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KINETIC ANALYSIS OF SIMULTANEOUSLY OCCURRING PROTON-SORBOSE SYMPORT AND PASSIVE SORBOSE TRANSPORT IN *SACCHAROMYCES FRAGILIS*

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Summary

Sorbose transport in *Saccharomyces fragilis* takes place both via an active sugar-H^+ symport system and via facilitated diffusion.

To establish whether the two modes of transport proceed via the same transporter or via two different carriers, the kinetic consequences of both models were investigated. The kinetic equations for initial transport were derived for three possible reaction sequences with respect to sugar and H^+ binding to the symport carrier: random binding and obligatory ordered binding with either sugar or H^+ binding first, yielding six sets of kinetic parameters.

Analysis of experimental data of sorbose transport in *S. fragilis* showed the existence of separate carriers for active, sorbose-H^+ symport and facilitated diffusion. Furthermore, it could be concluded that the symport carrier shows random binding of sugar and H^+.

In recent literature, a similar combination of active and passive sugar transport in *Rhodotorula gracilis* and *Chlorella vulgaris* was interpreted as two modes of action of the same carrier, viz., active symport via the protonated, and facilitated diffusion via the unprotonated carrier. Analysis of the experimental data according to the criteria presented in this paper showed, however, that this supposition is untenable and that two different carriers must also be involved in these micro-organisms.

Introduction

In yeast cells at least three different transport mechanisms for sugars are operative. Depending on the kind of sugar and on the yeast strain, the sugar

can enter the cell by means of energy-independent facilitated diffusion or via energy-coupled transport.

Energy-coupled transport in yeast can be divided into two types. In the first type the cells derive the energy, necessary for active transport, from a high-energy phosphate group. For example, *Saccharomyces fragilis* accumulates D-glucose and derivatives by means of this transport-associated phosphorylation [1].

The other mechanism seems to work according to the chemiosmotic hypothesis of Mitchell [2]. In this case, an electrochemical H^+ gradient across the cell membrane provides the energy for uphill transport. This so-called sugar-H^+ symport mechanism has been found not only in yeast [3–5], but also in bacteria [6,7] and algae [8,9]. In most cases, a H^+ : sugar stoichiometry of at least unity is found [6,7,10,11]. Usually it is assumed that the symport really takes place at a 1 : 1 H^+ : sugar ratio, higher values being caused by experimental artefacts. For sorbose transport in *S. fragilis*, however a stoichiometry of 0.5 has been measured [4].

To explain this observation it was suggested that besides active transport of sorbose, facilitated diffusion of this sugar took place simultaneously. This could be analogous to the situation that has been described for 6-deoxyglucose uptake by *Chlorella vulgaris* [11,12] and xylose transport by *Rhodotorula gracilis* [5]. In these organisms two transport mechanisms have been found: an active transport, operative at low pH values in the medium, and a passive uptake, working at high pH. It was assumed that both types of transport proceed via the same carrier. In the protonated form this carrier would have a high affinity for the sugar (active transport) and in the unprotonated form the affinity would be low (facilitated diffusion).

An alternative explanation would be symport and facilitated diffusion proceeding via different carriers. Discrimination between these two possibilities is possible by kinetic analysis of the initial transport velocity, taking into account the possible reaction types of the H^+-sugar carrier, viz., random binding of H^+ and sugar or obligatory ordered binding with either sugar or H^+ binding first. Based on these models six sets of equations were obtained.

As will be shown, the experimental data indicate that in *S. fragilis* sorbose transport occurs both via facilitated diffusion and via H^+-sugar symport, with two different carriers and with a random H^+ sugar binding to the symport carrier.

Theoretical Section

The general model is shown in Fig. 1, comprising several different possibilities.

In the case of facilitated diffusion, for instance, all kinetic constants are zero, except c_1, c_{-1}, g_1, g_{-1}, i and k. With the assumptions that: (1) $S_i = 0$ (initial influx kinetics), (2) the translocator is in equilibrium with ligands in solution, (3) the total number of translocator molecules at the membrane/solution interface is constant, and (4) translocation through the membrane is not affected by the surface potential [13], the rate equation for facilitated diffu-

