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A single half-turnover of the glucose carrier of the human erythrocyte

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Single half-turnovers of the glucose carrier of the human erythrocyte have been measured by recruiting carriers to the outward-facing conformation by (a) pre-exposing cells to extracellular maltose, or (b) pre-warming cells to 38 °C, before addition of D-[^{14}C]glucose at 0 °C. Based on these experiments estimates of the number of glucose carriers per red cell range from 124 000 to 190 000.

Membrane transport systems are usually described in terms of the 4-state carrier model, in which the carrier protein is envisaged as catalysing transport by alternating between inward- and outward-facing conformations. Most of the evidence for this model stems from analysis of the kinetics of transport systems including those for anions, nucleosides and glucose in the human erythrocyte. However, direct evidence for alternating conformations is limited to a few experiments which include measurements of fluorescence associated with substrate-binding to the glucose carrier [1,2], and a demonstration of a single half-turnover of the anion carrier [3]. In this report we describe two experiments which demonstrate the occurrence of a single half-turnover of the glucose carrier, and make it possible to estimate the number of glucose carriers per red blood cell. The results obtained are consistent with previous studies of both the kinetics of glucose transport and the number of glucose carriers per cell estimated from binding of cytochalasin B [4–7] and glucose [8].

The strategy for single turnover experiments was to equilibrate red blood cells under conditions in which a substantial proportion of carriers could be expected to be in the outward-facing conformation, then simultaneously alter these conditions and add D-[^{14}C]glucose so that transport could be measured during the period of carrier reorientation. This was carried out in two types of experiment, the details of which are given in the appropriate figure legends. In the first red blood cells were suspended in an isotonic solution containing phosphate-buffered saline and the non-transported disaccharide, maltose, at a concentration sufficient to trap a large proportion of carriers as the outward-facing maltose complex. This cell suspension was then diluted with a large excess of maltose-free isotonic phosphate-buffered saline containing D-[^{14}C]glucose. Transport was allowed to proceed for a fraction of a second before addition of the transport quenching solution, then processing of the cells for assay of radioactivity. In control experiments the red blood cells were pre-equilibrated in the absence of maltose, but the final conditions after addition of D-[^{14}C]glucose were made identical through inclusion of maltose at the appropriate concentration

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in the radioactive saline. In the second experimental design red blood cells were prewarmed to 38° C (a temperature expected to convert about 40% carriers to the outward-facing conformation [9]) before addition of ice-cold phosphate-buffered saline containing D-[^{14}C]glucose, and measurements of transport over a period of up to 80 ms were made in the same way as in the maltose experiments. Control cells for these experiments were pre-incubated at 0° C (when only 5–6% carriers are expected to be in the outward-facing conformation [9]) rather than 38° C.

The general methods used in preparing red blood cells and assaying glucose transport measurements were those described by Lowe and Walmsley [9,10], except that the transport quenching solution contained 5 μM cytochalasin B, in addition to 150 μM phloretin and 10 μM mercuric chloride in phosphate-buffered saline (pH 6.0), and an additional wash with this quenching solution was used in order to minimize 'zero time' D-[^{14}C]glucose uptake. In experiments using maltose, the maltose-pretreated cell suspension and the phosphate-buffered saline containing D-[^{14}C]glucose were mixed using the rapid reaction apparatus described by Lowe and Walmsley [10] with flow through the mixing chamber driven by motor-powered syringes, and with the solutions stored in ice-thermostatted sample-loops of Rheodyne valves before mixing. The maltose used in these experiments was pretreated with glucose oxidase (Sigma G6641) (by incubation of 0.32 M maltose in 5 mM phosphate-sodium (pH 5.1) with 50 units/ml glucose oxidase for 2 h at room temperature) to remove contaminating glucose, then freed from glucose oxidase by filtration through an Amicon UM10 ultrafiltration membrane. 'Temperature jump' experiments were carried out similarly, except that before mixing cells were thermostatted at 38° C in phosphate-buffered saline containing neither maltose nor glucose.

