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The one-site model of human erythrocyte glucose transport: testing its predictions using network thermodynamic computer simulations

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Network thermodynamic computer simulations were carried out using parameters experimentally derived by Lowe and Walmsley ((1987) Biochim. Biophys. Acta 903, 547–550) for two tests of the one-site model of human erythrocyte glucose transport. In the temperature-jump experiment, the simulations predicted the amplitude and relaxation time of accelerated uptake, but underestimated the net uptake due to an unexpectedly low measured basal rate. In the maltose-acceleration experiment, the dissociation constant of maltose was assessed at 0 °C by measuring the inhibitory effects of maltose on both cytochalasin B binding and on 3-*O*-methylglucose uptake, and using this value (52 mM) to calculate the dissociation constant (2.9 mM). The simulated experiment then did show a transient acceleration in uptake comparable in magnitude to that observed experimentally, except that the relaxation time was more than 10-fold longer in the simulations. Measurements of the temperature dependence of the inhibition of cytochalasin B binding by maltose and 3-*O*-methylglucose indicated that apparent sugar affinity is sensitive to carrier orientation at low temperatures, whereas at more physiologic temperatures the intrinsic dissociation constant predominated.

Introduction

Even in a relatively simple transport system, such as the one-site or alternating conformation model (Fig. 1) proposed for the human erythrocyte glucose carrier [1–3], the observed kinetic parameters are necessarily complex functions of both the substrate binding site affinity, and of the forces determining to which side of the membrane the binding site faces. This and technical difficulties associated with accurate measurements of initial flux rates have hampered attempts to elucidate the transport mechanism based on the rate constants of a particular model. However, Lowe and Walmsley [4,5], using a rapid stop-flow technique of flux measurement have provided a coherent description of transport parameters in terms of the one-site model. Both the model and several of the values for rate constants have been recently supported by data from Appleman and Lienhard [6,7] who used measurements of changes in intrinsic carrier fluorescence to derive the needed rate constants.

However, both Carruthers [8–10] and Naftalin [11,12] have challenged the description of the erythrocyte carrier by the one-site model. At least part of the controversy centers on the maltose-acceleration experiment, cited as evidence for the model and used to estimate the single half-turnover of the carrier and its binding site [5]. In this experiment, high concentrations of the disaccharide maltose, which binds to the outward-facing substrate binding site, but is not transported, are used to orient carriers to a predominantly outward-facing conformation in the initial phase of the experiment. The cells are then rapidly diluted into several volumes of maltose-free buffer containing a low concentration of radiolabeled glucose, the uptake of which is followed over short times with a stop-flow apparatus [5]. The dissociation of maltose upon dilution frees excess outward-facing carriers to bind and transport glucose, resulting in a transient acceleration of uptake as carriers relax to their thermodynamically favored inward-facing state at low temperatures [4]. As noted by Naftalin [11,12], a crucial but unmeasured parameter in this experiment is an estimate of the dissociation constant of

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maltose. Indeed, the absence of a substantial acceleration of glucose uptake in computer simulations using an inhibitory constant of 13 mM for maltose was cited as a reason for rejecting the one-site model by Naftalin [12]. In the present work the affinity of maltose for the erythrocyte carrier was measured at 0°C by two methods and was incorporated into network thermodynamic computer simulations of carrier function to determine whether and to what extent maltose-acceleration occurs under the proposed model. Additionally, the computer simulations are used to assess goodness of fit of the one-site model and its measured parameters in temperature-jump experiment also performed by Lowe and Walmsley [5].

Experimental Procedures

Erythrocyte preparation and transport assay. Human erythrocytes were drawn from volunteers and prepared for binding studies as previously described [13]. The uptake of an initial extracellular concentration of 55 μM 3-*O*-[^{3}H]methylglucose (New England Nuclear, 79 Ci/mmol) was performed at 0°C as previously described [14]. The inhibitory constant of maltose was measured over a range of five maltose concentrations (20–60 mM) and calculated as the negative *X*-axis intercept from a Dixon plot. Sucrose was added in appropriate amounts to bring the final osmolality of each sample to equal that of the sample containing 60 mM maltose. Maltose and sucrose were also included in the radioactive sugar solutions to equal concentrations outside cells.

