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Biosynthesis and Regulation of Fructose-1,6-Bisphosphatase and Phosphofructokinase in *Saccharomyces cerevisiae* Grown in the Presence of Glucose and Gluconeogenic Carbon Sources

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The mode of synthesis and the regulation of fructose-1,6-bisphosphatase (FBPase), a gluconeogenic enzyme, and phosphofructokinase (PFK), a glycolytic enzyme, were investigated in *Saccharomyces cerevisiae* after growth in the presence of different concentrations of glucose or various gluconeogenic carbon sources. The activity of FBPase appeared in the cells after the complete disappearance of glucose from the growth medium with a concomitant increase of the pH and no significant change in the levels of accumulated ethanol. The appearance of FBPase activity following glucose depletion was dependent upon the synthesis of protein. The FBPase and PFK were present in glucose-, ethanol-, glycerol-, lactate-, or pyruvate-grown cells; however, the time of appearance and the levels of both these enzymes varied. The FBPase activity was always higher in 1% glucose-grown cells than in cells grown in the presence of gluconeogenic carbon sources. Phosphoglucose isomerase activity did not vary significantly. Addition of glucose to an FBPase and PFK synthesizing culture resulted in a complete loss, followed by a reappearance, of FBPase activity and an initial increase, followed by a decrease, of PFK activity. In the presence of cycloheximide the disappearance of glucose and the changes in the levels of FBPase and PFK were decreased significantly. It is concluded that *S. cerevisiae* exhibits a more efficient synthesis of FBPase after the exhaustion of glucose compared to the activity present in cells grown in the presence of exogenous gluconeogenic carbon sources. Two metabolically antagonistic enzymes, FBPase and PFK, are present during the transition phase, but not during the exponential phase, of growth, and the decay or inactivation of these enzymes in vivo may be dependent upon a glucose-induced protease activity.

Glycolysis and gluconeogenesis are two key processes involved in carbohydrate metabolism (3, 5, 15, 27). Several investigators have shown that the synthesis of glycolytic enzymes in yeast is modulated by the kind and concentration of carbon source as well as by the concentration of intracellular metabolites (14, 19). The synthesis of phosphoglucose isomerase (EC 5.3.1.0) appears to be least sensitive to regulation, whereas the syntheses of fructose-bisphosphate aldolase (EC 4.1.2.13), triosephosphate isomerase (EC 5.3.1.1), and glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12) are most sensitive to regulation.

Catabolite regulation affects, in yeast and bacteria, the utilization of several carbohydrates other than glucose, the degradation of amino acids, the tricarboxylic acid cycle, and respiratory enzymes (18, 22). The synthesis of gluconeogenic enzymes including fructose-1,6-bisphosphatase (FBPase; EC 3.1.3.11) in yeast and bacteria have been shown to be under catabolic regulation (7, 11, 25). In the case of enteric bacteria, exogenous cyclic adenosine 3',5'-monophosphate (cAMP) reverses the glucose repression of a number of catabolite-sensitive enzymes (23). In addition to catabolic regulation, yeast FBPase has also been shown to be regulated by catabolite inactivation (8, 26, 30). This inactivation results in an irreversible loss of FBPase activity, within a relatively short period of time, when FBPase synthesizing cells are exposed to glucose, mannose, fructose, or sucrose. It has been suggested that an FBPase-specific protease may play a role in this inactivation process (13, 20).

Regulatory mechanisms of gluconeogenic and glycolytic enzymes incuding FBPase and phosphofructokinase (PFK; EC 2.7.1.11) have been examined in detail in animal systems (1, 3, 27). However, a simultaneous investigation of the synthesis and regulation of these two metabol-

ically antagonistic enzymes is lacking in microbial systems. Gancedo et al. (6) have reported no FBPase activity in *Saccharomyces cerevisiae* grown in the presence of 2% glucose. However, we have observed significant FBPase and PFK activities in cells grown in the presence of 1% glucose, fructose, or galactose (4). These observations prompted us to investigate the precise mode of synthesis and regulation of FBPase and PFK in glucose- and ethanol-grown cells. We report here for the first time the results of experiments demonstrating the mode of synthesis of FBPase and PFK in relationship to the growth phase, disappearance of glucose, and other physiological and metabolic changes in the culture. Results are also presented regarding catabolite repression and inactivation of FBPase in glucose-grown cells, and a simultaneous presence of FBPase and PFK in *S. cerevisiae* grown in the presence of several gluconeogenic carbon sources.

