Biochimica et Biophysica Acta, 1145 (1993) 180–182

BBAMEM 75885

Rapid Report

Misuse of graphical analysis in nonlinear sugar transport kinetics by Eadie-Hofstee plots

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(Received 9 November 1992)

Key words: Graphical analysis, misuse; Sugar transport kinetics, nonlinear; Kinetics; Eadie-Hofstee plot; (*S. cerevisiae*)

It has become common practice to analyse the sugar transport kinetics from initial uptake rates in *Saccharomyces cerevisiae* cells with Eadie-Hofstee plots. These plots often demonstrate a nonlinear behaviour. They have been resolved incorrectly into two quasilinear components indicating the presence of (at least) two uptake systems or components, with K_m values differing by a factor of about 10. This graphical analysis neglects the obvious additivity of the two hypothetical systems and is therefore in error. A more efficient way to determine kinetic parameters from initial uptake experiments is to use computer-assisted nonlinear regression analysis.

It has been postulated that sugar uptake in *Saccharomyces cerevisiae* may involve two different types of systems, distinguishable on the basis of apparent affinity for the sugar [1]. This assumption was made from sugar uptake experiments in *Saccharomyces cerevisae* cells using only 5-s uptake periods at 30°C over a range from 0.2 to 200 mM substrate concentrations. The data were plotted as initial velocity, v (nmol sugar/min per mg wet weight of cells) versus the initial velocity/substrate concentration (v/S) according to the Eadie-Hofstee equation:

$$v = -K_m \cdot (v/S) + V_m \quad (1)$$

A system with Michaelis-Menten kinetics would give a straight line with a slope equal to $-K_m$. It has become commonly the practice to analyse the kinetics from initial sugar uptake rates in *Saccharomyces cerevisiae* cells with Eadie-Hofstee plots by graphical estimation of the slope (apparent K_m value) and the interception with the ordinate (y), which corresponds to the V_{max} value. This method is applicable only at first as long as the initial uptake period for sugars is short enough preventing significant efflux by the facilitated diffusion system of the cell and at second as long as the slope obtained by this method is linear.

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However, it has been shown continuously and recently by many examples given in the literature [1–14] that very often Eadie-Hofstee plots from data of initial sugar uptake experiments in *Saccharomyces cerevisiae* are *not linear*, but quasi biphasic. By graphical analysis of two such quasilinear portions of the v vs. v/S plot the existence of two transport systems with a set of two different transport parameters were postulated. One prominent example (see inset of Fig. 1b) comes from initial glucose uptake rates in the *Saccharomyces cerevisiae* wild-type strain DFY1 [1]. The 'analysis', for which the Eadie-Hofstee plot was distinctly nonlinear, gave at least two different apparent K_m values, differing by a factor of about 10, with 1.5 ± 0.25 and 20 ± 8 mM.

Following this example [1] it has been widely accepted for *Saccharomyces cerevisiae* cells that the uptake of glucose is mediated by at least two types of transport, distinguishable as low-K_m around 2 mM (high-affinity) and high-K_m of about 20 mM (low-affinity) systems. The presence and the absence of the so called high-affinity and low-affinity slopes were taken as indications for the appearence and disappearance of visible functions of products of putative transporter genes [13–15].

The aim of this short communication is to demonstrate that the graphical analysis of the two quasilinear slopes constructed from nonlinear Eadie-Hofstee plots is in error. Therefore it is necessary to call attention to this kinetic approach, which unfortunately is practiced by many authors and working groups [1–15]. We have

