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Intermediary Metabolite Concentrations in Xylulose- and Glucose-Fermenting *Saccharomyces cerevisiae* Cells

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Glucose and xylulose fermentation and product formation by *Saccharomyces cerevisiae* were compared in batch culture under anaerobic conditions. In both cases the main product was ethanol, with glycerol, xylitol, and arabitol produced as by-products. During glucose and xylulose fermentation, 0.74 and 0.37 g of cell mass liter^{-1}, respectively, were formed. In glucose-fermenting cells, the carbon balance could be closed, whereas in xylulose-fermenting cells, about 25% of the consumed sugar carbon could not be accounted for. The rate of sugar consumption was 3.94 mmol g of initial biomass^{-1} h^{-1} for glucose and 0.39 mmol g of initial biomass^{-1} h^{-1} for xylulose. Concentrations of the intermediary metabolites fructose-1,6-diphosphate (FDP), pyruvate (PYR), sedoheptulose 7-phosphate (S7P), erytrose 4-phosphate, citrate (CIT), fumarate, and malate were compared for both types of cells. Levels of FDP, PYR, and CIT were lower, and levels of S7P were higher in xylulose-fermenting cells. After normalization to the carbon consumption rate, the levels of FDP were approximately the same, whereas there was a significant accumulation of S7P, PYR, CIT, and malate, especially of S7P, in xylulose-fermenting cells compared with in glucose-fermenting cells. In the presence of 15 μM iodoacetate, an inhibitor of the enzyme glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), FDP levels increased and S7P levels decreased in xylulose-assimilating cells compared with in the absence of the inhibitor, whereas fermentation was slightly slowed down. The specific activity of transaldolase (EC 2.2.1.2), the pentose phosphate pathway enzyme reacting with S7P and glyceraldehyde-3-phosphate, was essentially the same for both glucose- and xylulose-fermenting cells. It was, however, several orders of magnitude lower than that reported for a *Torula* yeast and *Candida utilis*. The presence of iodoacetate did not influence the activity of transaldolase in xylulose-fermenting cells. The results are discussed in terms of a competition between the pentose phosphate pathway and glycolysis for the common metabolite, glyceraldehyde-3-phosphate, which would explain the low rates of xylulose assimilation and ethanol production from xylulose by *S. cerevisiae*.

The fermentation of pentose sugars is of crucial economic importance for the production of ethanol from lignocellulosic biomass, in which pentoses can represent up to 40%. The major pentose sugar in lignocellulosic biomass is xylose. Xylose can be fermented to ethanol by yeasts, fungi, and bacteria (31). Bakers' yeast, *Saccharomyces cerevisiae*, cannot ferment xylose but can ferment its isomer, xylulose, to ethanol (35). The isomerization is catalyzed by the enzyme xylose (glucose) isomerase (EC 5.3.1.5), commercially used in the high-fructose syrup process. This is a bacterial enzyme which, in nature, functions as a xylose isomerase (8, 9). We have shown that it is possible to achieve ethanol yields, ethanol concentrations, and productivities in xylose fermentations comparable with those achieved in hexose fermentations when using commercial xylose isomerase and compressed bakers' yeast (17). We have also found that bakers' yeast in combination with xylose isomerase is superior to pentose-fermenting yeasts in terms of product yield when fermenting untreated lignocellulose hydrolysates, such as spent sulfite liquor (19).

In the present investigation we have compared levels of intermediary metabolites in glycolysis, in the pentose phosphate pathway (PPP), and in the tricarboxylic acid cycle in glucose- and xylulose-fermenting cells of *S. cerevisiae* in order to gain a better understanding of the metabolic regulation during anaerobic xylulose fermentation by *S. cerevisiae*. This approach has previously been described by us for studies of the metabolic regulation in *Streptococcus lactis* (20, 30) and *Candida tropicalis* (21, 32). The effects of iodoacetate (IA), an inhibitor of glyceraldehyde-3-phosphate (G3P) dehydrogenase (EC 1.2.1.12) (4, 6, 36), on metabolite levels and the specific activity of the PPP enzyme transaldolase (EC 2.2.1.2) were studied in order to elucidate the importance of the pool of G3P on the rate of xylulose fermentation in *S. cerevisiae*.

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MATERIALS AND METHODS

Organism. *S. cerevisiae* ATCC 24860 was maintained at 4°C on slants containing yeast extract (3 g liter^{-1}; Difco Laboratories, Detroit, Mich.), Bacto-Peptone (5 g liter^{-1}; Difco), agar (20 g liter^{-1}; Difco), malt extract (3 g liter^{-1}; Difco), and glucose or xylose (10 g liter^{-1}).

