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AN ANALYSIS OF THE APPARENT PARAMETERS OF THE GLUCOSE TRANSPORT SYSTEM IN THE RED CELL MEMBRANE

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SUMMARY

The pH dependence of glucose transport across the human red cell was studied in glucose exit experiments at 37 °C evaluated according to a somewhat modified Sen–Widdas method. It was found that the two parameters K_m and V varied inversely. While V showed a maximum at pH 7.1, K_m displayed a minimum at the same pH. It was shown that this inverse relationship is predicted from carrier kinetics if either different mobilities of free and loaded carrier are assumed or a finite reaction rate between substrate and carrier. In this case resistance terms related to mobilities and reaction rates enter into the apparent parameters in such a way that an exclusive or predominant effect of pH on D_{CS} or an effect of pH on the reaction rate can be the basis of the inverse variation.

INTRODUCTION

The pH dependence of glucose transport across the red cell membrane has been studied by several authors[1–3]. While Sen and Widdas[3] report a slight increase of both maximum rate and Michaelis constant with rising pH, Faust[1] observed a maximum for the transport rate at about pH 7, more pronounced at 37 °C than at 29 °C. A new study of the problem, therefore, was undertaken some time ago; its publication was delayed. In the meantime Lacko *et al.*[2] studied the pH dependence of equilibrium exchange rate and found a broad maximum near pH 7.4.

In our study a type of pH dependence resembling that reported by Faust[1] in the appearance of a minimum K_m near neutrality was found. Maximum rate, in contrast, was maximal at the same pH. The inverse relationship between K_m and V was tentatively ascribed to a preferential primary effect of pH on the mobility of the loaded carrier as distinct from that of the free carrier.

METHODS

The experiments were carried out on human blood received from the blood bank service of the University Clinics of Rome. The cells were received suspended in a medium containing glucose and citrate. They were washed 3 times with a phosphate-buffered saline of the desired pH. The experiments were carried out about 40 h after the blood had been taken from the donors.

For the determination of glucose transport an osmotic method introduced by Wilbrandt[4,5] was used. The cells were preloaded with 1.0 isotonic (300 mM) glucose. Exit of glucose from the preloaded cells into solutions of buffered saline containing low concentrations of glucose (0.025, 0.05. 0.075 and 0.1 isotonic= 7.5, 15, 22.5 and 30 mM, respectively) was followed. 1 vol. of the glucose-loaded cells was suspended in 20 vol. of the exit medium. The suspensions were incubated at 37 °C. Samples for the determination of osmotic fragility were taken at short time intervals. To minimise osmotic loss the fragility test was carried out at 5 °C. From the shift of the fragility curves the progress of penetration was calculated as described previously[4,6].

In some experiments (Fig. 2) radioisotope rather than osmotic methodology was used for the determination of glucose content of the cells. In these experiments the cell suspension was filtered under slight suction (9 cm Hg) through Millipore filters and the activity in the cells was measured on the filters with a Nuclear Chicago Counter Mark I.

For the evaluation of the experiments in terms of transport parameters Sen and Widdas[3] have introduced a graphical procedure involving a plot of reciprocal exit rate against external concentration. The abscissa intercept gives $-K_m$, the ordinate intercept $1/V$. We have first used this method of evaluation. Later, however, we turned to a somewhat modified procedure, in which $(S_1 - S_2)/v$ rather than $1/v$ is plotted against external concentration. This was motivated by the following reasons.

Let S_i, S_0 be internal and external sugar concentrations, respectively, v, rate of exit, V, maximum rate, K_m, Michaelis constant.

The Sen and Widdas method can be derived from the basic rate equation for exit rate

$$v = V\left(\frac{S_i}{S_i + K_m} - \frac{S_0}{S_0 + K_m}\right) \tag{1}$$

by introducing the simplifying assumption $S_i \gg K_m$ implying that $S_i/(S_i + K_m) \to 1.0$, and therefore, approximately,

$$\frac{1}{v} = \frac{1}{V} + \frac{1}{VK_m} \cdot S_0 \tag{2}$$

resulting in the possibility mentioned above to evaluate K_m and V from the intercepts of the plot of $1/v$ against S_0. The evaluation, however, actually rests on two simplifying assumptions: first (as mentioned before) $S_i \gg K_m$, but second, in addition, $S_i \gg S_0$. This is seen when Eqn 1 is rewritten in the form

$$\frac{1}{v} = \frac{S_i + K_m}{S_i - S_0}\left(\frac{1}{V} + \frac{1}{VK_m} \cdot S_0\right) \tag{3}$$

and compared with Eqn 2. For this reason $F = (S_i - S_0)/4$ rather than $1/4$ was plotted against the external concentration S_0, yielding a straight line according to the equation

$$F \equiv \frac{S_i - S_0}{v} = \frac{(S_i + K_m)}{VK_m} \cdot (S_0 + K_m) \tag{4}$$

with

$$S_0 = -K_m \text{ for } \frac{S_i - S_0}{v} = 0 \text{ (abscissa intercept)} \tag{4a}$$

and

$$\frac{S_i - S_0}{v} = \frac{(S_i + K_m)}{V} \text{ for } S_0 = 0 \text{ (ordinate intercept)} \tag{4b}$$

To avoid arbitrariness the evaluation was not carried out graphically but by calculation of the regression line and the intercepts directly from the experimental data.

