

Relationship of Glycolytic Intermediates, Glycolytic Enzymes, and Ammonia to Glycogen Metabolism During Sporulation in the Yeast *Saccharomyces cerevisiae*

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To identify the factors which control glycogen synthesis in *Saccharomyces cerevisiae*, we have studied the regulation of glycogen metabolism during sporulation, since in vivo glycogen has been reported to undergo significant changes in concentration during this process. We examined the concentration of a number of key glycolytic intermediates and enzymes in strains that sporulate at different rates and those that are deficient in sporulation. There were no significant changes found in the adenylate energy charge or cyclic AMP levels throughout sporulation. Although significant alterations occurred in the levels of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, phosphoenolpyruvate, and ATP during sporulation, only the fourfold increase in fructose-1,6-bisphosphate appeared to correlate with glycogen synthesis in all of the strains examined. Only limited changes occurred in the level of a number of glycolytic and gluconeogenic enzymes which were examined during this process. Intracellular glucose content underwent a dramatic 30- to 40-fold increase in sporulating cells. Comparison of strains with different rates of sporulation demonstrated that this increase in glucose content coincides with the time of glycogen degradation in each strain. Both the increase in glucose content and the degradation of accumulated glycogen were not observed in nonsporulating α/α strains, or in cells incubated in NH_4^+ supplemented sporulation medium. Although glucose appears to be the direct product of glycogen degradation, a 10-fold increase in a nonspecific alkaline phosphatase occurs at this time, which may be degrading phosphorylated sugars to glucose. All of the strains examined released extracellular glucose while suspended in acetate sporulation medium. It is concluded that most of the changes in the glycolytic pathway that occur during sporulation, with the exception of glycogen degradation and the concomitant increase in intracellular glucose pools, are a response to the transfer to sporulation medium and are independent of sporulation-specific processes. Inhibition of sporulation with ammonium ions resulted in a different pattern of change in all of the glycolytic intermediates examined, including a twofold increase in cyclic AMP levels. Ammonia did not interfere with glycogen synthesis, but prevented sporulation-specific glycogen degradation. The levels of the glycolytic enzymes examined were not affected by ammonia.

Sporulation in *Saccharomyces cerevisiae* provides an excellent model system for the study of the regulation of glycogen metabolism in eucaryotic cells. During sporulation, the cells first accumulate glycogen and subsequently degrade this stored glycogen (15, 17). Although the increase in glycogen occurs in both sporulating and nonsporulating strains of yeast, only sporulating strains degrade glycogen (15, 17). This degradation occurs immediately before spore wall formation (17). Although the glycogen content of yeast cells can be increased or decreased by modifying cultural conditions (27), the gly-

cogen content of sporulating cells first increases and subsequently decreases while the cells are incubated in the same nitrogen-free acetate medium (15, 17). Thus, this system offers an ideal opportunity for identifying the mechanism responsible for the regulation of glycogen metabolism. Although sporulating yeasts have been extensively examined with respect to macromolecular synthesis, there is very little information on the changes in intracellular metabolites and glycolytic enzymes during sporulation.

To define the metabolic factors responsible for the control of glycogen synthesis, we have

investigated the changes in the concentrations of several glycolytic intermediates and key metabolic enzymes. Special care was taken to use techniques which minimize artifacts resulting from the rapid turnover of metabolites and non-specific protein degradation.

Since ammonia has been found to block sporulation (22) and has been reported to be involved in glycogen metabolism (25, 27), the effect of ammonia on glycogen metabolism during sporulation was also examined.

MATERIALS AND METHODS

Yeast strains. Three strains of *S. cerevisiae*, SK-1, AP-1-a/ α , and AP-1- α / α , were used throughout this study. SK-1 was obtained from R. Roth (17). This strain is a homothallic prototroph which is capable of extensive and synchronous sporulation. Approximately 80% of these cells form asci between 8 and 9 h after transfer to sporulation medium. Strains AP-1-a/ α and AP-1- α / α were obtained from A. K. Hopper (15). AP-1-a/ α begins ascospore formation by 10 h, with 50% of the cells forming asci between approximately 12 and 14 h. The control strain, AP-1- α / α , which is isogenic with AP-1-a/ α except at the mating type locus, forms less than 0.01% asci within 24 h. These strains were routinely maintained on agar slants containing 1% yeast extract, 1% proteose peptone, and 1% of either glucose or galactose (YPDA or YPGal).

Growth and sporulation of yeast. The yeast were pregrown and sporulated by a modification of the procedures described by Roth and Halvorson (24), and Fast (8). The presporulation medium consisted of 1% yeast extract, 1% proteose peptone, and 1% potassium or sodium acetate. A 1-liter amount of presporulation medium contained in a 2-liter flask was inoculated with cells from either a YPDA or YPGal slant. The culture was incubated for 12 to 16 h at 30°C on an orbital shaker. The cells were harvested at a density of 2×10^7 to 4×10^7 cells per ml. The culture was centrifuged at 4,000 rpm ($3,290 \times g$) in a Sorvall GS-3 rotor for approximately 1 min at 4°C. The pellet of cells was washed twice with 10 ml of sterile distilled water and suspended in sporulation medium (1% potassium acetate) at a density of 3×10^7 to 4×10^7 cells per ml. For measurement of metabolite levels, the cells were suspended in 500 ml of sporulation medium in a 2-liter flask and incubated at 30°C on a reciprocal shaker water bath (160 1.5-inch [ca. 3.81-cm] strokes per min). Cultures used for measuring enzyme levels consisted of 300 ml of sporulation medium contained in a 2-liter flask and incubated at 30°C on an orbital shaker (1-inch [ca. 2.54-cm] orbit, 340 rpm). The rate, synchrony, and extent of sporulation were identical under both conditions.