Xylulose-xylose substrate. For the production of xylulose-xylose solutions, the method previously described by Chiang et al. (10, 11) was used with the following modifications. Xylose, purchased either from Fluka AG, Buchs, Switzerland or from Sigma Chemical Co., St. Louis, Mo. (350 g in 0.5 liter), was isomerized with 20 g of immobilized xylose isomerase (Maxazyme-GI; generously supplied by Gist-Brocades, Delft, The Netherlands) at 60°C for 24 h with continuous magnetic stirring. The enzyme was separated, and the liquid was concentrated to half the volume by vacuum evaporation at 55°C. One volume of absolute (99.9%) ethanol was added under a nitrogen atmosphere. In this solution, xylose crystallized at 4°C. The two phases were separated, and ethanol was removed from the liquid phase by repeated dilution with water followed by vacuum evaporation at 55°C. The ratio of xylulose to xylose was 17/83 after the isomerization and 54/46 after extraction in ethanol.

Preparation of inoculum. The inoculum was prepared in

two steps with the following medium in order to rapidly obtain a large cell mass: glucose (25 g liter^{-1}), xylose (25 g liter^{-1}), Bacto-Peptone (20 g liter^{-1}; Difco), and yeast extract (10 g liter^{-1}; Difco). The sugars were autoclaved separately. First, 100 ml of medium in a 500-ml shake flask was inoculated and cultivated for 12 h at 30°C in a shaking water bath (140 rpm). This culture was then used as the inoculum for four 1-liter shake flasks, initially containing 200 ml of medium (total culture volume, about 225 ml). These cultures were cultivated for 12 h as described above. The cells were harvested at 4°C by centrifugation at 6,000 × *g* for 25 min, suspended in 0.9% NaCl, and stored overnight at 4°C.

Fermentation. The fermentation was carried out in 160-ml bottles in a water bath at 30°C with magnetic stirring (300 rpm). The bottles containing glucose-xylose or xylulose-xylose solutions were flushed with nitrogen, sealed with rubber stoppers, and autoclaved. YNB medium (6.7 g liter^{-1}; Difco) filtered through a sterile 0.2-μm-pore-size (Millipore Corp., Bedford, Mass.) and the inoculum (final concentration, 16 g [dry weight] liter^{-1}) were added by using a syringe. The final sugar concentrations were 48 g (267 mM) of glucose, 40 g of xylose, 50 g of (333 mM) xylulose, and 40 g of xylose liter^{-1}. The syringe needle was left in the rubber stopper to allow the evacuation of carbon dioxide and sampling. When used, IA (E. Merck AG, Darmstadt, Federal Republic of Germany) was mixed with YNB medium added to the cultures as described above.

Analysis of substrates and products. Concentrations of glucose, xylose, xylulose, and the fermentation products ethanol, glycerol, and acetic acid were determined by high-performance liquid chromatography with one (17, 19) or two columns (Aminex HPX-87H; Bio-Rad Laboratories, Richmond, Calif.) (18). Even though the resolution was improved with the double-column system, xylitol and L- and D-arabitol could not always be sufficiently separated. Separate high-performance liquid chromatography standards for xylitol and L- or D-arabitol were similar, so that xylitol and L- and D-arabitol were accounted for as xylitol. Commercial reagent-grade preparations were used as standards, except for xylulose. This compound was purified (23), since it was found that the commercial preparations contained impurities of glucose and xylose.

Sampling and extraction of intracellular intermediary metabolites. Samples of 1.5 to 2 ml were taken when the consumption of sugar and the production of ethanol were linear, i.e., at about 2 and 24 h for glucose and xylulose fermentations, respectively. The samples were rapidly filtered (<2 s) and immediately frozen in liquid nitrogen as previously described (20, 32). Rapid filtration and immediate freezing are necessary in order to capture the in vivo metabolic situation. Metabolites were extracted as described earlier (20, 32). Six to eight samples were taken and extracted within 20 min during each fermentation. The cell extracts were stored at −80°C until analyzed.

Determination of intermediary metabolite concentrations. Metabolites were measured as soon as possible after sampling by using enzymatic assays coupled to spectrofluorometric determination of the consumption or formation of the cofactors NADH and NADPH (22). The emission was measured at 450 nm after excitation at 350 nm with a fluorescence spectrophotometer (Hitachi F-3000).

Fructose 1,6-diphosphate (FDP), pyruvate (PYR), citrate (CIT), malate (MAL), and fumarate (FUM) were assayed as previously described by using 5 μM NADH for the assays of PYR and NAD^+ (free acid, grade II) from Boehringer GmbH, Mannheim, Federal Republic of Germany, for the FUM and MAL assays (32).

