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The Absorption of Protons with α-Methyl Glucoside and α-Thioethyl Glucoside by the Yeast N.C.Y.C. 240

EVIDENCE AGAINST THE PHOSPHORYLATION HYPOTHESIS

By ROGER BROCKLEHURST, DAVID GARDNER and A. ALAN EDDY
Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.

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1. When yeast N.C.Y.C. 240 was grown with maltose in a complex medium based on yeast extract and peptone, washed cell preparations fermented α-methyl glucoside much more slowly than maltose. 2. The yeast absorbed α-methyl [^{14}C]glucoside from a 10mM solution in the presence of antimycin and iodoacetamide, producing [^{14}C]glucose, which accumulated outside the cells. The yeast itself contained hexose phosphates, trehalose, α-methyl glucoside and other products labelled with ^{14}C, but no α-methyl glucoside phosphate. 3. About 1 equiv. of protons was absorbed with each equivalent of α-methyl glucoside, and 1 equiv. of K^+ ions left the yeast. 4. α-Thioethyl glucoside was also absorbed along with protons. Studies by g.l.c. showed that the yeast concentrated the compound without metabolizing it. 5. The presence of trehalose, sucrose, maltose, L-sorbose, glucose or α-phenyl glucoside in each case immediately stimulated proton uptake, whereas fructose, 3-*O*-methylglucose and 2-deoxyglucose failed to do so. 6. The observations support the conclusion that α-thioethyl glucoside, α-methyl glucoside and maltose are substrates of one or more proton symports, whereas they seem inconsistent with the notion that the absorption of α-methyl glucoside involves the phosphorylation of the carbohydrate [Van Steveninck (1970) *Biochim. Biophys. Acta* **203**, 376–384].

The initial steps in the fermentation of α-methyl glucoside by yeast have been the subject of conflicting reports (Jennings, 1974). (1) Halvorsen and co-workers considered that the carbohydrate was absorbed intact by a specific permease, distinct from maltose permease, then hydrolysed by intracellular α-glucosidases such as isomaltase (Robertson & Halvorsen, 1957; Okada & Halvorsen, 1964). The same permease concentrated in the yeast α-thioethyl glucoside, an analogue of α-methyl glucoside that was not metabolized (Okada & Halvorsen, 1964; De Kroon & Koningsberger, 1970). (2) Van Steveninck (1970) proposed that α-methyl glucoside was absorbed through the maltose permease by a fundamentally different mechanism involving the phosphorylation of the carbohydrate (see Schemes 1*b* and 1*c* below). Polyphosphate, assumed to be located at the outer surface of the plasmalemma, was the suggested phosphate donor. Similar phosphotransferase systems have been proposed for glucose, 2-deoxyglucose and galactose (Van Steveninck, 1972; Kulaev, 1975), though the significance of the evidence has been questioned (Kotyk & Michaljaničová, 1974). One object of the present work was to examine again whether washed cells of the yeast strain studied by Van Steveninck (1970) in fact phosphorylated α-methyl glucoside when given this compound in the absence of other nutrients. (3) Work in our laboratories has shown that various yeast permeases absorbing respectively certain amino acids, carbohydrates and phosphate each involve protein symports (Cockburn *et al.*, 1975; Seaston *et al.*, 1973, 1976). For instance, after growth with maltose, a certain strain of yeast (N.C.Y.C. 74) absorbed both maltose and α-methyl glucoside together with protons (Seaston *et al.*, 1973). The strain (N.C.Y.C. 240) used by Van Steveninck (1970) has not previously been investigated from the standpoint of a possible symport involvement and forms the subject of the present report. Reference to Scheme 1 shows that whereas both the phosphorylation model (*b*) and the symport model (*a*) require that protons are absorbed with the carbohydrate, the neutralization of charge is achieved in different ways in the two cases. Thus the symport model characteristically involves a faster efflux of K^+, in the presence of the carbohydrate, when the proton pump is not recycling the absorbed protons. Further, the magnitude of the gradient of carbohydrate concentration, eventually formed across the plasmalemma, would depend on the magnitude of the corresponding proton

gradient, rather than on the availability of phosphate bond energy as the rival models (Schemes 1*b* and 1*c*) predict.

