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The kinetics of facilitated diffusion followed by enzymatic conversion of the substrate

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Abstract

The uptake of a substrate that is transported into the cell by facilitated diffusion and subsequently converted in a series of enzymatic reactions, measured as a function of the external concentration, does not usually show the rectangular hyperbolic function characteristic of Michaelis–Menten kinetics. Instead, a seemingly biphasic curve is observed, consisting of Michaelis–Menten kinetics at low concentrations and no further uptake at higher levels. By combining the equations for facilitated diffusion and an enzymatic reaction, we have derived an equation that describes the overall rate of uptake and metabolism of a substrate that is transported across the plasma membrane by facilitated diffusion. Modelling based on this equation simulated the kinetics found experimentally, as long as the kinetic parameters of the carrier were chosen to render it asymmetric. The overall rate was influenced by the kinetics of both reactions over a wide range of concentrations, confirming the principles of the 'Control Analysis' theory in an independent manner.

Key words: Enzyme kinetics; Facilitated diffusion; Membrane transport; Modeling

1. Introduction

Facilitated diffusion equilibrates the concentration of a compound on both sides of a biological membrane in a carrier mediated process. This transport mechanism does not require energy and is primarily found in eukaryotic cells for the uptake of substrates that are rapidly metabolized [1–3]. It is tempting to reason that the uptake of a substrate by facilitated diffusion is effective only when the subsequent metabolism keeps the internal substrate concentration low. In this view, the metabolic pathway acts as a concentrating mechanism. If so, the kinetics of this trapping step should influence the overall uptake rate.

In general, experimental work on transporters is based on a separation of the transport step and the subsequent metabolism. One way to achieve this separation is to use non-metabolizable analogues, that by definition have to differ from the real substrate and thus may give misleading results. In addition this approach may lead to difficulties in studies on facilitated diffusion, because the internal concentration can never exceed the external. Hence the signal is much smaller than in the case of active carriers that can concentrate substrates by a factor exceeding 10^4. Another strategy is to use ultra-short incubations, giving low signal as well. Therefore alternative methodology circumventing these problems is necessary. By combining the rate equations for uptake by facilitated diffusion and that step of the subsequent metabolism that exerts most of the flux control, an equation for the overall process was derived (see below). Modelling according to this equation showed that the flux control over a pathway can be shared between the transport and subsequent steps, with control coefficients varying with changing experimental conditions, as was demonstrated for amino acid metabolism [4]. This modeling provided a valid method for estimating the kinetics of the carrier and simulated the biphasic uptake curves observed experimentally for such systems [5–7].

In addition analysis of kinetic data using this approach is likely to provide new insights on systems under dispute, such as the human red blood cell glucose carrier [2,3]. Widely different mechanisms have

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been proposed to account for the change in kinetic properties of glucose transporters in yeasts [8]. These include one carrier with a K_m oscillating between 2 and 70 mM [9], three different carriers induced by glucose availability [10], or only two different carriers, one facilitated diffusion, one active transport [11]. Analysis of uptake data as we propose will distinguish between apparent changes of the kinetics of the transporter induced by adaptation of the metabolic pathway and the actual induction of a different type of carrier.

2. Materials and methods

Mathematical modeling was performed on a Sun microcomputer using standard Unix software tools in a program of our own design.

3. Results and discussion

3.1. Derivation of the equation

When cells have for some time been metabolizing a substrate that is present in more or less constant concentrations and is transported by facilitated diffusion, a steady state is reached in which the net rate of transport across the plasma membrane equals the metabolic rate:

$$V_t = V_e = V \tag{1}$$

where V_t is the net transport rate, V_e is the rate of the first metabolic step and V is the overall rate. The net transport rate (V_t) is given (see [2]) by:

$$V_t = \frac{K(S_1 - S_2)}{K^2R_{00} + KR_{12}S_1 + KR_{21}S_2 + R_{ee}S_1S_2} \tag{2}$$

where K is an affinity parameter, S_1 and S_2 are the external and the internal substrate concentrations respectively, R_{00} R_{12}, R_{21} and R_{ee} are resistance parameters determining the kinetics of the transporter, as defined in [2], p. 246, Eq. (4.6) and p. 250. When S_2 equals (almost) 0, the equation simplifies to:

