Effect of Serine Hydroxamate and Methyl α -D-glucopyranoside Treatment on Nucleoside Polyphosphate Pools, RNA and Protein Accumulation in *Streptomyces hygroscopicus*

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The accumulation of RNA and protein and the kinetics of nucleoside triphosphate and guanosine polyphosphate pools during amino acid starvation and carbon source downshift were investigated in *Streptomyces hygroscopicus*. RNA accumulation was controlled stringently during both amino acid starvation and carbon source downshift. The pool size of ppGpp increased dramatically under these conditions. However, the intracellular concentrations of nucleoside triphosphates were low and the concentration of guanosine polyphosphates was much lower than in *Escherichia coli*. The possible significance of this phenomenon in the regulation is discussed.

INTRODUCTION

In the preceding paper (Riesenberg & Bergter, 1984), a method for obtaining balanced growth of *Streptomyces hygroscopicus* was described. Balanced growth lasted for at least two doublings of biomass. This method enabled us to investigate the regulation of growth under balanced conditions, and under imbalance induced by various treatments.

Escherichia coli growing in a balanced manner accumulates guanosine 3'-diphosphate 5'-diphosphate (ppGpp) after perturbation, e.g. after amino acid starvation (Cashel & Gallant, 1969). ppGpp mediates the stringent response, which includes cessation of RNA accumulation. A wide range of other processes is influenced by ppGpp, which serves to re-orient the cell metabolism after metabolic shifts (Cashel, 1975; Nierlich, 1978; Gallant, 1979).

Similar regulatory patterns have been found in *Klebsiella pneumoniae* (Riesenberg & Kari, 1981; Riesenberg *et al.*, 1982) and *Bacillus subtilis* (Smith *et al.*, 1980). *Caulobacter crescentus* (Chiaverotti *et al.*, 1981) and *Rhizobium meliloti* (Belitsky & Kari, 1982) exhibit different regulatory patterns.

The occurrence of ppGpp in streptomycetes has been well documented (Hamagishi *et al.*, 1981). However, so far no effort has been made to investigate the stringent response and the possible role of ppGpp in a *Streptomyces* strain. Since we had worked out the conditions for balanced growth in *S. hygroscopicus* (Riesenberg & Bergter, 1984), the possible involvement of ppGpp in regulation following various shifts could be studied. In this paper we present the characterization of accumulation of RNA, protein, nucleoside triphosphate and guanosine polyphosphate pools during amino acid starvation and carbon source downshift.

Abbreviations: α MG, methyl α -D-glucopyranoside; SHX, DL-serine hydroxamate.

METHODS

Organisms, media and culture conditions. Conditions for *Streptomyces hygroscopicus* were given in the preceding paper (Riesenberg & Bergter, 1984). *Escherichia coli* CP78 (*arg his thr leu thi relA⁺*), kindly supplied by Dr N. Fiil (Copenhagen), was also used in these studies. Medium and culture conditions for *E. coli* were those of Kari *et al.* (1977).

Chemicals. DL-Serine hydroxamate (SHX), methyl α -D-glucopyranoside (α MG), yeast inorganic pyrophosphatase and 3'-nucleotidase were purchased from Sigma. Guanosine 5'-triphosphate 3'-diphosphate (pppGpp), guanosine 5'-diphosphate 3'-diphosphate (ppGpp), guanosine 5'-tetraphosphate (ppppG), adenosine 5'-triphosphate 3'-diphosphate (pppApp), adenosine 5'-diphosphate 3'-diphosphate (ppApp) and adenosine 5'-tetraphosphate (ppppA) were obtained from ICN (Irvine, Calif., USA). Guanosine 5'-monophosphate (pGp) was supplied by P-L Biochemicals (Milwaukee, Wisconsin, USA). Guanosine 5'-triphosphate (GTP), adenosine 5'-triphosphate (ATP), cytidine 5'-triphosphate (CTP), uridine 5'-triphosphate (UTP) and guanosine 5'-monophosphate (pG) came from Serva (FRG). Polyethyleneimine impregnated sheets (PEI and PEI-UV₂₅₄) were manufactured by Macherey & Nagel Co. (Düren, FRG). DEAE-A25 was from Pharmacia. $\text{KH}_2^{32}\text{PO}_4$ (carrier-free) was supplied by Isocommerz (GDR). $[8\text{-}^3\text{H}]\text{Guanosine}$ (370 GBq mmol⁻¹) and $[2\text{-}^3\text{H}]\text{adenine}$ (925 GBq mmol⁻¹) were purchased from Amersham and $[5\text{-}^3\text{H}]\text{uridine}$ (814 GBq mmol⁻¹) and $\text{L}\text{-}[^{14}\text{C}]\text{leucine}$ (7.8 GBq mmol⁻¹) were obtained from UVVVR Prague (CSSR).