Cytochalasin B binding assay. The binding of [^{3}H]cytochalasin B (New England Nuclear, 22 Ci/mmol) to intact erythrocytes was measured as previously described at 0°C [14]. At this temperature, at least 15 min was required for complete equilibration of binding. In subsequent assays 30 min equilibration times were used. Binding was measured over a range of cytochalasin B concentrations (10, 25, 50, 100, 200, and 3000 nM) and the apparent equilibrium dissociation constant and total cytochalasin B bound were calculated by the method of Scatchard according to Rosenthal [15]. Inhibition of 10 nM [^{3}H]cytochalasin B binding by maltose was measured for at least five inhibitor concentrations inclusive of the value for the apparent inhibitory constant, which was calculated from linear least-squares analysis of Dixon plots [2,13].

Computer simulations. Network thermodynamic computer simulations of glucose transport were performed as described previously [16,17] using the simulation program PSPICE (MicroSim Corp.) on a microcomputer. The one-site transport model (Fig. 1) used in the simulations was more complex than that employed previously [16,17], since it included the recently derived rate constants for carrier orientation in the loaded and unloaded state [4], and a circuit for inhibitor binding. A typical program used to derive the time-dependent simulation of glucose transport in the presence of 7.6 mM maltose is depicted in the Appendix.

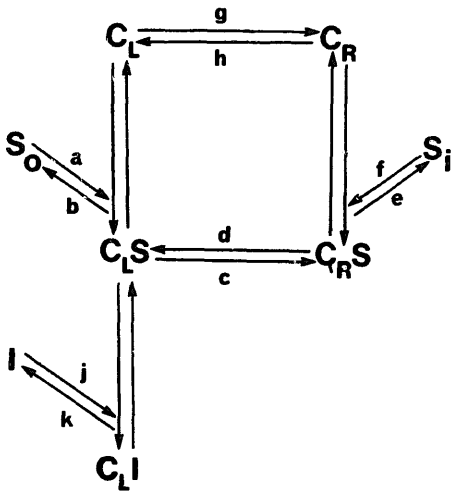

Fig. 1. One-site transport model. S_0, extracellular sugar; S_i, intracellular sugar; C_L, unloaded outward-facing carrier; C_R, unloaded inward-facing carrier; C_LS, loaded outward-facing carrier; C_RS, loaded inward-facing carrier; C_LI, maltose-bound to outward-facing carrier; *a–h*, *j*, *k*, rate constants for ligand binding and carrier orientation.

Results and Discussion

Two types of experiments were performed by Lowe and Walmsley [5] and considered to provide support for the one-site model of glucose transport, based upon changes in carrier binding site oritentation. In the temperature-jump experiment, erythrocytes incubated at 38°C (having 41% of carriers oriented outwardly [4,5,18]), were diluted into buffer containing D-[^{14}C]glucose to a final temperature of 6°C. A transient acceleration of uptake was observed in cells treated in this manner when compared to transport in cells maintained at 6°C [5,18]. Assuming equilibration of substrate with carriers to be very rapid compared to the actual transport process, the accelerated uptake was attributed to a 'half-turnover' of loaded carriers inwardly to the favored state of equilibrium at 6°C, in which 92–93% of carriers face inwardly, whether loaded or not [4]. If the model and its measured parameters are correct, transient computer simulations using experimentally derived parameters should mirror the observed effects. Network thermodynamic simulations of this experiment using a modification of the program listed in the Appendix, and the parameter values of Lowe and Walmsley [5,18], are shown in Fig. 2. The simulations were performed at a total number of carriers per cell calculated by Lowe and Walmsley to participate in the transport acceleration, which they noted to be similar to literature estimates [5]. It is apparent from Fig. 2 that there was a transient