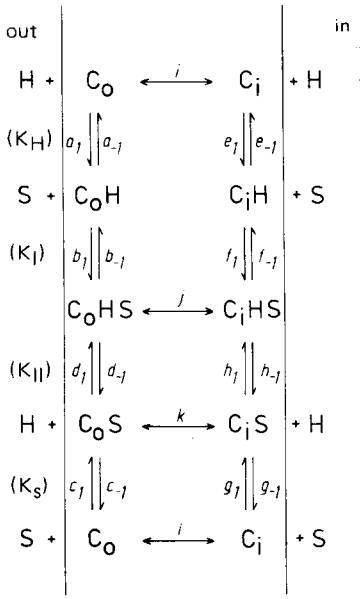

Fig. 1. General transport model, describing both H^+-sugar symport and facilitated diffusion. C_o and C_i, transporter, facing, respectively, the outside or inside of the cell; S, sugar; H, H^+; K_H, K_1, K_{11} and K_S, dissociation constants. a_1, a_{-1}, ... k, velocity constants.

sion is (see Ref. 14):

$$v = \frac{V[S]}{K_s + [S]}$$

These assumptions are not always justified, as shown, e.g., for sugar uptake in red blood cells via facilitated diffusion [14,15].

Without these assumptions similar Michaelis-Menten equations are obtained, however, in which the kinetic parameters are more complex [13,14,16]. For the sake of simplicity we will derive the rate equations under the simplified conditions. This is, in a first approximation, justified, as the main purpose of this approach is to evaluate if the influence of the H^+ concentration on the rate equation can be used to discriminate between the various models of simultaneous H^+-sugar symport and facilitated diffusion.

A. Passive and active transport proceed via different translocators

I. Passive transport. A separate translocator for facilitated diffusion is represented by the lower loop in Fig. 1. The rate equation for the simplified model has the well known form:

$$v_p = \frac{V_p[S]}{K_{s,p} + [S]} \tag{1}$$

or:

$$\frac{1}{v_p} = \frac{1}{V_p} + \frac{K_{s,p}}{V_p} \cdot \frac{1}{[S]} \tag{2}$$

where v = transport velocity = $k[C_0S]$, V_p = maximal transport velocity = kC_t, $C_t = [C_0] + [C_0S]$ and $K_{s,p} = c_{-1}/c_1$ = dissociation constant of the carrier-substrate complex (the index, p, indicates the facilitated diffusion system; the index, a, the symport system). This rate equation will be valid, irrespective of the mode of action, of a simultaneously operating H^+-substrate symport system.

II. Symport system. (A1) Fig. 1 represents a translocator, catalyzing only H^+-substrate symport, when $k = 0$. Assuming random binding of the ligands, the constants:

$$K_H = \frac{[C_0][H]}{[C_0H]} \tag{3}$$

$$K_{s,a} = \frac{[C_0][S]}{[C_0S]} \tag{4}$$

$$K_1 = \frac{[C_0H][S]}{[C_0HS]} \tag{5}$$

$$K_{11} = \frac{[C_0S][H]}{[C_0HS]} \tag{6}$$

have to be considered, with:

$$K_1K_H = K_{11}K_{s,a} \tag{7}$$

As

$$v = j[C_0HS] \tag{8}$$

and

$$V_a = jC_t \tag{9}$$

with

$$C_t = [C_0] + [C_0S] + [C_0H] + [C_0HS] \tag{10}$$

it follows that:

$$v_a = \frac{V_a[H][S]}{K_1(K_H + [H]) + [S](K_{11} + [H])} \tag{11}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a}\left(\frac{K_{11} + [H]}{[H]}\right) + \frac{K_1}{V_a}\left(\frac{K_H + [H]}{[H]}\right)\frac{1}{[S]} \tag{12}$$

rearrangement yields:

$$\frac{1}{v} = \frac{1}{V_a}\left(\frac{K_1 + [S]}{[S]}\right) + \frac{1}{V_a}\left(\frac{K_{11}[S] + K_1K_H}{[S]}\right)\frac{1}{[H]} \tag{13}$$

(A2) With obligately ordered binding, H^+ binding first, $[C_0S]$ will be infinitely low, reducing Eqn. 10 to:

$$C_t = [C_0] + [C_0H] + [C_0HS] \tag{14}$$

and

$$v_a = \frac{V_a[H][S]}{K_1(K_H + [H]) + [S][H]} \tag{15}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a} + \frac{K_1}{V_a}\left(\frac{K_H + [H]}{[H]}\right)\frac{1}{[S]} \tag{16}$$

which is equal to:

$$\frac{1}{v} = \frac{1}{V_a}\left(\frac{K_1 + [S]}{[S]}\right) + \frac{K_1 K_H}{V_a} \cdot \frac{1}{[S]}\frac{1}{[H]} \tag{17}$$