Materials and Methods

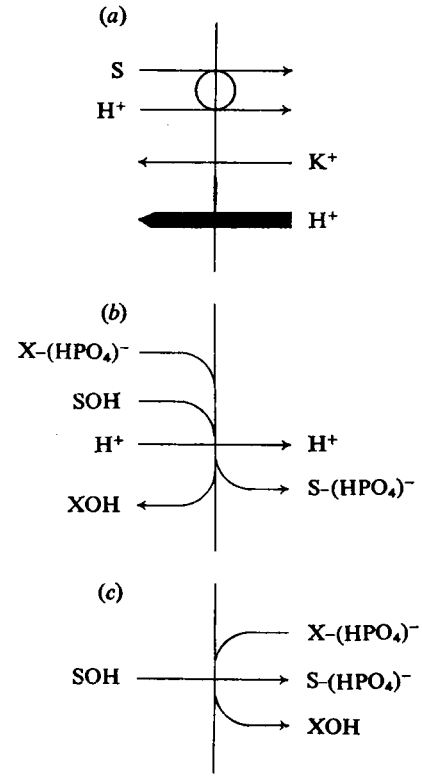
The strain of *Saccharomyces cerevisiae*, N.C.Y.C. 240 (British National Collection of Yeast Cultures, Nutfield, Surrey, U.K.), and the strain of *Saccharomyces carlsbergensis* (*uvarum*), N.C.Y.C. 74, were manipulated as described by Eddy & Nowacki (1971). The yeast was grown at 25°C in one of the following solutions (250ml): (1) a mixture comprising 3g of yeast extract (Difco Laboratories, Detroit, MI, U.S.A.), 5g of mycological peptone (Oxo Ltd., London S.E.1, U.K.), glucose (10g) and water to 1litre; (2) a similar solution in which maltose or α-methyl glucoside replaced glucose. The various cultures were held in a gyratory shaker (Orbital Incubator; Gallenkamp and Co. Ltd., Widnes, Cheshire, U.K.) and harvested after 17–40h.

Proton and K^+ movements

Proton uptake was assayed by using a suspension of the yeast (50mg dry wt.) in 5mM-Tris adjusted to pH5.0 with citric acid (5–8ml final volume) as described by Seaston *et al.* (1973). In specified cases 10μg of antimycin and 10mM-iodoacetamide were added to inhibit energy metabolism. A selected concentration of maltose, α-methyl glucoside or trehalose labelled with ^{14}C (4–100mCi/mol) was added 5min later. Samples (1ml) were taken at intervals during the next 4min and assayed as described below. The efflux of K^+ was monitored with a selective glass electrode (type C0150, from Chemetric, Twickenham, Middx., U.K.), connected to a high-impedance voltmeter constructed in this laboratory by Dr. R. Philo.

[^{14}C]Carbohydrate absorption

(1) To assay trehalose uptake, the above samples were each mixed with water (9ml) at 0°C. The yeast was separated by centrifugation at 1000*g* for 2min and washed with water (10ml) at 0°C. It was then mixed with water (0.5ml) and the scintillation fluid (5ml) described by Seaston *et al.* (1973). After 30min at room temperature (25°C), the cell debris was separated by centrifugation at 100*g* for 2min and mixed with a further portion (5.5ml) of aqueous scintillation fluid, which was subsequently separated by centrifugation from the cell debris and added to the first portion. The mixture was assayed for ^{14}C (Seaston *et al.*, 1973). (2) To assay uptake of maltose and α-methyl glucoside, the sample (1ml) of the yeast suspension was filtered [filter type AAWP 02500, 0.8μm pore size, from Millipore (U.K.) Ltd., London NW10 7SP, U.K.] and the yeast was washed with three successive portions (0.5ml) of water at 25°C. The filter and yeast were then extracted as above. The filtrate, including the washings, was assayed for glucose by using a glucose oxidase reagent (see below). Sugar uptake was computed in terms of the sum of the ^{14}C extracted from the yeast and the appropriate amount of glucose found in the filtrate (see the Results section).



Scheme 1. *Comparison of the symport and phosphorylation models*

(*a*) A proton symport for the solute S, neutralized either by the ejection of protons through the proton pump or by the efflux of K^+ ions (Seaston *et al.*, 1976). (*b*) Model showing how the phosphorylation mechanism proposed by Van Steveninck (1970), in which protons were not explicitly involved, might lead to the absorption of protons. An equivalent of these would be taken into the yeast with the solute (SOH = α-methyl glucoside) when the latter accepted a phosphate group from the polyphosphate [$X-(HPO_4)^-$] situated at the outer surface of the plasmalemma. The action of intracellular phosphatases on $S-(HPO_4)^-$ would lead to the accumulation of the solute (SOH) inside the yeast. (*c*) Solute phosphorylation at the expense of intracellular polyphosphate. No uptake of protons would be required.

T.l.c.

Other portions of the yeast suspension (50mg dry wt. in 5ml), containing 10mM-α-methyl [^{14}C]glucoside, were mixed with water (40ml) at 0°C. The yeast was separated by centrifugation, washed with water (3×5.0ml) at 0°C and then extracted with water (2ml) at 100°C for 10min. The cell debris was washed with water (2×2ml) and the total extract (6ml) was evaporated to dryness at 40°C. The residue was dissolved in water (0.4ml). (1) Portions (10μl) were applied to thin-layer cellulose sheets (type 13255; Kodak Ltd., Kirby, Liverpool, U.K.), which were developed by ascending chromatography for 3h with pyridine/ethyl acetate/acetic acid/water (36:36:7:21, by vol). The sheets were dried, cut into strips (10mm×25mm), which were each assayed for ^{14}C. (2) Further portions (20μl) of the above extract were incubated for 30min at 30°C with a solution (10μl) containing alkaline phosphatase (0.5μg) and 0.1M-Tris adjusted to pH8 with HCl. Portions (15μl) of the product were developed in parallel with (1) above. (3) Portions (1μl) of 100mM solutions of standard carbohydrates (1μCi/ml) were used as markers when radioautograms were to be prepared. The developed cellulose sheet was clipped to X-ray film (Eastman Kodak Co., Rochester, NY, U.S.A.) and left for several weeks in the dark before processing.