Sporulation in the presence of NH_4^+ . In experiments testing the effects of ammonium ions on sporulation, strain SK-1 was suspended in sporulation medium (1% potassium acetate) containing 10 mM $(\text{NH}_4)_2\text{SO}_4$. The minimal concentration of $(\text{NH}_4)_2\text{SO}_4$ required to cause 95% inhibition of sporulation in this strain was 7.5 mM (unpublished data).

Collection of metabolite samples. Samples for

the assay of intermediary metabolites were prepared by a modified procedure of Opheim and Bernlohr (23). A 25-ml amount of culture was rapidly pipetted onto a wet, 90-mm diameter, membrane filter (0.45- μm pore size, Millipore Corp.) over a vacuum of 65 cm of mercury. The filter was immediately removed and immersed in 15 ml of a solution containing 10% (wt/vol) trichloroacetic acid and 30% (vol/vol) methanol. This solution was contained in a glass petri plate cover resting on ice. These steps took approximately 8 s to complete. The filters were incubated in the solution for 1 h at 4°C. After removing the filter, the solution was extracted five times with 1 volume of water-saturated ether to remove the trichloroacetic acid. After centrifuging to remove the cells, the aqueous supernatant solution was removed and stored at -20°C. This procedure was used to prepare samples for the assay of all intermediates except ATP. Samples for ATP determinations were prepared by rapidly pipetting 2 ml of the culture into 1 ml of cold methanol containing 30% (wt/vol) trichloroacetic acid. These samples were incubated and extracted as described for the filtered samples. Samples taken from the pre-sporulation culture, except samples for ATP determination, were treated with 50 mg of acid-washed charcoal per ml to remove the high fluorescent background due to the yeast extract in the presporulation medium.

These sampling procedures were tested for alteration of metabolite levels, completeness of extraction, and recovery of intermediates. The levels of intermediates obtained by these procedures were in good agreement with the levels obtained by the addition of trichloroacetic acid and methanol (final concentration, 10% trichloroacetic acid and 30% methanol) directly to shaking cultures. Of the intermediates assayed, only ATP showed any change upon filtration, decreasing approximately 50%. Pipetting the sample directly into trichloroacetic acid and methanol prevented this decrease in ATP. Prolonged incubation or heating the cell suspension to 40°C (19) did not increase the yield. Extraction by 35% perchloric acid, followed by three cycles of freeze thawing in liquid nitrogen (19), yielded similar results. Greater than 90% recovery of all intermediates was obtained, and this was not affected by the charcoal treatment. Of the intermediates assayed, only glucose was found to occur in the medium. To correct the filtered samples for extracellular glucose retained by the filter, a value of 0.54 ml was determined as the amount of medium retained in the filter.

Assay of metabolites. The assays were performed in a Turner Model 111 fluorometer equipped with a 7-60 primary filter and a 2A secondary filter. The temperature was maintained at 30°C by a Lauda K-2/R (Brinkman Instruments) circulator. The change in fluorescence was monitored on a Sargent Welch model SR recorder.

The metabolites glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), glucose, and uridine diphosphoglucose were assayed by the method of Lowry and Passoneau (20). Fructose 1,6-bisphosphate (Fru 1,6-P₂) and phosphoenolpyruvate (PEP) were assayed by a modification of the procedures described by Lowry and Passoneau (20) as described below. Fru 1,6-P₂ was assayed in a reaction mixture containing 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride

ride (pH 8.1), with 1 mM sodium arsenate, 1 mM dithiothreitol, 100 μ M oxidized nicotinamide adenine dinucleotide (NAD⁺), 0.1 U (μ mol/min) of glyceraldehyde 3-phosphate dehydrogenase, and 2.0 U of triosephosphate isomerase. The reaction was initiated by the addition of 0.02 U of aldolase. The reaction mixture for the determination of PEP contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM MgSO₄, 5 mM hydrazine, 200 μ M ADP, 200 μ M reduced NAD (NADH) and 0.1 U of lactate dehydrogenase. Pyruvate kinase (0.3 U) was added to start the reaction. Adenylate nucleotides were measured with luciferase as described by Ball and Atkinson (1). In all of the assays described above, internal controls were performed to ensure that no inhibitors of the coupled enzyme reactions were present in the cell extracts. The samples were stored at -20°C and assayed within 1 week of collection. All intermediates were stable over this period.

Cyclic AMP (cAMP) was measured by the radioactive binding assay of Gilman (14), utilizing the Amersham cAMP assay kit. Since compounds which could interfere with cAMP binding may be produced during sporulation, internal standards were run in the presence of extract to make sure that binding was proportional to the amount of cAMP present. Samples of extract were also treated with purified beef heart phosphodiesterase. The enzyme was removed by precipitation with 5% trichloroacetic acid, and the amount of cAMP remaining in the samples was determined. Phosphodiesterase-sensitive cAMP levels are reported. Internal controls of added cAMP were run to confirm that all of the cAMP was degraded by this treatment.

Calculations of intracellular metabolite concentrations were based on the assumption that 1 g dry weight corresponds to 5 g wet weight, and that 1.67 g wet weight contains 1 ml of cell sap (6). There was no significant change in the protein concentration of the cells during sporulation. Multiple independent samples were obtained for analysis, and the standard error of the mean was calculated for each data point.

