulating carrier mechanism

R.J. Naftalin

Department of Physiology, King's College London, London (U.K.)

(Received 22 March 1988)

Key words: Carrier transport kinetics; Glucose; Erythrocyte; Carbohydrate transport

Simulation shows that the four-state mobile carrier model for sugar transport, in which the asymmetry arises from unequal rate constants of inward and outward translation of the free-carrier and carrier-sugar complex, does not fit with the observed data for pre-steady-state uptake recently obtained by A.G. Lowe and A.R. Walmsley ((1987) Biochim. Biophys. Acta 903, 547–550). The main reason for this discrepancy is that pre-steady-state fluxes are determined mainly by the dissociation constants K_s of glucose and maltose for the external sites, rather than the K_m (zero-trans$_{oi}$) of glucose and the K_i of maltose. The data are also inconsistent with other forms of asymmetric carrier but are fairly consistent with a symmetrical carrier with high-affinity sites for D-glucose or with a fixed site carrier model.

Introduction. Pre-steady-state uptake of ^{14}C-labelled D-glucose into human red cells at 0°C has been shown by Lowe and Walmsley [1] to be transiently increased following preincubation with the non-transported competitive inhibitor of glucose transport, D-maltose prior to exposure to D-glucose. This was demonstrated using a method for rapid mixing, quenching and separating red cell suspensions.

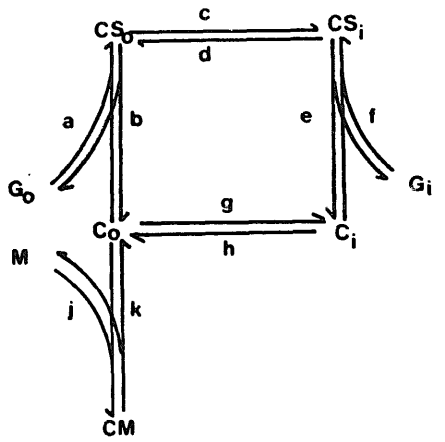
Preincubation of red cell suspensions with 150 mM maltose prior to dilution of the maltose to 7.56 mM and simultaneous exposure to 0.0947 mM ^{14}C-labelled D-glucose showed an enhancement of D-glucose uptake of approximately 1 μmol/l cells above that into cells which had not been exposed to maltose. The rate of sugar uptake falls to the unenhanced steady-state rate within 40 ms.

Correspondence: R.J. Naftalin, Department of Physiology, King's College London, Strand, London WC2R 2LS, U.K.

These data are important as they indicate that conformation changes within the sugar transporter are associated with transient changes in transport. Also, they give valuable new information into the relaxation processes within the transporter which can be used to test the consistency of transport models.

Lowe and Walmsley [1] claim that the observed pre-steady-state transients fit with their steady-state carrier model of sugar transport [2]. This four-state carrier model proposed that the asymmetry of the transport system is mainly due to inequality of the unidirectional rates of translation of loaded and unloaded carrier across the membrane. The dissociation constants K_s at the outside and inside (b/a and e/f, respectively, see Scheme I) are nearly symmetrical at 0°C, $K_{s(out)} \approx 10$ mM and $K_{s(out)}/K_{s(in)} = 0.74$.

The following equation derived by Deves and Krupka [3] on the basis of the four-state mobile carrier model shows the relationship between the dissociation constant, K_s, and the inhibition con-



Scheme I. The network of rate processes between states in the conventional mobile carrier model of glucose transport across the red cell membrane.

stant, K_i, of a nontransported inhibitor acting exclusively at external sites, e.g. maltose, which may be incorporated into the model.

$$K_i = K_s(1 + g/h) \quad (1)$$

Thus when the the ratio $g/h = 16.7$ [1,2] and $K_i \approx 13$ mM [1,2,4] it follows that K_s for maltose $= 0.734$ mM.

Although the K_s adopted for maltose is obtained from the $K_i = 13$ mM of maltose-dependent inhibition of glucose uptake into red cells, this is based on data obtained at a higher temperature than 0°C [5]. Fortuitously the K_i of maltose-dependent inhibition of D-glucose influx has been found to be independent of temperature in the range 0–20°C (Holman, G.D., private communication).

