Glucose Regulation of Cephamycin Biosynthesis in *Streptomyces lactamdurans* is Exerted on the Formation of α -Aminoadipyl-cysteinyl-valine and Deacetoxycephalosporin C Synthase

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Glucose exerted a concentration-dependent negative regulation on the biosynthesis of cephamycin C by *Streptomyces lactamdurans*. Formation of the cephamycin precursor $\delta(\alpha$ -aminoadipyl)-cysteinyl-valine was greatly decreased by excess glucose. The ring-expanding enzyme deacetoxycephalosporin C synthase was strongly repressed by glucose *in vivo*. Isopenicillin N synthase (cyclase) and isopenicillin N epimerase were not repressed by glucose. However, the activity of isopenicillin N synthase was inhibited *in vitro* by glucose 6-phosphate, and the activity of deacetoxycephalosporin C synthase was inhibited by inorganic phosphate, glucose 6-phosphate, fructose 2,6-diphosphate and fructose 1,6-diphosphate. The intracellular cAMP content decreased as growth proceeded and remained lower in glucose-supplemented cells than in control cultures. cAMP did not seem to be involved in glucose control of cephamycin biosynthesis.

INTRODUCTION

Carbon catabolite regulation is a general mechanism that controls the biosynthesis of antibiotics belonging to different biosynthetic groups (Martín & Demain, 1980). In some cases, glucose has been found to repress an enzyme of the biosynthetic pathway leading to the antibiotic. Moreover, glucose derivatives formed during transport or glycolysis may inhibit the activity of specific enzymes involved in antibiotic biosynthesis.

The biosynthetic pathways of β -lactam antibiotics are well understood, but only recently have specific enzymic steps been studied (Hollander *et al.*, 1984; Pang *et al.*, 1984; Ramos *et al.*, 1985). The biosynthesis of cephamycin in *Streptomyces* proceeds through the same general pathway as that of cephalosporin C in fungi. The tripeptide $\delta(\alpha$ -aminoadipyl)-cysteinyl-valine (ACV) is formed by condensation of the component amino acids and then cyclized by the enzyme isopenicillin N synthase (cyclase). The isopenicillin N formed is isomerized to penicillin N by the isopenicillin N epimerase, and finally the thiazolidine ring of penicillin N is expanded to a six-membered dihydrotiazine ring by the deacetoxycephalosporin C synthase (Martín & Liras, 1985) (Fig. 1). ACV formation, isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase have been characterized in *Streptomyces clavuligerus* (Jensen *et al.*, 1982, 1983, 1985) and *Streptomyces lactamdurans* (Castro *et al.*, 1985).

High glucose levels in the medium produced an *in vivo* accumulation of the penicillin N intermediate in the cephalosporin C biosynthetic pathway in *Acremonium chrysogenum* (Zanca & Martín, 1983), due to repression of the deacetoxycephalosporin C synthase (Behmer & Demain, 1983). The effect of glucose on penicillin G formation by *Penicillium chrysogenum* is due

Abbreviation: ACV, δ -L-(α -aminoadipyl)-L-cysteinyl-D-valine.

Fig. 1. Biosynthetic pathway of cephamycin C. A, Tripeptide (ACV) synthase; B, isopenicillin N synthase (cyclase); C, isopenicillin N epimerase; D, deacetoxycephalosporin C synthase (expandase); E, F, G, 'late' enzymes.

to a repression of penicillin-synthesizing enzymes (Revilla *et al.*, 1984) and, in particular, of the ACV-forming enzyme system (Martín *et al.*, 1982) and of the isopenicillin N synthase.

Glycerol regulation of cephamycin production has been reported in *S. clavuligerus* (Aharonowitz & Demain, 1978; Romero *et al.*, 1984) but the enzymes affected by carbon catabolite regulation have not been studied. To establish the mechanism of glucose regulation of β -lactam biosynthesis in actinomycetes, we have studied the effect of glucose on the formation of ACV, and on the activities of isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase in *S. lactamdurans*.

METHODS

Micro-organisms and culture conditions. *S. lactamdurans* NRRL 3802 var. JC1843, a strain with improved cephamycin production, was used (Castro *et al.*, 1985). Culture conditions and preparation of resting cell systems were as described by Cortés *et al.* (1984).

