NOTES

Effect of Ammonium Ions on Activity of Hydrolytic Enzymes During Sporulation of Yeast

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The addition of 10 mM ammonium sulfate to sporulation medium noncoordinately blocked the increases in protease C, protease B, α-mannosidase, and 1,4-amyloglucosidase activities which occur during normal sporulation of *Saccharomyces cerevisiae*, but had only a minor effect on the 10-fold increase in alkaline phosphatase activity.

Sporulation of *Saccharomyces cerevisiae* has long been known to be inhibited by the addition of a nitrogen source to sporulation medium (13). NH_4^+ blocks DNA, RNA, and protein syntheses (3, 4, 15), as well as the increase in protein (3) and glycogen degradation (5, 9) which occurs during normal sporulation. We have previously shown that NH_4^+ has only a minimal effect on energy and glycolytic pathway metabolites in cells incubated in sporulation medium supplemented with NH_4^+ (5). Therefore, we expected that the effects of NH_4^+ may be very specific. Since the differentiation during sporulation must necessitate significant amounts of intracellular macromolecular turnover, we examined the effect of NH_4^+ on a number of hydrolytic enzymes which may be involved in intracellular turnover.

S. cerevisiae strains SK-1 (9), AP-1-a/α, and AP-1-α/α (6) were used throughout this study. Yeast cells were sporulated in a manner similar to that used by Roth and Halvorson (16), as previously described (5). In experiments testing the effect of NH_4^+, 10 mM $(NH_4)_2SO_4$ was added to the sporulation medium. The minimum concentration of $(NH_4)_2SO_4$ required to cause 95% inhibition of sporulation of SK-1 was 7.5 mM (unpublished data). Sporulation was monitored by phase-contrast microscopy as previously described (5).

The cells were harvested and extracts were prepared as previously described (14). The addition of 2 mM phenylmethylsulfonyl fluoride had no effect on the levels of the non-proteolytic enzyme activities which were examined. Protease B activity was measured by the procedure of Juni and Heym (8), using Azocoll as substrate.

One unit of protease B activity causes an increase in optical density at 520 nm of 1.0 in 30 min. Protease C was measured by the method of Jones (7). One unit of protease C causes the release of 1 nmol of *p*-nitroaniline per min from benzoyl-tyrosine-*p*-nitroanilide. Proteases B and C were activated as described by Lenny (11) for 0 to 24 h. The maximum activities obtained during this activation procedure are reported. α-Mannosidase was measured as described by Opheim (14). One unit releases 1 nmol of *p*-nitrophenol per min from *p*-nitrophenyl-α-D-mannopyranoside. Amyloglucosidase was assayed as described by Colonna and Magee (2). One unit releases 1 μg of glucose per min from oyster glycogen. The nonspecific alkaline phosphatase (*phoH* gene product) was measured as described by Toh-e et al. (17) after precipitation with monospecific antiserum directed against purified alkaline phosphatase (Opheim, unpublished data). One unit releases one μmol of *p*-nitrophenol per min from *p*-nitrophenyl phosphatate. The protein concentration was determined by the method of Lowry et al. (12). Specific activities are expressed as units per milligram of protein. Glycogen was measured as described by Hopper et al. (6). All incubations were conducted at 30°C. None of the enzyme activities examined was affected by the addition of 20 mM $(NH_4)_2SO_4$ to the assay mixtures.