The internal concentration S_i was calculated from the intracellular amount of sugar, S (as obtained by the indirect osmotic method used) according to the equation

$$S_i = \frac{S}{S+1}(S_0 + 1) \tag{5}$$

This equation indicates osmotic equilibrium and assumes a reflection coefficient close to 1.0. It uses "cell units" according to Jacobs (unit of concentration = isotonicity, unit of volume = cell volume in isotonic medium).

Where numerical values of experimentally determined apparent parameters are given in this paper, these units are likewise used (in Figs 1 and 2).

Initial data ($t=0$) were used for v and for S_i. As in the experiments of Sen and Widdas[3] also with the indirect method used in our experiments the time-course of S initially approaches linearity sufficiently to allow satisfactory determination of v (ref. 7).

RESULTS

The numerical results of the determination of K_m and V are presented in Fig. 1. Each value is the mean from six experiments ± S.E. The pH dependence of the two parameters is inversely related, V showing a maximum, K_m a minimum at pH 7.4.

In view of the indirect nature of the osmotic method used for these experiments a set of experiments using isotope methodology was performed later. The external glucose concentrations in these experiments were zero and 0.1 isotonic. The method of evaluation was the same as that used in the osmitic experiments. The pH range was somewhat larger, the temperature 20 °C. The results are shown in Fig. 2. Between pH 6 and 9.6 the pH dependence of the apparent parameters "K_m" and "V" is again inversely related. At pH values <5 and >10 (not shown in the figure) the inverse relation between is "K_m" and "V" is not maintained.

These results are at variance with those of Sen and Widdas[3] who reported approximately linear increase of both K_m and V with rising pH. They resemble, however, those of Faust[1] and of Lacko *et al.*[2] in the observation of a maximum for V near pH 7.1.

The difference between our results and those of Sen and Widdas[3] can only be tentatively interpreted. The scatter of results obtained by osmotic methods is

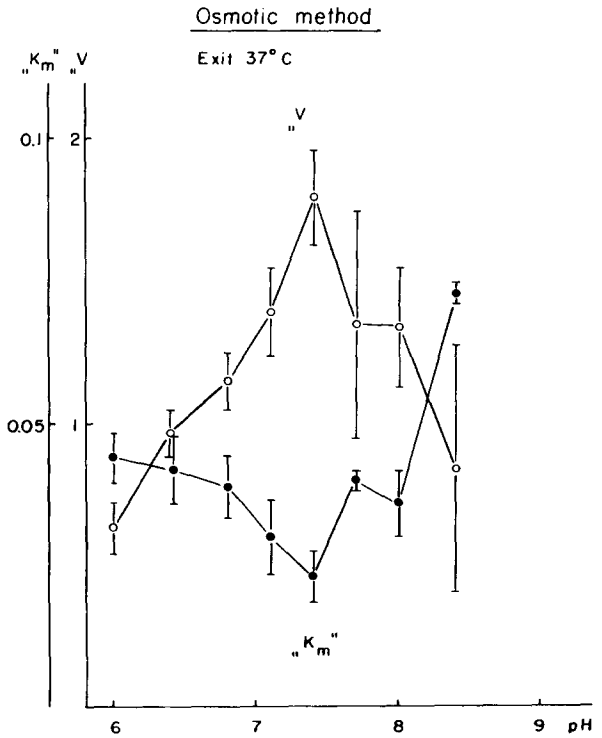

Fig. 1 Parameters of the glucose transport system in the human red cell membrane (as evaluated from exit experiments at 37 °C according to a modified Sen–Widdas method) as a function of external pH. The points give means from 6 experiments, each ±S.E. Units: multiples of isotonicity for K_m, multiples of isotonicity per min for V.

in general considerable. It is quite possible that the indirect method used in our experiments is, in this respect, somewhat more favourable than the direct method used by Sen and Widdas. But, as Fig. 1 shows, in the experiments performed with the indirect method the variation is also relatively high. We consider, therefore, the data given in Fig. 2 as a valuable confirmation of our results, not only because a different and independent method was used in these experiments but also because the variation in the experiments is less than in those with osmotic methodology. The fact that the variation of "K_m" with pH is inversely related to that of "V" would be an unlikely coincidence if "K_m" and "V" were independent entities, as they are tacitly thought to be in the assumptions underlying the derivation of Eqn 1. If, however, under expanded conditions, the two parameters are composite terms involving common components, an effect on one of these components may be the basis of inverse variation.