MATERIALS AND METHODS

Organism and growth conditions. Wild-type *S. cerevisiae* X2180 was grown in a nutritionally complete (nutrient) medium (carbon source; $MgSO_4$, 1 g; KH_2PO_4, 2 g; peptone, 6 g; yeast extract, 4 g; and distilled water, 1 liter). The medium was adjusted to an initial pH of 5.5 in all experiments with 2 N KOH or 5 N HCl, except in those where the initial pH of the medium was varied. Concentrations of glucose (wt/vol), ethanol, glycerol, and sodium lactate varied according to each experiment. Sodium pyruvate and alanine were added as carbon source to yield a final concentration of 2% (wt/vol). The gluconeogenic carbon sources were standardized on the basis of molar ratios, 2% ethanol being equivalent to a final concentration of 0.43 M. Culture flasks of 500-ml capacity, each containing 250 ml of medium with the appropriate carbon source, were inoculated with a standardized suspension of X2180 cells to an initial density of 1.5×10^6 cells per ml and incubated at 30°C in a Metabolyte water bath shaker (New Brunswick Scientific Co., Inc.) with 225 rpm. Cells were harvested by centrifugation in a Sorvall RC-5 Superspeed Refrigerated Centrifuge (DuPont Instruments) at 4°C after appropriate growth periods. In specific experiments, cycloheximide (100 μg/ml [10]) and glucose (1% wt/vol) were added to culture flasks, at appropriate times, before cell harvest.

Enzyme preparations. Crude enzyme preparations were obtained by disrupting cells in a Braun model MSK mechanical homogenizer as previously described (4). The supernatant from the disrupted cell mass was dialyzed for 18 h with three buffer changes and used as the source of enzymes. The protein was determined by the biuret method (9). Enzyme activities remained stable for at least 3 days when cell extracts were stored at 0°C.

Enzyme assays. All enzymes were assayed by coupling the particular step to the appropriate nicotinamide adenine dinucleotide- or nicotinamide adenine dinucleotide phosphate-linked reaction. The rate of production or the disappearance of reduced nicotinamide adenine dinucleotides was followed at 340 nm by using a Gilford 240 recording spectrophotometer (in cuvettes with a 1-cm light path) at 25°C. Enzyme activities are expressed as milliunits per milligram of protein at 25°C (1 mU is the enzyme activity that transforms 1 nmol of substrate in 1 min). Statistical analysis and controls for enzyme assays were as previously described (4).

FBPase. FBPase was determined according to the method of Gancedo et al. and Gancedo (7, 8). The activity of FBPase was followed by the glucose-6-phosphate dehydrogenase-dependent reduction of nicotinamide adenine dinucleotide phosphate (4).

PFK. PFK was determined according to the method of Sols and Salas (28). The activity of PFK was followed by the α-glycerophosphate dehydrogenase-dependent oxidation of NADH (4).

Phosphoglucose isomerase. Phosphoglucose isomerase activity was determined by the method of Maitra and Lobo (19). This activity was followed by the glucose-6-phosphate dehydrogenase-dependent reduction of nicotinamide adenine dinucleotide phosphate (4).

Glucose and ethanol determinations. The levels of glucose (milligrams/milliliter) in culture supernatants were determined by using the Sigma glucose assay kit no. 15-10 (Sigma Chemical Co.). In some cases, an initial 1:4 dilution of the supernatant was necessary to stay within assay limits. The levels of ethanol (milligrams per milliliter) in culture supernatants were determined by using the Sigma ethanol assay kit no. 331. An initial 1:12 dilution of the supernatant was necessary to stay within limits of the assay.

Measurement of growth and pH. At appropriate intervals, a portion of culture was removed aseptically and an American Optical Bright-Line hemacytometer was used to count cell number. The resulting cell counts were graphed as log functions. In specified experiments, the pH of the culture supernatant was determined in samples aseptically removed during incubation, or in samples obtained after cell harvest. The pH was determined using a Sargent-Welch pH meter (model NX).

Enzymes and chemicals. The coupling enzymes were obtained from Sigma Chemical Co., at the highest purity available. Dipotassium ethylenediaminetetraacetic acid was obtained from Eastman Organic Chemicals. All other fine chemicals were obtained from Sigma.

RESULTS

Relationship of the appearance of FBPase, PFK, and phosphoglucose isomerase with the disappearance of glucose from the growth medium. The activity of FBPase was first detected in 12-h cultures grown in the presence of 1% glucose, and this activity reached a maximum of 140 mU after 20 h (Fig. 1). A significant amount of PFK was present in 10-h cultures, and this activity increased to a maximum of 240 mU in 12-h cultures. In con-