Erythrose 4-phosphate (E4P) was determined by the method of Paoletti (24), with the following modifications. Standards and samples were diluted to an approximate concentration of 0.8 μM in buffer (6 g of triethanolamine, 16 mg of $NADP^+$, 10.3 mg of thiaminepyrophosphate, 2.5 mg of β-hydroxypyruvate [all from Sigma], 0.2 g of $MgCl_2 \cdot 6H_2O$ in 200 ml of water, pH 7.7); to both series of tubes were added 1.67 U of phosphoglucose isomerase (EC 5.3.1.9; from bakers' yeast, type III; Sigma) and 0.15 U of glucose-6-phosphate dehydrogenase (EC 1.1.1.49; from *Leuconostoc mesenteroides*, type XXIII; Sigma) ml^{-1}, and to one series of tubes, 0.1 U of transketolase (EC 1.1.2.2; from bakers' yeast, type X; Sigma) ml^{-1} was added. The difference in emission at 450 nm between two corresponding tubes is thus proportional to the E4P concentration.

Sedoheptulose 7-phosphate (S7P) was determined by the method of Paoletti (25) with the following modifications. Standards and samples were diluted to an approximate concentration of 0.8 μM in buffer in two series of tubes; to both series were added 0.9 U of fructose-6-phosphate kinase (EC 2.7.1.11, from rabbit muscle; Sigma) ml^{-1}. After incubation at 37°C for 30 min, the tubes were placed in boiling water for 3 to 4 min and then cooled at room temperature; to the first series of tubes, a solution of $NADP^+$, thiaminepyrophosphate, β-hydroxypyruvate, transketolase (EC 2.2.1.1), phosphoglucose isomerase (EC 5.3.1.9), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was added, and to the second series of tubes, the same solution including aldolase (EC 4.2.1.13; from rabbit muscle, type IV; Sigma) was added. The concentrations of the components of the reaction mixture were as follows: 21 μM NADP, 5.2 μM thiaminepyrophosphate, 24 μM β-hydroxypyruvate and 0.1 U of transketolase, 3.9 U of phosphoglucose isomerase, 0.13 U of glucose-6-phosphate dehydrogenase, and 0.241 U of aldolase ml^{-1}. The emission at 450 nm was measured after 90 min, and the difference in emission between two corresponding tubes was proportional to the S7P concentration.

With these modifications, it is possible to analyze one metabolite in 50 to 60 samples at one time. Using spectrofluorimetry for detection allows small sample volumes and makes it possible to measure the concentration of any number of metabolites in each extract. Each metabolite concentration is the mean of six to eight independently filtered and extracted samples during five independent and identical fermentation experiments (three in the case of xylulose fermentation with 15 μM IA). The metabolite concentrations and standard deviation were calculated from the emission measurements by using 0.334 g ml^{-1} for the ratio of dry weight to cell volume (13, 32).

Preparation of cell extracts and assay of transaldolase activity. Transaldolase (EC 2.2.1.2) was assayed in cell extracts from cells collected at the same time as the samples for the determination of intermediary intracellular metabolites. The cells were centrifuged at 10,000 rpm for 20 min at 4°C. The pellet was washed with 0.1 M phosphate buffer containing 0.5 mM EDTA (pH 7) and centrifuged again under the same conditions. The pellet was then diluted with a few milliliters of the same buffer as described above, also containing 5 mM mercaptoethanol, before being frozen at −20°C and pressed three times with an X-press. Extracts were stored at −80°C before analysis.

Transaldolase was assayed spectrophotometrically at 340 nm in triethanolamine-EDTA buffer, as described by Brand (3). Samples and standards (lyophilized transaldolase from

bakers' yeast; Sigma) were diluted to 5 to 50 U liter^{-1} with buffer diluted 10 times. The protein content in the samples was determined by the method of Bradford (2). One unit (U) was defined as the formation of 1.0 μmol of D-G3P from D-fructose-6-phosphate min^{-1} in the presence of D-E4P at pH 7.7 and 25°C in a coupled system with glycerophosphate dehydrogenase (EC 1.1.1.8)/triosephosphate isomerase (EC 5.3.1.1) and β-NADH.

Chemicals. All chemicals were of reagent grade.