Preparation of cell extracts. The preparation of the cell extracts for enzyme assays was initiated by the addition of approximately 200 g of ice to a 300-ml culture. The cells were collected by centrifugation at 5,000 rpm (5,140 \times g) in a Sorvall GS-3 rotor for 5 min at 4°C. The cell pellet was washed once with 5 ml of cold 20 mM imidazole-hydrochloride buffer (pH 7.0), containing 24 mM MgSO₄, 5 mM 2-mercaptoethanol, and 2 mM phenylmethyl sulfonyl fluoride. After washing, the cells were resuspended in 5 ml of the same buffer and either passed twice through a chilled French pressure cell with a modified valve (28) at 15,000 lb/in² or shaken for 10 min with 1 g of 0.3-mm diameter glass beads. Approximately 90% of the cells were broken by these treatments. Cell debris was removed by centrifugation for 5 min at 5,000 rpm (3,020 \times g) in a Sorvall SS-34 rotor at 4°C. The supernatant solution was immediately assayed for enzyme activity.

Enzyme assays. Glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), phosphofructokinase (PFK; EC 2.7.1.1), and fructose biphosphatase (FBPase; EC 3.1.3.11) were assayed by the method of

Gancedo and Gancedo (12). Hexokinase (EC 2.7.1) was assayed as described by Gancedo et al. (11). Phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.49) was assayed by the method of Gancedo and Schwermann (10). Pyruvate kinase (PK; EC 2.7.1.40) was assayed as described by Barwell and Hess (3). Phosphoglucotomutase was assayed as described by Daugherty et al. (7). Glycogen synthetase (EC 2.4.1.11) activity was determined as described by Huang and Cabib (16), modified according to Thomas et al. (26) for the measurement of labeled glycogen. Alkaline phosphatase activity was assayed by adding 20 μ l of the cell extract to 0.5 ml of 100 mM Tris-hydrochloride buffer (pH 8.5), containing 2 mM MgSO₄ and either 0.5 mM G6P or 0.5 mM glucose-1-phosphate. The phosphate released was determined as described by Fiske and Subbarow (9). Activities are expressed as μ moles of product produced per minute per milligram of protein. Glycogen was measured by the procedure described by Hopper et al. (15).

Protein was determined by the procedure of Lowry et al. (21), using desiccated bovine serum albumin (fraction V) as the standard.

Sporulation was determined by phase-contrast microscopy at \times 1,000. All buds were counted as cells, and approximately 200 cells were counted for each determination.

RESULTS

Levels of glycolytic intermediates. The concentration of the glycolytic intermediates G6P, F6P, Fru-1,6-P₂, and PEP have been investigated in yeast under various growth conditions (13). These intermediates show characteristic patterns during gluconeogenesis and glycolysis: G6P, F6P, and Fru-1,6-P₂ are at low levels, and PEP is at a high level during gluconeogenesis, whereas the reverse pattern occurs during glycolytic growth (2, 13). These intermediates were examined to determine if a gluconeogenic profile was maintained during sporulation.

Table 1 lists the levels of these intermediates in AP-1-a/ α , AP-1- α / α , and SK-1 during logarithmic growth in presporulation medium. These data showed the expected gluconeogenic pattern with high levels of PEP and lower levels of the other intermediates. All of the values were

TABLE 1. Metabolite levels of log-phase cells in presporulation medium^a

Strain	G6P	F6P	Fru-1,6-P ₂	PEP
AP-1-a/ α	5.5 \pm 0.5	2.0 \pm 0.1	0.21 \pm 0.02	17.9 \pm 1.8
AP-1- α / α	6.7 \pm 0.8	1.9 \pm 0.2	0.23 \pm 0.02	16.9 \pm 3.0
SK-1	5.4 \pm 0.4	1.5 \pm 0.1	0.65 \pm 0.04	14.7 \pm 2.2

^a Samples were taken from the presporulation culture immediately before centrifugation of cells for resuspension in sporulation medium. Values are the average of determinations on at least three separate cultures \pm the standard error of the mean. Metabolite levels are expressed in nanomoles per milligram of protein.

within the reported ranges for these intermediates (13). It is of interest to note that although G6P, F6P, and PEP have similar levels in all three strains, Fru-1,6-P₂ was threefold higher in SK-1 than in the AP-1 strains.

The levels of G6P, F6P, Fru-1,6-P₂, and PEP in AP-1-a/α during sporulation are shown in Fig. 1. The level of Fru-1,6-P₂ underwent major changes during sporulation. Its level increased over 500% within the first 4 h in sporulation media, plateauing at approximately 4 h and remaining at this level until 10 h, whereupon it began to decline. The changes in the levels of the other three intermediates were not as marked as the changes in Fru-1,6-P₂. The levels of these intermediates were compatible with gluconeogenesis being the net direction of metabolic flux throughout sporulation.

To determine whether the response seen in AP-1-a/α was specific to the sporulation process,

we investigated a nonsporulating strain, AP-1-α/α. The levels of metabolites in this strain are shown in Fig. 1. The changes in metabolite levels found in this strain were qualitatively similar to those found in AP-1-a/α. The level of Fru-1,6-P₂ underwent the same rapid increase and subsequent decline, although it did not increase to the same extent as it did in AP-1-a/α. The levels of G6P, F6P, and PEP differed only slightly. The level of PEP did not increase to the same degree as AP-1-a/α. UDP-glucose was measured in both AP-1-a/α and AP-1-α/α. No change in concentration (5.1 ± 0.4 nmol per mg of protein) was found during sporulation in AP-1-a/α or in AP-1-α/α incubated in sporulation medium. In general, it would appear that no major sporulation-specific changes occurred in these metabolites, and that the changes observed were a response to the pseudostarvation conditions of the sporulation medium.