α-Thioethyl glucoside absorption

The yeast (25mg dry wt.) was incubated at 30°C for 30min in a solution (5ml) containing the specified concentration of α-thioethyl glucoside at pH5 or 7 (see the Results section). The yeast cells were separated by centrifugation. They were washed with water (2×2ml) at 0°C, the washings and the original supernatant solution being mixed and evaporated to dryness at 70°C (sample A). The washed yeast was boiled with water (2ml) for 10min. The cell debris was separated by centrifugation, washed with water (2×2ml) and the total extract (6ml) was evaporated to dryness at 70°C (sample B). The amounts of α-thioethyl glucoside in samples A and B were assayed by g.l.c.

G.l.c.

This was based on the method of Sweeley *et al.* (1963). Samples A and B above were incubated with redistilled dry pyridine (1ml), hexamethyldisilazane (0.2ml) and trimethylchlorosilane (0.1ml) at 65°C for 30min. The precipitate that formed was separated by centrifugation at 1000*g* for 2min and discarded. Portions of the supernatant solution (1μl) were assayed in a GCD gas chromatograph (Pye Unicam, Cambridge, U.K.). The support phase was Gas-Chrom Q (85–100 mesh) and the liquid phase was OV 22, 5% (Phase Separation Ltd., Queensferry, Clwyd, Wales, U.K.). The injector and detector temperatures were both 250°C. The flow of N_2 carrier gas was 30ml/min. The temperature of the column was raised from 180 to 250°C at 4°C/min. The system was calibrated by peak-height analysis by using graded amounts of α-thioethyl glucoside and other appropriate standards.

Enzyme assays

The yeast (30mg dry wt. in 1ml) was agitated (Whirlimixer; Fisons Scientific Apparatus, Loughborough, Leics., U.K.) for 5min with 0.05ml of toluene/ethanol (1:4, v/v) to open the cells (Serrano *et al.*, 1973). The treated yeast was washed successively with 2×5ml of 0.1M-K_2HPO_4 adjusted to pH6.8 with HCl. To assay α-glucosidase activity, an appropriate weight of the treated yeast was incubated at 30°C with 20mM-α-methyl glucoside, 5mM-NaF to inhibit glucose fermentation, and 0.1M-K_2HPO_4 adjusted to pH6.8 with HCl. Controls lacking yeast or substrate were also prepared. Samples (1ml) were taken at intervals up to 20min, mixed with 0.2M-K_2HPO_4 (2ml), heated at 100°C for 3min and centrifuged at 1000*g* for 2min. Appropriate portions (up to 1ml) of the supernatant solution were mixed with a glucostat reagent (2.5ml). After 75min at 40°C, the value of A_{440} was determined and the glucose content of the sample calculated. The glucostat reagent contained, in a final volume of 100ml: (1) 120 units of glucose oxidase [type V, from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.]; (2) *o*-dianisidine (5mg) dissolved in methanol (0.5ml); (3) peroxidase (4mg) from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K.; (4) a mixture of 0.25M-KH_2PO_4 and 0.5M-Tris adjusted to pH7.6 with HCl. The Tris served to inhibit the endogenous glucosidase activity in the reagents (Porteous & Clark, 1965).

CO_2 formation

Conventional manometric techniques were used. The yeast (2–50mg dry wt.) was incubated at 30°C in the main compartment of a Warburg flask with antimycin (0.1μg/ml of yeast), 20mM-Tris adjusted to pH5 with citric acid and 10mM-α-methyl glucoside. The latter contained a negligible amount of free glucose.

Chemicals

The sources of some of the chemicals have been described previously (Seaston *et al.*, 1973). Trehalose, iodoacetamide, α-methyl glucoside, maltose (extra pure) and *o*-dianisidine were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. α-Thioethyl glucoside was synthesized by the method of Okada &

Halvorsen (1964). α-Methyl[U-^{14}C]glucose, [U-^{14}C]-glucose, [U-^{14}C]maltose and [U-^{14}C]trehalose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Alkaline phosphatase was obtained from the Boehringer Corp. (London) Ltd.

Results

Metabolism of α-methyl glucoside

Van Steveninck (1970) claimed that the strain no. 240 neither fermented nor assimilated α-methyl glucoside. However, our cultures of this yeast grew slowly in the standard yeast-extract/peptone medium (Seaston *et al.*, 1973) containing 1% (w/v) of α-methyl glucoside. About 40h was required to form the population (about 2mg/ml) that was reached in 22h when maltose was the principal source of carbon. Because the yield of yeast fell to 0.2mg/ml when the carbohydrate was omitted, there is no doubt that the α-methyl glucoside supported growth. Assays with glucose oxidase showed that the carbohydrate contained a negligible amount of free glucose.