In Fig. 1, the computed time-dependent changes in percentage probability of occurrence of each of the five possible carrier states (maltose-carrier complex is an extra state) and also the net uptake of sugar per litre cell are shown. The rate constants assigned for loaded and unloaded carrier movement across the membrane and dissociation constants for D-glucose binding are the those derived by Lowe and Walmsley [1,2]. The K_s for maltose is derived from Eqn. 1 on the basis of a $K_i = 13$ mM.

The net rate of glucose transport between inside sites and intracellular water is calculated on the assumption of 7 μmol carrier per litre cells [1,2]. During the initial 10 ms the carrier system is at equilibrium with 150 mM maltose in the exter-

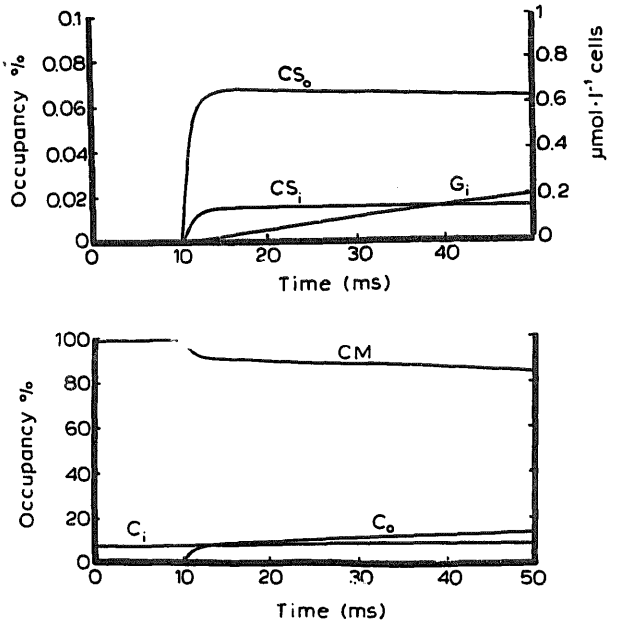


Fig. 1. Simulation of the time-dependent changes in percentage occurrence of each of the five possible membrane states. The simulations where carried out using fourth order Runge-Kutta numerical integration of the following six simultaneous differential equations:

$$d[C_o]/dt = [CS_o]\cdot b + [C_i]\cdot h + [C_m]\cdot k - [C_o]([M]\cdot j + [G_o]\cdot a + g) \quad (1)$$

$$d[CS_o]/dt = [C_o]\cdot a\cdot[G_o] + [CS_i]\cdot d - [CS_o](b + c) \quad (2)$$

$$d[CS_i]/dt = [CS_o]\cdot c + [G_i]\cdot f\cdot[C_o] - [CS_i](d + e) \quad (3)$$

$$d[C_i]/dt = [CS_i]\cdot e + [C_o]\cdot g - [C_i]([G_i]\cdot f + h) \quad (4)$$

$$d[C_m]/dt = [C_o]\cdot[M]\cdot j - [C_m]\cdot k \quad (5)$$

$$d[G_i]/dt = [T]([CS_i]\cdot e - [G_i]\cdot f\cdot[C_i]) \quad (6)$$

The values for the rate constants are the same as those assigned by Lowe and Walmsley [1,2] i.e. $a = 500\ s^{-1}\cdot mmol^{-1}$; $b = 5000\ s^{-1}$; $c = 1113\ s^{-1}$; $d = 90\ s^{-1}$; $e = 5000\ s^{-1}$; $f = 742\ s^{-1}\cdot mmol^{-1}$; $g = 12\ s^{-1}$; $h = 0.72\ s^{-1}$; $j = 140\ s^{-1}\cdot mmol^{-1}$; $k = 103\ s^{-1}$; [T] = 0.007 mM (where [T] is the concentration of all carrier forms mmol/l cells). $[G_o]$, $[G_i]$ and [M] refer to the concentrations of glucose in the external and internal solutions and maltose in the external solution, respectively. The step length of each interation was 50 μs. From time 0 to 10 ms [M] = 150 mM and $[G_o] = [G_i] = 0$ at time > 10 ms [M] = 7.56 mM and $[G_o] = 0.0947$ mM.

nal solution. After 10 ms a step change in the content of the external solution is simulated, so that the external solution contains 7.56 mM maltose and 0.0947 mM D-glucose.

