The Use of Phenolic Glycosides for Studying the Aerobic or Anaerobic Transport of Disaccharides into Yeasts

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Nitrophenolic glycosides have been used to characterize the kinetics of anaerobic transport into yeasts. This procedure overcomes the problem of providing energy for anaerobic transport, when using non-metabolizable sugar analogues. The glycosides were hydrolysed intracellularly, the glycon catabolized, and the nitrophenol rapidly expelled from the cells. Transport was the rate-limiting metabolic step and involved proton symport. Experiments are described that establish the validity of the method, which is simple, quick, cheap and reliable, and seems generally applicable to yeasts. The high absorption coefficients of the nitrophenols allow precise measurements to be made at low concentrations, at which the proton-conducting properties of the nitrophenols are minimal.

INTRODUCTION

In yeasts, the first step in the utilization of a disaccharide is usually its movement across the plasmalemma from the external medium into the cytosol, by means of a stereospecific carrier. This movement generally involves proton symport, the proton gradient being maintained by proton pumps associated with ATPase activity (reviewed by Eddy, 1982). The second step is the intracellular hydrolysis of the sugar into its component monomers, before their subsequent catabolism by way of the glycolytic pathway. However, some glycosidases act outside the plasmalemma, so that the monosaccharides formed by hydrolysis, rather than the disaccharide itself, are carried into the cell (reviewed by Barnett, 1981).

Certain difficulties in measuring the rates of sugar influx and efflux arise from the close interconnexion of transport with metabolism. These difficulties have usually been met by using non-metabolizable analogues, although this use may deprive the transport system of energy (cf. Barnett & Sims, 1976) and, hence, invalidate the experiment. Previously, Barnett & Sims (1982) have found, with three yeasts, that aerobic uptake of lactose and its analogue, methyl 1-thio-β-D-galactopyranoside (TMG), involves proton symport, the yeasts concentrating TMG at least 40-fold. Anaerobically, however, the yeasts, whether capable or incapable of fermenting lactose, did not concentrate TMG. The simplest explanation was that, under rigorous anaerobiosis, the functional energy supply was effectively depleted, so that there was no accumulation of TMG. Clearly, there are difficulties in interpreting the results of experiments using non-metabolizable sugar analogues for studying transport, when their uptake depends on unavailable endogenous reserves of carbohydrate, as may be particularly the case in anaerobic conditions.

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Abbreviations: CCCP, carbonyl cyanide m-chlorophenylhydrazone; DCCD, N,N'-dicyclohexylcarbodiimide; DESB, diethylstilboestrol; 4NPsGlc, 4-nitrophenyl α-D-glucopyranoside; 2NPβGal, 2-nitrophenyl β-D-galactopyranoside; 4NPβGal, 4-nitrophenyl β-D-galactopyranoside; TMG, methyl 1-thio-β-D-galactopyranoside.

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A possible method of studying the kinetics of anaerobic transport of glycosides is to employ a substrate of which only the glycon is metabolized, and to measure the release of the non-metabolizable aglycon. Nitrophenolic glycosides have already been used extensively for studying aerobic uptake by bacteria (e.g. Cohen & Monod, 1957; Wilson et al., 1982) and, hence, as they are easily obtainable, these glycosides would be convenient substrates. However, the validity of their use for critical kinetic studies depends on establishing that (i) transport of the glycoside, rather than its hydrolysis, is rate-limiting, and (ii) after hydrolysis in the cytoplasm, the nitrophenol is expelled rapidly from the cell. Clearly, it is also necessary to ascertain, for each yeast studied, first, that the relevant glycosidase is internal and, secondly, that the accumulation of the nitrophenol does not disrupt transport during the period of its measurement. Accordingly, such evidence is presented, from studies with several yeasts, validating the use of nitrophenolic compounds for kinetic investigations of the transport of glycosides, aerobically and anaerobically. This work was carried out with both α-D-glucopyranosides and β-D-galactopyranosides.