Van Steveninck (1970) studied the absorption of α-methyl glucoside by preparations of the yeast grown with maltose in a complex nutrient medium. Whereas he detected no fermentation of α-methyl glucoside under these conditions, we observed a very slow fermentation in the presence of 10mM-α-methyl glucoside. The rate was about 3nmol of CO_2/min per mg dry wt. after growth with maltose for 18h, and 18nmol/min per mg after growth for 24h. The fermentation of maltose was much faster, at about 140nmol/min per mg.

Preparations of the washed cells of strain no. 240 grown with maltose for 24h were treated with toluene to make them permeable to α-methyl glucoside (see the Materials and Methods section). These preparations released glucose from 20mM-α-methyl glucoside at the relatively low rate of about 10nmol/min per mg in the pH range 6–7, at 30°C. The K_m for the glucoside was about 10mM. The corresponding rate of maltose hydrolysis was 0.8μmol/min per mg, the K_m being about 17mM. The α-methyl glucosidase activity in similar preparations of strain no. 74 was about six times as large.

The products of α-methyl glucoside metabolism accumulating in the preparations of strain no. 240 grown for 17 or 24h were examined by t.l.c. Radioautography revealed the presence of at least seven fractions associated with ^{14}C. However, under the conditions described in the legend to Fig. 1, the ^{14}C was located mainly in the three bands, one of which represented α-methyl glucoside. A fourth band representing trehalose, a recognized product of carbohydrate metabolism in yeasts (Kotyk & Michaljaničová, 1974), was prominent in yeast preparations given α-methyl glucoside after 24h growth with maltose. The result of treating the cellular extract with alkaline phosphatase indicated that the fraction near the origin of the chromatogram mainly comprised hexose phosphates. No significant amount of α-methyl glucoside appeared to be released by the action of the phosphatase, either in the experiment illustrated in Fig. 1 or when the materials with R_F values smaller than that of glucose were eluted, treated with the enzyme, and fractionated a second time in the same solvent system. The material represented by the peak between A and B of Fig. 1 was not characterized. Because it was also prominent in extracts of the cells fed with [^{14}C]maltose, it was probably not a simple derivative of α-methyl glucoside. Qualitatively similar observations were made with preparations of yeast strain no. 74.

Clearly the observations illustrated in Fig. 1 further conflict with Van Steveninck's (1970) claim that α-methyl glucoside phosphate was the only labelled compound, besides α-methyl glucoside, that the yeast produced from exogenous α-methyl glucoside.

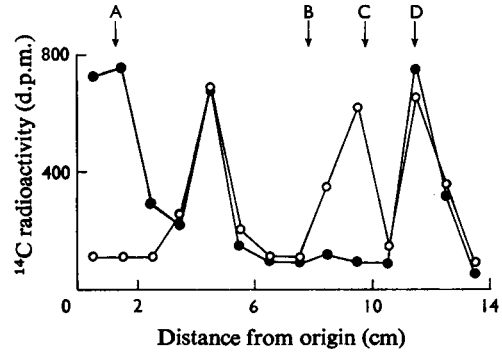


Fig. 1. *Chromatographic analysis of the cellular contents of strain no.* 240 *exposed to* 10mM-α-*methyl* [^{14}C]*glucoside for* 5*min at* 30°C

The yeast was grown with maltose for 17h to a population of 0.4mg dry wt./ml. The washed cells (10mg/ml) were suspended at pH5 in the presence of antimycin (10μg), 10mM-iodoacetamide and 10mM-α-methyl [^{14}C]glucoside (2μCi). After 5min the yeast was separated, washed and extracted. Portions of the extract were treated with alkaline phosphatase and then analysed; other portions were analysed directly (see the Materials and Methods section). The radioactivity (d.p.m.) associated with a given strip (1cm wide) is shown as a function of the distance of the mid-point of the strip from the origin of the chromatogram. ●, Original extract; ○, extract after treatment with alkaline phosphatase. The arrows mark the positions corresponding to: A, glucose 6-phosphate; B, trehalose; C, glucose; and D, α-methyl glucoside.