The following differences between the observed data and model predictions are seen (Fig. 1).

(a) There is no evidence of a rapid initial increase in D-glucose uptake followed by a sharp decrease in uptake at 30–40 ms, instead after a short delay there is an almost linear rate of sugar accumulation.

(b) Total sugar uptake at 40 ms is 0.2 μmol/l cells (i.e. less than 10% of that observed experimentally [1]).

The following analysis explains why the data of Lowe and Walmsley [1] are not in accord with the predictions of their four-state carrier model [2].

In transitional pre-steady-state conditions the mobile forms of carrier are not in steady-state 'equilibrium' with the carrier forms on the other side of the membrane. Steady-state distribution of carrier will only be reached when transport itself is at steady state. At steady state the distribution of carrier states within the membrane determines the steady-state kinetic parameters; i.e. the K_i for maltose and the appropriate K_m values for net and exchange transport of D-glucose. During the pre-steady-state transient, providing there is rapid equilibration of ligands with the carriers on the external membrane surface, the distribution of free and bound ligand will be determined by the dissociation constants of ligands K_s for the adjacent carriers.

Thus, in the pre-steady state condition sugar fluxes will be determined by: (a) the carrier distribution resulting from the initial conditions and (b) the K_s (not the $K_{m(zero\text{-}trans\ oi)}$ or K_i) of each ligand for the carrier on the adjacent membrane surface.

Maltose has access only to carrier on the external surface. Thus, when the sites on the external surface are in near equilibrium with ligands in the adjacent solution:

$$[M]\cdot j\cdot C_o = [CM]\cdot k$$

where j and k are the rates of association and dissociation of maltose (M) with external free carrier (C_o) and carrier-maltose complex (CM), respectively (see Scheme I) and $k/j = K_s$ is the dissociation constant of maltose. Thus

$$\frac{[M]}{K_s} = \frac{[CM]}{C_o}$$

However, the equilibrium distribution ratio of free carriers facing inwards and outwards is undisturbed by maltose. This ratio can be derived as follows:

$$C_o\cdot g = C_i\cdot h$$

Where g and h are the rate constants of inward and outward movement of free carrier C_o, C_i (see Scheme I);
Thus according to the model of Lowe and Walmsley [1,2]

$$\frac{g}{h} = \frac{C_i}{C_o} = 16.7$$

When [maltose] = 150 mM and $K_s = 0.734$ mM then;

$$\frac{[CM]}{C_o} = \frac{150}{0.734} = 204.3$$

The sum of all carrier forms is;

$$C_o + [CM] + C_i = 100\%$$

Hence

$$C_o\% = \frac{100\%}{1 + \frac{[CM]}{C_o} + \frac{C_i}{C_o}} = \frac{100\%}{1 + 204.3 + 16.7} = 0.45\%$$

Also CM% = 92.02% and C_i% = 7.51%. These initial values are graphed in the time interval 0–10 ms in Fig. 1a.
In the absence of maltose C_o% = 5.64% and C_i% = 94.34%.

Hence the percentage of extra carriers on the external surface induced by maltose is;
92.02% + 0.45% − 5.64% = 86.83% as correctly predicted [1].

However, the maximal percentage of the extra carriers involved in glucose transport in the pre-steady-state condition (assuming K_{so} for D-glucose is 10 mM and K_s for maltose is 0.734 mM and the concentrations of glucose and maltose in

the external solution immediately after mixing are 0.094 and 7.56 mM, respectively) is:

$$\frac{86.83\%}{1+(K_{soG}/[G_o])(1+[M]/K_{sM})}$$

$$=\frac{86.83\%}{1+(10/0.0947)(1+7.56/0.734)}=0.073\%$$

Lowe and Walmsley [1] erroneously calculated the proportion of extra carriers binding glucose on the basis of the steady-state affinity parameters K_{mG} = 0.145 mM and K_{sM} = 10 mM:

$$\frac{86.83\%}{1+(K_{mG}/[G_o])(1+[M]/K_{iM})}$$

$$=\frac{86.83\%}{1+(0.145/0.0947)(1+7.56/13)}=25.38\%$$

Because there are 340-fold fewer external sites binding glucose (CS_o) than previously predicted [1] there is no rapid decay in the fraction of sites binding glucose following the initial uptake, as there is a large excess pool of empty carrier C_o on the external surface which prevents the depletion of CS_o.