METHODS

Yeasts, growth and preparation of suspensions. The following yeasts were used: Candida utilis (Henneberg) Lodder et Kreger-van Rij NCYC 737, Debaryomyces polymorphus (Klöcker) Price et Phaff CBS 186, Kluyveromyces fragilis (Jörgensen) van der Walt NCYC 100 and Saccharomyces cerevisiae Meyen ex Hansen CBS 1171. They were maintained and cultivated as described by Barnett & Sims (1982).

Anaerobic conditions. Anaerobic incubations were under deoxygenated argon as described by Barnett & Sims (1982).

Measurements of transport. Transport was measured using cell suspensions of 10 ml, ~0.4 mg (aerobic) or 2.0 mg (anaerobic) dry weight of yeast ml⁻¹ in 'Universal' screw-capped bottles of 50 ml capacity. The bottles were held in a water bath at 25 °C and agitated by a submersible magnetic stirrer. Zero time for transport was that of adding the nitrophenyl glycoside, usually at a final concentration of 1.0 mM. Samples (0.75 ml) were taken at timed intervals, centrifuged in a minicentrifuge and equal volumes of 2.0 M-NaHCO₃ were added to the supernatant fluids. Absorbance was measured at 400 nm for 4-nitrophenol or 420 nm for 2-nitrophenol, against the appropriate blank.

Enzyme assays. Glycosidases were extracted either by disintegrating the yeasts with fine glass beads, as described by Ciriacy (1976), or by making the yeasts permeable with toluene and a detergent (BRIJ 35) (Sims & Barnett, 1978); the method used for measuring α-D-glucosidase was that given by Sims & Barnett (1978) and that for β-D-galactosidase by Barnett & Sims (1982).

Measurement of proton symport. The apparatus for measuring proton symport was calibrated and used as described by Eddy & Nowacki (1971). The relationship between pH and proton concentration was established by adding known amounts of 2.5 mM-HCl to a yeast suspension in 0.3 mM-Tris/citrate buffer (pH 4.5) (Barnett & Sims, 1982).

RESULTS AND DISCUSSION

The release of 4-nitrophenol into the medium by C. utilis, acting aerobically on 4NPzGlc, was measured at various pH values (Fig. 1). Yeast harvested from a culture medium at pH 3.5 was transferred to media adjusted to different values of pH; after this transfer, the release of nitrophenol was measured immediately (Fig. 1a). Alternatively, cells were harvested and transferred to media at pH 8 for 40 min, before measuring the release of 4-nitrophenol (Fig. 1b). The pH of the medium did not change during the experiments. Between pH 2 and pH 6, liberation of nitrophenol occurred immediately on adding 4NPzGlc; but at pH values of 7 and above, onset of release was progressively delayed, although at pH 7 and pH 8 the eventual rate was almost unaffected. After 5 h, the final concentration of 4-nitrophenol in the external medium at each pH value was similar (about 0.3 mM) which, in this experiment, corresponded to the hydrolysis of less than 15% of the glycoside provided. The accumulation of 4-nitrophenol in the medium eventually completely inhibited the hydrolysis or transport of 4NPzGlc, or both. This limiting concentration of 4-nitrophenol was independent of the external concentration of 4NPzGlc (up to ~4 mM-glycoside). Between pH 4 and pH 7, 4-nitrophenol output was initially linear with time (see also Fig. 1b), until the concentration in the medium reached about 0.15 mM. Had the α-D-glucosidase activity occurred outside the plasmalemma, 4-nitrophenol release
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Fig. 1. Effects of varying pH of suspending medium on release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside by maltose-grown Candida utilis, under aerobic conditions. (a) Yeast was harvested in the exponential phase of growth, washed and resuspended at about 900 μg dry wt ml⁻¹ with 2.0 mM 4NPaGlc in buffer at pH 3 (○), pH 4 (■), pH 5 (△), pH 6 (●), pH 7 (▲), pH 8 (◇), pH 9 (▼) or pH 10 (▲). At timed intervals, samples were centrifuged and 4-nitrophenol in the medium was estimated, as described in Methods. (b) Yeast prepared as in (a) was resuspended in buffer (i) at pH 3.5 + 2 mM 4NPaGlc (○), (ii) at pH 8.0 + 2 mM-4NPaGlc (●), (iii) at pH 8.0 + cycloheximide (50 μg ml⁻¹) (△), and (iv) at pH 8.0 alone (▲). In treatments (i) and (ii), 4-nitrophenol in the medium was estimated; simultaneously, the cells were extracted with 60% (v/v) ethanol and the internal 4-nitrophenol (▼) and glucoside (◇) estimated. For treatments (iii) and (iv), 4NPaGlc was added (to give 2 mM, final concentration) after 40 min and release of 4-nitrophenol into the medium followed with time.