RESULTS

Glucose fermentation. The sugar consumption and product formation in the anaerobic fermentation of the glucose-xylose mixture by *S. cerevisiae* are shown in Fig. 1a. All glucose was metabolized after 4 h, resulting in the formation of cell mass, ethanol, and the by-products glycerol, arabitol, and xylitol, as well as trace amounts of acetic acid and succinic acid. The mass balance of carbon moles after 2 h of fermentation when the consumption of sugar was constant (3.94 mmol g of initial biomass^{-1} h^{-1}) is given in Table 1. For the estimation of assimilated carbon moles converted to cell mass, the elemental composition formula $CH_{1.8}O_{0.56}N_{0.17}$ for *S. cerevisiae* was used (14, 29). The carbon dioxide was not measured. It was assumed that 1 mol of carbon dioxide was produced for every mol of ethanol produced. With this assumption, 93% of the consumed sugar carbon could be accounted for in the analyzed products.

Sampling and extraction of metabolites was performed after 90 to 120 min when the sugar consumption was constant (Fig. 1a). The concentrations of metabolites in glycolysis, PPP, and the tricarboxylic acid cycle are given in Table 2. The concentrations of FDP (≈2 mM), PYR (≈1 mM), CIT (≈1 mM), and MAL (≈0.5 mM) were in the same range as those reported earlier for *S. cerevisiae* metabolizing glucose anaerobically (15). The two metabolites measured in the PPP, E4P and S7P, could not be detected. The level of fumarate (FUM) was within the limits of the standard deviation.

Xylulose fermentation. The sugar consumption and product formation in the anaerobic fermentation of the xylulose-xylose mixture by *S. cerevisiae* are shown in Fig. 1b. Compared with the glucose consumption rate (Table 1), the xylulose consumption rate was one order of magnitude lower (0.39 mmol g of initial biomass^{-1} h^{-1}) at 24 h. The mass balance of carbon moles is summarized in Table 1. The production of cell mass and of ethanol was lower in xylulose- than in glucose-metabolizing cells. On the other hand, the production of pentitols was higher from xylulose than from glucose, while the amount of glycerol produced was about the same. Traces of acetic acid and succinic acid were also found. In xylulose-metabolizing cells, using the same assumptions as for glucose-metabolizing cells, about 25% of the utilized carbon could not be accounted for in the analyzed products.

Sampling and extraction of intermediary metabolites were performed after 24 h, when the sugar consumption rate was constant (Fig. 1b). The concentrations of the metabolites are summarized in Table 2. The levels of FDP (≈0.25 mM), PYR (≈0.5 mM), and CIT (≈0.5 mM) were lower than in glucose-metabolizing cells, whereas the level of MAL (≈0.5 mM) was about the same and the level of S7P was easily measurable (≈0.7 mM). The level of FUM was within the limits of the standard deviation.

Xylulose fermentation in the presence of IA. First, the effect of different concentrations (5 to 50 μM) of IA on the rate of xylulose fermentation with *S. cerevisiae* was studied (Fig. 2). At concentrations over 30 μM the fermentation was fully inhibited, and at concentrations lower than 10 μM the fermentation was not influenced. In the range of 15 to 20 μM the fermentation rate was reduced but not completely inhibited, and 15 μM was therefore chosen for the following experiment.