The dominant process reducing glucose influx after maltose dissociates is the slow inflow of free carrier, C_o from the external surface liberated after maltose dissociation. The rate of this process is determined mainly by the rate constant, g.

The absence of correspondence between observed data and the model predictions are sufficient grounds to reject this version of the mobile carrier model.

Before attempting to improve the fit by further testing of other models, it is worth considering whether the pre-steady-state data of Lowe and Walmsley [1] are reliable. The data of greatest interest are the maltose-dependent increase in D-glucose uptake in which stimulated uptake shows a rapid decrease.

The following points suggest its reliability:

(a) The concentration-dependence of the maltose effect on glucose uptake ($K_{1/2} \approx 25$ mM).

(b) The absence of any rapid component of uptake of D-glucose without maltose present indicates that the maltose-dependent acceleration is not due to some surface artefact.

The observed temperature-dependent effects on glucose uptake are more difficult to interpret as there must be considerable uncertainty regarding the precision of temperature regulation within the reaction chamber. Furthermore, interpretation of temperature jump effects in terms of any particular model is difficult, as all the rate constants are changed by temperature.

Nevertheless, these data must be regarded as provisional until they can be confirmed and extended by additional experiments with other kinds of initial conditions. In particular the possibility of contamination of the maltose solution with glucose must be eliminated, as unlabelled glucose in the preloading solutions could lead to uphill counterflow of labelled glucose into the cells during the initial period of exposure which could have a similar appearance to the apparent maltose dependent acceleration of pre-steady-state glucose uptake. Hence, no firm conclusions can be drawn with regard to the validity, or otherwise of the carrier model on the basis of this pre-steady-state data alone.

Alternative models. The following points can be made regarding improvement of the fit of carrier models to pre-steady-state uptake of glucose.

Maltose K_s. (a) The K_s for maltose must be greater than 0.73mM, predicted on the basis of the K_i = 13 mM for maltose-dependent inhibition of glucose uptake, otherwise reduction of the maltose concentration from 150 mM to 7.56 mM would have no significant effect on glucose uptake.

Eqn. 1 indicates that when $K_i \approx K_s$ for maltose then the ratio g/h must be $\leqslant 1$. This implies that the observed asymmetry of the glucose transporter does not arise from the asymmetry of movement of free carrier. (b) As the $K_{1/2}$ of maltose-dependent activation of D-glucose uptake is approx. 10–20 mM [1] the K_s of maltose must also be approx. 10–20 mM.

However, raising the K_s for maltose from 0.73 to 13 mM whilst retaining K_{so} of glucose at 10 mM, as Lowe and Walmsley [1] do, does not give any significant improvement in the fit of the predicted glucose inflow with the observed data, as the amount of carrier-sugar complex on the external surface, CS_o is still too small.

Glucose K_s. To simulate the observed rapid decay in pre-steady-state glucose inflow with the

mobile carrier model requires that at least half of the available binding sites on the external surface become liganded to glucose as maltose dissociates. It follows that the K_{so} of D-glucose must be considerably below 10 mM i.e. $\leqslant 1$ mM, otherwise 0.1 mM glucose will not bind to sufficient sites.

If the rates of ligand association with and dissociation from the carrier are not rate limiting then the following relationship used by Lowe and Walmsley [2] based on the analysis of steady state equations of the four-state carrier model derived by Lieb and Stein [5] can be used to obtain values for the unidirectional rates.

Thus:

$$K_m(\text{zero-trans}_{oi}) = K_{s(out)} \cdot \frac{(1+g/h)}{(1+c/h)} = 0.145 \text{ mM} \qquad (2)$$

Where $K_m(\text{zero-trans}_{oi})$ is the K_m for influx of D-glucose into cells containing zero sugar. If $K_{s(out)} \approx 10$ mM and $K_m(\text{zero-trans}_{oi}) = 0.145$ mM, then it follows from Eqn. 2 that $c > g$ and $c \gg h$, where c is the rate constant of movement of loaded carrier from outside to inside. The value chosen for c to give best fit to the steady-state data is $c = 1113\ s^{-1}$. This [1,2] predicts that the $t_{1/2}$ for equilibration of loaded carrier from outside to an inside is < 1 ms.