$$V_t = \frac{KS_1}{(K^2R_{00} + KR_{12}S_1)} \tag{3}$$

which can be rearranged to the form of a Michaelis–Menten equation:

$$V_t = \frac{1}{R_{12}} \frac{S_1}{(KR_{00}/(R_{12}) + S_1)} \tag{4}$$

where $1/R_{12}$ equals V_{max} and $K_m = KR_{00}/R_{12}$, therefore the kinetics for the zero-trans reaction (the internal substrate concentration is 0) can be described in the terms of a Michaelis–Menten equation.

The metabolic reaction trapping the substrate depends on S_2 according to Michaelis–Menten kinetics:

$$V_e = V_{e_{max}} \frac{S_2}{(K_m + S_2)} \tag{5}$$

Eq. (5) can be rearranged to solve for S_2 as follows:

$$S_2 = K_m(V/V_{e_{max}} - V) \tag{6}$$

Combining Eqs. (2 and 6) gives Eq. (7):

$$V = \left[KS - KK_m\left(V/(V_{e_{max}} - V)\right)\right] \Big/ \times \left[K^2R_{00} + KR_{12}S + KR_{21}K_m\left(V/(V_{e_{max}} - V)\right) + R_{ee}SK_m\left(V/(V_{e_{max}} - V)\right)\right] \tag{7}$$

where $S = S_1$.

Eq. (7) has been solved for V by standard algebraic methodology. The derivation is available from the authors on request. A comparable approach has been taken in [1]; however, these authors have not solved the equation for all cases. The generally valid outcome is the solution of a quadratic equation as follows:

$$V = \frac{1}{2}\left\{\frac{V_{max}(KR_{00} + R_{12}S) + S + K_m}{KR_{00} + R_{12}S - K_m(R_{21} + (R_{ee}S/K))} - \left[\left(\frac{V_{max}(KR_{00} + R_{12}S) + S + K_m}{KR_{00} + R_{12}S - K_m(R_{21} + (R_{ee}S/K))}\right)^2 - 4\frac{V_{max}S}{KR_{00} + R_{12}S - K_m(R_{21} + (R_{ee}S/K))}\right]^{1/2}\right\} \tag{8}$$

When $S = 0$, V must be 0 (no substrate, no transport, no metabolism). Substituting $S = 0$ gives:

$$V = 1/2\left[\frac{V_{max}KR_{00} + K_m}{KR_{00} + K_mR_{21}} \pm \sqrt{\left(\frac{V_{max}KR_{00} + K_m}{KR_{00} + K_mR_{21}}\right)^2}\right] \tag{9}$$

Because all parameters are positive and not zero, only the '−' version of the equation is biologically valid. The Hill–Whittingham equation, a similar equation describing the overall rate of a simple diffusion step followed by an enzymatic reaction [12] is also only valid in the '−' form.

3.2. Experimental applicability

Time course experiments for the uptake of labelled glucose by organisms that use facilitated diffusion as transport mechanism generally show three phases: (1) an initial rapid uptake caused by equilibration of the label inside and outside of the cell; (2) a steady increase in the amount of label incorporated due to conversion of glucose into metabolites and storage

carbohydrates. When utilizing metabolizable substrates, this situation occurs most often; (3) no further, or at least strongly reduced, uptake when all internal pools have been saturated and the efflux of end products equals the uptake of new label. The kinetics described by Eq. (8) is only observed in organisms in phase 2. Phase 1 yields the Michaelis–Menten kinetics of the carrier and phase 3 that of the long-term flux controlling step or of the combined steps exerting most control according to the flux control theory. Even when interpreting experiments measuring uptake in phase 2, it must be remembered that it is assumed that the trapping step is non-reversible. In those cases in which kinetics predicted by Eq. (8) have been observed [5–7], transport of glucose followed by phosphorylation by hexokinase in kinetoplastids, this assumption is correct, because the reaction forming glucose 6-phosphate is almost irreversible. This step also has been shown to control the glycolytic flux [13]. In other systems, the apparent kinetics of the second reaction may reflect the kinetics of the metabolic pathway as a whole, rather than one rate controlling step.

