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Role of D-Ribose as a Cometabolite in D-Xylose Metabolism by *Saccharomyces cerevisiae*

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The influence of D-ribose as a cosubstrate on the uptake and metabolism of the non-growth substrate D-xylose by *Saccharomyces cerevisiae* ATCC 26602 was investigated. Xylose was taken up by means of low- and high-affinity glucose transport systems. In cells exposed for 2 days to a mixture of xylose and ribose, only the high-affinity system could be detected. Glucose strongly inhibited the transport of xylose by both systems. Starvation or exposure to either xylose or ribose resulted in inactivation of xylose transport, which did not occur in the presence of a mixture of ribose and xylose. A constitutive non-glucose-repressible $NADPH_2$-dependent xylose reductase with a specific activity of ca. 5 mU/mg of protein that converted xylose to xylitol was present in a glucose-grown culture. No activity converting xylitol to xylulose or vice versa was found in crude extracts. Both xylose and ribose were converted to their corresponding polyols, xylitol and ribitol, as indicated by ^{13}C nuclear magnetic resonance spectroscopy. Furthermore, ethanol was detected, and this implied that pathways for the complete catabolism of xylose and ribose exist. However, the $NADPH_2$ required for the conversion of xylose to xylitol is apparently not supplied by the pentose phosphate pathway since the ethanol produced from D-[1-^{13}C]xylose was labelled only in the C-2 position. Acetic acid was produced from ribose and may assist in the conversion of xylose to xylitol by cycling $NADPH_2$.

Taxonomic and physiological studies have shown that *Saccharomyces cerevisiae* is unable to grow on D-xylose (18, 38), and consequently this yeast has not been investigated extensively in the search for xylose-fermenting yeasts. Recently, *S. cerevisiae* was reported to utilize xylose in conjunction with ribose, glucose, glycerol, or galactose (1, 38), although the rate of xylose utilization was considerably lower in *S. cerevisiae* than in other yeasts such as *Pichia stipitis*, *Pachysolen tannophilus*, and *Candida shehatae* (27). These latter yeasts have been investigated extensively for ethanol production from hemicellulose hydrolysates, but their poor ethanol tolerance and production rates in comparison with those of *S. cerevisiae* when grown on glucose will probably limit their commercial application (27). The components of xylose metabolism in *S. cerevisiae* have already been described. The ability to take up xylose by the use of the glucose transport system which has a broad specificity for pyranoses is well known (9, 15, 17). High- and low-affinity transport systems for glucose have been found in *S. cerevisiae* (3), and apparently both are used in xylose uptake (8, 32). However, the affinities of these systems for xylose are considerably lower than those for glucose, indicating that glucose is the primary substrate. Furthermore, one strain was found to have low activities of xylose reductase and xylitol dehydrogenase that should enable the strain to convert xylose to xylulose via xylitol (1). Xylulose is readily utilized by many *S. cerevisiae* strains (13, 34), although at a rate considerably lower than when glucose is used. However, it remained unclear why the utilization of xylose could occur only in the presence of a cosubstrate. In *Candida boidinii*, xylitol production was significantly increased by methanol supplementation, and the oxidation of the methanol was suggested by the authors to provide $NADH_2$ to enhance the conversion of xylose to xylitol (40). The purpose of this investigation was to determine the effect of ribose, the best cosubstrate previously shown to enhance xylose utilization in *S. cerevisiae* (38), on the kinetics of xylose uptake in whole cells and on xylose conversion in crude extracts. ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used to detect key intermediates and analyze how the distribution of labelled carbon may influence the redox balance in the cell.

MATERIALS AND METHODS

Organism. *S. cerevisiae* ATCC 26602 was used in all experiments and maintained on yeast-malt agar slants (41).

Yeast cultivation. A 48-h-old slant culture of *S. cerevisiae* ATCC 26602 was used to inoculate 150 ml of medium consisting of 6.7 g of yeast nitrogen base (YNB; Difco) per liter containing 10 g of D-glucose or 20 g of ethanol per liter as the carbon source in a 1-liter flask. The culture was incubated aerobically at 30°C on an orbital shaker (160 rpm; 27.5-mm throw).

Measurement of [^{14}C]xylose uptake. Glucose (10 g/liter)-grown cells were harvested in the mid-exponential growth phase. In some instances, the glucose-grown cells were resuspended in YNB medium at a dry mass concentration of 2 g/liter and exposed to D-xylose (5 g/liter) and/or D-ribose (5 g/liter) for 2 days or starved in YNB (without glucose) medium for 3 h or 2 days. All cells were harvested and washed twice with ice-cold distilled water, concentrated 100-fold by suspension in cold 0.1 M Tris-citrate buffer (pH 5) to a concentration of approximately 26 mg (dry weight) per ml, and held on ice. The cell suspension was incubated for 2 min at 30°C before the uptake of D-xylose was initiated by the addition of a stock solution of uniformly labelled D-[^{14}C]xylose (800 mmol/liter; Amersham; final specific activity, 9.25 MBq/mmol, as a result of dilution with unlabelled