Eqn. 2 requires that if K_{so} for glucose dissociation is reduced whilst the asymmetric properties of the transport system are retained, i.e. the relative rates g and h are held constant and $K_m(\text{zero-trans}_{oi}) = 0.145$ mM, then the rate constant c must also be reduced. The required reduction in the rate c will reduce carrier-sugar complex influx to such an extent that sugar inflow becomes too slow to match the observed rates.

A good fit of the four-state model to the influx data can be obtained if the asymmetry constraint is abandoned and it is assumed instead that transport is via a symmetrical carrier (see Fig. 2a). It is possible to simulate the observed inflows, where K_{so} of D-glucose $\approx K_{m(\text{zero-trans entry})} = 0.05$ mM and K_s of maltose $= 13$ mM $= K_i/2$ for maltose-dependent inhibition of glucose uptake.

On the basis of this model simulation of the maltose-dependent increase in glucose uptake has a relaxation time of approx. 10–15 ms. This is due to the rapid depletion of CS_o as it diffuses across the membrane as was predicted for the asymmetric model [1]. However, even in the absence of maltose a rapid decrease in the initial rate of glucose uptake is predicted due to depletion of CS_o as it diffuses inwards (Fig. 2b). As no rapid decay in glucose uptake has been reported the absence of maltose [1] this suggests that the symmetrical form of the mobile carrier model is also inconsistent with the observed data.

A fixed site model with maltose-dependent activation of glucose transport. An important aspect of the observed maltose-dependent stimulation of pre-steady-state glucose uptake [1] is that it gives qualitative support to the concept of a mobile carrier model, as it suggests a maltose-dependent accumulation of carrier on the external surface (Fig. 2a). This is not a direct prediction of fixed site models.

However, as carrier models do not give a good

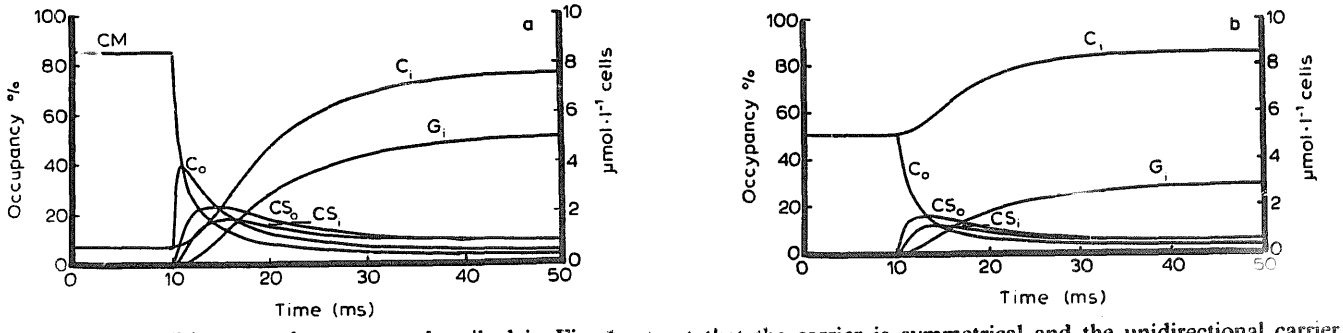
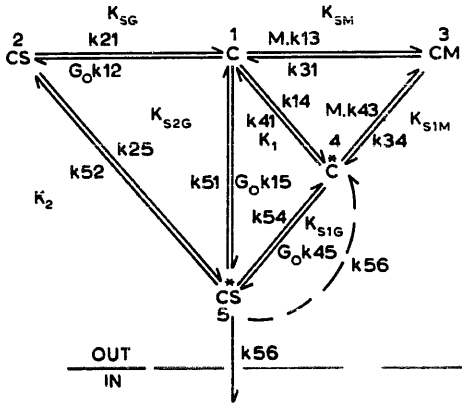