3.3. Modelling

A computer model based on Eq. (8) has been used to predict V as a function of S while varying the kinetic parameters, both of the transporter and of the enzymatic reaction (Figs. 1 and 2). A general constraint for facilitated diffusion carriers is that $R_{oo} + R_{ee} = R_{12} + R_{21}$ [2]. When $R_{oo} \neq R_{ee}$ and/or $R_{12} \neq R_{21}$, the carrier is asymmetric; the kinetics for influx and efflux differ, however, another constraint holds: $K_{m1}\ V_{max2} = K_{m2}\ V_{max1}$. The aim of the computer modeling was to simulate the seemingly biphasic curve for dependence of glucose uptake and metabolism on the external concentration observed experimentally in the parasitic protozoa *Trypanosoma brucei* [5,6] and *Trichomonas vaginalis* [14]. Michaelis–Menten kinetics were observed at low substrate concentrations and no addi-

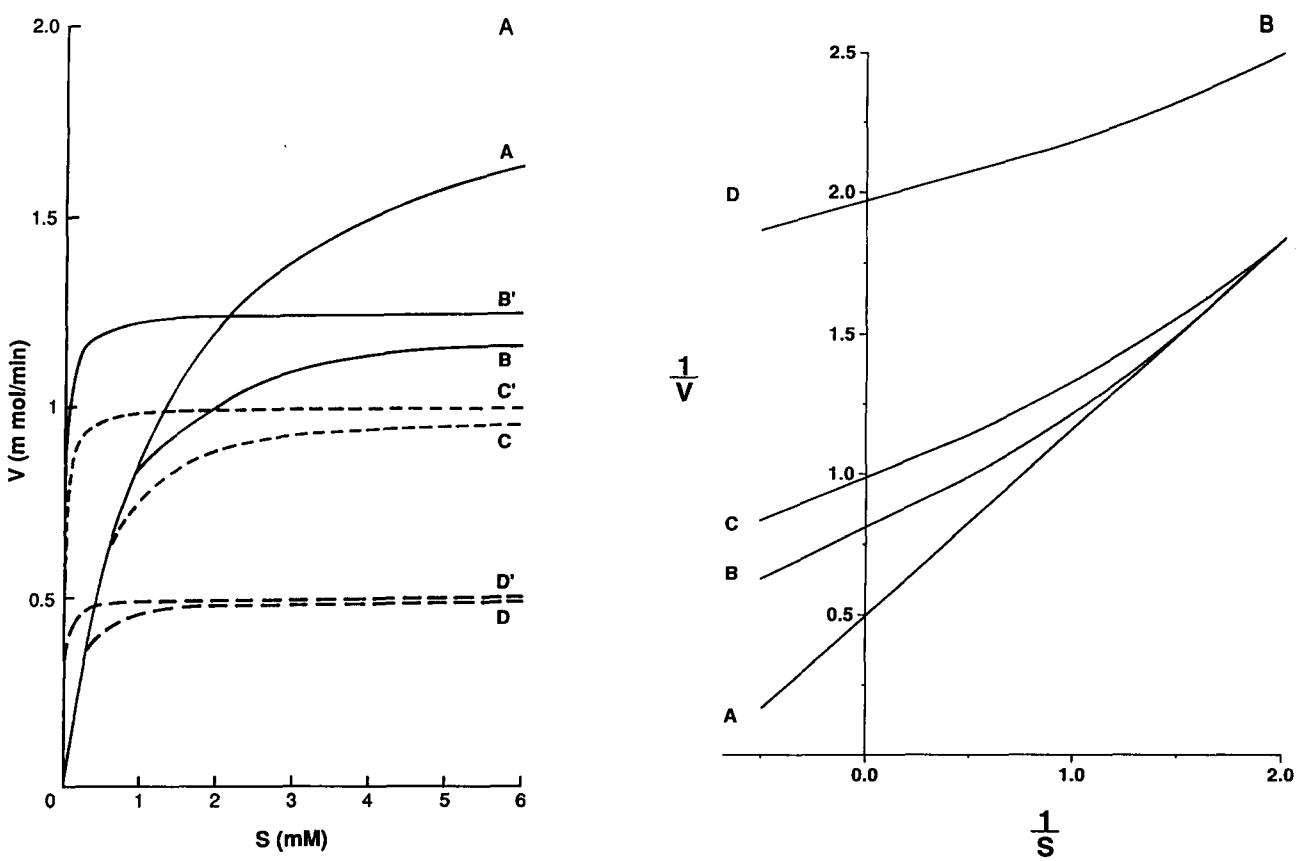

Fig. 1. (A) Model curves based on the kinetic properties of a facilitated diffusion carrier and an enzymatic reaction trapping the transported substrate. The resistance parameters and the V_{max} for zero-trans influx of the human red blood cell uridine transporter [15] were adopted for the carrier (R_{oo} = 2.25 g min/mmol, R_{12} = 0.505, R_{21} = 1.88, R_{ee} = 0.133, V_{max} = 1.98 mmol/min and K^m = 1.29 mM). The reaction trapping the substrate is assumed to obey Michaelis–Menten kinetics with a K_m of 0.02 mM. The V_{max} is varied from 2.5 (A), 1.25 (B), 1 (C) to 0.5 (D) mmol/min. The kinetics of the carrier for zero-trans uptake are featured in curve