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xylose) to a cell suspension at 30°C to achieve different final D-xylose concentrations. In some instances, 80 mmol of unlabelled D-ribose or D-glucose per liter was added to the assay mixture to investigate the effect on the rate of D-xylose uptake. The reaction was stopped after 0 and 10 s by dilution with 5 ml of ice-cold distilled water. Preliminary experiments showed that the uptake rate was linear for at least 20 s. The reaction mixtures were filtered immediately (Whatman GF/C glass microfiber filters; pore size, 1 μm), and the cells were washed twice with 5 ml of distilled water (4°C). The filters were placed in scintillation vials containing 10 ml of scintillation fluid (Aquagel; Chemlab, Randburg, South Africa), and the radioactivity was determined with a liquid scintillation counter (Beckman model LS 7800).

Measurement of D-xylose and D-ribose utilization and preparation of cell extracts for enzyme assays. Cultures grown on glucose or ethanol until the mid-exponential or stationary growth phase were harvested by centrifugation. The ability of these cultures (initial dry cell mass, 2 g/liter) to utilize a mixture of xylose (5 g/liter) and ribose (5 g/liter) was evaluated in YNB medium (150 ml in a 1-liter flask) at 30°C for 7 days. In some instances, cells cultivated on 10 g of glucose per liter were harvested in the mid-exponential growth phase, washed with cold distilled water, and exposed further to 5 g of xylose per liter or 5 g each of xylose and ribose per liter for 64 h. After being harvested, the cells were washed twice with pH 7.5 buffer (10 mM K_2HPO_4, 0.5 mM EDTA, 0.5 mM 2-mercaptoethanol) (11). A protease inhibitor, phenylmethylsulfonyl fluoride, was added at a concentration of 1 mM. The cells were disrupted with glass beads (0.5 to 1.0 mm) by vortexing for 20 cycles of 1 min each with 1-min intervals of cooling on ice. Cell debris was removed by centrifugation (28,000 × *g* at 4°C for 30 min).

Enzyme assays and cofactor specificity. Xylose reductase was assayed in a reaction mixture of 0.5 M K_2HPO_4 (pH 7.4) buffer, 50 mM D-xylose, 10 mM 2-mercaptoethanol, and 0.34 mM $NADPH_2$ or reduced NADH ($NADH_2$). Xylitol dehydrogenase was assayed in a reaction mixture of 75 mM Tris-HCl (pH 8.6) buffer, 50 mM xylitol, 0.25 mM $MgCl_2$, and 0.34 mM NAD^+ or oxidized NADP ($NADP^+$) (21). The enzyme reaction was monitored by determining the reduced cofactor spectrophotometrically at 340 nm with an LKB spectrophotometer. The reactions were started by the addition of cell extract sufficient to produce changes in absorbance of between 0.02 and 0.1 per min. Blanks containing all of the components except the substrate were used to correct the enzyme activity. Protein concentrations were determined by the method of Bradford (4) by using bovine serum albumin as the standard. The enzyme activity was expressed in micromoles of substrate converted per minute per milligram of protein at 30°C.

NMR spectroscopy studies. For NMR spectroscopy experiments, the glucose-grown culture was harvested in the mid-exponential growth phase once an absorbance of 1.0 (measured at 640 nm with an LKB spectrophotometer) was reached (100 ml of medium yielded 0.05 g of cell dry mass). In some instances, *S. cerevisiae* was cultivated in 3 liters of medium (10 g of glucose per liter) in 5-liter Erlenmeyer flasks at 30°C with stirring on a magnetic stirrer (700 rpm) until an absorbance of 1.3 was attained after 16 h. Concentrations of cells similar to that found under more-aerobic growth conditions were obtained. The cells were harvested aseptically by centrifugation at 2,500 × *g* for 5 min and washed twice. The culture was resuspended in 6.7 g of YNB to achieve a cell density of 60 g/liter. The experiment was started by adding 5 g each of D-[1-^{13}C]xylose (Omicron Biochemicals, Ithaca, N.Y.) and D-ribose per ml or 5 g of D-[1-^{13}C]ribose (Omicron) per ml to the cell suspension held at 30°C. Samples (1 ml) were withdrawn at appropriate times up to 6 h. One experiment was conducted under conditions of greater oxygen provision by reducing the cell concentration to 10 g/liter and adding 2.5 g of D-[1-^{13}C]xylose per liter and 2.5 g of D-ribose per liter to the culture in test tubes held in a slanted position at 30°C and rotated at 40 oscillations per min. At 0, 24, and 72 h, 3-ml samples were withdrawn. Each sample was rapidly filtered through a 0.45-μm-pore-size methanol-resistant membrane filter (Millipore Durapore HVLP 04700) and washed with 3 ml of a mixture of 50% (vol/vol) methanol in water held at −40°C (30). The cells were frozen in liquid nitrogen, and perchloric acid extracts were prepared as described by Den Hollander et al. (10). Filtrates and cell extracts were kept frozen until NMR spectroscopy could be conducted.