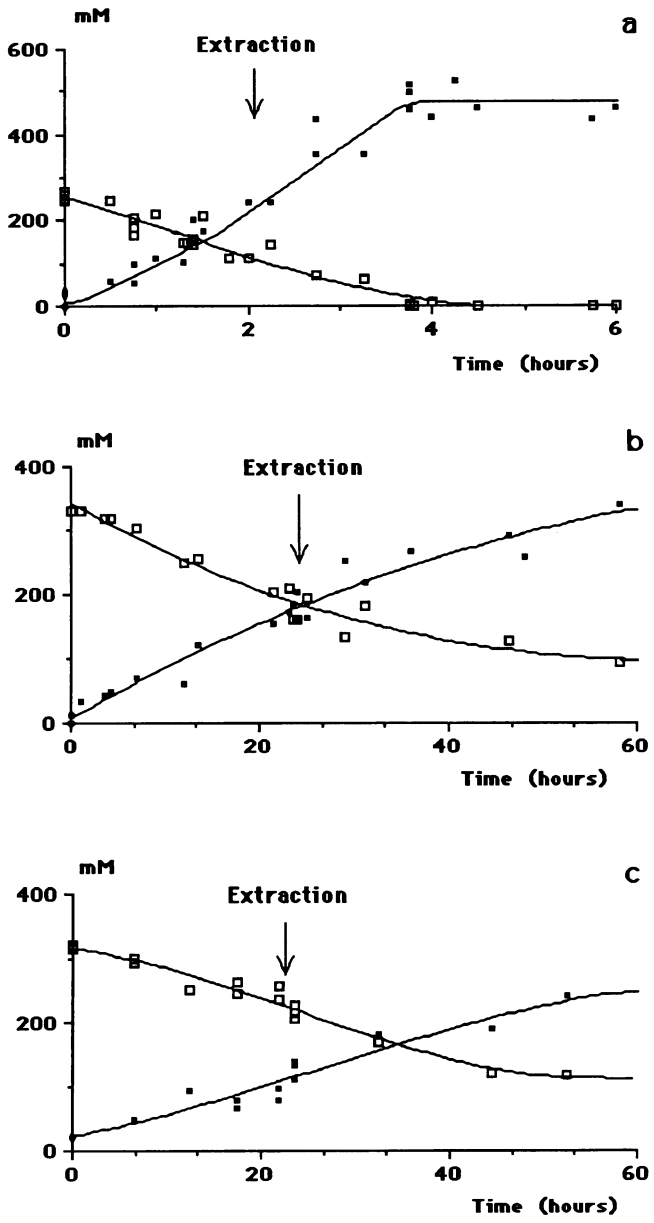

FIG. 1. Sugar substrate concentration (□) and ethanol concentration (■) (indicated on the *y* axis) during fermentation of glucose (a), xylulose (b), and xylulose with 15 μM IA (c) by *S. cerevisiae* in the presence of xylose. Data are from five (a and b) or three (c) independent and identical experiments.

The sugar consumption and product formation in the anaerobic fermentation of the xylulose-xylose substrate by *S. cerevisiae* in the presence of 15 μM IA are shown in Fig. 1c. At 24 h, the xylulose consumption rate was 0.26 mmol g of initial biomass^{-1} h^{-1}, compared with 0.39 mmol g of

TABLE 1. Carbon balance of the anaerobic fermentation of glucose and of xylulose (without or with 15 μM IA) in the presence of xylose by *S. cerevisiae* (initial biomass, 16 g liter^{-1})

Fermentation substrate	Consumed sugar (mM)		Substrate consumption rate (mmol g of initial biomass^{-1} h^{-1})	Main products (mM)				Carbon recovery (%)[a]	Analysis and extraction time (h)
	Substrate	Xylose		Cell mass[b]	Ethanol	Glycerol	Pentitols[c]		
Glucose	156	6.7	3.94	31	224	24	6.6	93	2
Xylulose	165	9.3	0.39	15	139	22	11.4	75	24
Xylulose[d]	110	NM[e]	0.26	≈0	114	17.8	4.7	80	24

[a] Assuming that 1 mol of carbon dioxide was formed for each mole of ethanol formed.
[b] Determined from the formula given by Roels and co-workers (see text and references 14 and 29).
[c] Accounted for as xylitol.
[d] With 15 μM IA.
[e] NM, Not measured.

initial biomass^{-1} h^{-1} in the absence of IA. The mass balance based on carbon moles is summarized in Table 1. Except for glycerol, the product concentrations were lower than in the absence of IA. Using the same assumptions as described above, approximately 20% of the utilized carbon could not be accounted for in the analyzed products. Sampling and extraction of intermediary metabolites were performed after 24 h, when the sugar consumption rate was constant (0.26 mmol g of initial biomass^{-1} h^{-1}) (Fig. 1c). Only the concentrations of FDP, PYR, and S7P were measured (Table 2). In comparison with the xylulose fermentation in the absence of IA, the level of FDP (≈1 mM) was higher and the levels of S7P (<0.1 mM) and PYR (≈0.2 mM) were lower.

Comparison of metabolite concentrations in glucose- and xylulose-metabolizing cells. The measurable metabolite concentrations were generally higher for the glucose-metabolizing cells than for the xylulose-metabolizing cells, except for S7P. There was, however, also a great difference in the sugar consumption rates between the two carbon sources: 3.94 mmol of glucose g of initial biomass^{-1} h^{-1} and 0.39 mmol of xylulose g of initial biomass^{-1} h^{-1}, respectively. The metabolite concentrations were normalized with respect to the carbon consumption rate, also taking into account the number of carbon atoms in each sugar molecule (Table 3). The FDP levels were similar for both carbon sources, whereas the levels of PYR, MAL, CIT, and in particular S7P, are higher in xylulose-metabolizing cells. The presence of IA increased the level of FDP and reduced the level of S7P by an order of magnitude in xylulose-fermenting *S. cerevisiae* cells.

Transaldolase activity in glucose- and xylulose-fermenting cells. The specific transaldolase activities for the different types of cells were of the same order of magnitude: 6.8 mU mg of protein^{-1} in cells fermenting glucose, 4.9 mU mg of protein^{-1} in cells fermenting xylulose, and 5.4 mU mg of protein^{-1} in cells fermenting xylulose in the presence of 15 μM IA.