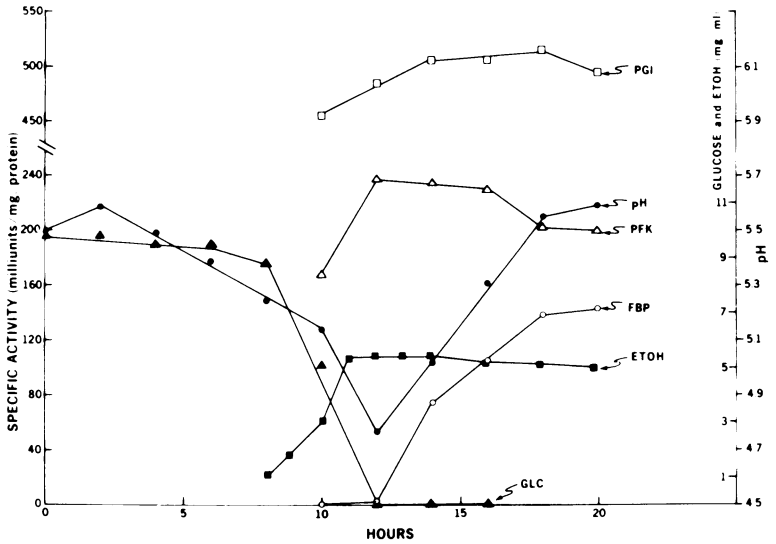

FIG. 1. *Enzymatic activities of PFK and phosphoglucose isomerase (PGI), and the mode of synthesis of FBPase in relation to the changes of pH, disappearance of glucose from, and the accumulation of ethanol in, the growth medium. S. cerevisiae X2180 was grown in the presence of 1% glucose with constant shaking. The glucose (▲) and ethanol (■) concentrations, and the pH (●) of the growth medium were determined at specified intervals. Cells were harvested from individual flasks after 10, 12, 14, 16, 18, and 20 h of growth, and extracts were prepared, dialyzed, and assayed for FBPase (○), PFK (△), and PGI (□) activities.*

trast, the phosphoglucose isomerase activity remained higher than the activities of PFK and FBPase. Results of additional experiments (not shown here) demonstrated that the levels of phosphoglucose isomerase were not significantly different in cells grown in the presence of different concentrations of glucose, fructose, and galactose; however, the specific activity of PFK was fivefold less in galactose-grown cells.

The appearance of FBPase activity coincided with the complete disappearance of glucose from, and a simultaneous increase in pH of, the growth medium. Unlike the synthesis of FBPase, the syntheses of PFK and phosphoglucose isomerase occurred before the disappearance of glucose. Accumulation of ethanol in the culture supernatant reached a maximum at 11 h, and did not change significantly in relation to the increase in the level of FBPase activity. An increase of pH of the culture supernatant in relation to the disappearance of glucose and the appearance of FBPase is particularly significant, but no significant change was observed in the accumulated acidic metabolites, acetate and pyruvate, in the culture supernatant after 8, 12, and 16 h of growth. There was, however, a slight reduction in the small amount of citrate accumulated through 8 h, after 12 h of growth.

Exogenous cAMP is known to reverse the glucose repression of catabolite-sensitive enzymes in bacteria (23). Such effects in yeasts and molds are not conclusive (2, 29). Attempts to demonstrate the synthesis of FBPase before the disappearance of glucose by supplementation of the growth medium with cAMP at 0, 8, and 10 h were unsuccessful. We have also harvested cells at 8 and 10 h and resuspended the cells in fresh medium containing cAMP but no glucose. No activity of FBPase was found after 2 h of incubation. The lack of any effect by exogenous cAMP may be due to the breakdown (decyclization) of cAMP, lack of cAMP permeation through the membrane, or ineffectiveness of cAMP in this particular yeast system.

Effect of cycloheximide on the synthesis of FBPase. The levels of FBPase did not increase after the addition of cycloheximide in either experiment (Fig. 2). Control cells exhibited FBPase activity to a level of 140 mU after 20 h of growth. Results from these experiments demonstrate that the synthesis of FBPase after the disappearance of glucose is blocked by 100 μg of cycloheximide per ml (10). Extracts from cells harvested after 8 or 10 h of growth failed to exhibit any inhibitory effect on the FBPase activity from cells harvested after 14 or 18 h, when the two extracts were added together in a 1:1 ratio.

Effect of different concentrations of glucose on the syntheses of FBPase and PFK. Different initial concentrations of glucose in the growth medium affected the time of appearance