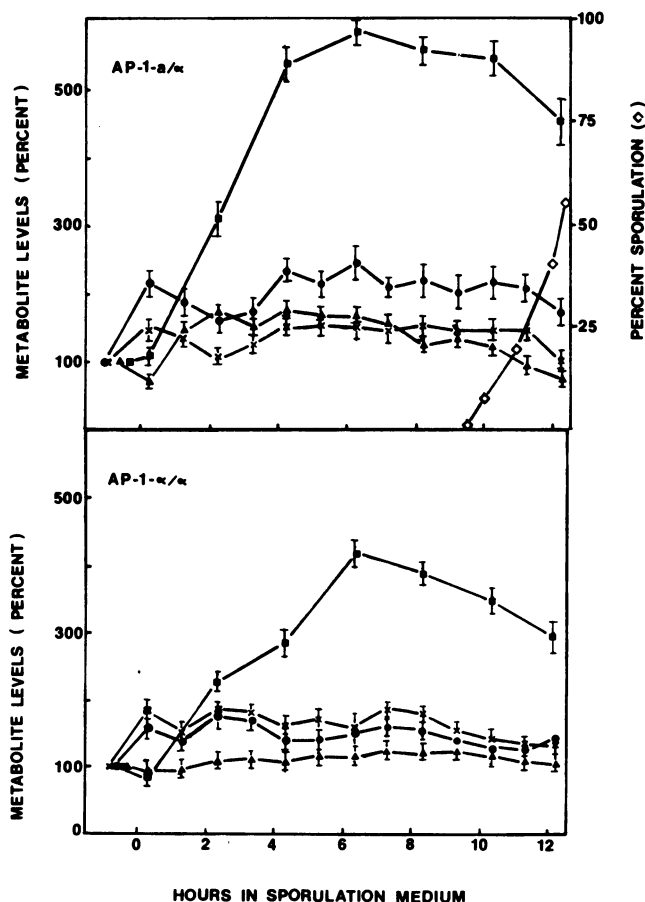


FIG. 1. Metabolite levels during sporulation in AP-1. AP-1-a/α and AP-1-α/α were pregrown and sporulated in 1% acetate medium as described in the text. Samples were collected and assayed for G6P (×), F6P (●), Fru-1,6-P₂ (■), and PEP (▲). Values are expressed as the percentage of the metabolite content of cells in presporulation medium. Bars indicate the standard error of the mean; percent sporulation is indicated by the open diamonds (◇).

If the changes observed in the AP-1 strains are a general metabolic response to the conditions of the sporulation medium, a similar pattern should be observed in other strains as well. This hypothesis was tested by using strain SK-1. As indicated in Fig. 2, a somewhat similar response occurred in SK-1 with respect to Fru-1,6-P₂. The level of Fru-1,6-P₂ increased early in sporulation, and subsequently decreased as spores were formed. F6P and G6P not only decreased in concentration immediately upon suspension in sporulation medium, as in AP-1, but they increased an additional 100% during sporulation and declined late in sporulation in a manner similar to Fru-1,6-P₂. In comparison to AP-1, a major difference was found in the timing of the change in Fru-1,6-P₂. The peak and subsequent decline occurred much sooner in SK-1 than in the AP-1 strains. This is of interest, since the rate of ascus formation was also more rapid

in SK-1 than AP-1- α/α . Thus, a qualitatively similar response to the pseudostarvation conditions of sporulation medium occurred in all three strains tested, although SK-1 appeared to respond more rapidly.

Optimal sporulation occurs under conditions of nitrogen starvation, and ammonia is a potent inhibitor of this process (22). We investigated the effects of NH₄⁺ on the levels of G6P, F6P, Fru-1,6-P₂, and PEP in SK-1 (Fig. 2). It is apparent from these data that the metabolic pattern was significantly altered. A rapid decline occurred in the levels of G6P, F6P, and PEP immediately after addition of cells to sporulation media. Furthermore, their levels remained low for the next 12 h. Although the level of Fru-1,6-P₂ increased, as it did in the absence of ammonia, its level did not increase to the same extent, and the kinetics of these changes were altered. It should be noted that Fru-1,6-P₂ increased during