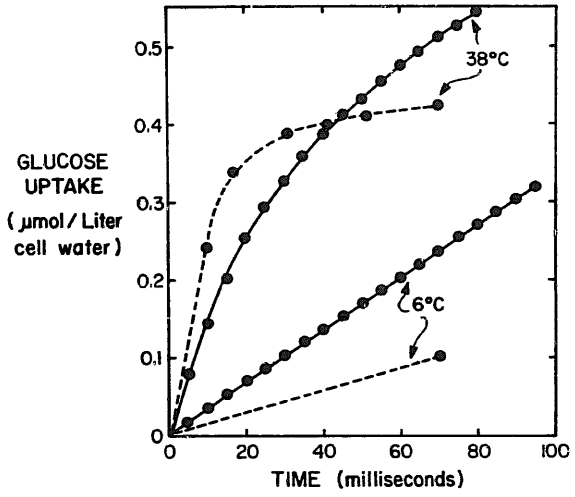

Fig. 2. Temperature-jump experiment. The data taken from Lowe and Walmsley [5,18] are shown by the dashed lines, computer simulations by the solid lines, with the pre-equilibration temperature as noted. The transport assay was initiated by diluting cells which had been pre-equilibrated at 38°C or at 6°C into buffer to a final temperature of 6°C. The simulation was performed using rate constants set to 6°C ($c=1503\ s^{-1}$, $d=208\ s^{-1}$, $g=40\ s^{-1}$, $h=3.7\ s^{-1}$) with carriers set initially in their orientation either at 38°C (41% outward-facing) or at 6°C (8.4% outward-facing) [5,18]. The number of carriers per liter of cell water (5.4 μM) was derived from measurements and calculations from this experiment [5].

acceleration of uptake in the simulation. Upon subtraction of basal transport at 6°C, the net uptake was 70–80% of that actually measured (not shown). Further, the effect relaxed with a half-time of 14 ms, compared to a measured value of about 10 ms. However, in spite of the reasonable approximation of the accelerated uptake, the simulated basal transport rate in the absence of a change in temperature was over 2-fold greater than actually measured in basal or temperature-equilibrated cells [5]. Thus the simulations using derived parameters of the temperature-jump experiment, although generally in accord with the data, do present discrepancies which need to be resolved.

In the second type of experiment, maltose in the extracellular space binds to the carrier and changes its orientation to an outward-facing predominance, even at 0°C when over 95% of unloaded carriers would naturally face inwardly [4]. Rapid dilution of cells into maltose-free buffer containing [^{14}C]glucose allows maltose to dissociate, glucose to bind, and a transient enhancement of uptake as glucose-bound carriers undergo a 'half-turnover' to the thermodynamically more stable inward-facing conformation.

In performing simulations of this maltose-acceleration experiment, a crucial missing parameter is the dissociation constant or K_{Sout} for maltose binding to the outward-facing carrier form. The measured K_{Iout} value does not suffice, since as shown by Devés and Krupka [3],

$$K_{Iout} = K_{Sout}(1 + g/h) \quad (1)$$

where K_{Iout} is the measured inhibitory constant of an externally bound ligand, K_{Sout} is its intrinsic dissociation constant, and g and h are rate constants for the unloaded carrier from Fig. 1. Thus the apparent K_{Iout} is a function both of the affinity of the carrier for the ligand, and of the forces governing availability of a carrier form which will bind ligand.

In order to obtain a reasonable estimate of the maltose K_{Sout}, its ability to inhibit both 3-*O*-methylglucose uptake and cytochalasin B binding were measured at 0°C. Inhibition of 3-*O*-methylglucose uptake resulted in linear Dixon plots with an apparent K_{Iout} value of 56 ± 7 mM (mean ± S.E.) in eight experiments. Similarly, equilibrium cytochalasin B binding was inhibited with an apparent K_{Iout} of 48 ± 3 mM in six experiments, again producing strictly linear Dixon plots (not shown). An average value of 52 mM was used to calculate a K_{Sout} value of 2.9 mM from Eqn. 1 and from the 0°C rate constants [4]. This value was incorporated into transient computer simulations of the maltose-acceleration experiment, which are shown along with approximations of the actual data from Lowe and Walmsley in Fig. 3 [5]. The number of carriers per liter of cell water (4.8 μM) was similar to that used in the temperature-jump experiment (5.4 μM). It was derived