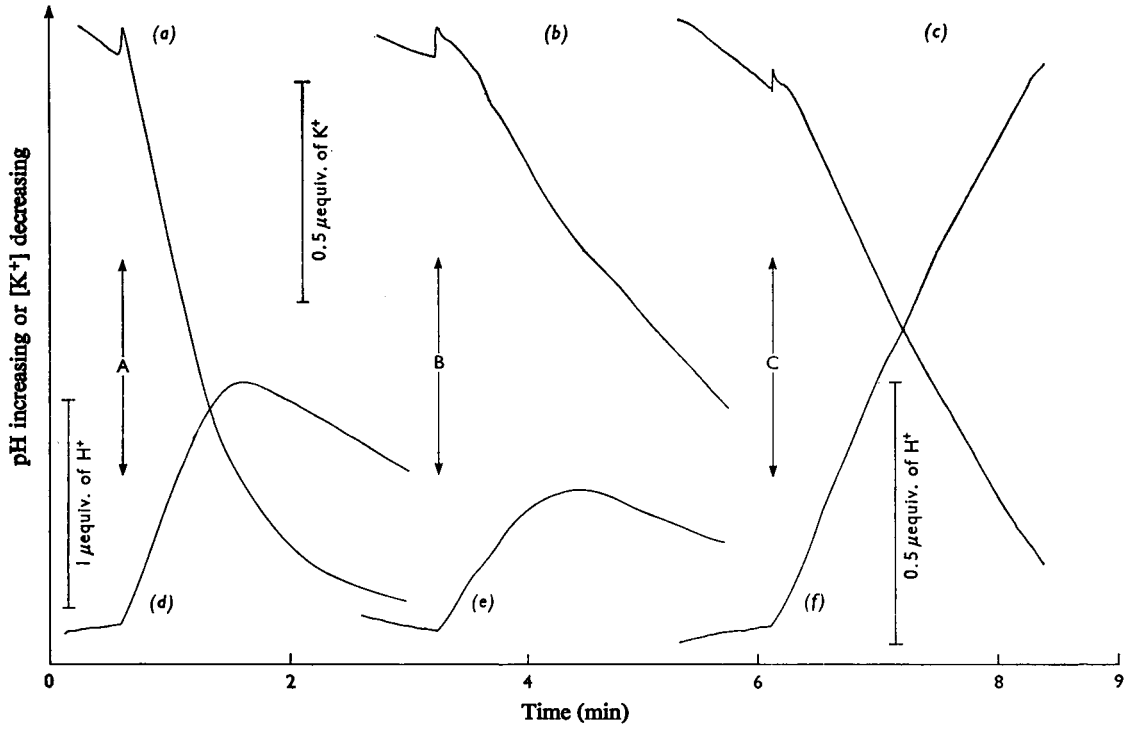


Fig. 2. *Displacements of protons into the yeast and of K^+ ions out of the yeast, caused by the addition of maltose, α-methyl glucoside or α-thioethyl glucoside to preparations of strain no.* 240

The lines represent recordings from the specific ion electrodes, obtained in three experiments each with a different sugar. The traces are depicted on the same time-scale. The same ordinate represents both the rise in recorded pH and the the fall in extracellular $[K^+]$. The systems were calibrated by the addition of small amounts of HCl or KCl. In each case the yeast was grown for 24h with 1% (w/v) maltose as the principal source of carbon. Portions (50mg dry wt.) of the washed yeast were suspended in the electrode vessel in 5mM-Tris adjusted to pH5 with citric acid. The carbohydrate was added 2min later at the indicated time: at A, 20mM-maltose; at B, 40mM-α-methyl glucoside; at C, 4mM-α-thioethyl glucoside. Traces (*d*), (*e*), (*f*) were from the pH electrode. The calibration for (*d*) is adjacent. That for (*f*) is adjacent and applies to trace (*e*) as well. Traces (*a*), (*b*) and (*c*) were from the K^+ electrode. The single calibration applies to all three traces.

Proton uptake and the efflux of K^+

Selected concentrations of maltose, α-methyl glucoside and α-thioethyl glucoside each initially accelerated the uptake of protons and the efflux of K^+ across the yeast cell membrane (Fig. 2). In three such assays 40mM-α-methyl glucoside caused an increase in proton uptake initially of 6.4 ± 0.9 (S.E.M.) nequiv./min per mg and an increase in K^+ efflux of 6.9 ± 0.8 nequiv./min per mg. Similarly α-thioethyl glucoside caused an extra flow of 8.6 ± 0.7 nequiv. of H^+/min per mg and a flow of 8.5 ± 0.5 nequiv. of K^+/min per mg. Maltose had a similar, though larger, initial effect on both flows, of about 20 nequiv./min per mg. Because the addition of 40mM-galactose or 40mM-3-*O*-methylglucose failed to change the rate of proton uptake by the yeast, the effects of the other compounds were not simply the result of increasing the osmotic pressure of the solution. The observations are thus consistent with Scheme 1(*a*) rather than Scheme 1(*b*).

Reference to Fig. 2 shows that, unlike α-thioethyl glucoside, the two substrates that the yeast fermented caused a net efflux of H^+ after about 1min. Our further studies (R. Brocklehurst, D. Gardner & A. A. Eddy, unpublished work) showed that the efflux of succinate accompanies the ejection of acid under these conditions, as Conway & Brady (1947) also observed. The fact that anions are being excreted may help to explain why the efflux of K^+, in the interval 1–2min after the glucoside was added, remained faster than in the period before the addition was made (Fig. 1).