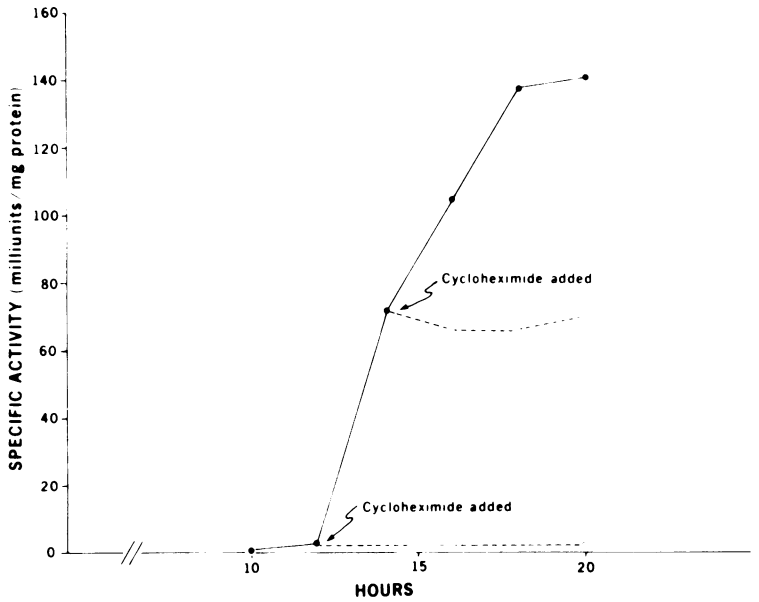

FIG. 2. *Effect of cycloheximide on the appearance of FBPase. S. cerevisiae X2180 was grown in the presence of 1% glucose in three identical flasks. Cycloheximide (100 μg/ml) was added to one flask at 12 h and to another flask at 14 h. Cells were harvested at 16, 18, and 20 h, and extracts were prepared, dialyzed, and assayed for FBPase (○) activity. The control experiment (●) contained no cycloheximide.*

as well as the maximum level of FBPase activity (Fig. 3A). The highest specific activity of FBPase was observed in 14- and 16-h cultures grown in the presence of 0.1% glucose. A decreasing order of this activity was in 0.5, 0.75, and 1% glucose-grown cultures. No FBPase activity was observed in the 14- and 16-h cultures grown in the presence of 2.5 and 5% glucose, respectively. The specific activity of PFK in 10-h cultures, grown in the presence of different concentrations of glucose, varied significantly (Fig. 3B), as did the maximum levels of PFK in the 20-h cultures. Particularly significant was the loss of PFK activity from 20-h cultures grown in the presence of 1, 2.5, and 5% glucose. Except in cells grown in the presence of 5% glucose, both FBPase and PFK activities were present simultaneously in all cultures grown with glucose as the carbon source. However, the initiation of the synthesis of FBPase varied depending upon the initial concentration of glucose in the growth medium. Maximum levels of FBPase and PFK were present between 12 and 16 h in cells grown in the presence of 1% glucose. This 12- to 16-h growth period represents a transition phase in the culture between exponential and stationary phase (Fig. 4). Although cell growth continued during this transition phase, the rate of growth was slower than that in the early or mid-exponential phase. Consistent with the lack of disappearance of ethanol from the growth medium, we did not observe any biphasic growth (11). Not only were FBPase and PFK activities present simultaneously in cells grown in the presence of 0.1% glucose, but the levels of these activities increased in a coordinate manner (Fig. 3A and B).

Effect of aeration and pH on the syntheses of FBPase and PFK. In cells grown in the presence of 1% glucose under low aeration, there was less than 10 mU of FBPase activity after 20 h of growth and approximately 50 mU after 40 h of growth (4). In highly aerated cultures, the synthesis of FBPase began at 12 h and reached a maximum level of approximately 140 mU after 20 h. The specific activity of PFK was not significantly affected by aeration.

The pH of the growth medium also affected the initiation of FBPase synthesis (Table 1). The least amount of FBPase activity was observed after 12 h in cells grown in medium with an initial pH of 5.0. Significantly higher levels of FBPase were found after 12 h in cells grown in media with an initial pH of 4.0 and 6.0. The synthesis of PFK was not significantly altered by the initial pH of the growth medium. Changes in the initial pH may affect the uptake and utilization of glucose and, consequently, the synthesis of FBPase.

Effect of gluconeogenic carbon sources on the syntheses of FBPase and PFK. There were approximately 500 mU of phosphoglucose isomerase activity, a small amount (40 mU) of