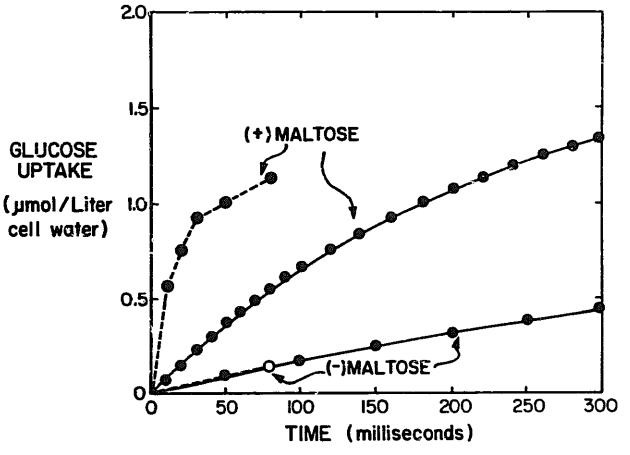

Fig. 3. Maltose-acceleration experiment. Data from Lowe and Walmsley [5] are shown by the dashed lines or by the single open circle (basal rate, no maltose), the computer simulations as solid lines. The simulations (see Appendix) were performed (1) (+)-maltose, with carriers oriented 76% outwardly upon binding of 150 mM maltose, followed by transport with maltose diluted to 7.56 mM; or (2) (−)maltose, preliminary equilibration followed by transport in the absence of extracellular maltose. Other experimental conditions and rate constants were those of Lowe and Walmsley at 0°C [4,5]. The K_{Sout} value for maltose was 2.9 mM as measured herein. The concentration of carriers (4.8 μM) again was derived from actual measurements of Lowe and Walmsley [5], and calculated as modified by Naftalin [20].

from calculations of Lowe and Walmsley [5], based on the observed amplitude of the single turnover in the maltose-acceleration experiment. However, this calculation also followed the modification of Naftalin [20], which in this experiment requires use of the K_{Sout} of maltose (2.9 mM), rather than its K_{Iout}. The transport rate in the absence of maltose was very similar to that determined experimentally. With 150 mM maltose present before dilution, the simulations showed an acceleration of uptake, the amplitude of which was 1.6 μmol/liter of cell water, when corrected for uptake which would occur in the presence of 7.56 mM extracellular maltose in both phases of the experiment (about 1.1 μmol/liter cell water per s). This compares fairly well with the observed amplitude attributed to the single turnover of 1.1 μmol/liter cell water [5]. The simulated half-relaxation time was 100 ms and was prolonged due to the need for maltose to dissociate following its dilution before glucose could bind and be transported inward. Thus the appearance of 'extra' carriers to participate in the single half-turnover [5] was not immediate, but gradual. The simulated value was more than 10-fold that actually observed, causing a comparable overestimate of net uptake [5]. The cause of this discrepancy is unknown, but could be partially explained by the sharp temperature dependence of the carrier rate constants. For example, using the rate constants observed at 6°C [4,5,18], the simulated time of half-relaxation falls to 60 ms (not shown). The discrepancy in the relaxation time has been brought out previously [11,12,19,20], and was among the reasons for rejection of the model by Naftalin [12,20]. Indeed, as suggested by one of the reviewers of this work, a prolonged relaxation time, as well as lack of a direct comparison with unstimulated uptake, could have contributed to Naftalin's failure to show obvious accelerated uptake in simulations performed over a 40 ms period [12]. The discrepancy in observed and simulated relaxation times has been acknowledged by Walmsley and Lowe [19], who proposed repeating the experimental protocol. Although this discrepancy is probably significant and will require further experiments to resolve, the overall qualitative results of these studies, as well as a wealth of transport [5,21,22], inhibitor [2,24], and fluorescence data [6,7] still generally favor the one-site over a two- or fixed-site model.

The K_{Sout} of maltose used in the simulations was important in determining the extent of maltose-induced acceleration. Under the conditions of the maltose-acceleration experiment, varying the maltose K_{Sout} in the simulations resulted in the biphasic curve shown in Fig. 4. At the K_{Sout} value of 2.9 mM corresponding to the measured K_{Iout} for maltose at 0°C (52 mM), the extent of acceleration was near-maximal, whereas at the K_{Iout} value of 13 mM taken by Lowe and Walmsley [5], from which Naftalin calculated a K_{Sout} value of 0.73 mM

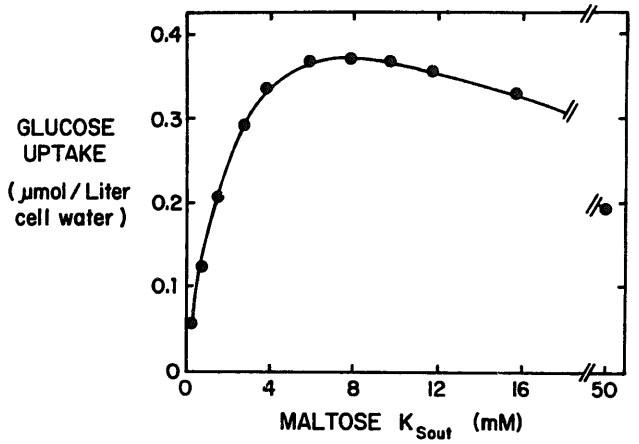

Fig. 4. Dependence of glucose uptake on the K_{Sout} of maltose. Simulations were performed using the parameters employed in Fig. 3, except that the K_{Sout} of maltose was varied as indicated. The extent of uptake at 40 ms is indicated on the *Y*-axis.

