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Inactivation of Glutamine Synthetase by Ammonia Shock in the Gram-Positive Bacterium *Streptomyces cattleya*

RICHARD WAX,†* LINDA SYNDER, AND LOUIS KAPLAN
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

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In cultures of the gram-positive bacterium *Streptomyces cattleya*, a rapid inactivation of glutamine synthetase was seen after ammonia shock. pH activity curves for ammonia-shocked and control cultures are shown. A peak of glutamine synthetase activity was seen during fermentation for production of the antibiotic thienamycin.

Gram-negative enteric bacteria respond similarly in regard to the regulation of glutamine synthetase (GS) (EC 6.3.1.2) activity when the ammonium ion concentration of the medium is raised (4, 8, 11). There is a covalent binding of one AMP residue to each of the 12 subunits (6), resulting in a rapid loss of GS activity as measured by the forward reaction but in relatively little change in activity as measured by the γ-glutamyl transferase (γGT) assay. The γGT assay does, however, show a change in the pH activity profiles of the adenylylated and a deadenylated forms of GS (8), and each enteric strain shows a characteristic GS isoactivity point.

Early studies have indicated that gram-positive bacteria differed from gram-negative bacteria in response to ammonia shock: in gram-positive bacteria, inactivation of GS, as measured by both the forward and the γGT assays, has not been observed to occur. However, loss of GS activity after ammonia shock in the gram-positive bacterium *Streptomyces clavuligerus* has been observed (Y. Aharonowitz, personal communication), and a rapid inactivation of GS in *S. cattleya* has been reported (R. Wax and L. Synder, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980. O29, p. 187). The loss of GS activity in *S. cattleya* has been confirmed, and evidence indicating that the GS of *S. cattleya* is modified by adenylylation has been presented (9).

Figure 1 shows GS activity as a function of pH in an extract of cells actively growing with lysine (2 g/liter) as the sole nitrogen source and in an extract of ammonia-shocked cells. GS activity was measured by the γGT assay, which showed a linear response with the extract concentration. Ammonia shock, γGT assays, and harvesting were carried out as described by Bender et al. (3), except that the temperature was 28°C. (We have shown that the effect of ammonia shock is

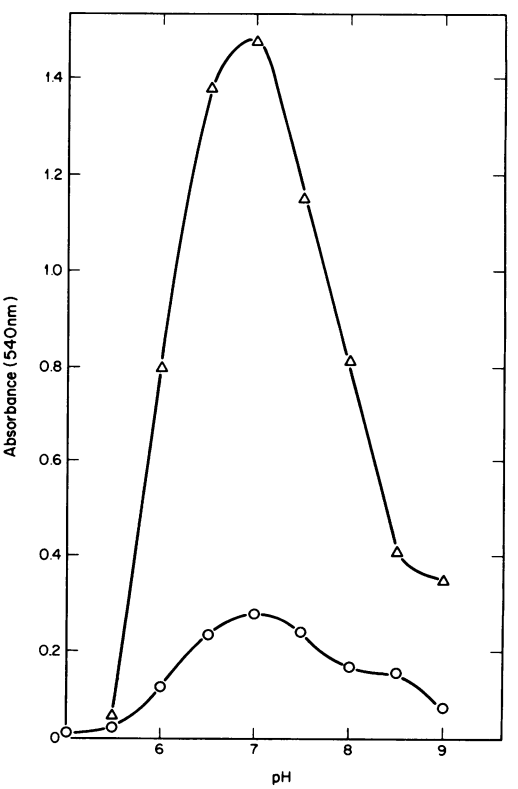

FIG. 1. pH profile showing the effect of $(NH_4)_2SO_4$ on the GS activity of *S. cattleya*. Cells were grown with 0.2% lysine as the sole nitrogen source, and then half of the culture was shocked with $(NH_4)_2SO_4$ (final concentration, 0.015 M). The absorbance (λ = 540 nm) indicates the amount of glutamyl hydroamate formed and thus is a measure of the GS activity. Symbols: △, growing cells that were not ammonia shocked; ○, ammonia-shocked cells.

† Present address: Fermentation Program, Frederick Cancer Research NCI-Facility, Frederick, MD 21701.

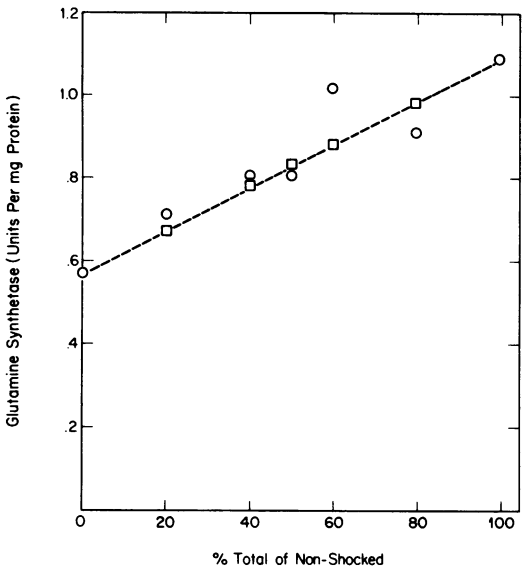

FIG. 2. GS activities in mixtures of extracts from a lysine-grown culture and extracts from a culture shocked with 0.015 M $(NH_4)_2SO_4$ for 20 min. Symbols: ○, actually measured values; □, values predicted from the simple dilution effect.

complete in <9 min at 28°C.) Cells were disrupted by sonic oscillation. Harvesting and assaying were always carried out on the same day. Protein was determined as described by Lowry et al. (7). The growth medium contained glycerol, NaCl, K_2HPO_4, $CaCo_3$, $MgSO_4 \cdot 7H_2O$, $FeSO_4$, and $CoCl_2$. Cultures were assayed for GS or ammonia shocked when an optical density of 100 to 200 Klett units was reached. The GS of *S. cattleya* was entirely intracellular. After sonic disruption and centrifugation at 23,000 × *g* for 30 min, only a trace of GS activity was found in the pellet.