Analyses. (i) NMR spectroscopy. ^{13}C proton-noise-decoupled NMR spectra were obtained overnight at 75 MHz on a Brucker AM-300 spectrometer at 20°C with a 5-mm tube. Samples were dissolved in D_2O, and 0.53% dioxane (67.7-ppm chemical shift) was used as the internal reference to compare data from the various spectra. The spectra of the medium were recorded after ca. 500 scans with 3-s delays, whereas for the extracts, ca. 2,000 scans were recorded. Manganese(II) chloride (0.8 mM) was added for relaxation enhancement and, together with gated broad-band decoupling of protons, used to cancel the nuclear Overhauser enhancement effect (25). The ^{13}C signals were identified by comparison with published data (5), and confirmation was obtained by comparison with spectra of pure unenriched metabolite reference samples.

(ii) Biomass, sugar, and ethanol determinations. The biomass of washed cells was determined gravimetrically at 105°C. Sugar concentrations were quantified by high-performance liquid chromatography and ethanol concentrations were determined by gas chromatography as described previously (38).

RESULTS

Characteristics of xylose transport. Fresh glucose-grown cells possessed only one system for xylose transport, with a K_m value (standard deviation of three determinations shown in parentheses) of 110 (±3) mM determined from Hanes-Woolf plots of the uptake data. In starved glucose-grown cells, however, two systems were observed, with K_m values of 49 (±7) and 228 (±5) mM. The V_{max} value of 281 (±7) μmol/min/g of the low-affinity system in starved cells was similar to the value of 266 (±5) μmol/min/g of the fresh cells, whereas the value of 119 (±9) μmol/min/g in the high-affinity system was more than twofold lower. Only one xylose transport system was found in cells incubated for 2 days in the presence of a mixture of xylose and ribose. This system had a K_m value of 53 (±5) mM similar to that of the high-affinity system but a V_{max} value of 221 (±9) μmol/min/g similar to that of low-affinity system. When the kinetic constants were determined by Lineweaver-Burk and Eadie-Hofstee plots, values similar to those determined by using Hanes-Woolf plots were obtained.

In comparison with the transport activity of fresh glucose-grown cells, starvation of glucose-grown cells for 3 h and 2 days resulted in 17 and 63% losses, respectively, of xylose-transporting activity (Table 1). Exposing glucose-grown cells for 2 days to xylose or ribose also resulted in decreases in transport activity. When glucose-grown cells were incubated

TABLE 1. Effect of different treatment conditions on the uptake rate of D-xylose (80 mmol/liter) by *S. cerevisiae* ATCC 26602 cultivated on glucose until mid-exponential growth phase

Treatment condition	Uptake rate (μmol/min/g)[a]	Relative rate (%)
None	108.3 (102.2–114.4)	100
Starvation for 3 h	83.5 (81.3–87.2)	77
Starvation for 2 days	37.2 (35.5–38.8)	34
Exposure to xylose for 2 days	47.9 (47.9–48.0)	44
Exposure to ribose for 2 days	38.1 (36.3–39.9)	35
Exposure to xylose and ribose for 2 days	117.6 (108.2–127.0)	109

[a] The values represent the means of duplicate independent determinations (ranges of data shown in parentheses).

TABLE 3. Specific activity of $NADPH_2$- and $NADH_2$-linked xylose reductase from crude extracts of *S. cerevisiae* cultivated under different conditions

Growth substrate[a]	Subsequent substrate exposure[b]	Sp act (mU/mg of protein)[c]	
		$NADPH_2$ linked	$NADH_2$ linked
Ethanol	None	3.3 (0.5)	0
Glucose	None	5.1 (0.3)	0.2 (0.1)
Glucose	Xylose and ribose	5.4 (0.4)	0.2 (0.1)
Glucose	Xylose	0.9 (0.1)	0.4 (0.2)

[a] The culture was grown on ethanol (20 g/liter) or glucose (10 g/liter) until mid-exponential growth phase.
[b] The culture was exposed to sugars (5 g each per liter) for 64 h at 30°C.
[c] The values represent the means (standard deviations in parentheses) of three replicate determinations.

in the presence of a mixture of ribose and xylose for 2 days, however, xylose was transported at a rate similar to that in fresh cells (Table 1).

The rate of xylose transport in the presence of glucose was only 11% of that in the absence of glucose in fresh glucose-grown cells, which indicated that glucose strongly inhibited xylose uptake. Ribose had a smaller inhibitory effect on xylose transport (Table 2). In glucose-grown cells exposed to a mixture of ribose and xylose for 2 days, glucose had a similar effect on the transport of xylose as it did in fresh glucose-grown cells, but the effect of ribose on these cells was negligible (Table 2).

D-Xylose and D-ribose metabolism. Approximately 25-fold-greater xylose reductase activity was demonstrated with $NADPH_2$ than with with $NADH_2$ as cofactor (Table 3). Subjecting the crude enzyme extract to dialysis failed to result in higher enzyme activity (data not shown), indicating that the low xylose reductase activity relative to that of other xylose-fermenting yeasts (6) was not the result of low-molecular-weight inhibitory compounds in the crude extracts. The enzyme activity of the reverse xylose reductase reaction, with 200 mM xylitol as substrate in 50 mM Tris-HCl (pH 8.6) and with 0.4 mM $NADP^+$ as cofactor, was approximately 10 times slower than the forward reaction (data not shown).