[12,20], the net acceleration was 3–4-fold less. In other words, at such a high affinity of maltose very few carriers are released to bind glucose with dilution of maltose to 7.56 mM.

In Fig. 5 a comparison is made for actual [5] and simulated data for the acceleration produced at 40 ms by various maltose concentrations (shown as those present before a 20-fold dilution in the assay). Several points are relevant. First, the concentrations of maltose at which glucose uptake was accelerated half-maximally are relatively similar, and correspond to a maltose concentration of 23.5 mM before dilution, or 0.63 mM during the uptake phase. Second, the extent of acceleration in the simulation actually decreased at the highest maltose concentration. This occurred whether glucose accumulation at 40 ms or the maximal rate of transport was plotted. It results from the fact that maltose, even when diluted from 150 mM to 7.6 mM, competes significantly with glucose for carriers under these condi-

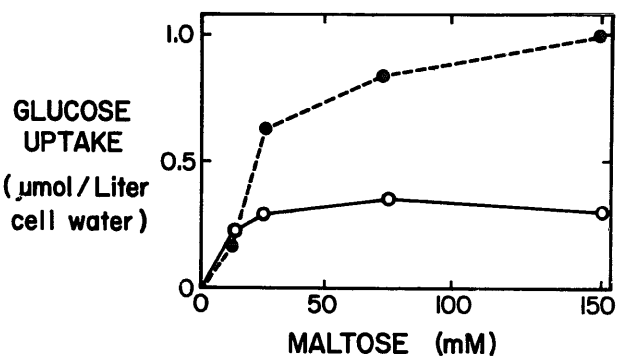

Fig. 5. Saturation of maltose-accelerated glucose uptake. The actual data from Lowe and Walmsley [5] are represented by the dashed line, the computer simulations by the solid line. Simulations were performed using a 20-fold dilution of the indicated initial extracellular maltose concentration, and expressed as the amount of glucose transported at 40 ms.

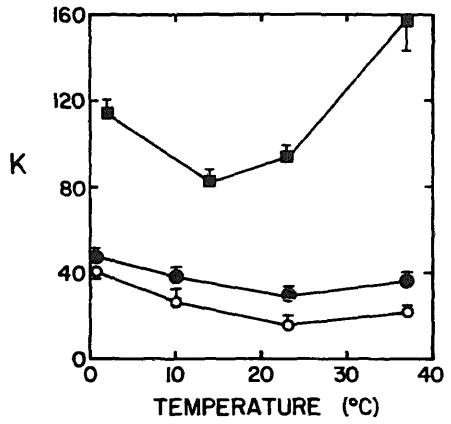

Fig. 6. Effects of temperature on the apparent affinity (K) of the carrier for cytochalasin B and sugars. Equilibrium cytochalasin B binding ($N = 3$ experiments, closed squares) was measured in intact erythrocytes at the indicated temperatures and the apparent dissociation constants expressed in units of nanomoles per liter. The abilities of maltose ($N = 6$ experiments, closed circles) and 3-*O*-methylglucose ($N = 2$ experiments, open circles) to inhibit cytochalasin B binding were also measured at the indicated temperature and shown as inhibitory constants derived from Dixon plots in units of millimoles per liter. Data are shown as mean ± standard error.

tions. Third, the net amount of simulated uptake at maltose concentrations over 25 mM was less than half that actually measured at 40 ms, due to the previously noted difference in relaxation time.

The effects of temperature on the apparent affinities of cytochalasin B and maltose measured in the cytochalasin B binding studies were also assessed, since these are relevant to the proposed temperature-dependence of carrier orientation [4]. In Fig. 6, it can be seen that the apparent affinity of the carrier for cytochalasin B displayed a substantial dependence on temperature, with a maximum at about 14° C. Decreased affinity was observed in the physiologic range of temperatures, as predicted if carriers are oriented 40% outwardly and unable to bind cytochalasin B under the one-site model [4]. However, contrary to predictions based solely on carrier orientation [4], affinity decreased at 3° C (Fig. 6). Assuming no change in carrier orientation, this effect must be due to a decline in the intrinsic affinity (or increase in the equilibrium dissociation constant) at very low temperatures, possibly related to unfavorable changes in membrane fluidity.