would probably have been linear with time and would have been more sensitive to changes of the external pH. Rather, the long-term adaptive responses shown, associated with a sudden change of external pH (and the consequent reduction in the chemical potential for H⁺) probably required some internal adjustment, perhaps involving the modulation of H⁺ pumping (Maloney, 1982). The amount of 4-nitrophenol measured in the external medium was little changed by including the amount extracted from the yeast with 60% (v/v) ethanol (Fig. 1 b). The nitrophenol was released immediately into the suspending medium and did not build up intracellularly.

The results shown in Fig. 2 confirm that the enzyme acted internally: neither cells made permeable, nor cell-free extracts, had α-D-glucosidase activity below pH 5, whereas the fastest excretion of 4-nitrophenol from intact cells was at pH 4. Furthermore, analysis of the ethanolic extracts (after treating with hot KOH) showed that there was no detectable intracellular accumulation of the 4-nitrophenyl glycoside (Fig. 1 b).

Broken cells released 4-nitrophenol much faster than intact cells (≈ 134 nmol min⁻¹ (mg dry wt)⁻¹ and ≈ 6 nmol min⁻¹ (mg dry wt)⁻¹, respectively, at pH 6); that is, the intact cells were hydrolysing 4NPaGlc at <5% of the potential catalytic rate. It is unlikely that this low rate
Fig. 2. Effect of varying pH on 4-nitrophenol release from 4-nitrophenyl α-D-glucopyranoside by intact
and broken cells of maltose-grown Candida utilis. •, Initial rates of release from intact cells, treated as
in Fig. 1(a); ○, activity of α-D-glucosidase of cells broken with ballotini (comparable results were
obtained with BRIJ 35/toluene-treated cells).

obtained with intact cells was the result of inhibition of the internal enzyme, since 0.5 mM 4-
nitrophenol did not inhibit the α-D-glucosidase activity of cell extracts. Hence, entry of the
glucoside into the cell, across the plasmalemma, seems to have been the rate-limiting step and,
moreover, the nitrophenol was not concentrated by the cells.

The use of more than one phenolic glycoside and more than one yeast helped to establish the
general applicability of the method, when glycosidic hydrolysis is intracellular. In S.
cerevisiae, under aerobic conditions, transport was not linear; there was an initial lag in the release of nitro-
phenol and the time courses only became linear after ~15 min. Such lags might be explained by
the inactivation of plasmalemma ATPase under conditions of energy deprivation (cf. Serrano,
1983a).

**Effects of oxygen and metabolic inhibitors**

The effects of inhibitors were studied on the release of nitrophenol from 4-nitrophenyl
glycosides by a number of yeasts, including some that fermented the glycoside anaerobically
(Table 1).