The initial phase of net proton absorption was studied further in the presence of metabolic inhibitors

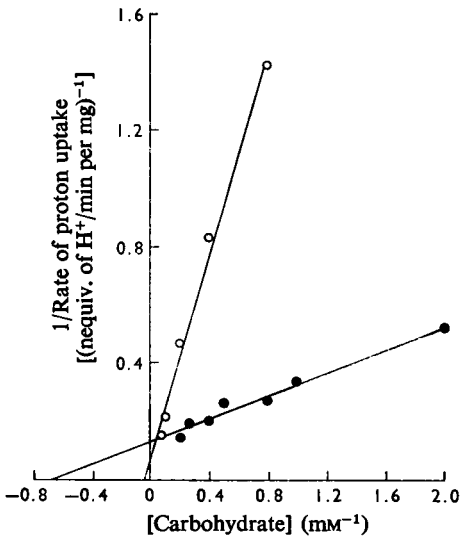


Fig. 3. *Double-reciprocal plots showing how the increase in the rate of proton uptake caused by α-methyl glucoside* (○) *or by α-thioethyl glucoside* (●) *increased with their respective concentrations*

The procedure described in the legend to Fig. 2 was used, except that the yeast suspension contained both antimycin (0.1 μg/mg dry wt. of yeast) and 2.5 mM-2-deoxyglucose. Four yeast cultures were assayed at a given carbohydrate concentration to obtain the results depicted.

that lower the cellular ATP content and stop proton ejection (Seaston *et al.*, 1973). When deoxyglucose and antimycin were used for that purpose with strain no. 240, the rate of proton uptake increased systematically with the concentration of α-methyl glucoside or α-thioethyl glucoside (Fig. 3). Hence the process appeared not to involve energy metabolism.

Proton stoicheiometry with α-methyl glucoside

The combination of antimycin and 10 mM-iodoacetamide also inhibited acid ejection in the presence of carbohydrates (cf. Seaston *et al.*, 1973). Table 1 compares the respective amounts of protons and of α-methyl glucoside absorbed under these conditions. Parallel assays with the glucostat reagent showed that glucose accumulated in the medium when either α-methyl glucoside (Table 1) or maltose was the substrate. Chromatographic analysis demonstrated that the glucose was associated with ^{14}C and was therefore formed from the added glucoside.

The glucose formed from maltose was probably not produced by damaged yeast cells (containing maltase) that had become exposed to the medium (R. Brocklehurst, unpublished work). The ratio (equiv. of H^+ absorbed/mol of glucoside absorbed) was accordingly computed in two ways. The glucose was assumed to be formed either (1) before or (2) after absorption of the α-methyl glucoside from which it was derived. Table 1 shows that only the second assumption led to a value of the stoicheiometric ratio that was roughly independent of the duration of the incubation. We tentatively conclude that the true ratio was probably 1.

Concentration of α-thioethyl glucoside by the yeast

The strain no. 240 was grown either with maltose or with α-methyl glucoside and the washed cells were then incubated with 0.5 mM-α-thioethyl glucoside at pH 5 for 30 min. Table 2 shows that these yeast preparations concentrated the glucoside whether or not antimycin was present to inhibit respiration. The process was inhibited by 0.2 mM-dinitrophenol and by raising the pH to 7. Virtually all the thioethyl glucoside (94–106% in ten assays) added to the system was recovered as such, in agreement with other reports about its metabolic inertness in yeast (Okada & Halvorsen, 1964; De Kroon & Koningsberger, 1970). The g.l.c. analysis showed that no trehalose was expelled from the yeast into the medium, so that α-thioethyl glucoside was evidently not simply exchanged with endogenous trehalose. Table 2 also shows that the yeast grown for 17 h accumulated less thioglucoside than did the preparations grown for 24 h. The rate of fermentation of α-methyl glucoside (see above), and the rate of absorption of protons with α-methyl glucoside, showed a similar dependence on culture age (see below).

The accumulation of α-thioethyl glucoside was inhibited by more than 80% in the presence of a 10 mM solution of one of the following: glucose, L-sorbose, α-methyl glucoside, maltose, sucrose, trehalose; or 2 mM-phenyl glucoside. In contrast, 100 mM-fructose was without effect on the accumulation of α-thioethyl glucoside. Similar observations, differing in some details, were made by Okada & Halvorsen (1964) with other yeast strains.

Proton absorption caused by other carbohydrates

It seemed possible that some of the above carbohydrates were either (1) substrates of a proton symport through which α-thioethyl glucoside and α-methyl glucoside were also absorbed, or (2) they were absorbed through other proton symports, thereby lowering the membrane potential and the uptake of thioethyl glucoside through its own system. The following observations are consistent with either of these interpretations. Each of a series of potential substrates was added to preparations of strain no. 240 grown for 24 h with maltose. (1) The

Table 1. *Proton absorption and glucose formation during the absorption of α-methyl glucoside by strain N.C.Y.C. no. 240*
The yeast was grown for 24h with maltose, washed, and portions (50mg) were incubated at pH5.0 with 10mM-iodoacetamide and antimycin (10μg) for 5min at 30°C. Then 20mM-α-methyl [^{14}C]glucoside (4mCi/mol) was added. The ^{14}C found inside the yeast, the glucose found outside the yeast and the sum of these two quantities (expressed in nmol of α-methyl glucoside/mg dry wt. of yeast) are compared with the extra number of protons absorbed by the yeast in the presence of α-methyl glucoside (see the Materials and Methods section). Mean values ± S.E.M. for four assays are shown. The stoicheiometric ratios are discussed in the text.