The inhibition of cytochalasin B binding by maltose and 3-*O*-methylglucose also varied with temperature, although to a lesser extent than the binding of cytochalasin B itself (Fig. 6). Clearly, the inhibition of cytochalasin B binding to the inward-facing carrier by extracellular maltose provides strong support for the one-site model. The temperature dependence of transport inhibition by maltose was assumed to be minimal by Naftalin [12], based on unpublished work by Holman. The present results, using indirect measurements of inhibition of cytochalasin B binding suggest that there is a significant, albeit small, biphasic dependence of maltose affinity on temperature (Fig. 6). However, the change in apparent affinity for maltose based on inhibition of cytochalasin B binding did not follow the prediction of Lowe and Walmsley [4] with regard to carrier orientation. If carrier orientation was a major determinant of maltose affinity, its affinity should have been maximal at 37° C, when about 40% of unloaded carriers should face outwardly [4]. What was observed was a consistent, albeit small, decrease in affinity at this temperature for both maltose and 3-*O*-methylglucose (Fig. 6). This was made even more pronounced by the fact that cytochalasin B affinity was at its lowest at this temperature (Fig. 6), suggesting that carriers are oriented outwardly, theoretically making it easier for maltose to displace bound cytochalasin B. If carriers are more outwardly oriented at physiologic than at lower temperatures [4], the maltose data suggest that the overall dissociation constant (K_{Sout}) must increase at 37° C. Significantly, a very similar temperature dependence was found by Levine et al. [25] for glucose inhibition of sorbose uptake, indicating that the present observation is not an artifact of using cytochalasin B-binding to assess sugar affinity. Whereas carrier orientation appears to be the major contributor to the apparent affinity of the outward-facing substrate binding site at low temperatures, at more physiologic temperatures intrinsic affinity is the dominant factor [25].

We had previously used network computer simulations to model the behavior of a simplified carrier in which sugar translocation was represented as a simple diffusion step [16,17]. With the availability of experimentally determined carrier rate constants [4], it was possible to upgrade the model, and to include an inhibitor binding step with simple modifications of the existing program. The theoretical basis and potential applications of network thermodynamic computer simulations using the circuit simulation program SPICE are detailed elsewhere [26–28], but the present work illustrates several advantages of this method. It is versatile, easily learned, capable of handling complex or coupled nonlinear systems, and it facilitates the interplay between experimentation and model or hypothesis formulation.

Appendix

SPICE Program (Fig. A-1) for transient simulation of *zero*-trans glucose influx in the presence of maltose, which produced the curve for maltose-acceleration shown in Fig. 3.

The definitions of the individual circuit elements are provided elsewhere [27]. The experimental conditions are identical to those used in the maltose-acceleration experiment by Lowe and Walmsley [4,5]: the extracellular space is 13.2-fold that of the intracellular glucose

```
*MALTOSE-ACCELERATION EXPERIMENT
CCL          1     0     1      IC=7E-8
CXL          3     0     1      IC=0
CXR          4     0     1      IC=0
CCR          2     0     1      IC=1.17U
CAL         11     0   13.18    0.095M
CAR         14     0     1      IC=0
CXLO        12     0     1      IC=0
CXRO        13     0     1      IC=0
CI          18     0     1      IC=3.56U
CINHF       19     0   13.18    IC=7.56M
CINHB       20     0     1      IC=3.56U
*VOLTAGE-CONTROLLED CURRENT SOURCES
GLOADA              11      7     POLY(2) 11    0   1   0   0   0   0 0   500K
GLOADD               8     11     12      0     5K
GULOADA             14     10     POLY(2) 14    0   2   0   0   0   0 0   500K
GULOADD              9     14     13      0     6.85K
GLA                  5     13     5       0     1113
GLD                 13     15     13      0     90.3
GCCL                 1     16     1       0     12.1
GCCR                16      1     16      0     0.726
GINHA                1     21     POLY(2) 1     0   19  0   0   0   0 0   140K
GINHD               22      1     18      0     0.41K
*AMMETERS
VLOADA               7     12     0
VLOADD              12      8     0
VULOADA             10     13     0
VULOADD             13      9     0
VTA                 17      5     0
VTB                  2     16     0
VTD                 15     17     0
VTS                 12     17     0
VIA                 21     18     0
VID                 18     22     0
CURRENT-CONTROLLED CURRENT SOURCES
FLOADA               1      3     VLOADA      1
FLOADD               3      1     VLOADD      1
FULOADA              2      4     VULOADA     1
FULOADD              4      2     VULOADD     1
FCTR                 3      4     VTA         1
FCTL                 4      3     VTD         1
FINHA               19     20     VIA         1
FINHD               20     19     VID         1
*CONTROL CARDS
.PRINT TRAN V(1) V(2)      V(3)     V(4)
.PRINT TRAN V(11) V(14)    V(18)    I(VTS)
.PLOT  TRAN (V11) V(14)    I(VTS)
.TRAN  10M 300M  UIC
.OPTIONS    ABSTOL=1E-15  VNTOL=1E-08  NUMDGT=4
.END
```