The xylose reductase appeared to be constitutive since cultivation of the organism on ethanol or D-glucose resulted in similar specific activities (Table 3). Furthermore, the activity remained similar when the glucose-grown cells were exposed to xylose and ribose, whereas exposure to xylose alone resulted in a decrease in xylose reductase activity, indicating a possible inactivation of the $NADPH_2$-dependent xylose reductase. The growth phase of cells grown on glucose or ethanol failed to affect the specific activity of the $NADPH_2$-dependent xylose reductase significantly (Table 4). No relationship between xylose reductase specific activity and the rate of xylose and ribose utilization was observed. However, cells grown on glucose and harvested in the exponential growth phase utilized both xylose and ribose to a greater degree than glucose-grown cells harvested in the stationary phase or ethanol-grown cells (Table 4).

Neither forward xylitol dehydrogenase activity with either NAD^+ or $NADP^+$ as cofactor nor reverse xylitol dehydrogenase reactions with xylulose as substrate and $NADH_2$ or $NADPH_2$ as cofactor were found in crude extracts. Significant background activity, however, was observed in the absence of substrate even in dialyzed samples which could have masked low activity if substrate was present (data not shown). Analysis by high-performance liquid chromatography showed that xylulose was neither produced nor removed (data not shown).

^{13}C NMR spectroscopy. In the presence of a high cell concentration (60 g/liter), D-[1-^{13}C]xylose and unlabelled ribose, peaks representing the β (98.8-ppm) and α (94.4-ppm) forms of the C-1 of xylose, were observed in the medium at 0 and 4 h (Fig. 1A and B). The ratio of the β to α peak heights was similar at 0 and 4 h, indicating that *S. cerevisiae* does not preferentially utilize either form of the sugar. Peaks representing the C-1 of xylose were also observed intracellularly after 4 h (Fig. 1D) but not after 6 h (Fig. 1E). The possibility that the xylose was detected as a result of inadequate washing of the cells was discounted by the absence of xylose peaks in the scan of the cell extract immediately after the addition of labelled xylose (Fig. 1C). Xylitol (64.8 ppm) was the major intermediate accumulated within the cell (Fig. 1D and E) and was also excreted into the medium (Fig. 1B). A

TABLE 2. Effect of D-ribose and D-glucose on the xylose transport rate by *S. cerevisiae* ATCC 26602 cultivated under different conditions

Sugars (80 mmol/liter) in transport assay	Xylose uptake rate (μmol/min/g)[a]	
	Fresh glucose-grown cells	Cells exposed to ribose and xylose (2 days)
Xylose	108.3 (102.2–114.4)[b] (100)[c]	117.6 (108.2–127.0) (100)
Xylose, ribose	81.0 (71.9–90.2) (75)	110.1 (104.6–115.6) (94)
Xylose, glucose	12.4 (12.2–12.6) (11)	9.3 (8.4–13.0) (8)

[a] The values represent the means of duplicate independent determinations.
[b] The values in the first set of parentheses indicate the range of data.
[c] The value is the second set of parentheses is the percent relative to the xylose uptake rate in the absence of other sugars in the assay mixture.

TABLE 4. $NADPH_2$-linked xylose reductase specific activity in crude extracts and percent utilization of D-xylose or D-ribose by *S. cerevisiae* grown on D-glucose or ethanol and harvested in different growth phases

Growth conditions	Xylose reductase sp act (mU/mg of protein)[a]	% Utilization over 7 days at 30°C[a]	
		D-Xylose	D-Ribose
Glucose-grown cells from mid-exponential phase	4.1 (0.2)	66 (1.6)	42 (0.8)
Glucose-grown cells from stationary phase	6.3 (0.4)	41 (2.2)	8 (1.2)
Ethanol-grown cells from mid-exponential phase	6.0 (0.4)	15 (2.2)	8 (1.3)

[a] Values represent the means (standard deviations in parentheses) of three determinations.

peak (19.1 ppm) representing the methyl group (C-2) of ethanol was observed in cell extracts at 4 and 6 h (Fig. 1D and E). This was confirmed by the addition of unenriched ethanol to the sample. No peaks representing ethanol were observed in the medium (Fig. 1B). A peak (17.5 ppm) in the 4-h cell extract could not be identified (Fig. 1D), but, from the position in the spectrum, a methyl group is indicated. The identity of the unknown compound in xylose metabolism is uncertain, although Ligthelm et al. (22) also observed an unknown peak in a similar position in their studies on xylose metabolism by *P. stipitis*.

In the presence of a lower cell concentration (10 g/liter) and under conditions of greater oxygen supply than those in the previously described experiment, only peaks representing the β and α C-1 of xylose and the C-1 of xylitol were observed in the medium. A single peak representing the C-1 of xylitol was found in the cell extract (data not shown).