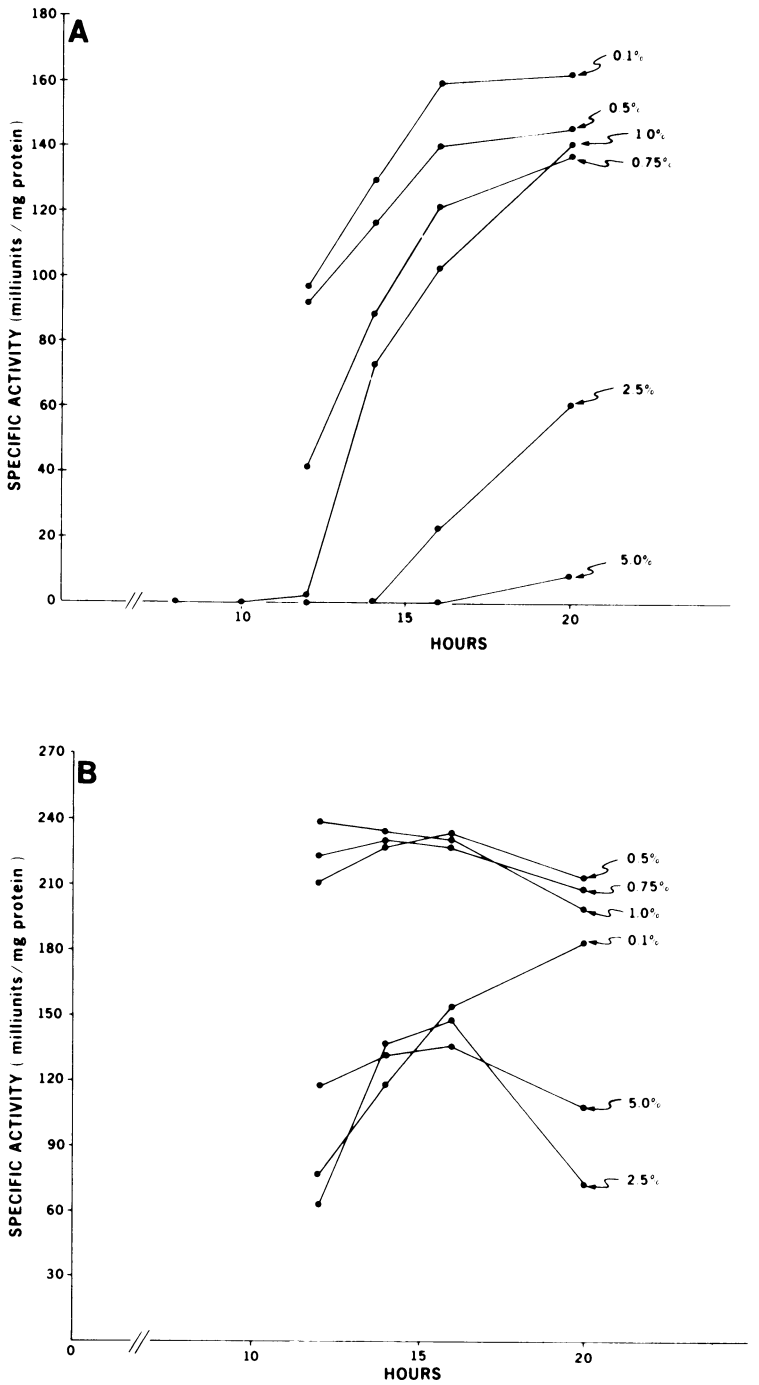

FIG. 3. *Effect of different concentrations of glucose on the syntheses of FBPase (A) and PFK (B). S. cerevisiae X2180 was grown under identical conditions except for the concentrations of glucose shown in the figure. Cells were harvested at 12, 14, 16, and 20 h and extracts were prepared, dialyzed, and assayed for FBPase and PFK activities.*

FBPase activity, and no PFK activity present in 15-h cultures grown in the presence of 1% (0.21 M) ethanol (Fig. 5). The activity of PFK was detected after 20 h, and this activity increased along with FBPase activity up to 30 h as the growth of the culture continued. This 20- to 30-

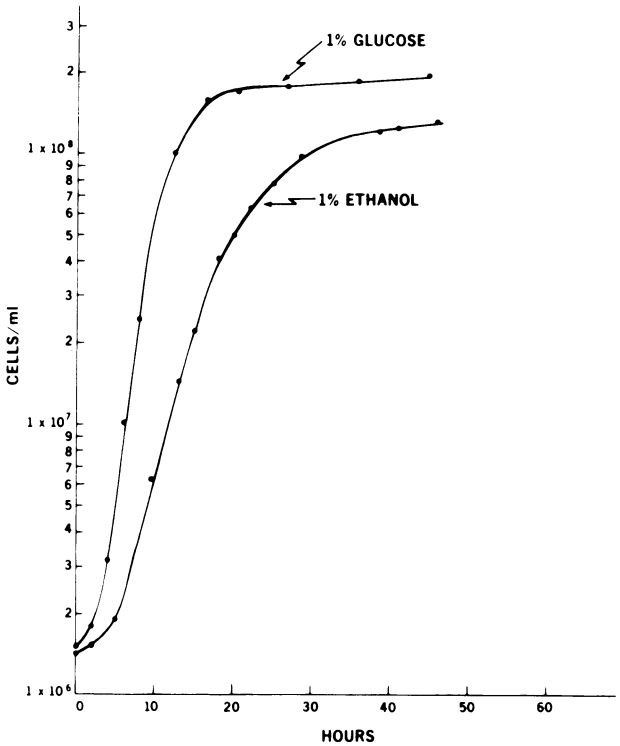

FIG. 4. *Comparison of the growth response of S. cerevisiae X2180 in 1% glucose- and 1% ethanol-containing media (initial pH 5.5) under constant shaking. Cultures were incubated at 30°C and cell counts were determined by using a hemacytometer, in portions removed at various times. Cell numbers were plotted as log functions.*