Fig. 1a illustrates the time-course at 0° C for D-[^{14}C]glucose uptake into glucose-free red blood cells which had been pre-exposed to 150 mM maltose. Comparing this with the uptake in non-maltose pretreated cells it is clear that there is an initial rapid (maltose-induced) uptake (which is largely complete within the time-resolution of the mixing device) followed by the much slower up-

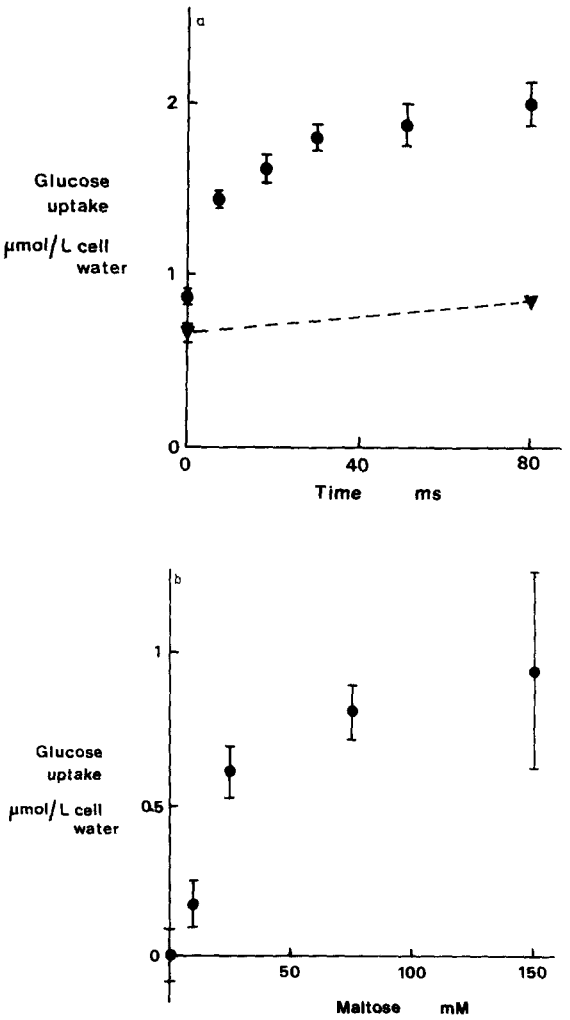

Fig. 1. In Fig. 1a glucose-free red cells at 70% hematocrit (1 volume, 0° C) were mixed with isotonic saline containing 0.1 mM D-[^{14}C]glucose with (●) or without (▼) 150 mM maltose (5.63 volumes, 0° C) using the rapid reaction apparatus of Low and Walmsley [10]. The flow rate after mixing was 1.86 ml/s and glucose transport was stopped with 15 ml quenching solution containing phloretin, mercuric chloride and cytochalasin B. All solutions were at 0° C and glucose uptake was calculated as described previously [9]. Uptakes of D-[^{14}C]glucose at zero time represent radioactivity remaining in the extracellular space (after washing) plus D-[^{14}C]glucose entering the cells during the washing procedure. Experiments in Fig. 1b were carried out similarly except that cells were pre-equilibrated with the indicated concentration of maltose. Points in Fig 1b were calculated as the difference between D-[^{14}C]glucose-uptake at 40 ms after pre-incubation with the indicated concentrations of maltose, and uptake at zero time. Each point is from a set of four measurements and in both figures error bars span 2 S.E.

take rate found in control cells. The very rapid rate of the maltose-induced D-[^{14}C]glucose-uptake is consistent with our previous description of the kinetics of the carrier [9], which predicts that the half time for reorientation of the glucose-loaded carrier from the outside- to the inside-facing conformation is of the order of 1 ms at 0 °C. As shown in Fig. 1b, the amplitude of the 'extra' influx of glucose associated with pre-exposure to maltose is a function of the maltose concentration, and this is consistent with the idea that the proportion of carriers poised in the outward conformation ready for inward transport of glucose is dependent on the extent of saturation of the carrier with maltose.