Identification of pppGpp and ppGpp. (i) **Culturing and radioactive labelling.** Spores of *S. hygroscopicus* were germinated and grown in 100 ml flasks containing 35 ml medium (with 0.15 mM- KH_2PO_4). Five ml was labelled with 11 MBq ^{32}P , ml⁻¹; 20 ml received 74 kBq ^{32}P , ml⁻¹. After further incubation for one doubling, the culture with the lower ^{32}P specific activity was distributed into three flasks as 5 ml samples for double labelling: the first contained $[5\text{-}^3\text{H}]\text{uridine}$ at 3 MBq (3.7 nmol) ml⁻¹, the second $[8\text{-}^3\text{H}]\text{guanosine}$ at 2.8 MBq (7.5 nmol) ml⁻¹ and the third $[2\text{-}^3\text{H}]\text{adenine}$ at 0.9 MBq (0.9 nmol) ml⁻¹. After 7.5 min SHX (2.5 mg ml⁻¹) was added to all cultures for induction of synthesis of pppGpp and ppGpp; 10 min after addition of SHX, all cultures were cooled in an ice bath.

(ii) **Extraction of cell pool.** Nucleotides were extracted with 1 M-formic acid in an ice bath for 30 min (Cashel *et al.*, 1969). After centrifugation of the extract, the supernatant was brought to pH 6.5 to 7.4 with NH_4OH . A small sample of the neutralized extract from cultures labelled with 11 MBq ^{32}P , ml⁻¹ was used for identification of nucleoside polyphosphates by thin layer co-chromatography, either with unlabelled UV-markers or with ^{32}P -labelled extracts of *E. coli*. The nucleoside polyphosphates of the remainder and of the neutralized extracts derived from double-labelled cultures were concentrated on a Sephadex DEAE-A25(HCO_3^-) column.

(iii) **Column concentration of nucleotides.** Each 5 ml supernatant was added to 13 ml H_2O and 8 ml Sephadex DEAE-A25(HCO_3^-) stock (2 vols H_2O :1 vol. Sephadex). After adsorption of nucleotides (10 min at room temperature, with occasional stirring) the gel was washed three times with 20 ml H_2O . After final decantation the sediment was stirred with 10 ml H_2O and then transferred to a column. The column was prewashed with 8 ml 0.2 M-triethylammonium bicarbonate and then the nucleotides were eluted with 8 ml 1 M-triethylammonium bicarbonate. The eluates were evaporated under reduced pressure at 30 °C. For complete removal of triethylammonium bicarbonate, evaporation was repeated four times using 50% (v/v) alcohol. Finally, the samples were dissolved in 200 μl H_2O .

The sample derived from the culture labelled with 11 MBq ^{32}P , ml⁻¹ was applied to a second DEAE-A25(HCO_3^-) column for separation of tentative pppGpp and ppGpp (see below).

The samples derived from $^{32}\text{P}/^3\text{H}$ labelled cultures were freeze-dried, dissolved in 20 μl H_2O and then used for thin layer chromatography (see below).

(iv) **Thin layer chromatography for the identification of nucleoside polyphosphates.** Two-dimensional resolution of nucleoside polyphosphates was achieved in the system of Cashel & Kalbacher (1970) (3.3 M-ammonium formate and 4.2% boric acid in the first dimension; 1.5 M- KH_2PO_4 in the second dimension) and the system of Pao & Gallant (1979) (4 M-formic acid and 1 M-lithium chloride in the first dimension; 1.5 M- KH_2PO_4 in the second dimension). Samples of 40 μl of neutralized formic acid extracts derived from cultures of *S. hygroscopicus* labelled with 11 MBq ^{32}P , ml⁻¹ were applied to each PEI-UV₂₅₄ plate (20 $\times$ 20 cm). The following UV-markers served for co-chromatography: pppGpp, ppGpp, ppppG, pppApp, ppApp, ppppA, GTP, ATP, CTP and UTP. Samples of 10 μl neutralized formic acid extracts of ^{32}P -labelled cells of *E. coli* CP78 were also applied for co-chromatography. The extracts were prepared as described by Török & Kari (1980). Nucleoside polyphosphates of the $^{32}\text{P}/^3\text{H}$ labelled and column-concentrated samples of *S. hygroscopicus* were separated applying the system of Cashel & Kalbacher (1970). After autoradiography the spots were cut out. Extraction of nucleotides, neutralization and quantification were done according to Török & Kari (1980).

(v) **Preparation of tentative pppGpp and ppGpp from *S. hygroscopicus*.** The nucleoside polyphosphates of the column-concentrated ^{32}P -labelled samples were separated by linear gradient elution for 25 h using DEAE-A25(HCO_3^-) column (1.3 $\times$ 10 cm) and 400 ml 0 to 1 M-triethylammonium bicarbonate. The expected fractions of tentative pppGpp and ppGpp identified by co-chromatography of unlabelled pppGpp and ppGpp (see above)

were concentrated by evaporation and were freeze-dried as described above.