TABLE 1. *Effect of initial pH on the appearance of FBPase and PFK activities*[a]

Initial pH of the medium	Enzyme activity (mU/mg of protein) after 12 h of growth[b]		pH of spent medium after 12 h of growth[d]
	FBPase (±SD)[c]	PFK (±SD)[c]	
3	1 ± 0.0	165 ± 3	2.92
4	18 ± 1	191 ± 1	3.79
4.5	14 ± 2	231 ± 6	4.28
5	2 ± 1	228 ± 5	4.51
5.5	4 ± 2	229 ± 1	4.75
6	22 ± 3	191 ± 5	5.10
7	21 ± 2	190 ± 1	6.20

[a] *S. cerevisiae* X2180 was grown in 1% glucose medium under identical conditions except for the initial pH of the medium.

[b] Cells were harvested after 12 h of growth, extracts were prepared and dialyzed, and specific activities of FBPase and PFK were determined.

[c] SD, Standard deviation.

[d] Mean determination.

h growth period represents the transition phase in the 1% ethanol-grown culture (Fig. 4). Both FBPase and PFK activity were present in 20-h cultures grown in the presence of glycerol, lactate (except in 0.86 M concentration), or pyruvate as the sole carbon source (Table 2). These activities increased significantly, in certain cases, after 40 h of growth. In contrast to glucose-grown cells, the activity of PFK appeared to be more stable in ethanol- and lactate-grown cells. Cells grown in the presence of 0.86 M ethanol, 0.43 M alanine, or 2% peptone exhibited very little PFK activity (10 mU) after 20 h of growth. Cells grown in the presence of 0.86 M sodium lactate had no appreciable FBPase or PFK activity. In this experiment there was very little cell yield. In all cases the cell yields were less for the gluconeogenic carbon sources than for 1% glucose. It is important to note that the specific activity of FBPase, in cells grown in the presence of 1% or less concentrations of glucose, was higher than in cells grown in the presence of any of the gluconeogenic carbon sources, including ethanol.

Effect of glucose disappearance and cycloheximide on the glucose inactivation of FBPase. Unlike previous studies with ethanol-, lactate-, or glycerol-grown cells, we used glucose-grown cells as the source of FBPase and examined the disappearance of glucose and the fate of FBPase and PFK in the presence and in

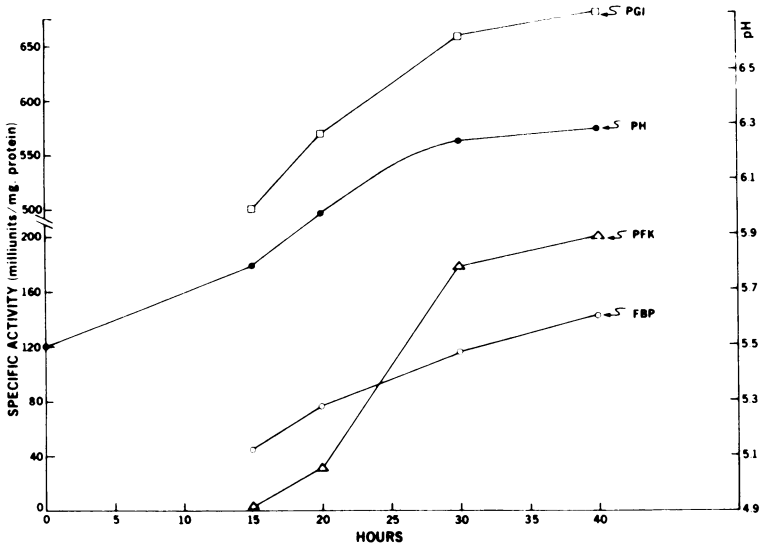

FIG. 5. *Enzymatic activities of FBPase (○), PFK (△), and phosphoglucose isomerase (PGI) (□) from S. cerevisiae X2180 grown in the presence of 1% ethanol with constant shaking. The pH (●) of supernatant from the growth medium was also determined. Cells were harvested at 15, 20, 30, and 40 h, and extracts were prepared, dialyzed, and assayed for FBPase, PFK, and PGI activity.*

TABLE 2. *FBPase and PFK activities from S. cerevisiae grown in gluconeogenic carbon sources*[a]