α-Mannosidase activity increased approximately ninefold during sporulation (Fig. 1A). The levels of enzyme activity were not affected during the first 2.5 h of incubation of cells in sporulation medium supplemented with 10 mM $(NH_4)_2SO_4$; thereafter, further increases in enzyme activity were reduced. α-Mannosidase ac-
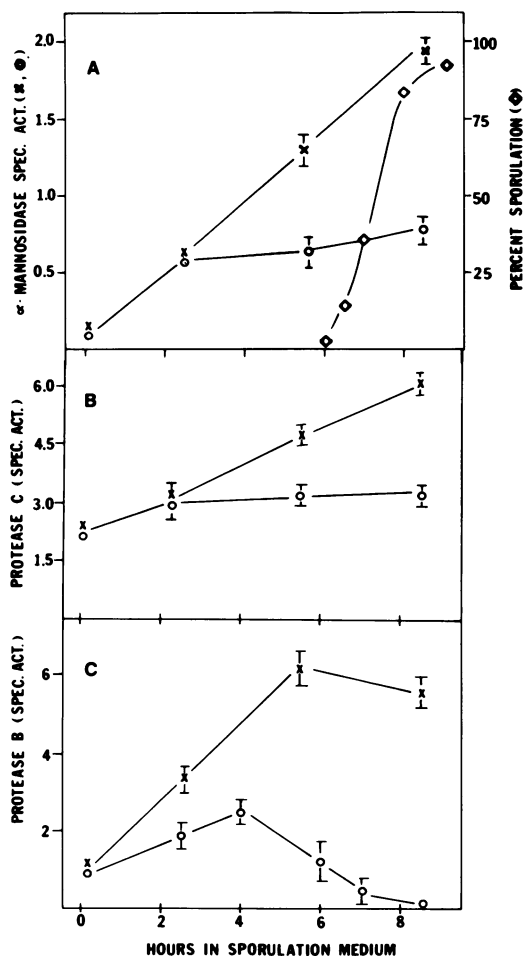
FIG. 1. Effect of NH_4^+ on α-mannosidase, protease C, and protease B activity during sporulation of strain SK-1. Extracts, prepared from SK-1 incubated in sporulation medium in the presence (○) and absence (×) of 10 mM $(NH_4)_2SO_4$, were assayed for α-mannosidase (A), protease C (B), and protease B (C). Sporulation in NH_4^+-free medium was monitored (A).

tivity increased fourfold during the next 6 h in the control strain and only 50% in the NH_4^+-supplemented culture.

Proteases C and B, which have been previously reported to specifically increase during sporulation (1, 10), increased 2.5- and 9-fold, respectively, during sporulation of SK-1 (Fig. 1B and C). In the presence of NH_4^+, protease C activity increased at its normal rate for 2.5 h of incubation and remained constant for the next 6 h (Fig. 1B). When the protein synthesis inhibitor cycloheximide (100 µg/ml) (7) was added after 2.5 h of incubation in sporulation medium supplemented with NH_4^+, the specific activity of both protease C and α-mannosidase remained

constant for the next 6 h (unpublished data), suggesting that the lack of increase in protease C and α-mannosidase activity in the presence of NH_4^+ is not due to an increased degradation rate. Protease B increased in specific activity at a rate slower than the control for the first 4 h of incubation in sporulation medium supplemented with NH_4^+. Thereafter, it decreased in specific activity (Fig. 1C).

The appearance of amyloglucosidase, which has been reported to occur during sporulation immediately before glycogen degradation (2), also increased in SK-1 during sporulation at approximately the same time as the onset of glycogen degradation (Fig. 2A and B). The addition of NH_4^+ to the sporulation medium appeared to block both the synthesis of amyloglucosidase (Fig. 2B), as well as the glycogen degradation that occurs during normal sporulation at 6 h (Fig. 2A). Similar results were obtained with AP-1-a/α when NH_4^+ was added to sporulation medium (unpublished data). Similar results were obtained when amylose (1 → 4 glucose) was used instead of glycogen in the amyloglucosidase assay (unpublished data), suggesting that glycogen degradation is due to an increase in α-1,4-amyloglucosidase activity.
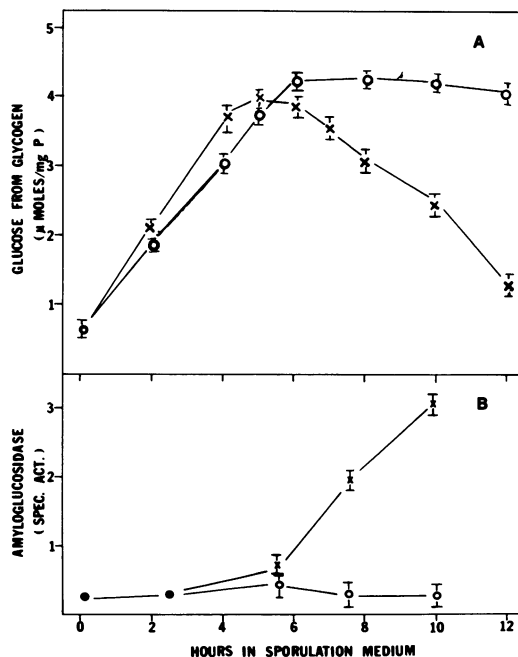

FIG. 2. Effect of NH_4^+ on glycogen degradation and amyloglucosidase activity during sporulation of SK-1. Samples, prepared from SK-1 incubated in sporulation medium in the presence (○) and absence (×) of 10mM $(NH_4)_2SO_4$, were assayed for glycogen content (A) and amyloglucosidase activity (B).