(vi) *Enzyme treatments.* In a modification to the method of Sy & Lipmann (1973), tentative pppGpp and ppGpp of *S. hygroscopicus* were treated with yeast inorganic pyrophosphatase and 3'-nucleotidase. A 100 µl portion of 15 mM-Tris/HCl (pH 7.2) containing 2 mM-ZnCl₂ and 20 units of yeast inorganic pyrophosphatase (solution 1) was added to freeze-dried samples of pppGpp and ppGpp, and the mixture incubated at 37 °C for 30 min. In a second experiment, 100 µl 15 mM-Tris/HCl (pH 7.2) containing 2 mM-ZnCl₂, 20 units of yeast inorganic pyrophosphatase and 0.01 units of 3'-nucleotidase (solution 2) was added to freeze-dried samples and the mixture incubated at 37 °C for 4 h. Samples (20 µl) before and after incubation were applied to PEI-UV₂₅₄ plates. The PEI plate with samples of solution 1 was co-chromatographed together with pGp as a UV-marker in 0.75 M-KH₂PO₄, pH 3.4 (Cashel *et al.*, 1969). Samples of solution 2 were run together with pG as a UV-marker, according to Randerath & Randerath (1964).

Determination of accumulation of RNA and protein. Cultures were double labelled according to Riesenbergs & Kari (1981) with minor modifications. The incorporation of [¹⁴C]leucine and [³H]uridine [9.3 KBq (41 nmol) ml⁻¹ and 185 KBq (20 nmol) ml⁻¹, respectively] into material precipitable by ice-cold 5% trichloroacetic acid was followed by filtration and washing of 0.1 ml samples (Whatman GF/C filters). Radioactivities were measured in a Packard Tricarb liquid scintillator and corrected for spill over (16% of ¹⁴C into the ³H-channel and 0.5% of ³H into the ¹⁴C-channel).

Determination of nucleoside polyphosphate pools. Spores of *S. hygroscopicus* were germinated and grown in tubes containing 5 ml medium (with 0.15 M-KH₂PO₄). After two doublings of biomass the culture was labelled with 14.8 MBq ³²P_i ml⁻¹. After a further doubling to equilibrate the pool, SHX or αMG was added. At the times indicated in the figures, samples (100 µl) were withdrawn, extracted at 0 to 4 °C in 1 M-formic acid for 30 min and then centrifuged. A sample of 50 µl of the formic acid extract was applied to PEI-plates in portions of 10 µl, with intermediate drying, and chromatographed with 0.75 M-KH₂PO₄ (pH 3.4) for separation of ATP, CTP and UTP, and with 1.5 M-KH₂PO₄ (pH 3.4) for separation of pppGpp, ppGpp and GTP. After autoradiography the spots were cut out and radioactivity was measured in a counter. The pool sizes were expressed as pmol (mg dry wt)⁻¹.

RESULTS

Identification of pppGpp and ppGpp from S. hygroscopicus

After inducing the accumulation of nucleoside polyphosphates by treatment with SHX, pppGpp and ppGpp were identified on the basis of the following criteria.

(i) Co-chromatography with standard UV-markers. The tentative ³²P-labelled spots of *S. hygroscopicus* were located at the same position on the chromatograms as the unlabelled UV-markers pppGpp and ppGpp after two-dimensional chromatography (Fig. 1a, b). Control markers such as pppApp, ppApp, ppppA, GTP, ATP, CTP and UTP did not co-migrate with pppGpp and ppGpp in the systems of Cashel & Kalbacher (1970) and Pao & Gallant (1979). In addition, ppppG moved more slowly in the first dimension of the system of Cashel & Kalbacher (1970) than the ³²P-labelled ppGpp spot of *S. hygroscopicus*.

(ii) Co-chromatography with ³²P-labelled markers from *E. coli*. Neutralized formic acid extracts from ³²P-labelled cultures of *E. coli* and of *S. hygroscopicus* were chromatographed separately and together. pppGpp and ppGpp of *E. coli* moved to the same position as the respective spots of *S. hygroscopicus*. Quantification of the spots from common and separate running confirmed the co-migration.

(iii) Labelling with purine and pyrimidine precursors. Cultures of *S. hygroscopicus* were labelled with low specific activity ³²P_i to detect nucleoside polyphosphates on autoradiograms after chromatography, and with high specific activity [³H]purine- and [³H]pyrimidine precursors to identify the nature of the nucleic acid bases (see Methods). The spots of *S. hygroscopicus* co-migrating with unlabelled pppGpp and ppGpp could be labelled with [8-³H]guanosine, but not with [2-³H]adenine and [5-³H]uridine. The spot located at the same position as unlabelled GTP was labelled with [8-³H]guanosine, but not with [2-³H]adenine and [5-³H]uridine. The ATP spot contained label of [2-³H]adenine and [8-³H]guanosine but not of [5-³H]uridine. The UTP- and CTP-control spots were labelled only by [5-³H]uridine.