When the cells (60 g/liter) were incubated in the presence

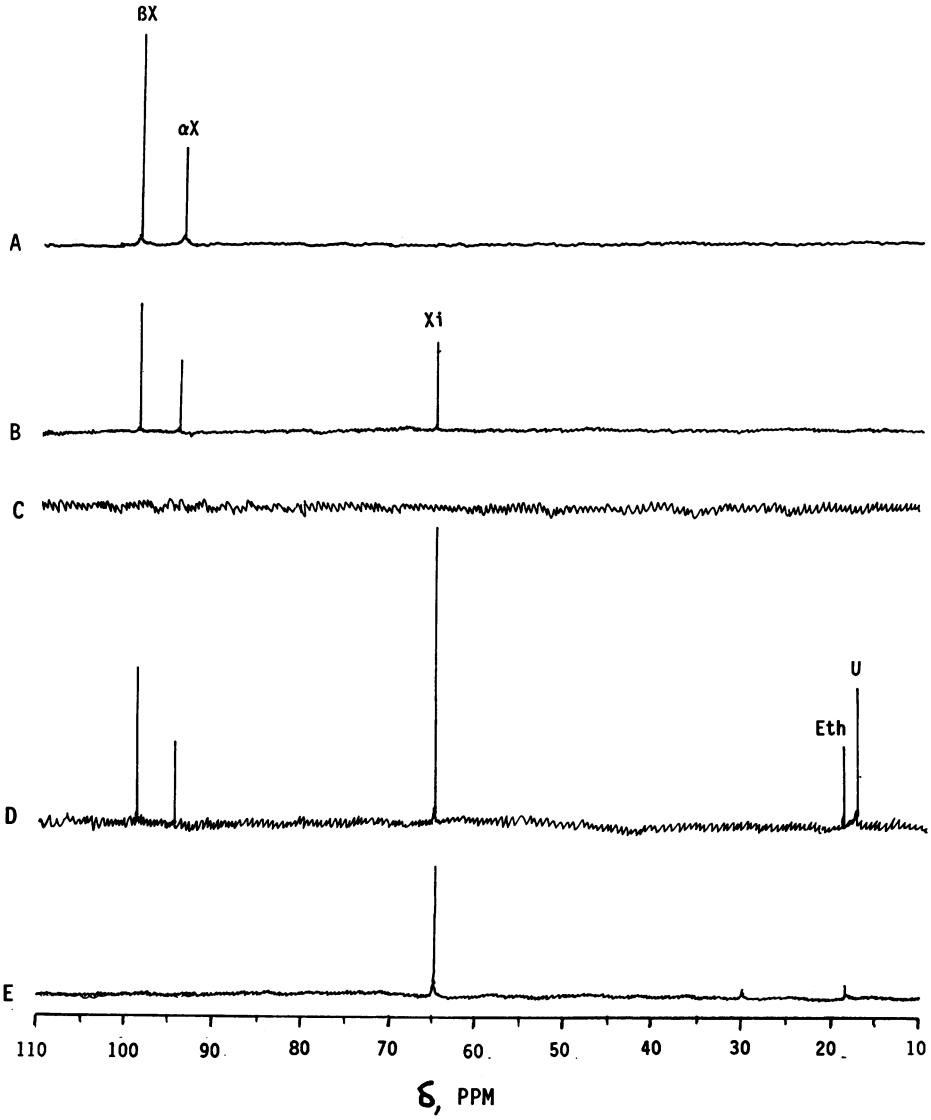

FIG. 1. ^{13}C NMR spectra of medium (A and B) and cell extracts (C, D, and E) prepared from *S. cerevisiae* ATCC 26602 cultivated on D-[1-^{13}C]xylose and D-ribose. Samples of medium were taken at 0 (A) and 4 (B) h. Cell extracts were prepared from samples taken at 0 (C), 4 (D), and 6 (E) h. Abbreviations: β-X, β-D-xylose; α-X, α-D-xylose; Eth, [2-^{13}C]ethanol; Xi, xylitol; U, the unknown compound.