The rate of release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside by intact cells of
C. utilis was sensitive to the oxygen concentration and, under highly anaerobic conditions, it was
only ~3% of that measured aerobically. Aeration of the anaerobic yeast suspension
immediately increased the rate of release of nitrophenol. Inhibitors of respiration and ATPase,
and proton conductors such as CCCP which act at the plasmalemma, when present at concen-
trations that did not affect α-D-glucosidase activity, virtually abolished the release of 4-
nitrophenol by S. cerevisiae in both aerobic and anaerobic conditions (Table 1), whereas
antimycin A affected only aerobic activity. So, aerobically, the ATP which provided energy for
transport appeared to be generated chiefly by oxidative phosphorylation; but DCCD and DESB
inhibited the proton pump of the plasmalemma, as Serrano (1980, 1983b) described, this being a
permeability barrier to such inhibitors (Sigler et al., 1981). These observations were consistent
with transport of the glycoside being the rate-limiting step to the release of 4-nitrophenol, so that
this release was impeded by any factor which slowed or retarded the uptake of the nitrophenyl
glycoside (aerobically or anaerobically).

**Kinetics of release of 4-nitrophenol from intact yeast**

Further evidence for the validity of the method was provided by kinetic studies. The release of
4-nitrophenol was amenable to analysis by methods based on the Michaelis-Menten equation, as
Fig. 3. Kinetics of aerobic uptake of 4-nitrophenyl \( \alpha \)-D-glucopyranoside by \textit{Candida utilis}. Dixon (1953) plot for four concentrations of 4NPaGlc: \( \bullet \), 0-091 mM; \( \square \), 0-45 mM; \( \blacksquare \), 0-91 mM; \( \Delta \), 2-27 mM (see Dixon \textit{et al.}, 1979). Yeast was prepared and 4-nitrophenol release followed as in the legend to Fig. 1, except that the yeast was suspended in 100 mM-K\( \text{H}_2\text{PO}_4 \) with various concentrations of 4NPaGlc (as indicated), with or without maltose. Rates were calculated from time courses of 4-nitrophenol release over 30 min.

Table 1. \textit{Effects of inhibitors on rates of transport of 4-nitrophenyl \( \alpha \)-D-glucopyranoside by \textit{Saccharomyces cerevisiae} and \textit{Candida utilis} under aerobic and anaerobic conditions}

All rates were measured with yeast freshly harvested from cultures growing exponentially on maltose as sole source of carbon, washed and resuspended in 0-1 mM-K\( \text{H}_2\text{PO}_4 \). Yeast suspensions were made anaerobic by bubbling deoxygenated argon through them for 15 min. For each experiment, 2 mM-4-nitrophenyl \( \alpha \)-D-glucopyranoside (final concentration) with or without inhibitor was added at zero time. Samples were removed at timed intervals and the rates shown were calculated from the period of linear uptake.

<table>
<thead>
<tr>
<th>Transport rate</th>
<th>( [\text{nmol 4NPaGlc min}^{-1} \text{ (mg dry wt yeast)}^{-1}] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. cerevisiae}</td>
<td>\textit{C. utilis}</td>
</tr>
<tr>
<td>Aerobic</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Control</td>
<td>31-8</td>
</tr>
<tr>
<td>+ 25 ( \mu \text{M}-\text{CCCP} )</td>
<td>9-5</td>
</tr>
<tr>
<td>+ 100 ( \mu \text{M}-\text{CCCP} )</td>
<td>0-6</td>
</tr>
<tr>
<td>+ 400 ( \mu \text{M}-\text{DESB} )</td>
<td>3-9</td>
</tr>
<tr>
<td>+ 175 ( \mu \text{M}-\text{Azide} )</td>
<td>2-2</td>
</tr>
<tr>
<td>+ 430 ( \mu \text{M}-\text{DCCD} )</td>
<td>1-8</td>
</tr>
<tr>
<td>+ 36 ( \mu \text{M}-\text{Antimycin} )</td>
<td>5-4</td>
</tr>
</tbody>
</table>

NM, Not measured.

in a Lineweaver-Burk plot for aerobic uptake of 4NPaGlc by \textit{C. utilis} and a Dixon (1953) plot for competition between 4NPaGlc and maltose (Fig. 3).