A, because S_2, the internal concentration, remains (close to) zero due to the high rates of the trapping reaction. The Michaelis–Menten kinetics of the trapping reactions with reduced V_{max} are shown as B', C' and D', respectively. B: The same curves as in Fig. 1A replotted in the double-reciprocal format. Note that the estimated kinetics depends on the concentration range of the measurement.

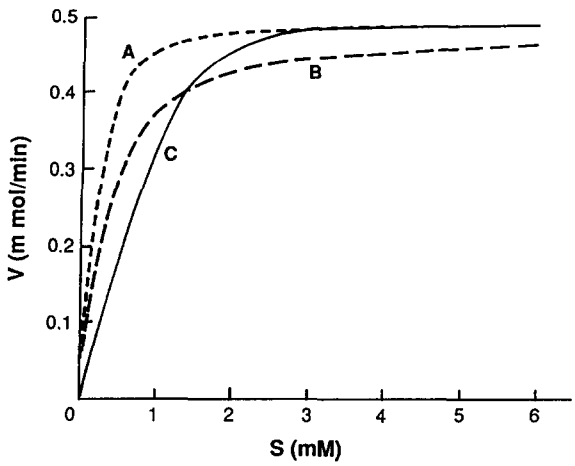

Fig. 2. Model curves demonstrating the different kinetics for the overall reaction while the V_{max} of the trapping reaction is kept constant. Curve B is derived from curve A (= curve D of Fig. 1) by a 5-fold increase of the K_m of the trapping reaction. Curve C is derived from curve A by a 5-fold lowering of the V_{max} of the carrier, while keeping the K_m constant.

tional uptake after a cross-over concentration had been reached. These kinetics were qualitatively interpreted as rate-limitation by the transport step at low concentrations and by the subsequent metabolism at increased levels. When the kinetic parameters of the carrier were chosen to render the carrier symmetric ($R_{oo} = R_{ee} = R_{12} = R_{21}$) that type of seemingly biphasic kinetics could not be simulated (data not shown). When the kinetic parameters of the uridine transporter of the human red blood cell membrane, which is asymmetric by a factor of 3.74 [15] were substituted, curves closely approaching the experimentally observed curves were obtained by assigning smaller K_m and V_{max} values to the second reaction (Figs. 1A and 2).