Antibiotic assays. Penicillin N, cephamycin C and deacetoxycephalosporin C were routinely measured by bioassay using *Escherichia coli* Ess 22-35 as the test strain (Castro *et al.*, 1985). Isopenicillin N was measured by bioassay against *Micrococcus luteus* ATCC 9341, a cephamycin-insensitive strain (Castro *et al.*, 1985). The different antibiotics were identified by HPLC, using a Varian 5000 chromatograph with a Micro-Pak mCH10 column, as described previously (Romero *et al.*, 1984). Antibiotic titres are given as μg cephamycin C.

Enzyme extracts. Partially purified cell-free extracts for assays of isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase were prepared by sonicating cells grown for 72 h (Cortés *et al.*, 1984). Ultracentrifuged extracts (100 000 g for 2 h, Beckman LB-70) were treated with 0.2% protamine sulphate, and the clear supernatant was precipitated with ammonium sulphate (30–50% saturation for isopenicillin N epimerase and 50–80% saturation for isopenicillin N synthase and deacetoxycephalosporin C synthase).

Enzyme assays. Isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase were assayed in reaction mixtures prepared as described previously (Cortés *et al.*, 1984; Castro *et al.*, 1985), supplemented with 1 mM-phenylmethylsulphonyl fluoride (PMSF). Specific enzyme activities are given as nkat (g protein)⁻¹.

Intracellular glucose 6-phosphate content. Glucose 6-phosphate was extracted from washed mycelium with 10% (w/v) TCA. The protein precipitate was discarded and the TCA present in the supernatant was removed by extracting twice with an equal volume of diethyl ether. Residual ether in the aqueous fraction was removed under vacuum. The glucose 6-phosphate content in the sample was determined by measuring the reduction of NADP by

glucose-6-phosphate dehydrogenase at A_{340} ; 1 μ mol glucose 6-phosphate produced an increase of 6.02 (Horecker & Wood, 1957). The reaction mixture contained, in a final volume of 1 ml: $MgCl_2$, 10 mM; NADP, 0.13 mM; glucose-6-phosphate dehydrogenase, 1.25 units; Tris/HCl buffer pH 7.5, 15 mM.

cAMP determination. Mycelium was extracted with 10% TCA as indicated above, and cAMP was determined by the competition between cAMP in the sample and exogenous $[8\text{-}^3H]cAMP$ for the cAMP-binding protein, using a cAMP assay kit (Amersham) according to the manufacturer's instructions. The bound radioactivity was measured in a Philips 4700 liquid scintillation counter. The kit was calibrated with pure cAMP (Sigma).

Determination of ACV and glutathione. ACV and glutathione were extracted with 7.5% (w/v) TCA from samples of mycelium (approximately 250 mg dry weight) taken at various times of culture, and purified as described by Loder & Abraham (1971). After oxidation with performic acid, the sulphonated peptides were derivatized with *o*-phthalaldehyde and separated in a Varian 5000 HPLC chromatograph equipped with a Micro-Pak mCH10 reverse-phase column using a mixture of 10 mM-phosphate buffer, pH 7, and methanol (9:10, v/v) as solvent. The intracellular content of these tripeptides was quantified by using pure ACV or glutathione as standards (Cortés *et al.*, 1984).

Determination of glucose, protein and inorganic phosphate. Glucose was determined by the glucose oxidase method, protein by the Lowry method and inorganic phosphate by the method of Fiske and Subbarow as described by Leloir & Cardini (1957).

Chemicals. Glucose 6-phosphate, fructose 1,6-diphosphate, fructose 2,6-diphosphate and glucose-6-phosphate dehydrogenase (type XII from *Torula*) were obtained from Sigma. The isopenicillin N and ACV were a gift from Dr P. Van Dijck (Gist Brocades, Delft, The Netherlands) and penicillin N was kindly supplied by Dr H. H. Peter (Ciba Geigy, Basel, Switzerland). All other chemicals were of reagent quality.

RESULTS

Effect of glucose and phosphate on cephamycin C production in batch cultures. Maltose, glucose (at low concentrations) and galactose supported good antibiotic production by *S. lactamdurans* when added to nutrient broth/yeast extract (NY) base medium, whereas glycerol, lactose, sucrose and starch did not support cephamycin production.

S. lactamdurans grew similarly in NYG medium supplemented with 165 mM (high) or 55 mM (low) glucose, probably because in both cases growth was limited by phosphate. Glucose was exhausted from control cultures (containing 55 mM-glucose) at 72 h of incubation, but remained at high concentration throughout the fermentation in high glucose cultures (Fig. 2). The pH of the culture medium increased slightly during the fermentation but it was not modified as a result of addition of high concentrations of glucose.