(A3) With obligately ordered binding, sugar binding first, $[C_0H]$ will be infinitely low and thus Eqn. 10 reduces to:

$$C_t = [C_0] + [C_0S] + [C_0HS] \tag{18}$$

and thus:

$$v_a = \frac{V_a[H][S]}{K_{11}K_{s,a} + [S](K_{11} + [H])} \tag{19}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a}\left(\frac{K_{11} + [H]}{[H]}\right) + \frac{K_{11}}{V_a}\frac{K_{s,a}}{[H]} \cdot \frac{1}{[S]} \tag{20}$$

which can be rearranged to:

$$\frac{1}{v} = \frac{1}{V_a} + \frac{K_{11}}{V_a}\left(\frac{K_{s,a} + [S]}{[S]}\right)\frac{1}{[H]} \tag{21}$$

B. Passive and active transport proceed via the same translocator

I. Passive transport. (B1) The velocity of the facilitated diffusion component will be given by $v = k[C_0S]$. Assuming random binding of the ligands for the symport system, it follows from Eqns. 3, 4, 6 and 10, that:

$$v_p = \frac{V_p[S]}{K_s\{(K_H + [H])/K_H\} + [S]\{(K_{11} + [H])/K_{11}\}} \tag{22}$$

or:

$$\frac{1}{v_p} = \frac{1}{V_p}\left(\frac{K_{11} + [H]}{K_{11}}\right) + \frac{K_s}{V_p}\left(\frac{K_H + [H]}{K_H}\right)\frac{1}{[S]} \tag{23}$$

(B2) Assuming obligately ordered binding for the symport system with the H^+ binding first, reaction between C_0S and H^+ will not be possible and thus K_{11} has no meaning. From Eqns. 3—5 and 10 it then follows that:

$$v_p = \frac{V_p[S]}{K_s\{(K_H + [H])/K_H\} + [S]\{(K_H K_1 + K_s[H])/K_H K_1\}} \tag{24}$$

or:

$$\frac{1}{v_p} = \frac{1}{V_p}\left(\frac{K_H K_1 + K_s[H]}{K_H K_1}\right) + \frac{K_s}{V_p}\left(\frac{K_H + [H]}{K_H}\right)\frac{1}{[S]} \tag{25}$$

(B3) Assuming obligately ordered binding for the symport system with the sugar binding first, only K_s and K_{11} have to be considered, whereas the conservation equation reduces to:

$$C_t = [C_0] + [C_0S] + [C_0HS] \tag{26}$$

From Eqns. 4, 6 and 26 it follows that the rate equation of facilitated diffusion is given by:

$$v_p = \frac{V_p[S]}{K_s + [S]\{(K_{11} + [H])/K_{11}\}} \tag{27}$$

or:

$$\frac{1}{v_p} = \frac{1}{V_p}\left(\frac{K_{11} + [H]}{K_{11}}\right) + \frac{K_s}{V_p}\cdot\frac{1}{[S]} \tag{28}$$

II. Symport system. Along the same lines of reasoning the transport velocity of the symport system, utilizing the same transporter as facilitated diffusion, can be calculated. This yields:

(B1) random binding:

$$v_a = \frac{V_a[H][S]}{K_1(K_H + [H]) + [S](K_{11} + [H])} \tag{29}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a}\left(\frac{K_{11} + [H]}{[H]}\right) + \frac{K_1}{V_a}\left(\frac{K_H + [H]}{[H]}\right)\frac{1}{[S]} \tag{30}$$

which is equal to:

$$\frac{1}{v} = \frac{1}{V_a}\left(\frac{K_1 + [S]}{[S]}\right) + \frac{1}{V_a}\left(\frac{K_{11}[S] + K_1K_H}{[S]}\right)\frac{1}{[H]} \tag{31}$$

(B2) H^+ binding first:

$$v_a = \frac{V_a[H][S]}{K_1(K_H + [H]) + [S]\{(K_1K_H + K_s[H])/K_s\}} \tag{32}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a}\left(\frac{K_s[H] + K_1K_H}{K_s[H]}\right) + \frac{K_1}{V_a}\left(\frac{K_H + [H]}{[H]}\right)\frac{1}{[S]} \tag{33}$$

or:

$$\frac{1}{v} = \frac{1}{V_a}\left(\frac{K_1 + [S]}{[S]}\right) + \frac{K_1K_H}{V_a}\left(\frac{1}{K_s} + \frac{1}{[S]}\right)\frac{1}{[H]} \tag{34}$$

