Stimulation of the Differential Rate of Exoenzyme Formation in *Bacillus amyloliquefaciens* by Streptolydigin, an Inhibitor of RNA Chain Elongation

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(Received 13 September 1974)

INTRODUCTION

Bacillus amyloliquefaciens secretes a number of degradative enzymes into the external environment. A low level of extracellular enzymes occurs during the exponential phase of growth, then the rate of enzyme secretion increases to a high linear value which is maintained into the stationary phase (Coleman, 1967).

A model to account for these characteristics involves two separate effects, which may be superimposed; both would contribute to increased exoprotein mRNA formation and hence exoprotein itself. The first is an increase in RNA polymerase available for exoenzyme gene transcription on cessation of ribosomal RNA synthesis, after the end of exponential growth. The second is an increase in the concentration of the substrate for this enzyme resulting from ribosomal RNA turnover during the post-exponential phase of the growth cycle (Coleman, Brown & Stormonth, 1974). If the proposed model is correct, it might be possible to separate these two effects. Therefore the effect on *B. amyloliquefaciens* of a number of inhibitors of RNA synthesis; including streptolydigin, was examined. Streptolydigin specifically inhibits the rate of transcription by reducing the rate of phosphodiester bond formation during RNA chain elongation without affecting the initiation or termination processes (Cassani, Burgess, Goodman & Gold, 1971).

METHODS

Organism. The aerobic spore-forming bacillus used in this work was *Bacillus amyloliquefaciens* strain T (Welker & Campbell, 1967).

Growth conditions. The composition of the medium was: $(\text{NH}_4)_2\text{HPO}_4$, 34 mM; KCl, 5 mM; MgSO_4 , 1 mM; FeCl_3 , 0.5 mM; CaCl_2 , 0.125 mM; ZnSO_4 , 0.0125 mM; sodium citrate, 2.125 mM; 'trace metal ion' solution (Coleman & Elliott, 1965), 0.25 ml/l; vitamin solution, 50 ml/l; a mixture of equal amounts (by weight) of 19 naturally-occurring L-amino acids (cysteine omitted) to give a total amino acid concentration of 0.5%; maltose, 1% (w/v). The medium was adjusted to pH 7.2 with 10% (v/v) phosphoric acid. The vitamin solution consisted of 0.1 mg riboflavin, and 1.0 mg each of inositol, biotin, choline chloride, folic acid, nicotinamide, calcium pantothenate, pyridoxal-5-phosphate and thiamine hydrochloride in 1 l water. Batches (50 ml), contained in 250 ml conical flasks, were loop-inoculated from an aqueous spore suspension. The cultures were incubated at 30 °C in a gyrotory incubator-shaker (Model G25, New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.).

Washed bacterial suspension experiments. Late exponential phase cultures (20 h) were centrifuged at 5000 *g* for 2 min and the supernatant fraction was discarded. The bacterial

Table 1. *Effect of streptolydigin on Bacillus amyloliquefaciens*

Standard deviations are given in parentheses

	No streptolydigin	Streptolydigin (10^{-5} M)
Differential rate of enzyme formation (units formed/mg dry wt increase in bacterial mass)		
α -Amylase	4 (0.5)	10 (1)
Protease	40 (4)	70 (8)
Ribonuclease	8 (1)	18 (2)
'Nucleotide' pool size (μ g/mg cell material)	6.1 (0.4)	11.0 (0.7)
Rate of increase in bacterial density (mg dry wt/ml culture/h)	0.42 (0.02)	0.25 (0.01)

pellet was washed by resuspension in fresh growth medium and then resuspended to the original density in the same medium, which was maintained at 30 °C to prevent the bacteria from suffering a temperature shock, in the presence and absence of 10^{-5} M-streptolydigin (a gift from the Upjohn Co., Kalamazoo, Michigan, U.S.A.). The resulting suspensions were then incubated with shaking (50 ml/250 ml conical flask) at 30 °C, and samples taken for assay at 30 min intervals.

Extracellular enzyme assay. α -Amylase, protease and ribonuclease were determined in bacterial suspension supernatant fractions as described by Coleman & Elliott (1962), May & Elliott (1968) and Coleman & Elliott (1965), respectively.

Bacterial density determination. Bacterial densities were determined by the method of Stormonth & Coleman (1972).

'Nucleotide' pool size. This was measured as described by Stormonth & Coleman (1974).

RESULTS

Late exponential phase bacteria were resuspended in fresh 1 % maltose-0.5 % L-amino acid mixture defined medium and increase in bacterial density, exoenzyme secretion and 'nucleotide' pool size were monitored during incubation in the presence and absence of streptolydigin.

It was found that in the presence of streptolydigin at a concentration of 10^{-5} M the bacteria continued to grow at a constant rate which was, however, only 60 % of that achieved in the absence of inhibitor (Table 1). This reduction in growth rate was accompanied by a twofold increase in differential rate of α -amylase, protease and ribonuclease formation (Table 1) compared with the tenfold increase observed during the transition from minimum to maximum rate of exoenzyme formation or the exponential to the post-exponential condition (Coleman, 1967). This partial effect was in turn accompanied by a twofold increase in 'nucleotide' pool size without change in the distribution of the individual components which retained the pattern described by Stormonth & Coleman (1974).

DISCUSSION

During the experiment, since growth took place and hence ribosomal RNA continued to be formed in the presence of 10^{-5} M-streptolydigin, extensive release of RNA polymerase for increased exoenzyme mRNA synthesis would not be possible. However, since the rate of utilization of precursors diminished, it is possible that a higher 'steady state' nucleoside triphosphate level provided an increased substrate concentration for exoprotein mRNA formation (Coleman *et al.* 1974).

The fact that exoenzyme formation increased in proportion to the increase in 'nucleotide' pool size is consistent with the idea that exoenzyme mRNA synthesis depends on the concentration of mRNA precursor material.

These results provide the first evidence for a separation of the two parameters which, it has been suggested, are implicated in exoenzyme regulation, and provide additional evidence in support of the model proposed by Coleman *et al.* (1974).

D.A.S. thanks the Science Research Council for a Research Studentship.

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