DISCUSSION

Xylulose is an extremely expensive substrate. A method has recently been described for the production of pure xylulose (28). This method was, however, not available at the beginning of the present study. We therefore prepared a mixture of xylulose and xylose by using a modification of the method described by Chiang et al. (10, 11). With the assumption that xylose was not metabolized by *S. cerevisiae*, we then used xylulose-xylose and glucose-xylose mixtures to compare xylulose and glucose fermentations with *S. cerevisiae*. In both fermentations about 10 mM of xylose was assimilated and pentitols were produced, indicating the presence of the enzyme xylose reductase in *S. cerevisiae* and possibly also the enzyme xylitol dehydrogenase (Fig. 3). This has previously been reported for the assimilation of galactose by *S. cerevisiae* in the presence of xylose. Anaerobic formation of xylitol and arabitol from xylose as well as from xylulose has also been previously reported (16). The formation of xylitol has been ascribed to a redox imbalance (Fig. 3) (5, 33).

The rates of sugar consumption and of ethanol production in the fermentation of xylulose by *S. cerevisiae* were much lower than for the fermentation of glucose by *S. cerevisiae*, as was also observed in previous investigations (11, 35). This could be due to differences in the sugar transport systems and/or to differences in the metabolic regulation in glucose- and xylulose-assimilating *S. cerevisiae* cells.

There are no specific reports available on the transport system(s) for xylulose. Wang and Schneider (34) suggested that xylulose might not be transported by an active process in *S. cerevisiae* on the basis of the fact that acyclic polyols enter by a fast, nonactive mechanism (7) and that there are structural similarities between xylulose and these acyclic polyols. In a study on the fermentation of xylulose-xylose mixtures with bakers' yeast, no time lag was found between the consumption and the utilization of xylulose (10).

TABLE 2. Intracellular concentrations of intermediary metabolites in *S. cerevisiae* cells (without or with 15 μM IA) in the presence of xylose

Fermentation substrate	Concn (mM ± SD) of intermediary metabolites of:						
	PPP		Glycolysis		Tricarboxylic acid cycle		
	S7P	E4P	FDP	PYR	CIT	FUM	MAL
Glucose	ND[a]	ND	2.17 ± 0.085	0.79 ± 0.09	0.82 ± 0.04	0.06 ± 0.04	0.39 ± 0.01
Xylulose	0.77	ND	0.26 ± 0.015	0.46 ± 0.08	0.42 ± 0.03	0.05 ± 0.01	0.31 ± 0.05
Xylulose[b]	0.06 ± 0.02	NM[c]	1.16 ± 0.05	0.17 ± 0.01	NM	NM	NM

[a] ND, Not detectable.
[b] With 15 μM IA.
[c] NM, Not measured.

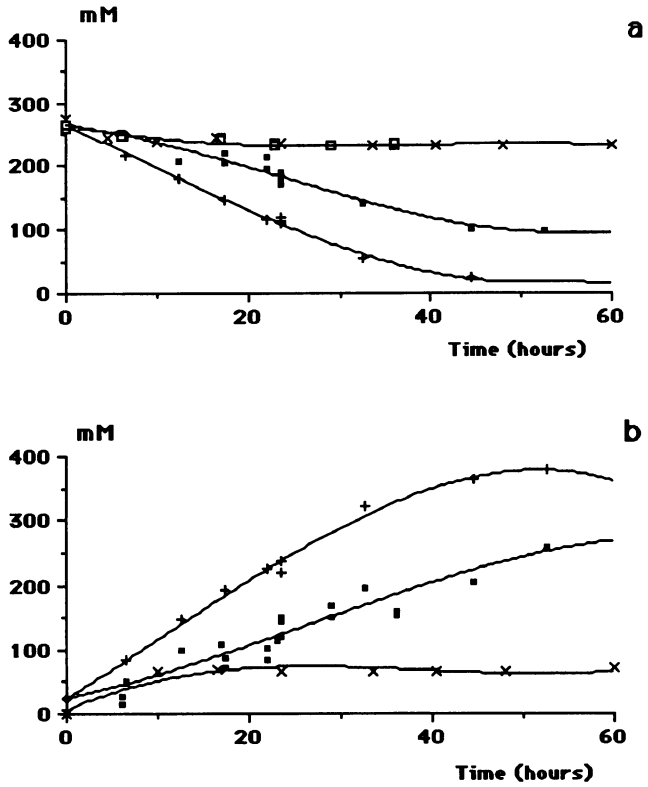

FIG. 2. Effect of different IA concentrations (0 [+], 15 [■], 30 [□], and 50 [×] μM) on xylulose (a) and ethanol (b) concentrations (indicated on the *y* axis) during xylulose fermentation by *S. cerevisiae* in the presence of xylose.