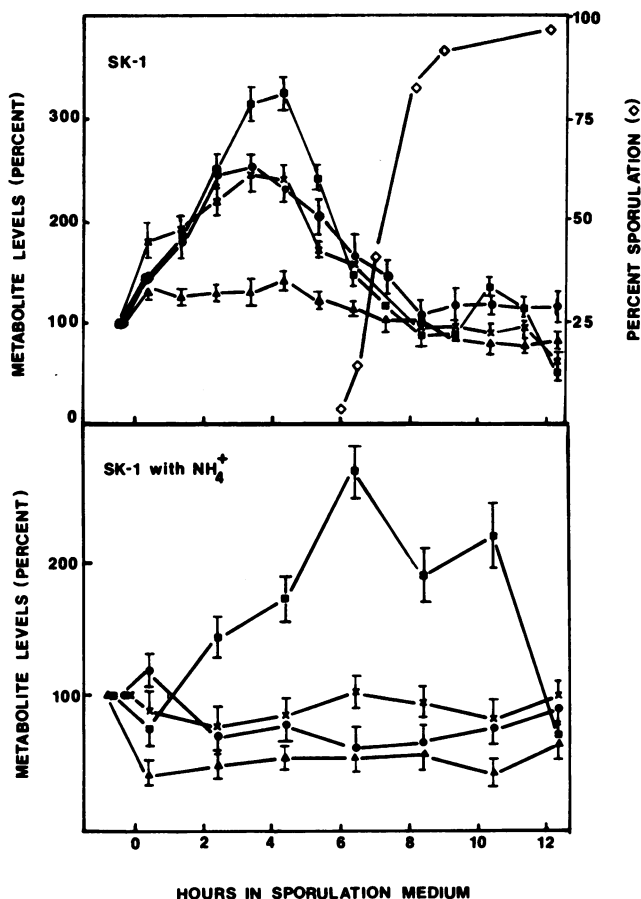


FIG. 2. Metabolite levels in SK-1 during sporulation in the presence and absence of ammonia. SK-1 was pregrown and sporulated in either 1% acetate or 1% acetate with 10 mM ammonium sulfate. Samples were collected and assayed for G6P (×), F6P (●), Fru-1,6-P₂ (■), and PEP (▲). Other symbols are as described in the legend to Fig. 1.

glycogen synthesis (see below), both in the presence and absence of ammonia.

Adenylate nucleotides and energy charge. There was a major difference between ATP levels in the AP-1 strains and strain SK-1, (Fig. 3). In both AP-1 strains, the level of ATP gradually increased to approximately 200% of its initial level. In SK-1, on the other hand, the ATP level remained at about the same level over the first 4 h of sporulation and then began to decline. The results indicate that the changes in ATP levels are neither sporulation-specific nor a general response to the culture conditions. The significance of these changes is not clear from these data. In the presence of NH_4^+ , the ATP level rapidly declined to 13 nmol per mg of protein, and then increased to 25 ± 3 nmol per mg of protein at 6 h into sporulation.

The concentrations of ADP and AMP were also measured during sporulation in strain SK-1 in the presence and absence of ammonia. We could find no significant changes in the levels of these intermediates (ADP = 0.35 ± 0.09 mM; AMP = 0.010 ± 0.004 mM) during sporulation. In the presence of ammonia, the concentration of ADP doubled in 8 h ($P < 0.01$). The energy charge of the cell was calculated to be 0.89 ± 0.02 and was not significantly altered during sporulation. The change in ATP levels during sporulation caused a slight drop in energy charge, but this was statistically insignificant, due to the high levels of ATP relative to ADP and AMP, and the variability in the values for ADP and AMP. The addition of ammonia to sporulating cells caused a minor decrease in the energy charge to 0.83 ± 0.04 .

It was of interest to examine whether cAMP levels changed in these cells when the cell metabolism changed from glycogen synthesis to glycogenolysis. There was no significant change in cAMP level throughout sporulation in SK-1 in samples taken at 1-h intervals. Ammonia appeared to significantly increase the cAMP concentration from 18 pmol per mg of protein to 41 pmol per mg of protein ($P < 0.005$).

Glucose levels. It has been previously demonstrated that the glycogen content of sporulating cells begins decreasing shortly before spore formation (15, 17). Since the pattern of glycolytic intermediates did not seem to indicate a sudden reversal of flux at this time, it appeared that the products of glycogen degradation were not entering the glycolytic pathway. This could be explained by several hypotheses, one being that glycogen is degraded to free glucose, which could not be phosphorylated and enter glycolysis. The data in Fig. 4 appear to substantiate this hypothesis. In AP-1-a/ α , the intracellular glucose

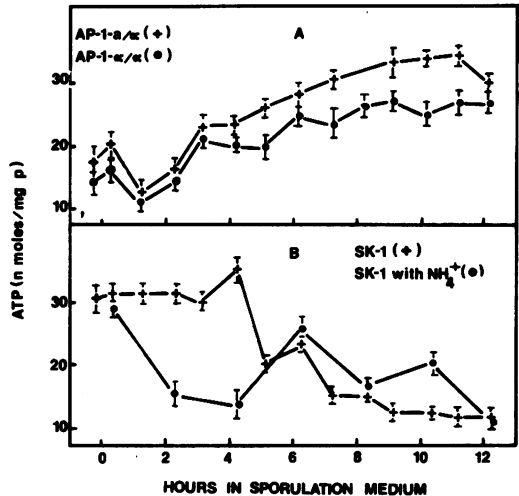


FIG. 3. Level of ATP in sporulating cells in the presence and absence of ammonia. (A) Samples from AP-1-a/ α (+) or AP-1- α/α (●) cells, sporulated as described in the text, were assayed for ATP. (B) In a similar manner, SK-1 cells sporulating in either 1% acetate (+) or 1% acetate with 10 mM ammonium sulfate (●) were assayed for ATP.

remained at a constant, low level until approximately 7 h into sporulation. Between 8 and 10 h, a rapid 30-fold increase occurred in the level of glucose, reaching a peak intracellular concentration of approximately 5 mM. The time of this increase coincided with the reported period of glycogen breakdown in this strain (15). In strain AP-1-a/ α , glycogen is not degraded (15), and there was no increase observed in the intracellular glucose levels (Fig. 4). Further support of this hypothesis is gained from data presented for SK-1 (Fig. 5). In this strain, glycogen degradation was initiated at approximately 5 to 6 h into sporulation, as has been previously reported (17). The observed increase in glucose levels occurred approximately at the time of glycogen breakdown. Furthermore, in NH_4^+ -repressed cells which do not sporulate, the level of intracellular glucose remained constant and glycogen was not appreciably degraded (Fig. 5). Since treatment of rabbit muscle glycogen, trehalose, glucose-1-phosphate, G6P, uridine 5'-diphosphoglucose, *N*-acetyl-glucosamine or glucosamine with 10% trichloroacetic acid-30% methanol did not release free glucose (unpublished data), it is concluded that the observed rise in intracellular glucose was not an artifact of sample preparation, but was a product of cellular metabolism and may have resulted from the sporulation-specific degradation of glycogen.