Fig. 2. The conditions are the same as described in Fig. 1 except that the carrier is symmetrical and the unidirectional carrier constants are $a = 6000\ s^{-1} \cdot mmol^{-1}$; $b = 300\ s^{-1}$; $c = 1000\ s^{-1}$; $d = 1000\ s^{-1}$; $e = 300\ s^{-1}$; $f = 6000\ s^{-1} \cdot mmol^{-1}$; $g = 2\ s^{-1}$; $h = 2\ s^{-1}$; $j = 100\ s^{-1}\ mmol^{-1}$; $k = 1300\ s^{-1}$; $T = 0.007$ mM. i.e. $K_{soG} = K_{siG} = 0.1$ mM and $K_{soM} = 13$ mM. In Fig. 2a from time 0 to 10 ms [M] = 150 mM and $[G_o] = [G_i] = 0$ at time > 10 ms [M] = 7.56 mM and $[G_o] = 0.0947$ mM. In Fig. 2b from time 0 to 10 ms and $[G_o] = [G_i] = 0$ at time > 10 ms $[G_o] = 0.0947$ mM.



Scheme II. The network of rate processes between the various states of the external site of the fixed site sugar transporter. Where C(1) is the inactive empty state; C*(4) is the activated empty state; CM(3) is the state in which maltose is bound; CS(2) is the inactive state in which glucose is bound and CS*(5) is the activated state in which glucose is bound and transport occurs. The equilibrium dissociation constants for the various states of the transporter are: $K_1 = k_{14}/k_{41}$; $K_2 = k_{25}/k_{52}$; $K_{sG} = k_{21}/k_{12}$; $K_{s1G} = k_{54}/k_{45}$; $K_{s2G} = k_{51}/k_{15}$; $K_{sM} = k_{31}/k_{13}$; $K_{s1M} = k_{34}/k_{43}$.

match to the data a fixed site model will be examined. The main advantage of fixed site models of glucose transport is that the parameters of exchange are independent of those determining net flux [6]. The observed asymmetric kinetic properties of the red cell transport system are explained on the basis of the testable hypothesis that glucose binding to the inner surface is modified by interaction with haemoglobin [6] or by ATP [7].

A possible model of maltose-dependent activation of glucose inflow with the fixed site transporter is as follows:

(a) The external binding site exists either in an inactive state, C or activated state, C* which equilibrate slowly. Transport across the membrane occurs only when sugar binds to the activated form, CS* and not when bound in the inactive form, CS (Scheme II).

(b) Following dissociation of maltose, the empty site passes to the activated state more rapidly than to the inactive state. Hence dissociation of maltose generates a transient increase in the proportion of activated sites, C* (Fig. 3a). This increase in number of activated sites will transiently increase the rate of glucose uptake (Fig. 3b).

However, without prior exposure to maltose, transport of sugar proceeds at a constant rate (Fig. 3c) as is observed at 0 °C.

At equilibrium the principle of microscopic reversibility requires that inflows and outflows at all nodal points in a network occur with equal frequency, thereby preventing cyclic equilibria [8]. This constraint requires that at equilibrium the following relationships in the network depicted in Scheme II must hold:

e.g. $K_{s1G}/K_{s2G} = K_{sG}/K_2$ and $K_{siM}/K_1 = K_{sM}$

$[C^*]/[C] = K_1$; $[C^*S]/[CS] = K_2$;

$[CS]/[C] = [G_o]/K_{sG} = [G_o]K_1/(K_{s1G} \cdot K_2)$;

$[C^*S]/[C^*] = [G_o]/K_{s1G}$; $[C^*S]/[C] = [G_o]K_1/K_{s1G}$;

$[CM]/[C] = [M]K_1/K_{s1M}$; $[CM]/[C^*] = [M]/K_{s1M}$.

Since in the fixed site model the total number of binding sites, T remains constant on each side of the membrane it follows that

$$[C] = T_o/(1 + [C^*]/[C] + [CS]/[C] + [C^*S]/[C] + [CM]/[C])$$

$$= T_o/(1 + K_1 + [G_o](1 + K_2)/K_{s1G} + [M]/K_{sM})$$

also

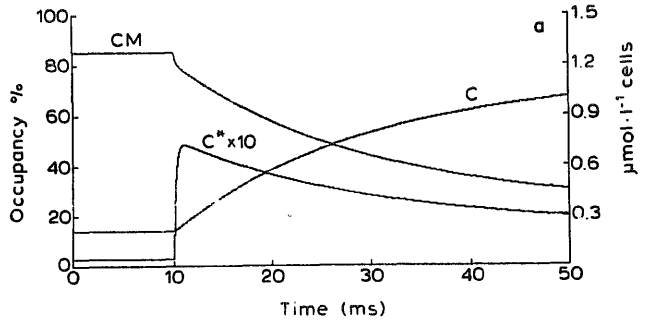
$$C^*S = T_o K_{sG}/(1 + K_2)/\{K_{s1G}(1 + K_1)/(1 + K_2) + [G_o] + K_{sG}[M]/K_{sM}(1 + K_2)\}$$