In 'Metabolic Control Analysis' [16,17] a so called 'Control coefficient' is assigned to each step of the pathway studied. This control coefficient is defined as the ratio of the change of the rate of the overall pathway and the (small) change in the rate of the step in question, the conditions for all other steps remaining unaltered. The coefficient equals 0 if no change in the rate of the overall pathway occurs and 1 if the relative change of this one step and the pathway are equal. In Fig. 1A only the V_{max} of enzymatic reaction that follows the transport step and thus traps the substrate in the cell is altered. The model curves suggest that at low external concentrations the kinetics of the transporter exert most control over the overall rate, at higher concentrations the overall rate approaches the V_{max} of the enzymatic step (Fig. 1A). At the concentration where the rate of each of the two steps, and thus their control coefficients, would have been equal, the overall rate of the transport and metabolic step combined is up to 20% lower than the slowest of the separate steps. In most of the range, both steps share the control over the metabolic flux, while the coefficients change from almost 1 for the transport step and 0 for the metabolism at low concentrations to almost 0 and 1 respectively at the higher concentrations. This system therefore illustrates and confirms the principles of 'Control Analysis' theory [16,17] by a complementary and independent demonstration that the control of a metabolic flux can be shared between steps. Eq. (8) solves for one particular case, which can explain experimental results [5,6,14] in a manner that agrees with the biology of the system. Modeling according to Eq. (8) does allow reasonably precise estimates of the kinetics of the transporter, as long as sufficient measurements in the relevant range of concentrations are available. It is more difficult to estimate the K_m of the enzymatic reaction, which in reality may represent the kinetic properties of the overall metabolic pathway, rather than one rate controlling step.

The data of Fig. 1A are replotted in Fig. 1B using the double reciprocal plot. Curve A yields a straight line giving the kinetics of the transporter, while the other plots are curved. Given the scatter of experimental data, it will in most cases be possible to fit a straight line. The estimated K_m and V_{max} will always be lower than those of the transporter and higher than those of the rate-controlling metabolic step. The relative contribution of each will depend on the range of concentrations used in the measurements. In the lower range the kinetics of the transporters makes the dominant contribution, in the higher range the metabolic step. This may explain the low K_m values found for the glucose transporter of the bloodstream of *Trypanosoma brucei* with glucose as substrate [18,19], but not when the non-metabolizable analogue 6-deoxy-D-glucose is used [18], or when correcting for the influence of the metabolic step at higher concentrations [5].

The curves of Fig. 2 illustrate how other changes in the kinetics of both steps influence the overall metabolic flux. Decreasing the affinity of the second step by increasing the K_m 5-fold drastically decreased the total flux, even at the lower concentrations, where the carrier is supposed to be rate limiting (Fig. 2). This effect is due to an increase of S_2, the internal concentration of the non-metabolized substrate, which increases the counterflow through the carrier, thus reducing the net flow. If the maximum rate of the carrier is reduced while keeping its K_m constant, a curve differing even more distinctly from the rectangular hyperbole is obtained (Fig. 2 curve C). This mutual influence of the trapping step and the facilitated diffusion process indicates that trapping of the substrate by a high-affinity enzymatic reaction can give the combined process the properties of active transport. This in turn suggests

that facilitated diffusion is particularly effective for the uptake of rapidly converted substrates, because low internal concentrations result in high net transport rates.

3.4. Applicability to other systems

The kinetics of glucose uptake by yeasts are reportedly unusual and complicated, with carriers whose "apparent K_m would oscillate between 2 and 70 mM " [9] or three different transport systems varying with culture conditions [10]. Some of these findings may now be understood as one type of facilitated diffusion carrier [8] interacting with the subsequent metabolism of glucose. For this it must be assumed that both the transport and the enzymatic activities can be adapted to the culture conditions, as was found in protozoa [6]. The observation that the high affinity transport depended on the ability to phosphorylate the sugar [20] is strong supporting evidence for this conclusion. Many of the Eadie–Hofstee plots for glucose uptake by yeasts show a discontinuity e.g. [10,20,21], which can possibly be explained by the type of kinetics predicted by Eq. (8). When modelling according to Eq. (8) cannot give a satisfactory fit to the experimental data, other mechanisms, such as the induction of different carriers must be assumed.

In conclusion, we suggest that the interaction of facilitated diffusion and the subsequent metabolism of a substrate is a common phenomenon, that has not been analyzed as such. Eq. (8), which is based on purely theoretical considerations, predicts exactly the kinetics observed for substrates that are taken up by facilitated diffusion and rapidly metabolized after entering the cell. The outcome of this study suggests that facilitated diffusion is particularly suitable for substrates that are rapidly converted into metabolites that can no longer exit the cell.

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