It was shown previously that the assumption of unequal mobilities for free and loaded carrier results in the appearance of "apparent parameters", K_m and V involving the ratio of the two mobilities[8]. This raises the question whether such apparent parameters may under certain condition show inverse variation and what these conditions are.

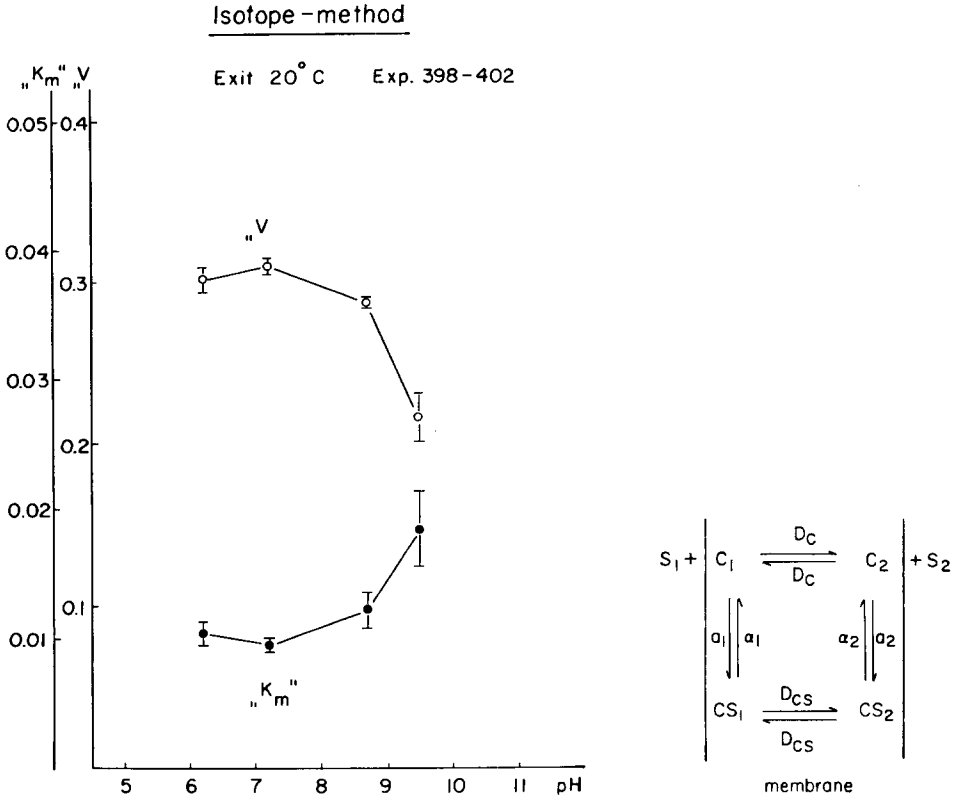

Fig. 2. Results of experiments resembling those presented in Fig. 1 but carried out with radioisotope methodology at 20 °C. Means from 5 experiments ± standard error of the mean. Units as in Fig. 1.

Fig. 3. Scheme of the carrier system under general conditions. Concentrations: S=substrate, CS=carrier substrate complex, C=free carrier. Rate constants: D_C and D_{CS} mobilities of free and loaded carrier. α and a, rate constants of the reaction between substrate and carrier.

In the following discussion a more general treatment[9] will be given which includes the possible effect of reaction rates (frequently assumed to be high, and therefore neglected).

In the derivation of Eqn 1 two special conditions were assumed: equal mobilities of free and loaded carrier ($D_C = D_{CS} = D$) and rapid reaction between substrate and carrier ($\alpha \gg D$) (fig. 3). Relaxing both these simplifying conditions, according to the scheme shown in Fig. 3, yields for the transfer rate in the steady state instead of Eqn. 1:

$$v = 2C_t \frac{S'_1 - S'_2}{\frac{2}{\alpha_1}(S'_2+1) + \frac{2}{\alpha_2}(S'_1+1) + \frac{1}{D_{CS}}(S'_1+1+S'_2+1) + \frac{1}{D_C}[S'_1(S'_2+1)+S'_2(S'_1+1]} \quad (6)$$