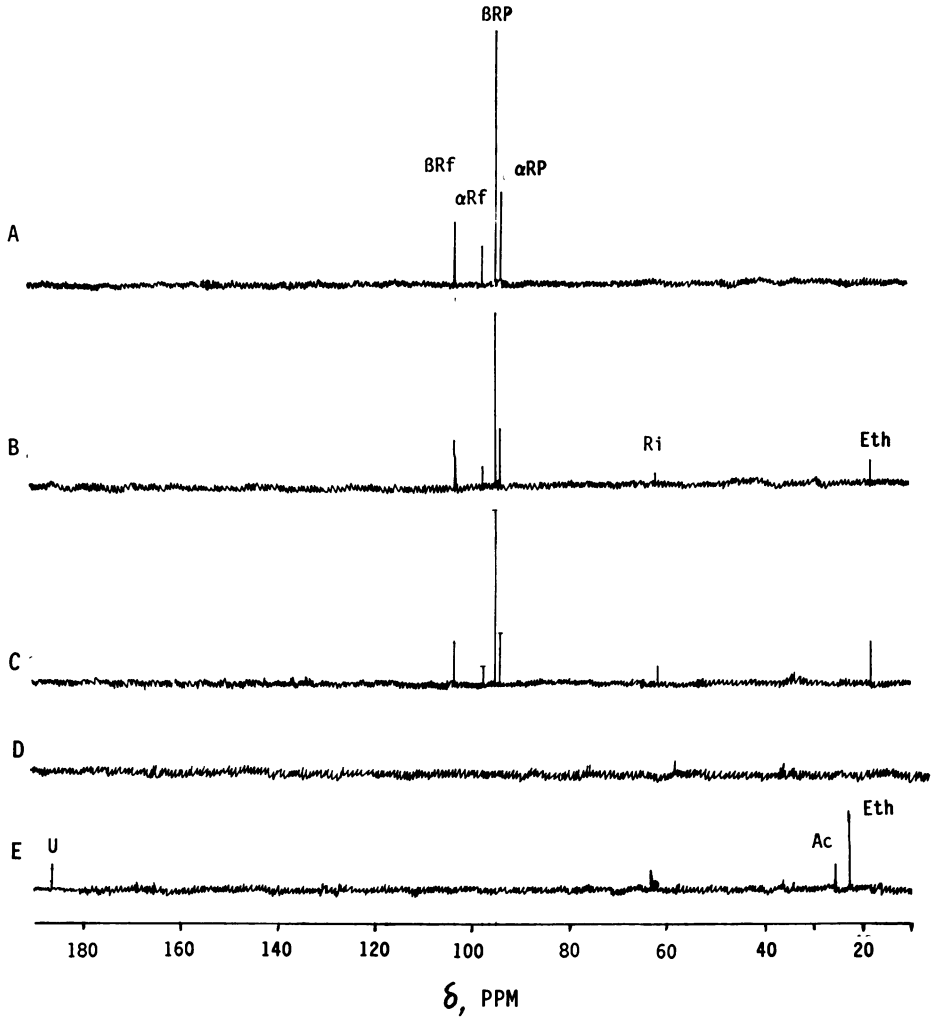

FIG. 2. ^{13}C NMR spectra of medium (A, B, and C) and cell extracts (D and E) prepared from *S. cerevisiae* ATCC 26602 cultivated on D-[1-^{13}C]ribose. Samples of medium were taken at 0 (A), 3 (B), and 5 (C) h. Cell extracts were prepared from samples taken at 0 (D) and 5 (E) h. Abbreviations: βRf, β-D-ribofuranoside; αRf, α-D-ribofuranoside; βRp, β-D-ribopyranoside; αRp, α-D-ribopyranoside; Eth, [2-^{13}C]ethanol; Ac, [2-^{13}C]acetate; Ri, [1-^{13}C]ribitol or [5-^{13}C]ribitol; U, the unknown compound.

of D-[1-^{13}C]ribose, peaks representing the α and β forms of both ribopyranose (96.0 and 97.7 ppm) and ribofuranose (98.4 and 103.1 ppm) were observed in the medium at 0, 3, and 5 h (Fig. 2A to C) but not in the cell extracts (Fig. 2D and E). Peaks representing the C-1 of ribitol (64.6 ppm) and the C-2 of ethanol (19.1 ppm) were observed at 3 and 5 h in the medium samples (Fig. 2B and C). The presence of ribitol was confirmed by the addition of the unenriched compound, whereas ethanol was also confirmed by gas chromatography (0.2 g/liter at 5 h). Cell extracts of the 3- and 5-h samples revealed peaks representing the methyl group (C-2) of acetate (25.6 ppm) and ethanol (22.7 ppm). The peaks of the C-2 group of acetate and ethanol were shifted to a greater degree than those observed in the medium. The identities of these peaks were confirmed by the addition of the unenriched compounds. An unknown peak was observed at 186.4 ppm in the 5-h extract. However, the identity of this peak could not be confirmed by the addition of unenriched acetate or acetyl phosphate, which has chemical shifts in this region. The position of the peak in the spectrum indicated that the peak represented a carboxyl group (33). The failure to observe ethanol and acetate marked in the C-1 position indicates that no scrambling of the label occurred during ribose metabolism.