Figure 2 shows the result of mixing extracts from a lysine-grown culture in various proportions with extracts of the same culture after shocking for 20 min with 0.015 M $(NH_4)_2SO_4$. The low-activity sample had no effect on the high-activity sample beyond simply diluting it, thus supporting the previous finding (9) that reduction in GS activity is due to modification of the enzyme rather than to synthesis of an inhibitor.

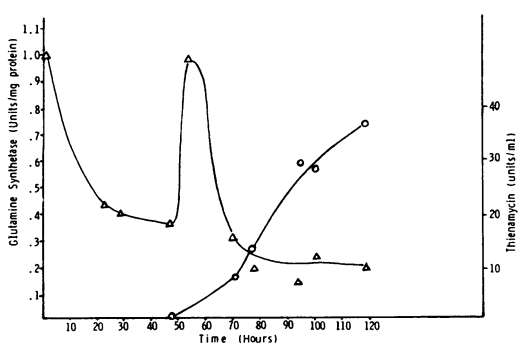

FIG. 3. GS activity as a function of time during thienamycin production. Symbols: △, GS; ○, thienamycin.

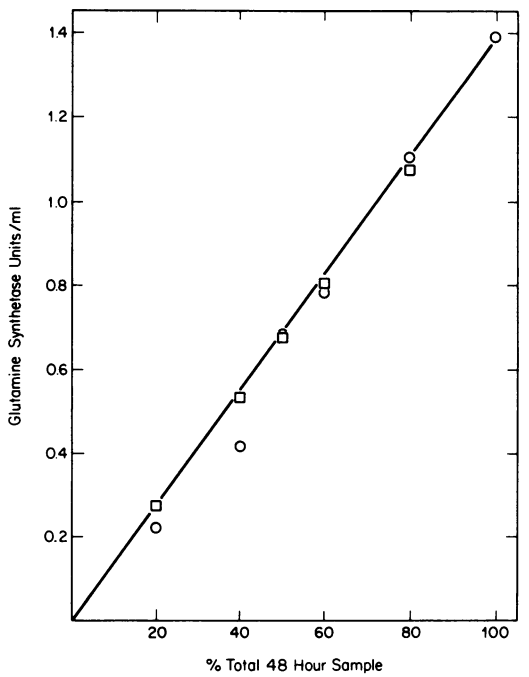

FIG. 4. Mixtures of extracts from a thienamycin production culture with a high GS activity (48 h) and extracts from a production culture with a very low activity (72 h). Symbols: ○, actually measured values; □, values predicted from the simple dilution effect.

The rapid decrease in enzyme activity, as measured by the γGT assay, without a change in the pH activity profile showed that there was a difference between the GS of *S. cattleya* and the GSs of both the gram-negative bacteria and the other gram-positive bacteria. Our results are similar to those seen for *S. clavuligerus* (Aharonowitz, personal communication), thus hinting that there is a similarity among streptomycetes in regard to GS.

Aharonowitz and Demain (1, 2) have shown a correlation between nitrogen metabolism and antibiotic production in *S. clavuligerus*. Since *S. cattleya* produces the antibiotic thienamycin (5), we were able to investigate the GS activity throughout a fermentation for production of this secondary metabolite. There was a sharp peak of GS activity at about the time that antibiotic synthesis commenced, followed by a rapid loss of activity (Fig. 3).

Vegetative mycelia were grown in a seed medium containing sucrose, distiller solubles, yeast extract, and corn gluten meal. The presterilization pH was adjusted to 7.5 with NaOH. Portions were stored at −80°C and then grown again in the same seed medium for each experiment. After 48 h at 28°C, the contents of three seed medium flasks were pooled, and 1-ml portions were inoculated into 27 flasks, each containing 20 ml of a production medium that included glycerol, monosodium glutamate, NH_4Cl, NaCl, $MgSO_4$, $FeSO_4$, $CoCl_2$, L-isoleucine, and MES (morpholinoethanesulfonic acid), pH 7.0. The thienamycin yield was determined as described by Kahan et al. (5).

Fermentation samples with peak GS activities were mixed with 72-h samples in various proportions (in this experiment, the GS level by 72 h had dropped to an undetectably low level). The loss of activity by 72 h was not due to synthesis of an inhibitor, but resulted from dilution of the high-activity sample (Fig. 4).

At this time, we have no explanation for the peak of GS activity which occurred 48 to 54 h into the production stage of the fermentation. The timing may be incidental, or there may be a correlation between this peak and the onset of antibiotic production.

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