(with $S'=S/K_m$ and rate constants according to Fig. 3), or, substituting m_1 for $1/\alpha_1 C_t$, m_2 for $1/\alpha_2 C_t$, x for $1/D_{CS}C_t$ and y for $1/D_C C_t$ and adapting to the plot F *vs* S_2 (*cf.* Eqn 4) in our evaluation:

$$F \equiv \frac{S_1 - S_2}{v} = S_2\left(\frac{x+y}{2} + m_1 + y\frac{S_1}{K_m}\right) + S_1\left(\frac{x+y}{2} + m_2\right) + K_m(x+m_1+m_2) \qquad (7)$$

This equation shows that F again is a linear function of S_2. The meaning of the intercepts, however, is more complex. The abscissa intercept ($F=0$) now is

$$\text{“}K_m\text{”} = K_m \frac{S_1\left(\frac{x+y}{2} + m_2\right) + K_m(x+m_1+m_2)}{S_1 y + K_m\left(\frac{x+y}{2} + m_1\right)} \qquad (8)$$

(with “K_m”, apparent constant as obtained by the evaluation and K_m, true constant) and the ordinate intercept ($S_2=0$) is

$$S_1\left(\frac{x+y}{2} + m_2\right) + K_m(x+m_1+m_2) \qquad (9)$$

In order to make these complex terms more useful it is convenient to recall that for D-glucose in our experiments $S_1 \gg K_m$ and to neglect the additive terms with K_m yielding the relations analogous to Eqns 4a and 4b:

$$\text{“}K_m\text{”} = K_m \frac{\frac{x+y}{2} + m_2}{y} \qquad (10)$$

and

$$\frac{1}{\text{“}V\text{”}} = \left(\frac{x+y}{2} + m_2\right) \qquad (11)$$

If the mobilities are different ($D_C \neq D_{CS}$) but the rate of reaction high ($m_2 \ll x, y$) the equations reduce further:

$$\text{“}K_m\text{”} = K_m \frac{x+y}{2y} \qquad (12)$$

$$\text{“}V\text{”} = \frac{2}{x+y} \qquad (13)$$

On the other hand for $D_C = D_{CS} = D$ and $1/C_t D = u$

$$\text{“}K_m\text{”} = K_m \cdot \frac{u+m_2}{u} \qquad (14)$$

$$\text{“}V\text{”} = \frac{1}{m_2+u} \qquad (15)$$

As Eqns 12 and 14 as well as Eqns 13 and 15 show, the two cases give formally similar expressions for the apparent parameters. They can, therefore, not be distinguished without further data.

From Eqns 10 and 11 the following situation results. If one (and only one) of the four resistance terms m_1, m_2, x and y is changed, the effect of this change on "K_m" and "V" is different depending on which term is changed, as shown in the following table (Table I).

TABLE I

Change in resistance term	*Effect on apparent parameters*	
	"K_m"	"V"
Increase in m_1	No change	No change
Increase in m_2	Increase	Decrease
Increase in x	Increase	Decrease
Increase in y	Decrease	Decrease
Increase in x and y (x/y=constant)	No change	Decrease

Thus, a change in m_1 ("*cis*" reaction resistance) has no effect, a change in m_2 ("*trans*" reaction resistance) or in x (translocation resistance of loaded carrier) elicits opposite changes in "K_m" and "V" and a change of y (translocation resistance of free carrier) parallel changes. Finally a simultaneous change in x and y such that x/y remains constant will affect "V" but not "K_m".

Therefore, the results reported here and shown in Figs 1 and 2 can not be due to a pH dependence of y or of m_1 but could well arise from a pH effect on x or on m_2, *i.e.* on the mobility of loaded carrier or on the reaction rate on the outside of the membrane (or both). With the reserve in mind that a change in m_2 cannot be ruled out the following considerations will be confined to the condition $D_C \neq D_{CS}$, $m_2 \to 0$.

DISCUSSION

The results of this study indicate an inverse relation between the variation of "K_m" and "V" with pH as evaluated by a modified Sen–Widdas method. It was shown that this result is predicted from kinetics if either unequal mobilities of free and loaded carrier are assumed or a rate limiting reaction velocity between substrate and carrier (or both), and if, furthermore, either the reaction rate α_2 or the mobility D_{CS} (Fig. 3) is pH dependent. In the case of D_{CS} the pH effect must mot necessarily be exclusive but a possible simultaneous effect on D_C must be at least less pronounced than that on D_{CS}. In the case of m_2, however, any simultaneous pH effect on m_1 is compatible with the data since m_1 does not enter into the apparent parameters at all. Thus, *e.g.* the effect on the reaction rate may well be symmetrical.

It should be kept in mind that predictions as to apparent parameters depend on the method of evaluation. Thus, Table 1 holds for evaluation by the Sen–Widdas method, but not, in general, by other procedures.

The conclusion reached with respect to a possible pH effect on D_{CS} is in accordance with the result of a thermodynamic analysis of the temperature dependence of the same parameters, as shown in a previous publication[10].

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