DISCUSSION

These data indicate that *S. cerevisiae* ATCC 26602 possesses components of the pathways necessary to metabolize D-xylose and D-ribose although the organism is unable to grow on either sugar (Fig. 3) (38). The metabolism of xylose has been studied extensively in a number of yeasts, but ribose metabolism has not been covered in the literature. Evidently, both sugars are transported into *S. cerevisiae*. The presence of only a low-affinity system for xylose uptake in fresh cells but both low- and high-affinity systems in starved cells indicates that xylose is transported by both glucose-transporting systems as shown previously under certain conditions (8, 32). The K_m values of xylose transport reported here are considerably higher than values typically reported for glucose uptake by *S. cerevisiae* (8, 9, 32) and for xylose uptake by *P. stipitis* (14). However, the K_m values for

xylose uptake by the facilitated diffusion system of *C. shehatae* (23) and the low-affinity system of *Pichia heedii* (12) were similar to the values reported here. Since the completion of our work, two systems for xylose transport in *S. cerevisiae* have been reported, with K_m values of 1.5 M and 190 mM, respectively (16). The substrate concentration range (up to 400 mM) used in our experiments probably would not allow identification of the low-affinity system reported by these authors. This may indicate the presence of three xylose-transporting systems in *S. cerevisiae*, one of which appears only on starvation of cells in the absence of a growth substrate. V_{max} values ranging between 58 and 368 μmol/min/g were reported in or calculated from the literature (8, 9, 16, 17, 20, 32), and those reported here fall well within this range.

The regulation of xylose transport also appears to be similar to that of glucose. The high-affinity system for glucose is repressed and inactivated in the presence of high glucose concentrations (2, 3, 28), and this is reflected by the presence of only the low-affinity system in fresh glucose-grown cells. The low-affinity system is constitutive, but its activity is inhibited when the high-affinity system is derepressed (28). This results in a progressive decline in low-affinity activity during carbon source starvation. Under fully repressed conditions, therefore, only the low-affinity system is present, whereas only the high-affinity system can be detected under fully derepressed conditions (28). In our experiments, the cells were probably incompletely derepressed after 3 h of starvation, resulting in the simultaneous operation of both systems. After 2 days in the presence of ribose and xylose, however, only one system was detected that had characteristics similar to those of the high-affinity system observed in cells starved for 3 h.

Glucose strongly inhibited xylose transport in fresh glucose-grown cells, probably as a result of competition by both sugars for the low-affinity carrier (Table 2). Ribose also inhibited low-affinity xylose transport but to a lesser degree than glucose. The high-affinity system, operative in cells exposed to xylose and ribose for 2 days, was similarly inhibited by glucose but was not significantly inhibited by ribose. This indicates that ribose apparently competes with xylose for low-affinity transport but not with the high-affinity transport.

The decay in the xylose uptake during starvation or in the presence of either xylose or ribose (Table 1) may be due to the fast turnover of the glucose transport system (19). Inactivation of the glucose uptake system was observed under nitrogen starvation and the presence of a fermentable sugar (8). In the absence of a carbon source as well as in the presence of ethanol or cycloheximide, glucose transport was stable for at least 30 h (8). With respect to inactivation during prolonged starvation, therefore, xylose transport appears to behave differently from glucose transport. The presence of a mixture of xylose and ribose, on the other hand, seemed to protect xylose transport against inactivation over a 2-day period of starvation (Table 1). ^{13}C NMR studies reported here show that ribose is metabolized to C-2 compounds. One possible mechanism by which ribose could effect the protection of xylose transport is through more efficient utilization of ribose in the mixture, which could supply the maintenance energy required to prevent loss of the transport systems through protein turnover in the cells. This aspect, however, requires further investigation.

These data indicate that the slow utilization of xylose is unlikely to be limited by the rate of uptake. Calculations based upon the results of the utilization of xylose in the presence of ribose (5 g of each per liter) previously reported (38) show that the maximum rate of xylose utilization was only 1.1 μmol/min/g of cells, whereas calculations from the kinetic constants reported here show that the maximum rate of xylose transport after incubation in the presence of xylose alone for 2 days at the same xylose concentration would still be 85 μmol/min/g of cells. This implies that xylose transport probably is not rate limiting, and the improvement in the rate of xylose transport in the presence of ribose cannot account for the stimulation of xylose utilization observed.

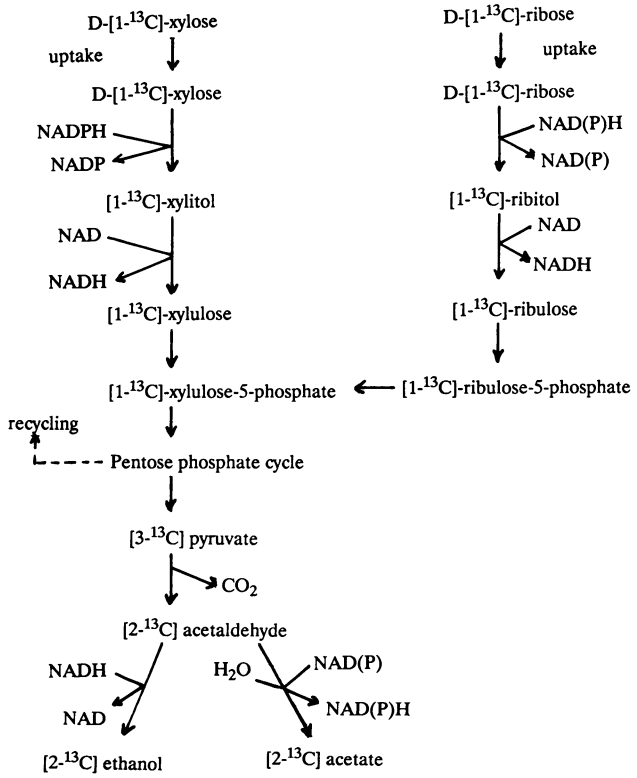

FIG. 3. Possible metabolic pathways for the utilization of xylose and ribose by yeasts and distribution of label of D-[1-^{13}C]xylose or D-[1-^{13}C]ribose.