Carbon Source	Enzyme activity after 20 h of growth[b]		pH of spent medium after 20 h of growth[d]	Enzyme activity after 40 h of growth[b]		pH of spent medium after 40 h of growth[d]
	FBPase (±SD)[c]	PFK (±SD[c])		FBPase (±SD)[c]	PFK (±SD)[c]	
0.217 M Ethanol	77 ± 8	58 ± 8	5.90	—	—	
0.434 M Ethanol	76 ± 7	43 ± 10	5.84	134 ± 5[c]	58 ± 10	6.47
0.868 M Ethanol	28 ± 1	6 ± 1	5.65	—	—	
0.217 M Glycerol	39 ± 4	97 ± 9	5.94	—	—	
0.434 M Glycerol	42 ± 5	85 ± 12	5.91	85 ± 9	211 ± 9	5.95
0.868 M Glycerol	43 ± 3	94 ± 4	5.88	—	—	
0.217 M Na Lactate	58 ± 7	116 ± 15	6.55	—	—	
0.434 M Na Lactate	71 ± 6	105 ± 9	5.93	87 ± 6	91 ± 14	6.94
0.868 M Na Lactate	6 ± 1	9 ± 1	5.41	—	—	
0.434 M Na Pyruvate	23 ± 7	106 ± 2	6.74	—	—	
0.434 M Alanine	41 ± 2	10 ± 3	5.99	62 ± 2	33 ± 2	6.88
2% Peptone	37 ± 2	7 ± 4	5.87	78 ± 4	15 ± 1	6.18

[a] *S. cerevisiae* X2180 was grown under identical conditions except for the kind and concentration of gluconeogenic carbon source.
[b] Enzyme activity (mU/mg of protein) was determined in dialyzed cell extracts after 20 and 40 h of growth.
[c] SD, Standard deviation.
[d] Mean determination.

the absence of cycloheximide. When glucose (1%) was added to a growing culture at 14 h, the glucose disappeared completely from the medium within 2 h (Fig. 6A). This disappearance of glucose was much faster because of the larger quantity of induced cells in this experiment as compared with the slower disappearance of glucose shown in Fig. 1. With the addition of glucose at 14 h, the FBPase activity rapidly disappeared and then, by 17 h began to reappear (Fig. 6B). The pH of the growth medium decreased up to 16 h and then increased along with the activity of FBPase. The FBPase activity observed between 17 and 18 h is most likely the result of newly synthesized enzyme after the disappearance of glucose. The activity of PFK increased rapidly during the first hour after the addition of glucose, and then the activity decreased (Fig. 6C). When 1% glucose and cycloheximide (100 μg/ml) were added simultaneously to a 14-h culture, the disappearance of glucose from the medium was much slower and the FBPase activ-

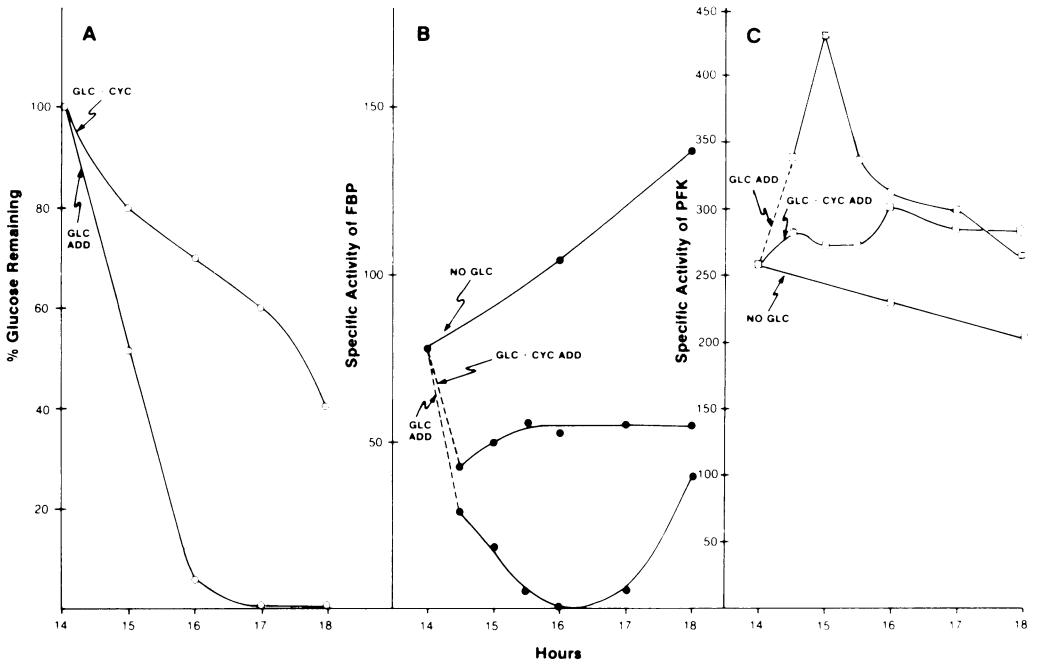

FIG. 6. *Effect of cycloheximide on the glucose inactivation of FBPase and PFK in vivo. Cultures of S. cerevisiae were grown in glucose medium for 14 h in a series of flasks under identical conditions. Fresh glucose, at a 1% final concentration, was added aseptically after 14 h of growth to one group of flasks. Glucose (GLC, 1%) and cycloheximide (CYC, 100 μg/ml) were added to a second group of flasks after 14 h of growth. No addition was made to the control flasks. The amount of GLC remaining in the growth medium was determined at specified time intervals after the addition of GLC or GLC plus CYC at 14 h (A). Cells were harvested at specified intervals after the addition of GLC or GLC plus CYC, extracts were prepared and dialyzed, and the specific activities of FBPase (B) and PFK (C) were determined.*