Since the hydrolases measured normally increase dramatically during sporulation, it is probable that they function during this period of time to produce the precursors required for new synthesis. The inhibitory action of NH_4^+ on the synthesis of these hydrolases may, therefore, be responsible for the inhibition of glycogen and protein degradation observed in NH_4^+-supplemented sporulation medium. The kinetics of inhibition appear to be unique for each enzyme, suggesting that the synthesis of each enzyme is independently regulated by NH_4^+.

To examine whether NH_4^+ causes a general inhibition of protein synthesis or derangement of cell metabolism we examined the nonspecific alkaline phosphatase which increases significantly during sporulation (Fig. 3A and B). This increase in activity is not sporulation specific, since it occurred in AP-1-α/α, a nonsporulating strain (Fig. 3A). The increase in activity was
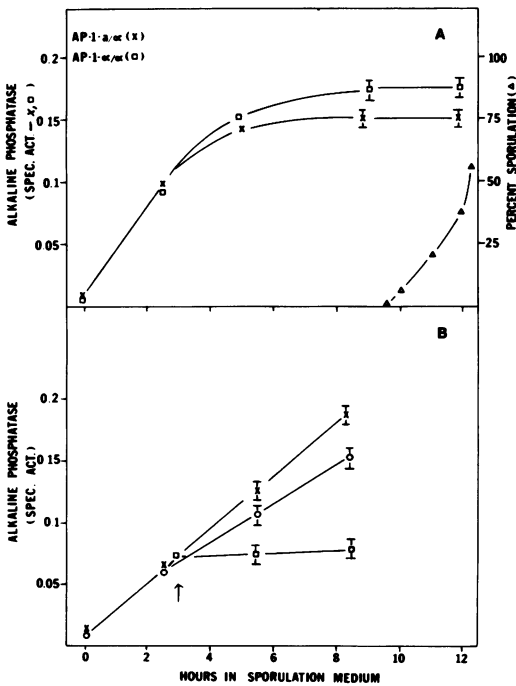

FIG. 3. *Effect of NH_4^+ on alkaline phosphatase activity during sporulation. (A) Extracts, prepared from AP-1-a/α (×) and AP-1-α/α (□) incubated in sporulation medium, were assayed for alkaline phosphatase activity. Sporulation was monitored in AP-1-a/α (△). (B) Extracts, prepared from SK-1 incubated in sporulation medium in the presence (○) and absence (×) of 10 mM $(NH_4)_2SO_4$, were assayed for alkaline phosphatase. Cyclohexamide (100 μg/ml) was added to control cultures without NH_4^+ at 3 h, and extracts were prepared and assayed for alkaline phosphatase activity (□).*

dependent on protein synthesis, since the addition of the protein synthesis inhibitor cyclohex-imide immediately arrested further increase in activity (Fig. 3B). The addition of NH_4^+ to sporulating SK-1 cells has only a minor effect on the synthesis of alkaline phosphatase. At 8 h, there is 80% as much alkaline phosphatase in NH_4^+-supplemented cultures as in control cultures (Fig. 3B). These data indicate that NH_4^+ does not function by nonspecifically inhibiting protein synthesis and that the inhibition of the other hydrolases examined is due to the specific inhibition of their synthesis by NH_4^+ or some metabolite of NH_4^+ (15).

In summary, these data indicate that NH_4^+ specifically blocks the increase in activity of certain hydrolytic enzymes which normally occurs during sporulation of yeast. I suggest that this may be partially responsible for the lack of protein (3) and complex carbohydrate degradation (5) observed in cells incubated in NH_4^+-supplemented sporulation medium.

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