Extracellular glucose. Kane and Roth have shown that both sporulating and nonsporulating

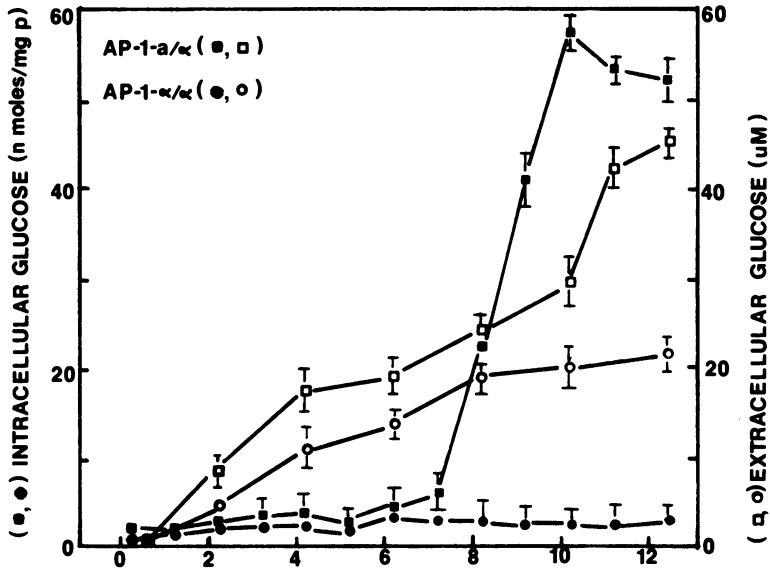


FIG. 4. Glucose production by sporulating cells. Intracellular levels of glucose in AP-1-a/α (■, internal; □, external) and AP-1-α/α (●, internal; ○, external). The strains were pregrown and transferred to sporulation medium as described in the text. The intracellular level of glucose is expressed as n moles of glucose per milligram of protein. The concentration of glucose in the medium is expressed in micromolar units.

strains release carbohydrates into the medium during sporulation (16). The results in Fig. 4 and 5 substantiate these observations and identify a portion of the extracellular carbohydrate as glucose. Although both sporulating and nonsporulating strains released glucose into the medium, the rate of release declined after 8 h in the nonsporulating strain, AP-1-α/α. In contrast, the rate of glucose release did not decrease in AP-1-a/α or SK-1, and appeared to increase in AP-1-a/α as intracellular glucose increased. It is also of interest to note that even in the presence of NH_4^+ , SK-1 released a small amount of glucose into the medium.

Enzyme levels. The levels of the glycolytic enzymes were of interest because the metabolic changes we observed could be caused by a change in enzyme activities. Since proteolytic activity increases significantly during sporulation (4, 18), major changes in the level of glycolytic enzymes could be occurring late in sporulation due to the degradation of specific enzymes. Therefore, we examined a number of glycolytic enzymes throughout sporulation, including hexokinase, phosphoglucomutase, glycogen synthetase (both G6P-dependent and -independent forms), G6PDH, PFK, pyruvate kinase, PEP carboxykinase, and FBPase. The only enzyme to demonstrate any significant change in specific activity was FBPase, which gradually declined to 40% of its original activity by 12 h in all of the strains tested. The addition

of ammonia had no effect on the level of these enzymes.

Since intracellular glucose levels increased during sporulation, without changes in any of the glycolytic enzymes examined, it was considered that an alkaline phosphatase may have been induced during sporulation, causing an increase in the degradation of hexose phosphates to glucose. Alkaline phosphatase was therefore measured, using G6P or glucose-1-phosphate as substrate. We found that this enzyme increased continuously during sporulation, resulting in an eight- to nine-fold increase in specific activity (unpublished data).

DISCUSSION

This work provides the first metabolic profile of sporulating yeast cells with respect to both intermediary metabolites and key glycolytic enzymes. Investigation of both sporulating and nonsporulating strains, as well as strains which require different lengths of time for ascospore formation, demonstrated several interesting relationships.

Since no major changes were observed during sporulation in any of the key glycolytic enzymes measured, it would appear that changes in metabolic intermediates may be responsible for the regulation of glycogen metabolism. There are no changes in ATP, energy charge or cAMP which correlate with glycogen synthesis or breakdown.