(B3) Sugar binding first:

$$v_a = \frac{V_a[H][S]}{K_s K_{11} + [S](K_{11} + [H])} \tag{35}$$

or:

$$\frac{1}{v_a} = \frac{1}{V_a}\left(\frac{K_{11} + [H]}{[H]}\right) + \frac{K_s}{V_a} \cdot \frac{K_{11}}{[H]} \cdot \frac{1}{[S]} \tag{36}$$

or:

$$\frac{1}{v} = \frac{1}{V_a} + \frac{K_{11}}{V_a}\left(\frac{K_s + [S]}{[S]}\right)\frac{1}{[H]} \tag{37}$$

Thus, the total transport velocities are:
A1, from Eqns. 1 and 11:

$$v_t = \frac{[S]\{V_p K_1(K_H + [H]) + K_{s,p} V_a[H]\} + [S]^2\{V_p(K_{11} + [H]) + V_a[H]\}}{K_{s,p} K_1(K_H + [H]) + [S]\{K_1(K_H + [H]) + K_{s,p}(K_{11} + [H])\} + [S]^2(K_{11} + [H])} \tag{38}$$

A_2, from Eqns. 1 and 15:

$$v_t = \frac{[S]\{V_a K_{s,p}[H] + V_p K_1(K_H + [H])\} + [S]^2[H](V_a + V_p)}{K_1 K_{s,p}(K_H + [H]) + [S]\{K_1(K_H + [H]) + K_{s,p}[H]\} + [S]^2[H]} \tag{39}$$

A_3, from Eqns. 1 and 19:

$$v_t = \frac{[S](K_{s,p} V_a[H] + V_p K_{11} K_{s,a}) + [S]^2\{V_a[H] + V_p(K_{11} + [H])\}}{K_{11} K_{s,a} K_{s,p} + [S]\{K_{11} K_{s,a} + K_{s,p}(K_{11} + [H])\} + [S]^2(K_{11} + [H])} \tag{40}$$

B_1, from Eqns. 22 and 29 and assuming that binding of one ligand to the translocator does not influence the affinity for the second ligand ($K_s = K_1$ and $K_H = K_{11}$):

$$v_t = \frac{[S](V_a[H] + V_p K_H)}{K_1(K_H + [H]) + [S](K_H + [H])} \tag{41}$$

or

$$\frac{1}{v_t} = \frac{K_H + [H]}{V_a[H] + V_p K_H} + \frac{K_1(K_H + [H])}{V_a[H] + V_p K_H} \cdot \frac{1}{[S]} \tag{42}$$

B_2, from Eqns. 24 and 32:

$$v_t = \frac{[S](V_a K_s[H] + V_p K_1 K_H)}{K_1 K_s(K_H + [H]) + [S](K_1 K_H + K_s[H])} \tag{43}$$

or:

$$\frac{1}{v_t} = \frac{K_1 K_H + K_s[H]}{V_a K_s[H] + V_p K_1 K_H} + \frac{K_1 K_s(K_H + [H])}{V_a K_s[H] + V_p K_1 K_H} \cdot \frac{1}{[S]} \tag{44}$$

B_3, from Eqns. 27 and 35:

$$v_t = \frac{[S](V_a[H] + V_p K_{11})}{K_s K_{11} + [S](K_{11} + [H])} \tag{45}$$

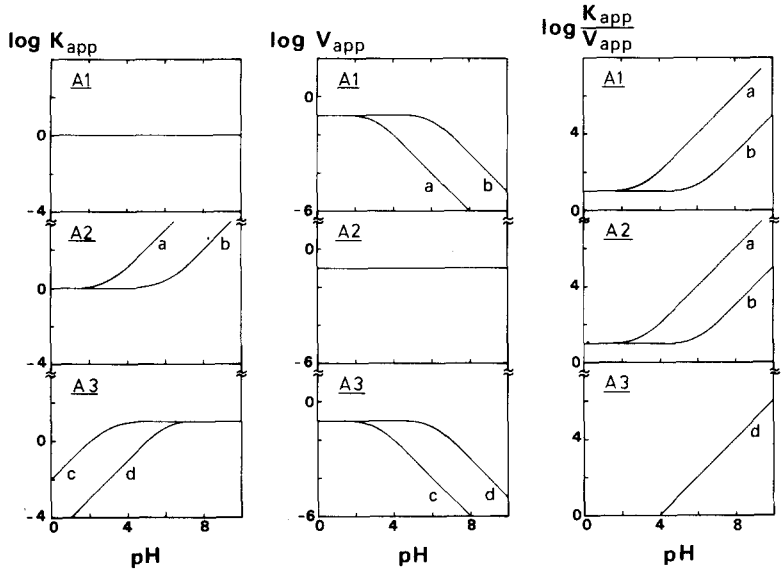

Fig. 2. pH dependence of the apparent kinetic constants for the H^+-sugar symport models A. K_1 = 1 mM; V_a = 0.1 mmol sugar/g cells per h. (a) K_H = 1 mM; (b) K_H = 0.001 mM; (c) $K_{s,a}$ = 10 mM, K_{11} = 1 mM; (d) $K_{s,a}$ = 10 mM, K_{11} = 0.001 mM.