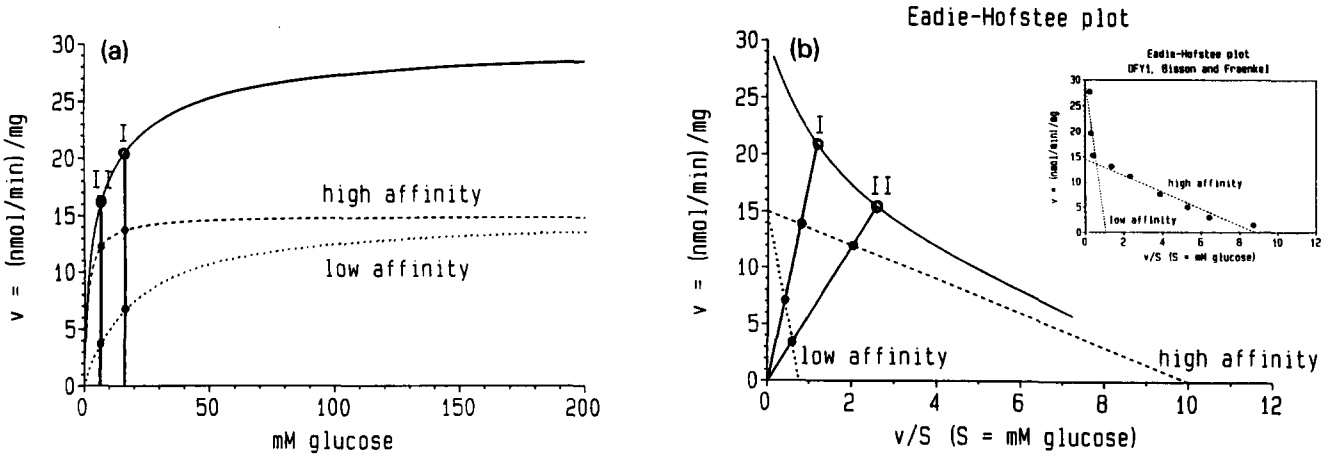

Fig. 1. (a) Initial sugar uptake rate versus glucose concentration. The dashed line represents the high-affinity system ($K_m = 1.5$ mM and $V_m = 15$ nmol min^{-1} mg^{-1}), the dotted line the low-affinity system ($K_m = 20$ mM and $V_m = 15$ nmol min^{-1} mg^{-1}) and the solid line both systems together. (b) The transport models of Fig. 1a in the Eadie-Hofstee form. The radial lines I and II depict two different glucose concentrations. The corresponding concentrations are also shown in Fig. 1a. (Inset) Initial glucose uptake data of the wild-type DFY1 (taken from Bisson and Fraenkel [1], Fig. 3A).

expressed this concern previously [16] and discussed the matter at respective specialist meetings [17,18].

By assuming that two Michaelis Menten systems are operative for transport the following simple equation is constructed:

$$v = [V_{max1} \cdot S/(K_{m1} + S)] + [V_{max2} \cdot S/(K_{m2} + S)] \quad (2)$$

By conversion of Eqn. 2 to the Eadie-Hofstee form Eqn. 3 it is already obvious that no simple two linear slopes can be expected:

$$v = -K_{m1} \cdot (v/S) + V_{m1} + V_{m2} \cdot [(K_{m1} + S)/(K_{m2} + S)] \quad (3)$$

In Fig. 1a a graphical demonstration of Eqn. 2 is given. By addition of the high-affinity system ($K_{m1} = 1.5$ mM and $V_{max1} = 15$ nmol/min per mg, dashed line) to the low-affinity system ($K_{m2} = 20$ mM and $V_{max2} = 15$ nmol/min per mg, dotted line) the solid line is obtained. Fig. 1b shows the correponding example for the Eadie-Hofstee form calculated from Eqn. 3. Notice that the result is a curve! By drawing a line through the quasilinear steep and the shallow part of the solid curve the errors are recognized at a glance. Both new slopes obtained are distinctly different from those of which each affinity system has been separately constructed (dashed and dotted line). In order to demonstrate the additivity in the Eadie-Hofstee form two radial lines representing two different substrate concentrations due to the transformation of the abscissa are depicted (from the origin to points I and II on the curve; the corresponding lines are also drawn in Fig. 1a). By the method proposed by Rosenthal [19] it can be shown that the length of each radial line I and II is the sum of the lengths from origin to the intersections at the dashed and dotted line.

The method of Rosenthal is superseded by computer assisted nonlinear regression analysis (Ref. 20, for example). The analysis of the experimental data for glucose uptake in the DFY1 wild type strain [1] can be fitted exactly by two Michaelis-Menten terms. The analysis is consistent with an apparent K_m value of 1.4 mM and a second one with a few M! This value is indeed far away from the socalled low-affinity K_m of about 20 mM. We assume as a working hypothesis that this high K_m value of a few M represents rather likely a diffusion process. We have analysed so far many initial sugar uptake experiments in *Saccharomyces cerevisiae* cells in our own laboratory and also data taken from the literature. Best fitting in a great number of experiments was accomplished with one Michaelis-Menten and one diffusion term. The so-called low affinity system of about 20 mM advocated by Bisson and Fraenkel [1] does probably not exist in reality and has resulted from an underestimation of K_m values derived from an erroneous graphical analysis of nonlinear Eadie-Hofstee plots. Until there is no other explanation we have to assume that nonspecific diffusion accounts to the socalled low-affinity transport.

This work was supported by the Deutsche Forschungsgemeinschaft and by Fonds der Chemischen Industrie.

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