Time with α-methyl [^{14}C]glucoside (min) ...	0.5	1.0	2.0	4.0
^{14}C retained (nmol/mg)	11.1±3.0	18.6±3.2	25.4±3.4	29.4±6.2
Glucose found (nmol/mg)	5.2±1.3	4.2±1.7	16.0±6.0	32.8±7.8
Glucose found+^{14}C retained (nmol/mg)	16.3±3.3	22.8±3.6	41.4±6.9	62.2±9.5
Protons absorbed (nequiv./mg)	14.7±2.1	26.8±2.9	40.0±3.3	52.9±1.7
Ratio (H^+ absorbed/^{14}C retained)	1.4±0.1	1.6±0.1	1.7±0.1	2.0±0.3
Ratio [H^+ absorbed/(glucose+^{14}C retained)]	0.9±0.1	1.2±0.1	1.1±0.2	0.9±0.2

Table 2. *Ionic and other factors influencing the accumulation of α-thioethyl glucoside by yeast N.C.Y.C. no. 240 grown with maltose or α-methyl glucoside*
The yeast was grown with 1% (w/v) of the indicated carbon source, washed and incubated for 30min at 30°C with α-thioethyl glucoside. The pH of the salt solution was adjusted to the indicated value by the addition of citric acid. In Series 1–4 the concentrations of α-thioethyl glucoside in the cells ([cells]) and in the solution phase ([medium]) were determined (see the Materials and Methods section). In Series 5 the concentration in the cells was determined directly and the concentration in the medium by difference. The results of one of at least two similar sets of assays are shown.

		Incubation conditions		Distribution of α-thioethyl glucoside at 30min		
Series	Carbon source (culture age)	Buffer constituents	Initial glucoside concentration (mM)	[cells] (mM)	[medium] (mM)	[cells]/[medium]
1	Maltose (17h)	0.01M-K_2HPO_4 (pH5)	0.25	2.2	0.21	10
2	Maltose (24h)	0.01M-K_2HPO_4 (pH5)	0.5	4.6	0.02	230
3	Maltose (24h)	0.01M-K_2HPO_4 (pH7)	0.5	0.5	0.49	1
4	α-Methyl glucoside (22h)	0.01M-K_2HPO_4 (pH5)	0.2	9.7	0.05	194
5	Maltose (24h)	0.1M-Na_2HPO_4 (pH5)	0.5	17.5	0.15	116
		0.1M-Na_2HPO_4 (pH5)+0.2M-KCl	0.5	13.0	0.24	54
		0.1M-Na_2HPO_4 (pH5)+antimycin (2μg/ml)	0.5	15.1	0.17	90
		0.1M-Na_2HPO_4 (pH5)+antimycin (2μg/ml)+0.2mM-dinitrophenol	0.5	6.0	0.38	16
		0.2M-Na_2HPO_4 (pH7)	0.5	0.5	0.49	1

presence of a 40mM solution of one of the following caused no immediate increase in the rate of proton uptake: D-xylose, D-galactose, 3-*O*-methylglucose, 2-deoxyglucose and fructose. Fructose nevertheless caused a rapid proton ejection after about 1min. (2) A 40mM solution of one of the following was added to a given yeast preparation. The rate of proton uptake increased in this and another similar experiment to the extents (nequiv. of H^+/min per mg) shown in parentheses: L-sorbose (17); α-methyl glucoside (15); sucrose (17): trehalose (13); turanose (20); glucose (12) and 2mM-α-phenyl glucoside (17). Smaller concentrations of glucose (2mM) failed to stimulate proton uptake, in agreement with Seaston *et al.* (1973) The fact that fructose neither inhibited α-thioethyl glucoside uptake, nor caused a faster proton uptake, whereas glucose, sorbose and the other carbohydrates examined affected both processes, is consistent with the symport model. (3) The carbohydrates that stimulated proton uptake comprised two groups. The first group (trehalose, maltose and turanose) caused roughly the same proton uptake whether the yeast was grown for 17 or 24h, although the magnitude of the uptake varied with the carbohydrate (three experiments). The second group (α-phenyl glucoside, α-methyl glucoside, sucrose) caused a larger proton uptake in the older yeast preparations. For instance, the rate with 2mM-

sucrose was $3.3 \pm 0.7(3)$ nequiv. of H^+/min per mg after 17h of growth and $9.2 \pm 1.0(4)$ after 24h growth. Preliminary work suggested that α-thioethyl glucoside, glucose and sorbose belonged to the second category.