The biosynthesis of cephamycin C, however, was greatly dependent on the glucose concentration (27.5, 55, 110 or 165 mM) of the medium. Production of antibiotic in control cells grown in 55 mM-glucose started at about 48 h of fermentation (Fig. 2*b*). The onset of antibiotic production was delayed as the glucose concentration was increased from 27.5 mM to 110 mM. No antibiotic was detected in batch cultures supplemented with 165 mM-glucose. Addition of inorganic phosphate (25–50 mM) to the cultures containing 55 mM-glucose stimulated growth and produced an early onset of cephamycin C biosynthesis without inhibiting the production of the antibiotic. The same results were obtained in three different experiments.

The antibiotic formed was insensitive to penicillinase (as are cephalosporin and cephamycin) in all cases. No accumulation of any penicillinase-sensitive antibiotic (e.g. isopenicillin N or penicillin N) as a result of the inhibition of cephamycin C biosynthesis by glucose was observed.

Control by glucose and its phosphorylated derivatives of cephamycin C biosynthesis by resting cells. To study the glucose effect under controlled conditions avoiding the indirect effect of growth, resting cells of *S. lactamdurans* in MOPS buffer were prepared from cells grown in NYG medium supplemented with high or low glucose (Fig. 3). Glucose produced a concentration-dependent reduction of cephamycin biosynthesis. Cells grown in high glucose showed a delayed onset of cephamycin C production; however, when cephamycin synthesis commenced it proceeded at about the same rate as in control cells. Final levels of cephamycin were lower than half those produced by cells grown in low glucose (Fig. 3). When the resting cells were supplemented with an additional 165 mM-glucose, formation of antibiotic was still further reduced (see Discussion).

Fig. 2.

Fig. 3

Fig. 2. Effect of glucose on cephamycin biosynthesis by batch cultures of *S. lactamdurans*. (a) Growth in NYG medium (Δ , $\blacktriangle$) and residual sugar in cultures ($\square$, $\blacksquare$) with 55 (Δ , $\square$) or 165 ($\blacktriangle$, $\blacksquare$) mM-glucose. (b) Cephamycin produced in medium with 55 ($\circ$) or 165 ($\bullet$) mM-glucose, and pH in glucose-supplemented or unsupplemented cultures ($\blacklozenge$). Results are from a representative experiment of four determinations.

Fig. 3. Effect of glucose on the biosynthesis of cephamycin C by resting cells of *S. lactamdurans*. Resting cells were prepared from cultures grown in (a) 55 mM-glucose or (b) 165 mM-glucose. In each case, washed resting cells were supplemented ($\bullet$) or not ($\circ$) with additional 165 mM-glucose. Note that the higher production of cephamycin per ml by resting cells is due to the fact that cells were concentrated four-fold, with respect to the cell density of the NYG culture. Results are from a representative experiment of five determinations.

Fig. 4. Kinetics of protein synthesis by resting cells of *S. lactamdurans* at 0, 4, 8, 12 and 24 h. Protein synthesis is expressed as the percentage of [¹⁴C]valine taken up by the cells that was incorporated into protein; results are means of two determinations. Uptake of [¹⁴C]valine was 60% of the total radioactivity added to the culture (10^5 c.p.m. ml⁻¹), and did not change significantly during the 24 h of incubation.

To establish if the delayed onset of cephamycin biosynthesis by resting cells obtained from 165 mM-glucose-grown cultures was due to late derepression of cephamycin-forming enzymes, the protein turnover in resting cells was determined (Fig. 4). Protein synthesis was observed in cells suspended in a carbon-, nitrogen- and phosphate-free buffer. That a significant protein synthesis takes place in resting cells suggested that the enzymes involved in cephamycin

Fig. 5. Intracellular cAMP pool of cells grown in batch cultures with 55 (○) or 165 (●) mM-glucose. Results are means of three determinations.

biosynthesis might be repressed in cells grown in high glucose at the time the cells were collected to prepare the resting cell system.