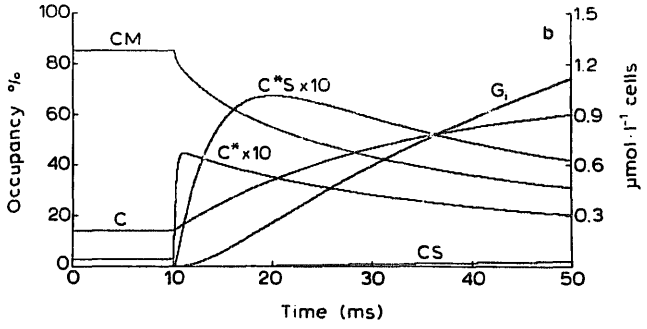
As the rate of zero-trans glucose influx is dependent on the proportion of transporter on the external surface in the state C*S, it follows that the K_i for maltose dependent inhibition of zero-trans glucose influx $= K_{sM}(1 + K_2)$ and the apparent K_m (for glucose uptake) $= K_{s1G}(1 + K_1)/(1 + K_2)$. This model can easily be adapted to describe the observed temperature jump effects on glucose uptake [1] if it is assumed that the equilibrium between the activated and inactive forms of carrier is shifted by temperature.

The possible role of non-transported sugars as partial activators has been previously discussed [9]. The fixed site model above could explain how the sugars like maltose and 6-*O*-pentylgalactose have lower K_i values for inhibition of low-affinity

sugar transport like sorbose than for inhibition of glucose transport [9,10].

It should be noted that the model describes only half of the transport system. Inflow of sugar across the membrane ($[CS^*] \cdot k_{56}$) is accompanied by a return of the transporter to the unliganded activated state C*. As the model describes only the zero-trans condition no return flow from the inside to outside is modelled. It is evident that more pre-steady-state data are required before any useful model of exchange and events occurring at the inner surface can be accurately defined.





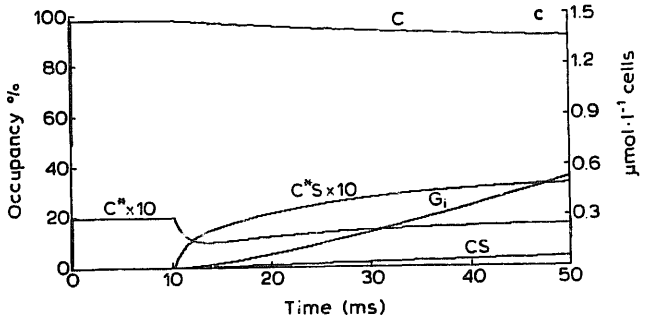


Fig. 3. Simulation of the time dependent changes in percentage occurrence of the five possible membrane states on the external surface of a fixed site carrier. The unliganded external binding site exists either in an inactive state, C or activated state. C* the binding site liganded to glucose CS also can exist in the activated and inactive state. Transport across the membrane occurs only when sugar binds to the activated form, CS* (Scheme II). The simulations were carried out using fourth order Runge-Kutta numerical integration of the following six simultaneous differential equations;

$$d[C]/dt = [CS] \cdot k_{21} + [CM] \cdot k_{31} + [C^*] \cdot k_{41} + [CS^*] \cdot k_{51} - [C]([G_o] \cdot k_{12} + [M] \cdot k_{13} + k_{14}) \quad (1)$$

$$d[CS]/dt = [C] \cdot [G_o] \cdot k_{12} + [CS^*] \cdot k_{52} - [CS](k_{21} + k_{25}) \quad (2)$$

$$d[CM]/dt = [C] \cdot [M] \cdot k_{13} + [C^*] \cdot [M] \cdot k_{43} - [CM](k_{31} + k_{34}) \quad (3)$$