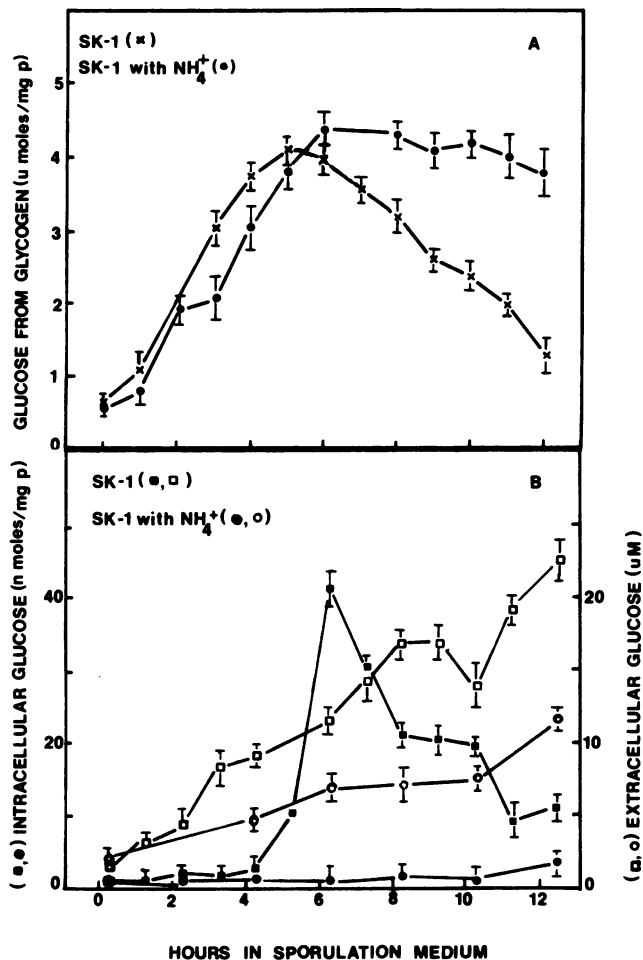


FIG. 5. Glycogen and glucose levels in sporulating SK-1 cells. (A) Samples from cells sporulating in either 1% acetate (x) or 1% acetate with 10 mM ammonium sulfate (●) were assayed for glycogen content. (B) The same cells were assayed for the amount of intracellular (■, ●) and extracellular (□, ○) glucose as described in the text.

The only intermediate which was found to change during glycogen synthesis was Fru-1,6-P₂, which increased three- to fivefold in every strain tested during glycogen synthesis independent of changes in G6P or F6P (Fig. 1 and 2), suggesting that Fru-1,6-P₂ may activate glycogen synthesis in yeast. These data suggest that the role of Fru-1,6-P₂ in the regulation of glycogen synthesizing enzymes should be examined more extensively. Changes in the level of G6P do not appear to regulate glycogen synthesis, as suggested by previous work (25).

The reason for this change in Fru-1,6-P₂ is unknown. PFK, which produces Fru-1,6-P₂, remains constant during sporulation, and FBPase, which degrades Fru-1,6-P₂, gradually decreases to 40% of its original value in a manner which

does not correlate with the change in Fru-1,6-P₂. The effectors of FBPase and PFK, AMP, and ATP, either do not change, or show no relationship to the change in Fru-1,6-P₂ levels or glycogen synthesis in these strains of yeast (Fig. 3). Ammonia, which has been suggested to affect glycogen synthesis in yeasts through its action on PFK (25), did not block glycogen synthesis or the increase in Fru-1,6-P₂ pools (Fig. 2).

The glycolytic intermediates G6P, F6P, Fru-1,6-P₂, and PEP underwent similar changes in both AP-1-a/α and AP-1-α/α. Since both sporulating and nonsporulating strains exhibited similar metabolic patterns, it would appear that the metabolic response of these cells, with the exception of glycogen degradation, is not specific to the sporulation process and probably repre-

sents a general response to the pseudostarvation conditions of the sporulation medium.

The changes in pool levels reported in SK-1 are interesting in that there was in general a coordinate change in the levels of the glycolytic intermediates examined, increasing early in sporulation and later declining (Fig. 2). This pattern suggests that gluconeogenesis remains the major metabolic flux, even during the period of glycogen breakdown (Fig. 5). If a shift to glycolysis was occurring, an increase in Fru-1,6-P₂ and a decrease in PEP would be expected to occur (2). In addition, the direction of metabolic flux as determined by the calculation of G6P/F6P ratios (2) supports this conclusion (unpublished data).

The degradation, but not the synthesis, of glycogen appears to be sporulation specific (15, 17), in that glycogen degradation does not occur in the nonsporulating strain, AP-1- α/α (11, 22). Our data on intracellular glucose levels show a strong temporal relationship with glycogen degradation (Fig. 5). In strains SK-1 and AP-1- α/α , the concentration of intracellular glucose increased dramatically, concomitant with glycogen breakdown in these strains, whereas in AP-1- α/α the intracellular glucose level remained at the low initial level, suggesting that the glucose is a result of glycogen breakdown. Since the cell has a high concentration of nonspecific alkaline phosphatase at the late stages of sporulation, it is not possible to tell whether glycogen is degraded to glucose, or to hexose phosphate, which is then degraded to glucose and inorganic phosphate. The latter seems less probable, since there was no increase in G6P or F6P when glycogenolysis occurred (Fig. 1 and 2). In addition, Colonna and Magee (5) have reported the appearance of a glycogenolytic activity in sporulating cells capable of releasing free glucose from glycogen. This activity may be responsible for the increased intracellular glucose observed at the time of glycogen degradation. The glucose released into the medium during sporulation by these cells accounts for less than 1% of the glycogen degraded during this period, suggesting that the glucose produced from glycogen degradation is used elsewhere in the cell.

SK-1, as well as AP-1- α/α and AP-1- α/α , released carbohydrate in the form of glucose. The amount of glucose released by SK-1 could account for roughly 25% of the total carbohydrate reported by Kane and Roth (17) to be released during sporulation in this strain. Thus, it would appear that carbohydrates other than glucose are also being released. It should be noted that glucose was released by both sporulating and nonsporulating strains, suggesting that the re-

lease of glucose is not a sporulation specific event and may have no function in the sporulation process. Radioactive glucose incorporation studies indicated that the extracellular glucose was utilized by sporulating SK-1 cells at a rate of approximately 5% per h from the 4th to the 8th h of sporulation (unpublished data).