Inorganic phosphate (10 mM), when added either to control or glucose-supplemented resting cells, did not modify the yield of antibiotic. Several phosphorylated derivatives of glucose (5–10 mM) were studied as possible effectors. Glucose 6-phosphate (10 mM) and 2-deoxyglucose 6-phosphate (5 mM) completely blocked cephamycin C production in resting cells. Indeed, these glucose derivatives had a greater regulatory effect than glucose at the same concentration. This trend was consistently observed in four different experiments. The regulatory effect produced *in vivo* by glucose 6-phosphate may be exerted at the membrane level, since it is not clear whether this compound is taken up in the intact form.

Effect of glucose on intracellular cAMP content. The intracellular cAMP content in *S. lactamdurans* was very high early in the growth phase of the culture when the glucose content of the medium was also high (Fig. 5), but it decreased later, reaching a steady low level after 48 h incubation. The concentration after 48 h was two to three times lower in glucose-supplemented cells than in control cultures after 48 h incubation when the antibiotic was being produced. This pattern does not support the involvement of cAMP in glucose control of cephamycin biosynthesis (see Discussion).

Intracellular ACV and glutathione levels. The pool of ACV was determined in control and glucose-supplemented cells. ACV content in control cells was very high early in the fermentation (24–36 h) just before antibiotic production began, and then decreased rapidly (Fig. 6a). The pool of ACV in cultures supplemented with 165 mM-glucose was about 25% of that in control cells, suggesting that either the supply of precursor amino acids for ACV synthesis was lower, or the ACV synthase was repressed in cells grown in high glucose concentrations.

The intracellular content of glutathione (γ -glutamyl-cysteinyl-glycine), a structural analogue of ACV that is not a precursor of penicillin, was stimulated by addition of a high glucose concentration to the cells (Fig. 6b). However, glutathione has many other poorly known cellular functions and its level in cells may have little to do with antibiotic production. The pool of glutathione increased considerably between 24 and 48 h in control and glucose-supplemented cultures and then decreased, but in all cases it was higher in cells grown in high glucose concentrations than in control cultures.

Isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase. Both isopenicillin N synthase and isopenicillin N epimerase activities of *S. lactamdurans* cells grown in high glucose concentrations were slightly greater than those in control cells, although formation of the isopenicillin N synthase in glucose-supplemented cells was delayed with respect to control cultures (Fig. 7a, b). Deacetoxycephalosporin C synthase ('expandase') activity increased steadily throughout the fermentation in control cells. In cells grown in high glucose concentrations, the enzyme was not detected before 48 h of fermentation, a delay of

Fig. 6

Fig. 7

Fig. 6. Intracellular content of (a) ACV and (b) glutathione in cells grown in batch cultures with 55 (○) or 165 (●) mM-glucose. Results are means of three determinations.

Fig. 7. Time course of the specific activities of (a) isopenicillin N synthase, (b) isopenicillin N epimerase and (c) deacetoxycephalosporin C synthase of cells grown in 55 (○) or 165 (●) mM-glucose. Results are means of three determinations.

more than 24 h with respect to control cells; but later a low level of activity was observed (Fig. 7c). These results suggest that the expandase activity was strongly repressed and/or inhibited by glucose (see Discussion).

Inhibition of cephamycin biosynthetic enzymes by glycolytic intermediates and inorganic phosphate. Several glycolytic intermediates, ATP and phosphate were tested as possible effectors on the *in vitro* activities of isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase (Table 1). Glucose 6-phosphate (1 mM) produced a 53% inhibition of the activity of deacetoxycephalosporin C synthase and a strong inhibition (83%) of isopenicillin N synthase. Fructose 1,6-diphosphate also inhibited deacetoxycephalosporin C synthase (46%) and fructose 2,6-diphosphate produced a similar effect. None of the other glycolytic intermediates tested had a significant effect on cyclase and expandase. Isopenicillin N epimerase was partially inhibited only by fructose 1,6-diphosphate and 2-phosphoglycerate.

Inorganic phosphate (1 mM) inhibited deacetoxycephalosporin C synthase but not isopenicillin N epimerase or isopenicillin N synthase. Inorganic phosphate and the sugar phosphates exerted a concentration-dependent inhibition of deacetoxycephalosporin C synthase (Fig. 8).

Fig. 8

Fig. 9

Fig. 8. Effect of increasing concentrations of glucose 6-phosphate (▲), fructose 1,6-diphosphate (■) and inorganic phosphate (○) on the activity of deacetoxycephalosporin C synthase. Results are means of two determinations. The activity of the control (uninhibited) reaction was $0.47 \text{ nkat (g protein)}^{-1}$.