$$d[C^*]/dt = [C] \cdot k_{14} + [CM] \cdot k_{34} + [CS^*] \cdot (k_{54} + k_{56}) - [C^*] \cdot (k_{41} + [M] \cdot k_{43} + [G_o] \cdot k_{45}) \quad (4)$$

$$d[CS^*]/dt = [C] \cdot [G_o] \cdot k_{15} + [CS] \cdot k_{25} + [C^*] \cdot [G_o] \cdot k_{45} - [CS^*] \cdot (k_{51} + k_{52} + k_{54} + k_{56}) \quad (5)$$

$$d[G_i]/dt = [T] \cdot [CS^*] \cdot k_{56} \quad (6)$$

The values of the rate constants used in the simulations are: $k_{12} = 100\ s^{-1} \cdot mmol^{-1}$; $k_{21} = 10\ s^{-1}$; $k_{13} = 10\ s^{-1} \cdot mmol^{-1}$; $k_{31} = 250\ s^{-1}$; $k_{34} = 2500\ s^{-1}$; $k_{43} = 500\ s^{-1} \cdot mmol^{-1}$; $k_{25} = 2\ s^{-1}$; $k_{52} = 100\ s^{-1}$; $k_{45} = 50000\ s^{-1} \cdot mmol^{-1}$; $k_{54} = 5000\ s^{-1}$; $k_{14} = 50\ s^{-1}$; $k_{41} = 2500\ s^{-1}$; $k_{15} = 20\ s^{-1} \cdot mmol^{-1}$; $k_{51} = 100\ s^{-1}$; $k_{56} = 100\ s^{-1}$; [T] = 0.007 mM. ([T] is the concentration of all carrier forms in mmol/l cells). $[G_o]$, $[G_i]$ and [M] refer to the concentrations of glucose in the external and internal solutions and maltose in the external solution, respectively. The integration step size 2.5 μS.

Hence the K_{s1G} of glucose for the activated site = k_{54}/k_{45} = 0.1 mM. The K_s of maltose for the activated site = k_{34}/k_{43} = 2.5 mM. The K_s of glucose for the inactive site is k_{21}/k_{12} = 0.1 mM and the K_{sM} of maltose for the inactive site = 25 mM and the K_i for steady-state maltose-dependent inhibition of glucose uptake = 25(1 + 0.04) = 26 mM. The K_m for steady-state glucose uptake is $K_{s1G}(1 + K_1)/(1 + K_2)$ = 0.098 mM. In Fig. 3a from time 0 to 10 ms [M] = 150 mM and $[G_o] = [G_i] = 0$; at time > 10 ms [M] = 7.56 mM and $[G_o] = 0$ mM. In Fig. 3b from time 0 to 10 ms [M] = 150 mM and $[G_o] = [G_i] = 0$; at time > 10 ms [M] = 7.56 mM and $[G_o] = 0.1$ mM. In Fig. 3c from time 0 to 10 ms [M] = 0 mM and $[G_o] = [G_i] = 0$; at time > 10 ms [M] = 0 mM and $[G_o] = 0.1$ mM.

References

1 Lowe, A.G. and Walmsley, A.R. (1987) Biochim. Biophys. Acta 903, 547–550.
2 Lowe, A.G. and Walmsley, A.R. (1986) Biochim. Biophys. Acta 857, 146–154.
3 Deves, R. and Krupka, R.M. (1984) Biochim. Biophys. Acta 769, 455–460.
4 Krupka, R.M. and Deves, R. (1980) Biochim. Biophys. Acta 589, 134–144.
5 Lieb, W.R. and Stein, W.D. (1974) Biochim. Biophys. Acta 373, 178–196.
6 Naftalin, R.J., Smith, P.M. and Roselaar, S. (1985) Biochim. Biophys. Acta 820, 235–249.
7 Carruthers, A. (1986) Biochemistry 25, 3592–3602.
8 Thomsen, J.S. (1953) Phys. Rev. 91, 1263–1266.
9 Naftalin, R.J. and Holman, G.D. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 257–300, Academic Press, London.
10 Barnett, J.E.G., Holman, G.D., Chalkley, R.A. and Munday, K.A. (1975) Biochem. J. 145, 417–429.