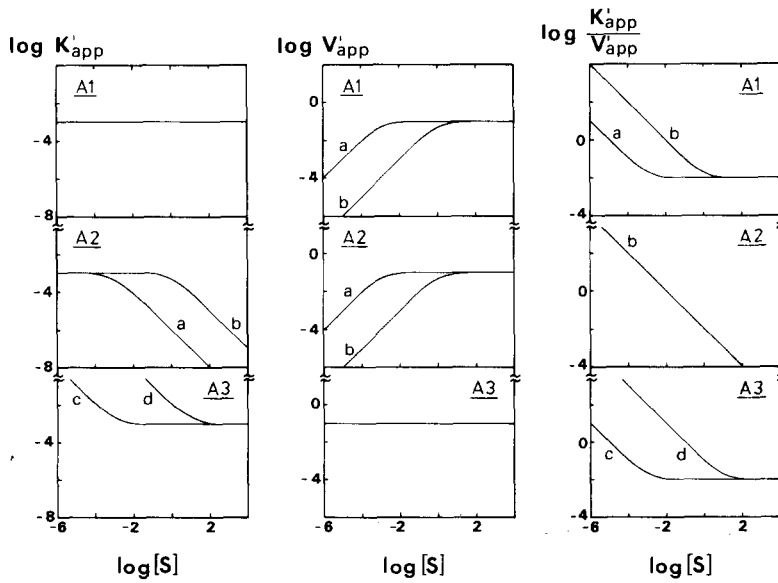

Fig. 3. Dependence of the apparent kinetic constants for the H^+-sugar symport models A on the log[sugar] based on Eqns. 13, 17 and 21. $K_H = K_{11}$ = 0.001 mM; V_a = 0.1 mmol sugar/g cells per h.

or:

$$\frac{1}{v_t} = \frac{K_{11} + [H]}{V_a[H] + V_p K_{11}} + \frac{K_s K_{11}}{V_a[H] + V_p K_{11}} \cdot \frac{1}{[S]} \quad (46)$$

From these equations it is clear that a two-segment Lineweaver-Burk plot indicates the involvement of two separate carriers (model A), whereas H^+-sugar symport and facilitated diffusion via the same carrier yields a linear Lineweaver-Burk plot (model B).

In the model A system, the reaction type of the symport carrier can be established by calculation of the apparent kinetic constants of the active component, obtained, e.g., by computer splitting of the experimental data, under different experimental conditions. Simulation curves are shown in Figs. 2 and 3.

Apparently, it is not possible to discriminate between a strictly coupled symport system and a mixed H^+-sugar symport plus facilitated diffusion, proceeding via the same carrier, by kinetic analysis at one, fixed pH value of the medium. Discrimination is possible, however, by taking advantage of the fact that the apparent kinetic constants respond differently to pH changes, as shown in Fig. 2 for the single symport system and in Fig. 4 for the mixed system. The reaction type of the symporter can be deduced from these plots.