Fig. 9. Changes in the intracellular content of glucose 6-phosphate during batch culture in medium containing 55 (○) or 165 (●) mM-glucose. Results are means of three determinations.

Table 1. Effect of glycolytic intermediates, ATP and phosphate on the *in vitro* activity of isopenicillin N synthase, isopenicillin N epimerase and deacetoxycephalosporin C synthase of *S. lactamdurans*

Enzyme extracts were obtained from 72 h cultures by sonication and purified as described in Methods. Results are means of three determinations. The values in parentheses are percentages of the control.

Addition (1 mM)	Specific activity [nkat (g protein) ⁻¹]		
	Isopenicillin N synthase	Isopenicillin N epimerase	Deacetoxycephalosporin C synthase
None	4.27 (100)	1.17 (100)	0.26 (100)
Glucose	4.27 (100)	1.17 (100)	0.26 (100)
Glucose 6-phosphate	0.72 (17)	1.17 (100)	0.12 (47)
Fructose 6-phosphate	4.27 (100)	1.17 (100)	0.26 (100)
Fructose 1,6-diphosphate	4.27 (100)	0.78 (66)	0.14 (54)
Fructose 2,6-diphosphate	4.27 (100)	1.17 (100)	0.09 (35)
2-Phosphoglycerate	4.27 (100)	0.78 (66)	0.26 (100)
3-Phosphoglycerate	4.27 (100)	1.17 (100)	0.26 (100)
2,3-Diphosphoglycerate	4.27 (100)	1.17 (100)	0.26 (100)
Phosphoenolpyruvate	4.27 (100)	1.17 (100)	0.26 (100)
Pyruvate	3.73 (87)	1.17 (100)	0.24 (94)
Phosphate	4.27 (100)	1.17 (100)	0.15 (60)
ATP	ND	ND	0.26 (100)

ND, Not determined.

Inorganic phosphate, glucose 6-phosphate or fructose 1,6-diphosphate, each at 2 mM, produced a 50% inhibition of deacetoxycephalosporin C synthase.

The phosphate effect may have been due to the sequestration of Fe^{2+} ions required for deacetoxycephalosporin C synthase activity. However, no reversal of the phosphate effect by increasing concentrations of Fe^{2+} was obtained in several experiments. To test if the effect of glucose 6-phosphate was due to the presence of free phosphate released by hydrolysis, both free glucose and inorganic phosphate were determined in the reaction samples containing glucose

6-phosphate. No hydrolysis of glucose 6-phosphate was detected, indicating that glucose 6-phosphate itself was the inhibitory effector.

Intracellular glucose 6-phosphate content. Since glucose 6-phosphate inhibited at least two enzymes of the cephamycin biosynthetic pathway, its intracellular concentration was determined in cells grown in either high or low glucose concentrations (Fig. 9). Glucose 6-phosphate followed a similar pattern to that of cAMP: it was high early in the fermentation and decreased at between 24 and 48 h of growth. No difference in the intracellular concentration of glucose 6-phosphate per mg dry weight was observed as a result of glucose supplementation. However, if the glucose 6-phosphate concentration was calculated in relation to the approximate volume of the cells, the intracellular concentration was high enough to produce an 'in vivo' inhibition of cyclase and expandase at early times in the fermentation (Fig. 9).

The intracellular volume of *S. lactamdurans* has been estimated as 2.0 μ l per mg dry wt, which compares well with the reported values for *Streptococcus salivarius* (2 μ l per mg dry wt) (Vadeboncoeur & Trahan, 1982), *Zymomonas mobilis* (3.3 μ l per mg dry wt) (Dimarco & Romano, 1985) and *Streptomyces antibioticus* (1.3–1.7 μ l per mg dry wt) (J. Salas, personal communication).

DISCUSSION

Glucose exerted a strong negative regulation on cephamycin biosynthesis by *S. lactamdurans*, similar to the repressive effect of glucose on penicillin formation by *P. chrysogenum* (Revilla *et al.*, 1984), and more intense than the effect of glucose on cephalosporin biosynthesis by *A. chrysogenum* (Zanca & Martín, 1983; Behmer & Demain, 1983).