A comparable series of results were also obtained with two lactose-utilizing yeasts, \textit{K. fragilis} and \textit{D. polymorphus}, and 4-nitrophenyl \( \beta \)-D-galactopyranoside. Of the kinetic constants obtained, the values for \( V_{\text{max}} \) were the same aerobically and anaerobically, but those for the \( K_m \) differed. During the aerobic transport of 4NP\( \beta \)Gal, a low concentration of CCCP again had little effect on \( V_{\text{max}} \) and gave an increased value for the apparent \( K_m \). Values of kinetic constants, based on the liberation of 4-nitrophenol, are compared in Table 2 with constants determined by other methods. With \textit{K. fragilis}, close agreement was found between the \( K_m \) for 4NP\( \beta \)Gal transport determined by nitrophenol release and that calculated directly by measurements of
Table 2. Kinetic constants of uptake and glycosidase activities

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Aerobic uptake</th>
<th>Anaerobic uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candida utilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPαGlc)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-maltose)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPαGlc min^-1 (mg dry wt yeast)^-1]</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>α-D-Glucosidase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPαGlc) at pH 5.5</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>at pH 8.5</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-maltose) at pH 5.5</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>at pH 8.5</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPαGlc min^-1 (mg dry wt yeast)^-1]</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td><strong>Saccharomyces cerevisiae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPαGlc)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-maltose)</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPαGlc min^-1 (mg dry wt yeast)^-1]</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><strong>Kluuyveromyces fragilis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPβGal)* from 4-nitrophenol release</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-lactose) against 4NPβGal</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-lactose) against TMG</td>
<td>1.2†</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-lactose) from H^+ symport</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPβGal min^-1 (mg dry wt yeast)^-1]</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 2NPβGal min^-1 (mg dry wt yeast)^-1]</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td><strong>Debaryomyces polymorphus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPβGal)</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPβGal min^-1 (mg dry wt yeast)^-1]</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>β-D-Galactosidase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong> (mm-4NPβGal)*</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong> (mm-lactose) non-competitive</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 4NPβGal min^-1 (mg dry wt yeast)^-1]</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td><strong>Vmax</strong> [nmol 2NPβGal min^-1 (mg dry wt yeast)^-1]</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

* Apparent K_m was the same for 2NPβGal as for 4NPβGal.
† Barnett & Sims (1982).

Cells harvested in the late exponential phase of growth usually gave values for K_m within 5% and V_max within 20% of those given above.

Proton uptake elicited by adding 4NPβGal to the yeast. Similarly, there is close agreement in the estimates of the apparent affinity of K. fragilis for lactose, based directly on lactose-elicited proton symport (determined as the K_m lactose), the competitive effect of lactose on the transport of [^14C]TMG (K_lactose) and the effect on the release of 4-nitrophenol from 4NPβGal. In all three examples shown in Table 2, the apparent affinity of the transport system for the 4-nitrophenyl glycoside was of the same order as its affinity for the glycosidase, although the maximal rate of release from the cell (V_max) was only a fraction of the maximal activity of the enzyme. The high affinity (low K_m) and high rate (V_max) obtained with 4NPβGal (Table 2), relative to the corresponding constants obtained with methyl 1-thio-β-D-galactopyranoside (Barnett & Sims, 1982), favoured the use of the phenolic glycoside. The same was true for 4NPαGlc.
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Fig. 4. Effect of varying concentration of exogenously supplied 4-nitrophenol on rates of proton exchange between maltose-grown *Candida utilis* and the suspending medium, under aerobic conditions. Yeast was suspended at 4 mg dry wt ml⁻¹ in 0.3 mM-Tris/citrate buffer (pH 4.5) with 4-nitrophenol. Changes of pH were measured for ~3 min after adding either 2.5 mM-D-glucose or 2.5 mM-maltose. ●, Uptake of protons with maltose; ○, output of protons with D-glucose.