Using the conventional 4-state carrier model the proportion of glucose carriers present as the unloaded outward- and inward-facing conformations and as the maltose-bound outward-facing conformation can be calculated before and after mixing with D-[^{14}C]glucose from the K_i for maltose (13 mM [11]) and the equilibrium between the inward- and outward-facing carrier conformations [9]. This information was used to calculate the number of glucose carriers per cell from the single-turnover (STO) data in Fig. 1a as follows.

$T = 0\,°C$, $g = 0.0809\ s^{-1}$, $h = 0.00484\ s^{-1}$ [9], Initial maltose concentration [M] = 150 mM, final [M] = 7.6 mM, final glucose concentration [G] = 0.947 mM, K_m (glucose) = 0.145 mM [9], number of red cells/litre cell water = $1.72 \cdot 10^{13}$.

% Carriers binding maltose (150 mM) (assuming $K_i = 13$ mM [11])

$$= 100 - \frac{100}{(1 + ([M]/K_I))} = 92.02\%$$

% Outward-facing free carriers (C_o)

$$= (100 - 90.02) \cdot (h/(h+g)) = 0.45\%$$

Hence total % outward-facing carriers

$$[C_o + (CoM)] = 92.47\%$$

% Outward-facing free carriers in absence of maltose

$$= 100 \cdot (h/(h+g) = 5.64\%$$

Hence% 'extra' outward-facing carriers in presence of 150 mM maltose

$$= 92.47 - 5.64 = 86.83\%$$

% of the 'extra' carriers involved in the STO after mixing with 0.1 mM glucose

$$= \frac{86.83}{(1 + (K_m/[G])(1 + [M]/K_i)}$$

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

$$= 25.4\%$$

Number of carriers per cell

$$= \frac{\text{amplitude of STO}}{0.254} \cdot \frac{\text{(Avogadro's number)}}{\text{(number of cells/l cell water)}}$$

$= 124000$ (taking the STO amplitude (0.9 μM) as the difference between the overall glucose uptake with 150 mM maltose back-extrapolated to zero time, and the measured uptake with 150 mM maltose at zero time)

or $= 152000$ (taking the STO amplitude (1.1 μM) as the difference between the overall glucose uptake with 150 mM maltose (back-extrapolated to zero time), and the uptake at zero time in the absence of maltose).

Fig. 2 illustrates the results of a single turnover experiment in which cells at 38 °C (with carriers mainly in the outward-facing conformation) were mixed with a larger volume of ice-cold D[^{14}C]glucose. The number of carriers per cell was calculated from the single-turnover experiment in Fig. 2 as follows, using values of rate constants g and h according to Ref. 9.

At 6 °C $g = 64.5\ s^{-1}$, $h = 52.7\ s^{-1}$,

K_m (glucose) = 0.4 mM, final [G] = 0.0947 mM.

At 38 °C $g = 0.270\ s^{-1}$, $h = 0.0249\ s^{-1}$.

% Outward-facing free carriers at 38 °C $= 100 \times (h/(h+g))$

$$= 41.1\%$$

% Outward-facing free carriers at 6 °C $= 100 \times (h/(h+g))$

$$= 8.38\%$$

Hence $41.1 - 8.38 = 32.72\%$ carriers are available for the STO.

$$\% \text{ Carriers used in the STO} = \frac{32.72}{(1 + \{K_m/[G]\})} = 6.26\%$$

Number of carriers per cell

$$= \frac{\text{amplitude of STO}}{0.0627} \cdot \frac{\text{(Avogadro's number)}}{\text{(number of cells/l cell water)}}$$

= 190000 (taking the STO amplitude (0.34 μM) as the difference between the overall uptake of glucose into cells pre-incubated to 38° C (back-extrapolated to zero time) and the zero-time uptake into the cells)

The preceding calculations of numbers of carriers per cell depend not only the accuracy of the experiments themselves, but also on K_m for glucose, K_i for maltose and the values used for rate constants g and h in the 4-state carrier model. As a result the overall uncertainty in the calculations is probably of the order of ±50% in the both the maltose- and temperature-jump experiments. Nevertheless the values obtained are in reasonably good agreement with the lower range of previous estimates of 180000–350000 carriers/cell (based on cytochalasin B binding [4–7]) and 150000–250000 carriers/cell (based on glucose binding in the presence of ammonium sulphate [8]).