With glucose-fermenting *S. cerevisiae* cells, the assimilated carbon moles were accounted for in the products. The carbon balance was based on the assumption that 1 mol of carbon dioxide was produced for every mole of ethanol. With the same assumption for xylulose-fermenting cells, both in the presence and in the absence of IA, 20 to 25% of the assimilated carbon could not be accounted for. Since no other products were detected with high-performance liquid chromatography, the missing carbon was most probably lost as carbon dioxide. The enhanced formation of pentitols in xylulose-fermenting cells might suggest that additional carbon dioxide was produced in the PPP. This does, however, not apply to xylulose-fermenting cells in the presence of IA, in which case fewer pentitols were produced.

TABLE 3. Intracellular concentrations of intermediary metabolites normalized to the carbon assimilation rate in *S. cerevisiae* cells fermenting glucose and xylulose (without or with 15 μM IA) in the presence of xylose

Fermentation substrate	Concn (μM mM assimilated carbon^{-1} h^{-1}) of intermediary metabolites of:				
	PPP	Glycolysis		Tricarboxylic acid cycle	
	S7P	FDP	PYR	CIT	MAL
Glucose	ND[a]	5.7	2.0	2.2	1.0
Xylulose	24.4	8.2	14.6	13.4	9.8
Xylulose[b]	2.8	55.2	8.0	NM[c]	NM

[a] ND, Not detectable.
[b] With 15 μM IA.
[c] NM, Not measured.

Our results indicate that the slow rates of sugar consumption and of ethanol production for cells fermenting xylulose compared with cells fermenting glucose could be caused by a block in the PPP, since there was an accumulation of S7P in xylulose-fermenting cells which was not observed in glucose-fermenting cells (Fig. 3). Neither of the two measured metabolites in the PPP (S7P and E4P) accumulated in glucose-metabolizing cells, as previously reported (12).

In the PPP, the enzyme transaldolase catalyzes the reaction of S7P with G3P, an intermediate of the glycolytic pathway, to produce fructose-6-phosphate and E4P (Fig. 3). For xylulose-fermenting cells, the fact that we could not detect E4P but measured high levels of S7P may be due to either an inefficiently acting transaldolase or, as suggested by Ciriacy and Porep (12), the possibility that the pool of G3P does not match that of S7P because of the efficiency of the subsequent glycolytic enzymes. In addition, these authors also observed an accumulation of E4P in *S. cerevisiae* cells growing on xylulose, which we did not find. This could be due to strain differences as well as to differences in cultivation conditions.

The specific transaldolase activity was of the same order of magnitude for both glucose- and xylulose-fermenting *S. cerevisiae* cells, indicating that it is a constitutive enzyme and that xylulose assimilation does not induce increased amounts of enzyme. To the best of our knowledge, the specific transaldolase activity in *S. cerevisiae* has not yet been reported. The presently found transaldolase activities were several orders of magnitude lower than those reported for a *Torula* yeast (0.52 U mg^{-1} [26]) and for *Candida utilis* (0.2 U mg^{-1} [27]), both considered to be very good xylose assimilators. Together with the fact that it has been estimated that only 0.9% of the assimilated glucose goes through the PPP under aerobic conditions (16), the low transaldolase activities might indicate that the PPP is not very well developed in *S. cerevisiae*.

In preliminary studies with glucose-fermenting *S. cerevisiae* cells, the levels of G3P were too low to be detected by an adaptation of the FDP assay (22) (data not shown), which is in good agreement with the published data (4, 15). In order to elucidate the suggestion that the accumulation of S7P is due to an insufficient pool of G3P (12), we also measured the levels of S7P and FDP in xylulose-fermenting *S. cerevisiae* cells in the presence of the metabolic inhibitor IA (35) (Fig. 3). IA is an alkylating agent which reacts with sulfhydryl groups and inhibits dehydrogenases. At low concentrations, it mainly inhibits the glycolytic enzyme G3P dehydrogenase (EC 1.2.1.12) (36) and has been used for specific G3P dehydrogenase inhibition studies of intact cells and of cell extracts (4, 6). When G3P dehydrogenase, which converts G3P to 1,3-diphosphoglycerate in glycolysis, is inhibited by IA in intact xylulose-fermenting cells, G3P is expected to accumulate and become available to react with S7P in the PPP. In fact, the presence of IA decreased the S7P levels, and the accumulation of FDP would support the suggestion that the presence of IA causes an accumulation of glycolytic intermediates. The presence of IA did not influence the specific activity of transaldolase in xylulose-fermenting *S. cerevisiae* cells.