Ammonia has been previously reported to be a strong inhibitor of sporulation (22). The metabolic pattern observed during normal sporulation is drastically altered by the addition of ammonium ions. Although there were significant changes in Fru-1,6-P₂, G6P, F6P, PEP, ATP, and cAMP, all of the glycolytic enzymes studied remained unchanged, as did the energy charge and the synthesis of glycogen. Glycogen degradation, however, was blocked, as was the rise in intracellular glucose which occurred at this time in the absence of NH₄⁺ (Fig. 2). Since there were so many changes in the metabolic parameters examined, it seems probable that NH₄⁺ acts at a number of sites in the metabolism to block sporulation. These data suggest that the addition of ammonia to sporulating cells does not decrease the energy state of the cell or the concentration of the glycolytic precursors of the major carbon pathways to a level which would be expected to limit metabolism.

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LITERATURE CITED

1. Ball, W. J., and D. E. Atkinson. 1975. Adenylate energy charge in *Saccharomyces cerevisiae* during starvation. *J. Bacteriol.* **121**:975-982.
2. Barwell, C. J., and B. Hess. 1971. Regulation of pyruvate kinase during gluconeogenesis in *Saccharomyces cerevisiae*. *FEBS Lett.* **19**:1-4.
3. Barwell, C. J., and B. Hess. 1972. Application of kinetics of yeast pyruvate kinase *in vitro* to calculation of glycolytic flux in the anaerobic yeast cell. *Hoppe-Seyler's Z. Physiol. Chem. Bd.* **353**:1178-1184.
4. Betz, H., and U. Weiser. 1975. Protein degradation and proteinases during yeast sporulation. *Eur. J. Biochem.* **62**:65-76.
5. Colonna, W. J., and P. T. Magee. 1978. Glycogenolytic enzymes in sporulating yeast. *J. Bacteriol.* **134**:844-853.
6. Conway, E. J., and M. Downey. 1950. An outer metabolic region of the yeast cell. *Biochem. J.* **47**:347-355.
7. Daugherty, J. P., W. Draemer, and G. Joshi. 1975. Purification and properties of phosphoglucomutase from Fleischmann's yeast. *Eur. J. Biochem.* **57**:115-126.
8. Fast, D. 1973. Sporulation synchrony of *Saccharomyces cerevisiae* grown in various carbon sources. *J. Bacteriol.* **116**:925-930.
9. Fiske, C. H., and Y. Subbarow. 1952. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375-400.
10. Gancedo, C., and K. Schwerzmann. 1976. Inactivation by glucose of phosphoenolpyruvate carboxykinase from *Saccharomyces cerevisiae*. *Arch. Mikrobiol.* **109**:221-225.

11. Gancedo, J. M., D. Clifton, and D. G. Fraenkel. 1977. Yeast hexokinase mutants. *J. Biol. Chem.* **225**: 4443-4444.
12. Gancedo, J. M., and C. Gancedo. 1971. Fructose-1,6-diphosphatase, phosphofructokinase and glucose-6-phosphate dehydrogenase from fermenting yeasts. *Arch. Microbiol.* **76**:132-138.
13. Gancedo, J. M., and C. Gancedo. 1973. Concentrations of intermediary metabolites in yeast. *Biochimie* **55**: 205-211.
14. Gilman, A. G. 1970. A protein binding assay for adenosine 3',5'-monophosphate. *Proc. Natl. Acad. Sci. U.S.A.* **67**: 305-312.
15. Hopper, A. K., P. T. Magee, S. K. Welch, M. Friedman, and B. D. Hall. 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. *J. Bacteriol.* **119**:619-628.
16. Huang, K., and E. Cabib. 1973. Yeast glycogen synthetase in the glucose 6-phosphate independent form: a case of cold lability without major changes in molecular size. *Biochim. Biophys. Acta* **302**:240-248.
17. Kane, S., and R. Roth. 1974. Carbohydrate metabolism during ascospore development in yeast. *J. Bacteriol.* **118**:8-14.
18. Klar, A. J. S., and H. O. Halvorson. 1975. Proteinase activities of *Saccharomyces cerevisiae* during sporulation. *J. Bacteriol.* **124**:863-869.
19. Kopperschlager, G., and H. W. Augustin. 1967. Fehlermöglichkeiten bei der bestimmung von metabolitgehalten in hefezellen. *Experientia* **23**:623-624.
20. Lowry, O. H., and J. V. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press Inc., N.Y.
21. Lowry, O. H., N. Rosebrough, A. Farr, and P. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
22. Miller, J. J. 1963. The metabolism of yeast sporulation. V. Stimulation and inhibition of sporulation and growth by nitrogen compounds. *Can. J. Microbiol.* **9**:259-277.
23. Opheim, D., and R. W. Bernlohr. 1973. Purification and regulation of glucose-6-phosphate dehydrogenase from *Bacillus licheniformis*. *J. Bacteriol.* **116**:1150-1159.
24. Roth, R., and H. O. Halvorson. 1969. Sporulation of yeast harvested during logarithmic growth. *J. Bacteriol.* **98**:831-832.
25. Rothman, L. B., and E. Cabib. 1969. Regulation of glycogen synthesis in the intact yeast cell. *Biochemistry* **8**:3332-3341.
26. Thomas, J. A., K. Schlender, and J. Lerner. 1969. A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including and improving biosynthesis of UDP-¹⁴C-glucose. *Anal. Biochem.* **25**:486-499.
27. Trevelyan, W. E., and J. Harrison. 1956. Studies on yeast metabolism. 7. Yeast carbohydrate fractions. Separation from nucleic acid, analysis, and behavior during anaerobic fermentation. *Biochem. J.* **63**:23-32.
28. Walters, J. R., and D. P. Stahly. 1968. Modification of the valve of the French pressure cell. *Appl. Microbiol.* **16**:1605.