Glucose transport and/or catabolism repressed both ACV formation and the ring expansion catalysed by deacetoxycephalosporin C synthase until the glucose concentration decreased below a threshold level. Repression of ACV formation by glucose has also been found in *P. chrysogenum* (Martín *et al.*, 1982; G. Revilla & J. F. Martín, unpublished). Deacetoxycephalosporin C synthase (expandase) was also sensitive to glucose repression in *A. chrysogenum* (Behmer & Demain, 1983; Zanca & Martín, 1983; Heim *et al.*, 1984). On the contrary, the isopenicillin N synthase and the epimerase of *S. lactamdurans* were not sensitive to repression by glucose (Fig. 7). Similarly, the cyclase of *A. chrysogenum* was less sensitive to carbon catabolite regulation (Heim *et al.*, 1984). In general, it seems that ACV biosynthesis and ring expansion are very sensitive to carbon catabolite regulation.

Nitrogen source repression of cephamycin biosynthesis in *S. lactamdurans* is exerted coordinately on four enzymes of the biosynthetic pathway [tripeptide-forming enzyme(s), cyclase, epimerase and expandase] (Castro *et al.*, 1985). Carbon catabolite regulation of cephamycin formation in this streptomycete does not appear to be exerted coordinately on these enzymes (Figs 6 and 7). Rather, it seems that two of the steps of the biosynthetic pathway (formation of ACV and ring expansion) are particularly sensitive to carbon catabolite regulation. It is interesting that carbon catabolite regulation in *S. lactamdurans* specifically affected the formation of a peptide involved in penicillin biosynthesis, and did not repress the biosynthesis of glutathione (Fig. 6), a tripeptide that is also formed by a non-ribosomal enzyme system probably similar to that responsible for ACV biosynthesis (Fawcett & Abraham, 1975; López-Nieto *et al.*, 1985).

Glucose also had a drastic effect on cephamycin biosynthesis by resting cells in short-term experiments (Fig. 3), which might be explained, at least in part, by the fact that protein synthesis, and therefore the *de novo* formation of cephamycin synthases, is intense in resting cells (Fig. 4). Our results suggest that inhibition of the activity of preformed enzymes may also play an important role in glucose control of cephamycin biosynthesis. In effect, the expandase activity was strongly inhibited *in vitro* by glucose 6-phosphate, fructose 1,6-diphosphate and inorganic phosphate and the cyclase was inhibited by glucose 6-phosphate. Fructose 2,6-diphosphate, which has been described as a regulatory effector of glycolysis in higher cells (Hers *et al.*, 1982), had a significant inhibition effect on expandase but showed no effect on the other two enzymes (Table 1).

A decreased O₂ availability as a result of glucose addition has been suggested by Braña *et al.* (1983). In our conditions, especially in the resting cells, addition of glucose did not increase growth. Therefore, it seems unlikely that depletion of O₂ occurs, although we cannot exclude this possibility.

The role of inorganic phosphate in the control of cephamycin biosynthesis in *S. lactamdurans* is less intense. Inorganic phosphate, alone or when added to glucose-supplemented fermentations, did not affect total cephamycin biosynthesis *in vivo*; this effect is different from that observed in *A. chrysogenum* and *P. chrysogenum* (Martín *et al.*, 1982), where inorganic phosphate enhances the regulation exerted by glucose. Lubbe *et al.* (1984) have suggested that phosphate inhibits the expandase activity of *S. clavuligerus* by sequestering Fe²⁺ ions required for activity of this enzyme. However, we were unable, in repeated experiments, to reverse the inhibition by phosphate of the expandase of *S. lactamdurans* by addition of high Fe²⁺ concentrations.

cAMP is involved in carbon catabolite regulation by glucose in enterobacteria (Perlman *et al.*, 1969), but such involvement in other micro-organisms is, so far, not clear. The time-course of intracellular cAMP accumulation during fermentation, which shows a sharp decrease in parallel with glucose depletion (in contrast to the situation in enterobacteria), indicates that carbon catabolite regulation of cephamycin biosynthesis is not mediated by cAMP. These results agree with similar observations made by Vining and coworkers, who also concluded that cAMP was not involved in the onset of streptomycin biosynthesis in *Streptomyces griseus* (Regan & Vining, 1978) or in carbon catabolite repression of β -galactosidase in *Streptomyces venezuelae* (Chatterjee & Vining, 1981). cAMP in *Streptomyces* follows the same pattern as intracellular ATP and other nucleotides involved in the control of phosphate-sensitive antibiotic biosynthesis (Martin & Demain, 1977; Martin *et al.*, 1978), but does not appear to be involved in carbon catabolite regulation of antibiotic production.

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