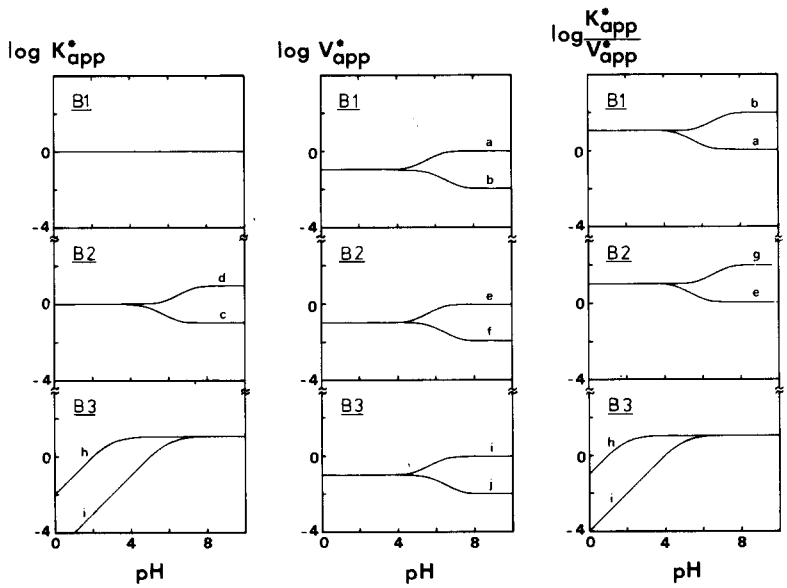

Fig. 4. pH dependence of the apparent kinetic constants for the total transport model B, i.e., when H^+ symport and facilitated diffusion are working via the same carrier. K_1 = 1 mM; K_H = 0.001 mM and V_a = 0.1 mmol sugar/g yeast per h. (a) V_p = 1 mmol/g per h; (b) V_p = 0.01 mmol/g per h; (c) K_s = 0.1 mM; (d) K_s = 10 mM; (e) K_s = 1 mM, V_p = 1 mmol/g per h; (f) K_s = 1 mM, V_p = 0.01 mmol/g per h; (g) K_s = 100 mM, V_p = 1 mmol/g per h; (h) K_s = 10 mM, K_{11} = 1 mM, V_p = 1 mmol/g per h; (i) K_s = 10 mM, K_{11} = 0.001 mM, V_p = 1 mmol/g per h; (j) K_{11} = 0.001 mM, V_p = 0.01 mmol/g per h.

Materials and Methods

S. fragilis was grown, with glucose as carbon source, and harvested and washed as described before [3].

Transport studies, utilizing ^{14}C-labeled sorbose, were performed in yeast cell suspension, buffered with 100 mM Tris-maleate at pH values between 3.8 and 7.8. Sugar uptake was measured as described before [1], at 25°C under aerobic conditions.

At each medium pH value, the data were computer-analysed according to the program of van Wielink et al. [17]. Analysis was performed with the formula:

$$v = \frac{V_1[S]}{K_1 + [S]} + \frac{V_2[S]}{K_2 + [S]} + \ldots + \frac{V_n[S]}{K_n + [S]}$$

According to the method of non-linear least squares, the optimum values of K_{app} and V_{app} were obtained and used for further kinetic analysis as described in the theoretical section.

^{14}C-labeled sorbose was obtained from the Radiochemical Centre (Amersham).

Results

Sorbose uptake in *S. fragilis* appeared to be linear with time for at least 5 min. Transport velocities were calculated from the slope of the uptake plots over the period from ½ to 3 min (Fig. 5). The plots indicate some aspecific, initial adsorption. This initial adsorption was temperature independent and not related to transmembrane transport (see, e.g., Ref. 18).

Previous studies have shown the presence of a mixed transport mechanism for sorbose in this yeast strain [4] consisting of a high-affinity H^+-sugar symport system and a low-affinity facilitated diffusion. Graphs of $1/v$ against $1/[S]$ yielded two-segment Lineweaver-Burk plots at all pH values studied (Fig. 6).

Via computer simulation, as described under Materials and Methods, the

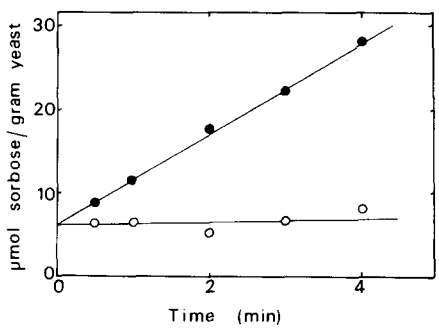

Fig. 5. Initial uptake of sorbose by *S. fragilis* at 25°C (●) or at 3.5°C (○) under aerobic conditions. Yeast concentration 10% (w/v), initial concentration 400 mM; 100 mM Tris-maleate (pH 5.5). Uptake was carried out as described under Materials and Methods.

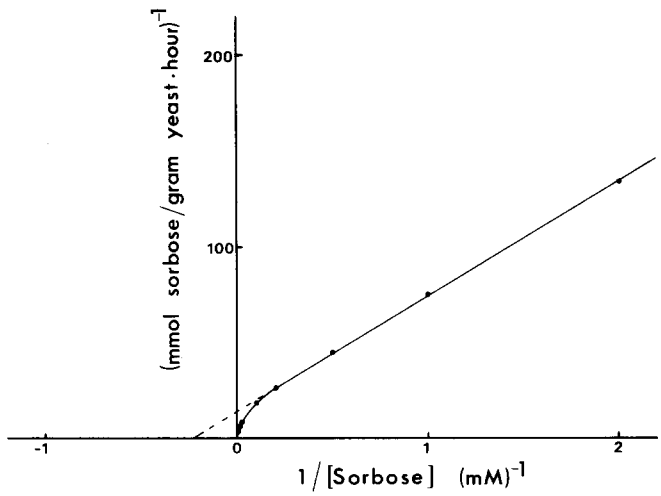

Fig. 6. Lineweaver-Burk plot of the initial uptake of sorbose by *S. fragilis*. Yeast concentration 10% (w/v), 100 mM Tris-maleate (pH 4). Uptake was carried out as described under Materials and Methods.

apparent K and V values for both transport systems were calculated at ten different medium pH values between 3.8 and 7.8.