Fig. A-1.

distribution space; the total carrier concentration is 4.8 μM; carriers are oriented 76% outwardly and mostly bound to maltose, the extracellular concentration of which has been diluted from 150 mM to 7.6 mM; the equilibrium dissociation constant of maltose is 2.9 mM; the K_{Sout} and K_{Sin} values for glucose are 10 mM and 13.7 mM, respectively [4,12]; the initial extracellular glucose concentration is 0.095 mM; and the rate constants for carrier orientation (*c*, *d*, *g* and *h* of Fig. 1) are 1113 s^{-1}, 90.3 s^{-1}, 12.1 s^{-1}, and 0.726 s^{-1}, respectively, as derived by Lowe and Walmsley [4].

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References

1 Vidaver, G.A. (1966) J. Theor. Biol. 10, 301–306.
2 Gorga, F.R. and Lienhard, G.E. (1981) Biochemistry 20, 5108–5113.
3 Devés, R. and Krupka, R.M. (1984) Biochim. Biophys. Acta 769, 455–460.
4 Lowe, A.G. and Walmsley, A.R. (1986) Biochim. Biophys. Acta 857, 146–154.
5 Lowe, A.G. and Walmsley, A.R. (1987) Biochim. Biophys. Acta 903, 547–550.
6 Appleman, J.R. and Lienhard, G.E. (1985) J. Biol. Chem. 260, 4575–4578.
7 Appleman, J.R. and Lienhard, G.E. (1989) Biochemistry 28, 8221–8227.
8 Carruthers, A. (1986) J. Biol. Chem. 261, 11028–11037.
9 Helgerson, A.L. and Carruthers, A. (1987) J. Biol. Chem. 262, 5464–5475.
10 Carruthers, A. (1988) Trends Biochem. Sci. 13, 426–427.
11 Naftalin, R.J. (1988) Trends Biochem. Sci. 13, 425–426.
12 Naftalin, R.J. (1988) Biochim. Biophys. Acta 946, 431–438.
13 May, J.M. (1988) J. Biol. Chem. 263, 13635–13640.
14 May, J.M. (1988) Biochem. J. 254, 329–336.
15 Rosenthal, H.E. (1967) Anal. Biochem. 20, 525–532.
16 May, J.M. and Mikulecky, D.C. (1982) J. Biol. Chem. 257, 11601–11608.
17 May, J.M. and Mikulecky, D.C. (1983) J. Biol. Chem. 258, 4771–4777.
18 Lowe, A.G. (1989) Biochem. Soc. Trans. 17, 435–438.
19 Walmsley, A.R. and Lowe, A.G. (1988) Trends Biochem. Sci. 13, 427–428.
20 Naftalin, R.J. (1988) Trends Biochem. Sci. 13, 423.
21 Brahm, J. (1984) J. Gen. Physiol. 339, 339–354.
22 Wheeler, T.J. (1986) Biochim. Biophys. Acta 862, 387–398.
23 Krupka, R.M. and Devés, R. (1981) J. Biol. Chem. 256, 5410–5416.
24 Devés, R. and Krupka, R.M. (1978) Biochim. Biophys. Acta 510, 339–348.
25 Levine, M., Levine, S. and Jones, M.N. (1971) Biochim. Biophys. Acta 225, 291–300.
26 Mikulecky, D.C. and Thomas, S.R. (1979) J. Franklin Inst. 308, 309–325.
27 White, J.C. and Mikulecky, D.C. (1982) Pharmacol. Ther. 15, 251–291.
28 Mikulecky, D.C. (1983) Am. J. Physiol. 245, R1–R9.