Fig. 5. Anaerobic uptake of 4-nitrophenyl β-D-galactopyranoside by lactose-grown *Kluyveromyces fragilis*. Yeast at 4 mg dry wt ml⁻¹ was incubated with 2.5 mM-4NPβGal and no inhibitor (●), 0.5 mM-DESB (○), or 50 μM-CCCP (□). 4-Nitrophenol release and ethanol formation were estimated as described in Methods.

**Effects of 4-nitrophenol on H⁺ movement**

The effects of varying the concentration of 4-nitrophenol on proton movements (uptake on addition of maltose or output with D-glucose) are shown in Fig. 4. At the range of concentrations of 4-nitrophenol produced in the kinetic experiments, in which linear release of nitrophenol was observed, there was little inhibition of either proton symport or extrusion. Furthermore, there was close agreement between the concentration of 4-nitrophenol that abolished proton symport and that which accumulated before the excretion of 4-nitrophenol was stopped (Fig. 1).

**Findings with nitrophenyl glycosides**

The method used here, for studying the transport of glycosides into yeasts, depends on the following: (i) rate-limiting uptake of intact glycoside; (ii) rapid intracellular hydrolysis, so that the glycoside does not accumulate; (iii) expulsion of the nitrophenolic aglycon. Unlike the entry of the glycoside into the cells, expulsion of the nitrophenol is not carrier-mediated and is not rate-limiting. At the internal pH of a yeast, nitrophenols exist predominantly as nitrophenolate. This lipophilic anion, like 2,4-dinitrophenolate (Duncan & Croghan, 1970), can diffuse freely across the plasmalemma, the membrane potential favouring rapid expulsion from the cell.

The action of nitrophenolate as a proton conductor might well have invalidated the method. However, despite its consequent limitations, the results showed the method to be reliable. Nitrophenol has a high absorption coefficient, so that low concentrations, at which there is no marked inhibition of proton symport, can be measured precisely. Further, transport was rate-limiting and glycoside was not accumulated intracellularly; this was because the glycosidases had high activities towards, and affinities for, the nitrophenolic glycosides (Table 2). In addition, the glycons provide a continuous source of energy, so that transport can be studied over much longer periods than can be achieved either with non-metabolizable analogues anaerobically or by measuring proton symport.
Clearly, anaerobic transport of the glycosides involved proton symport, as this transport was inhibited by the proton conductor, CCCP, and the ATPase inhibitor, DESB (Table 1). Fermentation of the glycon sustained anaerobic transport of the phenolic glycosides; this contrasted with the anaerobic uptake of methyl l-thio-β-D-galactopyranoside (TMG) (Barnett & Sims, 1982), which also entered K. fragilis by proton symport but whose uptake was not sustained, the internal equilibrating quickly with the external thiogalactoside. So anaerobic transport limits the rate of fermentation of the glycon: ethanol production and the release of 4-nitrophenol coincided (Fig. 5) stoichiometrically. This was also true when the ATPase was partly inhibited by DESB.

Changes between aerobic and anaerobic conditions can alter the kinetics of transport. Over an eightfold range of concentrations, the maximum transport velocity, \( V_{\text{max}} \), for entry of 4NPβGal into K. fragilis was unaffected; nor was \( V_{\text{max}} \) changed in the presence of CCCP. So the mechanism and rate of glycoside uptake appear to be the same aerobically and anaerobically in this fermenting yeast. However, in each case, there was a marked change in \( K_m \). These findings could be variously interpreted; they are analogous to those of Baker & Carruthers (1981), who reported that changes in the concentration of internal ATP in giant axons of Loligo affect the apparent \( K_m \), but not \( V_{\text{max}} \), for the uptake of 3-O-methyl-D-glucose. However, such changes might also be explained in terms of alterations of the membrane potential, as discussed by van den Broek & van Steveninck (1982) for K. fragilis.

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