The relationship between the logarithm of the apparent kinetic constants and pH is shown in Figs. 7 and 8. Plots of the logarithm of the apparent kinetic constants of the symport system against log[S], with [H] as independent variable are shown in Fig. 9. These results correspond to model A1, with, as best fitting constants, $K_1 = 5.01$ mM, $K_H = 0.40$ μM, $K_{s,p} = 603.5$ mM, $V_a = 0.063$ mmol/g yeast per h and $V_p = 0.718$ mmol/g yeast per h.

Discussion

The kinetic analysis of sorbose transport in *S. fragilis*, as presented in this paper, is in accordance with the previous conclusion of two transport systems: a high-affinity uphill H^+-sorbose symport and a low-affinity facilitated diffusion. However, the suggestion that both transport mechanisms proceed via the same carrier [4] is contradicted by the present results. The biphasic Lineweaver-Burk plot (Fig. 6) can only be interpreted as a two-component system, utilizing separate carriers (model A), as discussed in Theoretical Section. In accordance, the kinetic constants for the facilitated diffusion component, obtained by computer splitting of the experimental data, appear to be pH independent.

From Fig. 7 it is clear that K_{app} of the symport carrier is pH independent, whereas V_{app} decreases at increasing pH. Comparison of Fig. 7 with Fig. 2 shows that the experimental results can only be reconciled with model A1, viz., a symport carrier with random sugar and proton binding and facilitated diffusion via a separate transporter. Comparison of Figs. 9 and 3 confirms this conclusion.

Simultaneously occurring H^+-sugar symport and facilitated diffusion has also been described for xylose in *R. gracilis* [5] and 6-deoxyglucose in *C.*