The presence of ^{13}C NMR spectroscopy peaks representing xylitol and ribitol indicates that both sugars are initially reduced to their corresponding polyols (Fig. 3) (24). The enzyme catalyzing the conversion of xylose to xylitol is apparently constitutive and not subject to glucose repression since activities were similar irrespective of growth on glucose or ethanol. This is in contrast with the data of Batt et al. (1), who reported an inducible xylose reductase in *S. cerevisiae* A364A. In other xylose-fermenting yeasts, the enzyme was also found to be inducible (21, 39). The specific activities of the xylose reductase in crude extracts of *S. cerevisae* (ca. 5 mU/mg of protein) were lower than the values reported in the xylose-fermenting yeasts *P. tannophilus* (72 to 280 mU/mg) (11, 21), *C. shehatae* (480 mU/mg) (6), and *P. stipitis* (920 mU/mg) (29). This observation corresponds with the considerably lower rate of xylose utilization in *S. cerevisiae* than in the xylose-fermenting yeasts (38). However, the rate of xylose conversion by purified aldose reductase of *S. cerevisiae* (37) was 9.5 μmol/min/g of cells, which is considerably greater than the in vivo rate of 1.1 μmol/min/g of cells observed (38). The

activity of this enzyme is apparently regulated either by inactivation of the enzyme (Table 3) or by inhibition due to a redox imbalance. The presence of ribose in both instances appears to prevent enzyme inactivation and to alleviate the redox imbalance.

The failure to observe xylitol dehydrogenase activity in crude extracts was surprising since ^{13}C NMR spectroscopy indicated that ethanol was produced intracellularly from labelled xylose and this implied that the complete metabolic pathway was present (Fig. 3). Batt et al. (1) found that their strain produced ethanol from xylose when galactose was present, indicating the conversion of xylose by the oxidoreductive pathway via xylitol. An alternative pathway for the production of xylitol from xylose was described in *C. boidinii* and *S. cerevisiae* (31, 40). This includes the conversion of xylose to xylulose via an isomerase and then to xylitol via the reverse xylitol dehydrogenase reaction. Our data did not provide any evidence to support this route for xylitol production nor any other alternative route.

These results indicate that the reasons for the inability of *S. cerevisiae* to utilize xylose except in the presence of other substrates such as ribose may be connected to metabolic regulation rather than limitations in the capacity of the metabolic steps involved. The ability to convert ribose to ethanol and acetate would indicate that a number of key intermediates or cofactors could be produced that may assist xylose conversion to xylitol. Purified aldose reductase apparently has a specific requirement for $NADPH_2$ (37), and ribose metabolism may supplement the requirement for this cofactor. In addition, the $NADP^+$ that would accumulate as a result of xylose conversion strongly inhibits the activity of the aldose reductase (37), and its removal as a result of cometabolism of ribose would alleviate this inhibition. In yeasts, the oxidative step of the pentose phosphate pathway is a major source of $NADPH_2$ by cycling of carbon via glucose 6-phosphate (35). *Candida utilis*, which also converts xylose to xylitol by an $NADPH_2$-specific aldose reductase, meets the requirement for $NADPH_2$ by recycling intermediates through the oxidative step of the pentose phosphate pathway and by $NADP^+$-linked oxidation of isocitrate (7). In *S. cerevisiae*, a similar situation should prevail. Apparently, this did not occur in this strain under the conditions of these experiments as we failed to detect ethanol labelled in position C-1, which would be indicative of cycling via the pentose phosphate pathway (Fig. 3). Furthermore, ethanol did not originate from ribose.

Acetic acid production is observed during ethanol fermentation by *S. cerevisiae* when an excess of glucose is present. This accumulation has been ascribed to bottlenecks in respiration and the activity of the tricarboxylic acid cycle, leading to a redox imbalance (35, 36). Although overproduction of $NADH_2$ also occurs during acetic acid formation (35), the apparent specificity of the aldose reductase for $NADPH_2$ (37) would preclude $NADH_2$ meeting the needs of this reaction, and, furthermore, the ability to interconvert $NADH_2$ and $NADPH_2$ by a transhydrogenase is absent in yeasts (35). Recently, Postma et al. (26) reported in *S. cerevisiae* that acetate production is catalyzed by an acetaldehyde dehydrogenase with dual specificity for NAD^+ and $NADP^+$ and suggested a possible biosynthetic role for this reaction as was also observed in *C. utilis* when grown on ethanol or with methylamine as the nitrogen source (7). Therefore, excess $NADP^+$ produced by the aldose reductase reaction may be reduced by means of the aldehyde dehydrogenase, resulting in a cycling of $NADPH_2$ and $NADP^+$ (Fig. 3).

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