ity initially decreased, but then the activity leveled off, retaining approximately 70% of the original activity, and the PFK activity did not change significantly. It is important to note that more than 50% of the added glucose was taken up in the presence of cycloheximide, but the FBPase activity remained relatively unaffected. The addition of glucose to the in vitro reaction mixture had no effect on FBPase activity. These results indicate that glucose itself is not responsible for the loss of FBPase activity in vivo; instead, new protein synthesis, after glucose addition, is necessary for a direct or indirect effect on FBPase activity as well as the observed decrease of PFK activity.

DISCUSSION

Results of this investigation demonstrate clearly that cells grown in 1% or less glucose possess the highest level of FBPase activity, and synthesis of FBPase is initiated only after the disappearance of glucose from the growth medium. The fact that the specific activity of FBPase is increased linearly after the disappearance of glucose suggests that this synthesis is dependent upon an inducer. We believe that the pH change of the growth medium is related to the utilization of certain acidic metabolites and a simultaneous induction of FBPase. However, the exact nature of such an inducer has not been determined. Individual gluconeogenic carbon sources, when provided exogenously at equimolar concentrations, induce less FBPase than is found in cells grown in the presence of 1% glucose. It thus appears that glucose-grown cells may provide the most insight into the biosynthesis of FBPase and the interplay between glycolytic and gluconeogenic regulations as exemplified by PFK and FBPase.

Results presented here reinforce the view (14, 19) that the synthesis of phosphoglucose isomerase is constitutive in nature and is not affected by the nature or the concentration of the carbon source. In contrast, the synthesis of PFK is affected both by the nature and by the concentration of the glycolytic or gluconeogenic carbon source. The PFK also appears to be unstable, and the inactivation may be mediated by a glucose-induced protease. However, a modification of the enzyme other than proteolysis cannot be

ruled out. Increased inactivation of PFK and other glycolytic enzymes in glucose-supplemented cultures has been observed by Maita and Lobo (19). But cycloheximide (10 μg/ml) apparently had no effect on the inactivation of these enzymes.

The term inactivation-repression (8, 26, 30) suggests that glucose, in some manner, inactivates preexisting FBPase in ethanol- or lactate-grown *S. cerevisiae*. We have investigated the inactivation of FBPase in glucose-grown *S. cerevisiae*. Results presented in this report indicate clearly that the inhibition of new protein synthesis, after glucose addition, also inhibits the inactivation of FBPase. Since the inactivation of FBPase by a specific protease has been demonstrated in rabbit liver (24) and in vitro by Molano and Gancedo (20), such an inactivation by FBPase by a glucose-induced protease is also possible in vivo. Similar inactivation of enzymes by intracellular proteases is well recognized in microorganisms (13).

For the purpose of metabolic efficiency and to conserve cellular energy, it seems appropriate to postulate a mutually exclusive mode of synthesis of FBPase and PFK to prevent a futile cycle (16, 17, 21). Evidence in support of this view has come from the observations that glucose-grown cells lack FBPase activity and ethanol-grown cells possess a significantly reduced PFK activity (6). Although it is true that, depending upon the kind and the concentration of carbon source used, the degree of aeration, and the time of harvest of culture, one may observe the presence of either FBPase or PFK, but not both (Fig. 1, Fig. 5), it does not mean, however, that these two metabolically antagonistic enzymes are never present simultaneously in cells grown in the presence of either glycolytic or gluconeogenic carbon sources. To the contrary, results presented in this report from well-defined experiments, using glucose- or ethanol-grown cells, demonstrate clearly that both of these enzymes are present at some phase during the growth of *S. cerevisiae*. We also believe that the same is true for glycerol-, lactate-, and pyruvate-grown cells (Table 2). PFK and FBPase have also been shown to be present in both glycolytic and gluconeogenic tissues in animals (1, 3, 27). To what extent these metabolically antagonistic enzymes are active in vivo remains to be determined. It is known that PFK is a cytosolic enzyme, and recently it has been shown that FBPase is also a cytosolic enzyme (12). This rules out compartmentalization as a possible basis for the post-translational regulation of FBPase and PFK. It should be noted that, depending upon the carbon source used, during the early and mid-exponential phase of culture growth, there is present in cells only one of these activities, thus avoiding a futile cycle. However, during the transition phase (between the exponential and the stationary phase) both enzymes are present irrespective of the carbon source used. At this point post-translation regulations may play a vital role in preventing a futile cycle. One such post-translational mechanism that would suppress futile cycling has been suggested by Koerner et al. (17).

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