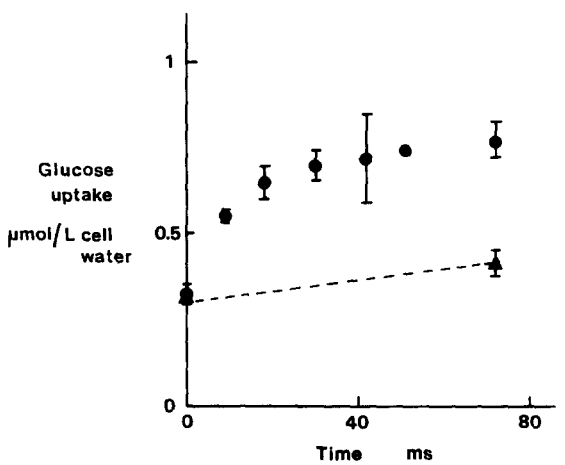

Fig. 2. Glucose-free red cells at 38° C and 70% hematocrit (1 volume) were mixed with isotonic saline at 0° C containing 0.1 mM D-[^{14}C]glucose (5.63 volumes) using the rapid reaction apparatus (●) and glucose uptake was assayed as indicated in Fig. 1a/b. The final temperature after mixing was approximately 6° C. In control experiments (▲) cold red cells were mixed with isotonic saline to give the same final temperature and glucose uptake was measured in the same way. Error bars span 2 S.E. for measurements based on sets of four determinations.

As shown above single half-turnovers of the glucose carrier can be readily accounted for by the conventional 4-state carrier model, whereas transport mechanisms such as that suggested by Carruthers [12] in which inward- and outward-facing glucose binding sites exist simultaneously, are more difficult to reconcile with temperature-jump studies and are possibly inconsistent with maltose-induction of single half-turnovers. While the results presented here do not exclude more complex models of glucose transport they add to the weight of evidence, which includes many measurements of steady state glucose transport [9,13–15], inhibitor studies [16,17] and measurements of changes in fluorescence during transport [2], supporting the conventional 4-state carrier model.

References

1 Gorga, F.R. and Lienhard, G.E. (1982) Biochemistry 21, 1905–1908
2 Appleman, J.R. and Lienhard, G.E. (1985) J. Biol. Chem. 260, 4575–4578
3 Jennings, M.L. (1982) J. Gen. Physiol. 79, 169–185
4 Taverna, R.D. and Langdon, R.G. (1973) Biochem. Biophys. Res. Commun. 54, 593–599
5 Lienhard, G.E., Gorga, F.R., Orasky, J.E. and Zoccoli, M.A. (1977) Biochemistry 16, 4921–4926
6 Jung, C.Y. and Rampal, A.L. (1977) J. Biol. Chem. 252, 5456–5463
7 Basketter, D.A. and Widdas, W.F. (1977) J. Physiol. 265, 39P
8 Kahlenburg, A., Urman, B. and Dolansky, D. (1971) Biochemistry 10, 3154–3162
9 Lowe, A.G. and Walmsley, A.R. (1986) Biochim. Biophys. Acta 857, 146–154
10 Lowe, A.G. and Walmsley, A.R. (1985) Anal. Biochem. 144, 385–389
11 Krupka, R.M. and Deves, R. (1980) Biochim. Biophys. Acta 598, 127–133
12 Carruthers, A. (1984) Prog. Biophys. Mol. Biol. 43, 33–69
13 Lacko, L., Wittke, B. and Kromphardt, H. (1972) Eur. J. Biochem. 25, 447–454
14 Wheeler, T.J. (1986) Biochim. Biophys. Acta 862, 387–398
15 Brahm, J. (1984) J. Gen. Physiol. 339, 339–354
16 Krupka, R.M. and Deves, R. (1981) J. Biol. Chem. 256, 5410–5416
17 Gorga, F.R. and Lienhard, G.E. (1981) Biochemistry 20, 5108–5113