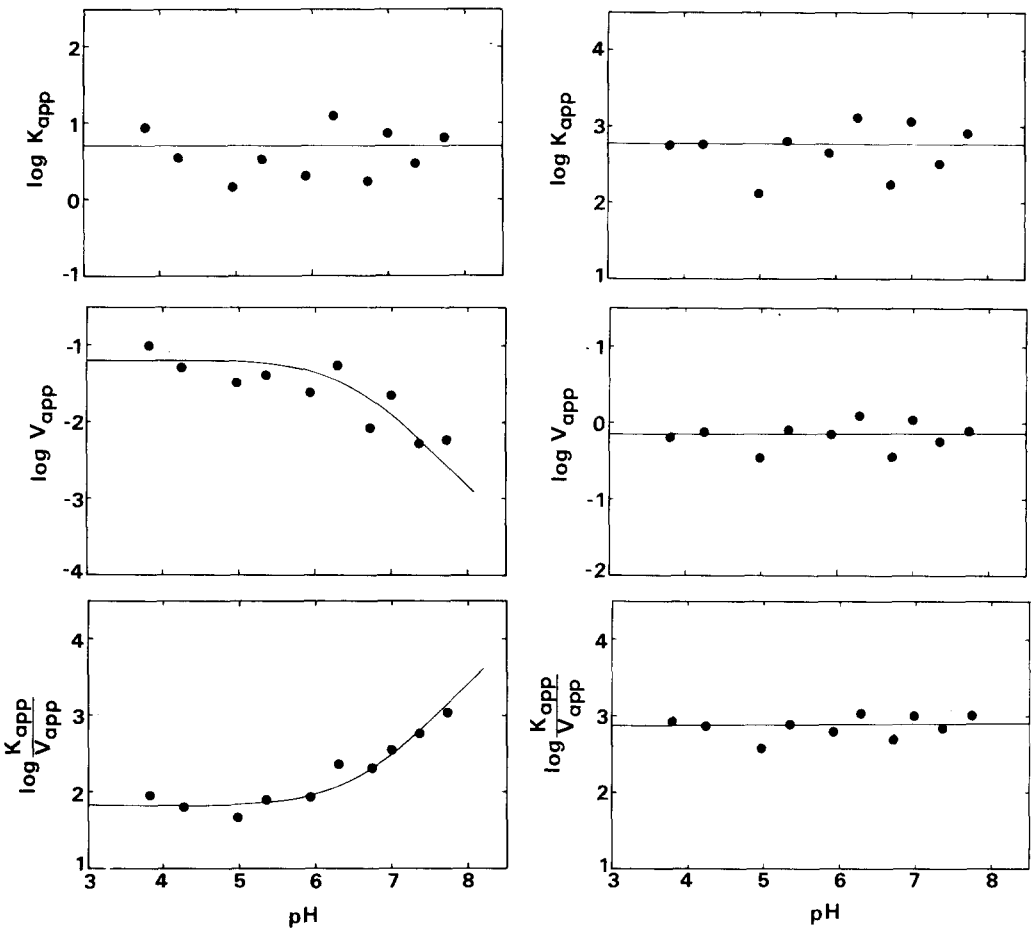

Fig. 7. Relationship between the logarithm of the apparent kinetic constants and pH for the H^+-sorbose symporter. Data on the constants are obtained after computer simulation as described in Materials and Methods. K_{app} values in mM, V_{app} values mmol/g per h.

Fig. 8. Relationship between the logarithm of the apparent kinetic constants and pH for facilitated diffusion. Data on these constants are obtained after computer simulation as described in Materials and Methods. K_{app} values in mM, V_{app} values mmol/g per h.

vulgaris [11,12]. In both cases, it was suggested that only one carrier was involved. The unprotonated carrier would accomplish low-affinity facilitated diffusion, whereas protonation of the carrier would change the system into an uphill, high-affinity symport, implicating an obligatory ordered binding, H^+ first, for the symport system. Our results imply, however, that these conclusions are presumably untenable. In both cases, two-segment Lineweaver-Burk plots were found, indicating transport via separate carriers. Furthermore, the pH dependence of the kinetic constants for 6-deoxyglucose uptake in *C. vulgaris* showed a pH-insensitive K_{app} and a pH-dependent V_{app}, in accordance with random binding, rather than with the suggested obligatory ordered binding.

The finding of random binding of sugar and H^+ to the symport carrier is

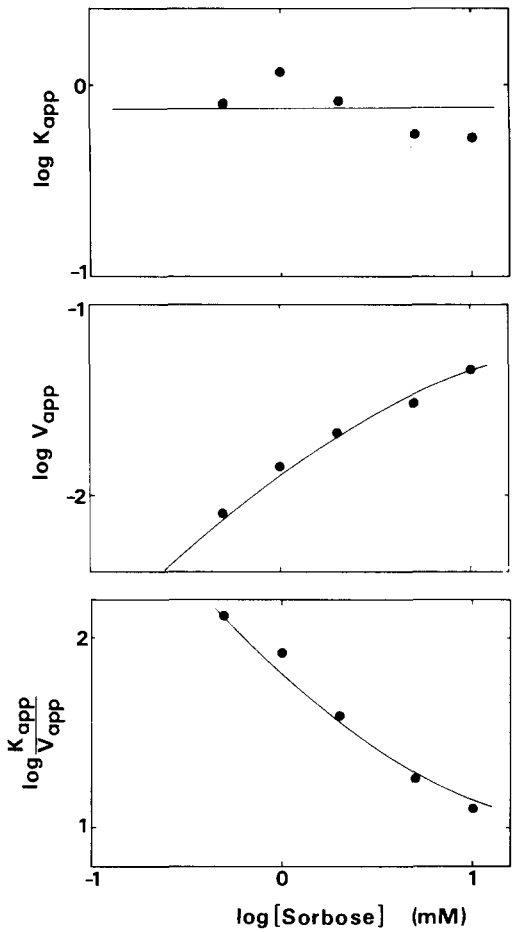

Fig. 9. Relationship between the logarithm of the apparent kinetic constants and the logarithm of sorbose concentration. Data were obtained from Lineweaver-Burk plots after correction of the experimental data for facilitated diffusion (with K = 603.5 mM and V_p = 0.718 mmol/g per h. K_{app} values in μM; V_p values mmol/g per h.

of significance for further studies on the mode of action of symport systems. It indicates that the activity of the symport system is controled by a 'double key' function of both H^+ and sugar, rather than by an H^+-induced increase of the affinity of the carrier for sugar. In this context, it will be of great significance to establish if other symport systems (e.g., in bacteria) also exhibit random binding.

The binding constant for H^+ to the carrier (0.4 μM) indicates an H^+-binding group with a pK_H of about 6.4, close to the pK_H of the imidazole ring of histidine. This suggests an essential role of a histidine residue in H^+ binding to the symport carrier. This suggestion is supported by recent studies on the H^+-lactose symport carrier of *Escherichia coli* [19]. These studies, with a completely different experimental approach, also indicated the involvement of histidine in H^+ binding to the symport carrier.

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