Therefore, the slow metabolism in xylulose-fermenting *S. cerevisiae* cells concomitant with the accumulation of S7P could be due both to a poorly developed PPP, here displayed as a very low transaldolase activity, and a competition for G3P between glycolysis and the PPP. The observation that

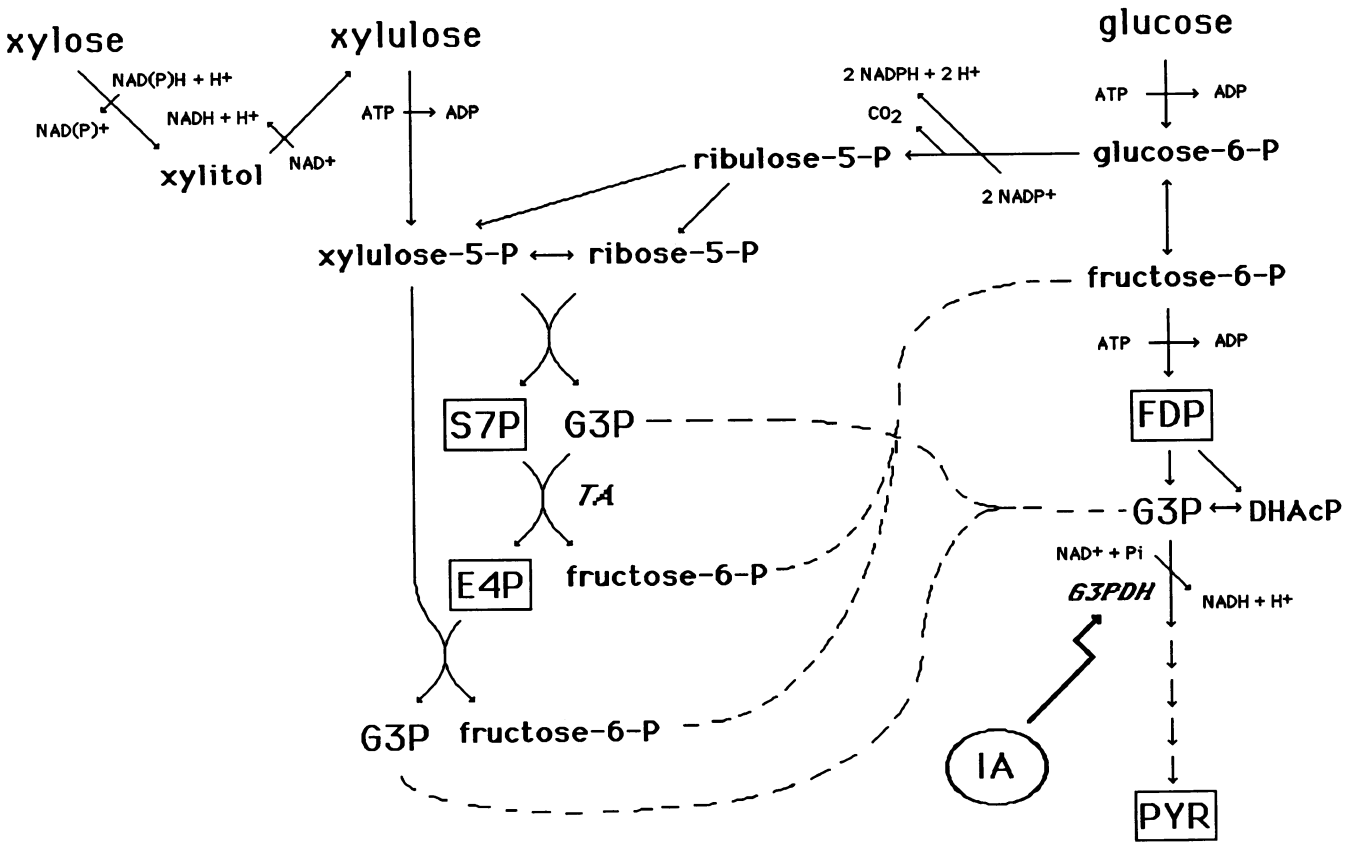

FIG. 3. Scheme of the PPP and of glycolysis in yeast. Abbreviations are as given in the text. DHAcP, Dihydroxyacetone phosphate. TA, Transaldolase (EC 2.2.1.2).

the carbon balance could not be closed for the xylulose-fermenting *S. cerevisiae* cells would suggest that additional pathways, not operating in glucose-fermenting cells, are active when *S. cerevisiae* metabolizes xylulose.

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