Dual modes of absorption of α-methyl glucoside

When strain no. 240 was grown with 1% α-methyl glucoside for 23h, the addition of 20mM-α-methyl glucoside to the washed cells, in the presence of deoxyglucose and antimycin, caused an increase in proton uptake of about 12nequiv./min per mg. α-Thioethyl glucoside had a similar effect. These preparations concentrated α-thioethyl glucoside (Table 2). Similar preparations of strain no. 74, also grown with α-methyl glucoside, failed to absorb protons faster in the presence of α-methyl glucoside (six experiments). Nevertheless a fast proton ejection was initiated when the glucoside was metabolized (cf. Fig. 2). Interestingly, preparations of the strain no. 74 grown with maltose absorbed protons faster both in the presence of α-methyl glucoside (Seaston *et al.*, 1973) and, to the extent of 15nequiv./min per mg, in the presence of 5mM-α-thioethyl glucoside. We conclude that strain no. 74 exhibits two modes of α-methyl glucoside absorption, one of which involves a proton symport and superficially resembles the mode used by strain no. 240.

Discussion

In our hands the yeast no. 240 behaved towards α-methyl glucoside in a manner quite different to that reported by Van Steveninck (1970). The nature of the labelled metabolites produced from α-methyl [^{14}C]glucoside (Fig. 1 and Table 1), the slow fermentation of the compound and its slow utilization for growth are each inconsistent with the claim (Van Steveninck, 1970) that α-methyl glucoside metabolism was limited to two specific reactions. These were believed to comprise (1) the phosphorylation reaction forming α-methyl glucoside phosphate, a version of which is shown in Scheme 1*b*, and (2) a dephosphorylation reaction forming intracellular α-methyl glucoside from the methyl glucoside phosphate. Because the first of the above reactions precedes the second, the methyl glucoside phosphate would be labelled before the cellular methyl glucoside when the yeast was exposed to methyl [^{14}C]glucoside, as indeed Van Steveninck (1970) reported. However, the phosphorylated compounds that we detected were quite clearly not phosphorylated methyl glucoside but hexose phosphates, so we have not studied the matter further. One unresolved question is whether the system of chromatography that Van Steveninck (1970) used in fact separated glucose from α-methyl glucoside.

Besides the lack of evidence for the formation of a phosphorylated derivative of either α-methyl glucoside or α-thioethyl glucoside in our preparations, there are other observations that are difficult to explain in terms of the phosphorylation mechanism (Scheme 1). (1) The influx of H^+ and the accompanying efflux of K^+, caused by both α-methyl glucoside and α-thioethyl glucoside (Fig. 2), are inconsistent with the proposed transfer of a phosphate group into the yeast (Scheme 1*b*). The group-transfer model requires no efflux of K^+ to neutralize the influx of H^+, even when the free sugar is the main cellular product as a result of dephosphorylation. (2) The phosphorylation hypothesis supposes that the accumulation of α-thioethyl glucoside is driven mainly, or exclusively, by phosphate-bond energy rather than by the proton gradient acting across the plasmalemma. Nevertheless, (*a*) the presence of the proton conductor dinitrophenol and (*b*) the use of pH7 instead of pH5 both lowered the uptake of the glucoside, possibly by lowering the proton gradient (Table 2).

The phenomena based on (1) and (2) above are consistent, however, with the symport model (Scheme 1*a*). Moreover, the observed influx of H^+ and the efflux of K^+ resemble the movements of these ions seen during the absorption of other substrates that yeasts concentrate. The statement applies to various amino acids absorbed by certain strains of *Saccharomyces cerevisiae* (Seaston *et al.*, 1973), as well as to the absorption of phosphate (Cockburn *et al.*, 1975), maltose, α-methyl glucoside and various amino acids by yeast no. 74 (Eddy & Nowacki, 1971; Seaston *et al.*, 1973, 1976). The symport model provides a common basis for these effects, which represent a means of coupling the influx of the organic solutes to the free energy inherent in the flow of protons across the plasmalemma (Mitchell, 1970).

The rate of fermentation of α-methyl glucoside, the extent to which α-thioethyl glucoside is concentrated, as well as the rate of proton uptake that both compounds caused were each smaller in the yeast preparations grown with maltose for 17h as opposed to 24h. In contrast, the proton uptake with maltose was slightly faster in the younger cell preparations. Such behaviour is consistent with genetic evidence that the α-methyl glucoside permease and the maltose permease are distinct entities (Hawthorne, 1958; Okada & Halvorsen, 1964; ten Berge, 1972). The presence of one of three pairs of genes (Hawthorne, 1958) and, at least in strain no. 74, of an additional gene (ten Berge, 1972) is required for the fermentation of α-methyl glucoside. Further work with strains of defined genotype is needed to relate these observations in the literature to the two modes of methyl glucoside absorption found in the preparations of strain no. 74 grown with maltose or α-methyl glucoside respectively. The

effects that other carbohydrates causing proton absorption, such as glucose and sorbose, produced on the uptake of α-thioethyl glucoside by strain no. 240 suggests that these compounds may be substrates of the α-methyl glucoside permease. Glucose and sorbose are also absorbed by facilitated diffusion (Heredia *et al.*, 1968).

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