(iv) Enzymic hydrolysis of spots co-migrating with (p)ppGpp. Tentative ³²P_i-labelled pppGpp and ppGpp were completely converted into pGp by treatment with Zn²⁺-activated inorganic pyrophosphatase from yeast. The hydrolysed labelled compound moved to the same position on the chromatogram as unlabelled pGp-UV-marker. In reaction mixtures which contained 3'-nucleotidase and inorganic pyrophosphatase, the tentative ³²P-labelled pppGpp

Fig. 1. Combined autoradiogram and fluorogram of formic acid extracts of ^{32}P -labelled and SHX-treated mycelia of *S. hygroscopicus* and of unlabelled UV-markers pppGpp, ppGpp, pppApp, ppApp (spots marked by dotted line), after two-dimensional chromatography according to (a) Cashel & Kalbacher (1970), and (b) Pao & Gallant (1979). Experimental conditions are described in Methods.

Fig. 2. Effect of SHX treatment (a, b) and αMG treatment (c, d) on pools of ATP ($\circ$), GTP ($\bullet$), ppGpp ($\square$) and pppGpp ($\blacksquare$) pools in *S. hygroscopicus*. Experimental details are described in Methods.

and ppGpp were completely degraded to pG on the basis of co-chromatography with unlabelled pG-UV-marker (for experimental details see Methods). The enzymic tests proved that a pyrophosphoryl group was present in the 3'-position of guanosine 5'-triphosphate and of guanosine 5'-diphosphate. We concluded therefore, that *S. hygroscopicus* contained both pppGpp and ppGpp.

Fig. 3. Immediate response of ppGpp (□) and pppGpp (■) pools in *S. hygroscopicus* after SHX treatment. Experimental details are described in Methods.

Effect of SHX treatment on nucleoside polyphosphate pools, and RNA and protein accumulation

Accumulation of pppGpp and ppGpp occurred quickly after addition of SHX (2.5 mg ml^{-1}) to exponentially growing mycelia of *S. hygroscopicus* (Fig. 2). The pool of pppGpp increased earlier than that of ppGpp (Fig. 3). The maximum concentration of pppGpp was reached about 10 min after SHX addition (Fig. 2). The peak was sharp, followed by a relatively rapid decrease. A plateau was established about 50 min after the treatment. ppGpp reached a plateau 20 min earlier, and no sharp peak was observed. The plateau levels of pppGpp and ppGpp were very low, i.e. 5 and 18 pmol (mg dry wt)⁻¹. No pppGpp and ppGpp could be measured in samples from exponentially growing mycelium, although faint spots could be seen on the autoradiographic films.

The pool sizes of all four ribonucleoside triphosphates decreased after SHX-treatment. The pool size of ATP decreased slowly but continuously from 1000 pmol (mg dry wt)⁻¹ to about 250 pmol during 90 min (Fig. 2). The pool size of GTP of exponentially growing mycelium amounted to about one-third of that of ATP. In contrast to ATP, the GTP pool decreased quickly; the pool size decreased to 50% of its original value after 10 min of SHX action. The GTP pool became constant at 25% of its initial value after 40 min (Fig. 2). The decrease in the GTP pool [more than 200 pmol (mg dry wt)⁻¹] was greater than the increase of pppGpp and ppGpp pools [less than 50 pmol (mg dry wt)⁻¹]. The pool size of CTP of exponentially growing mycelia was about 200 pmol (mg dry wt)⁻¹ and the pool size of UTP was about 260 pmol (mg dry wt)⁻¹. The kinetics of the CTP pool size after SHX treatment resembled that of GTP; 40 min after SHX treatment the new level of CTP was about 40% of the control value. The UTP pool decreased rapidly after addition of SHX, reaching a minimum of about 20% of the control pool size after 20 min. Subsequently, the pool size increased slowly, reaching about 65% of the control level after 70 min (data not shown).

Accumulation of RNA and protein stopped after treatment of the culture with SHX (Fig. 4). This stringent control was relieved by simultaneous addition of SHX (2.5 mg ml^{-1}) and chloramphenicol (0.2 mg ml^{-1}) (data not shown). The intensity of the light scattered by the culture as a measure of biomass remained constant immediately after addition of SHX. The small increase 30 min later was probably due to changes in mycelial morphology, since microscopic observation revealed that the mycelia showed a tendency to fragment.

Effect of α MG treatment

The response of *S. hygroscopicus* to carbon source shift down, imposed by α MG treatment of a glucose-grown culture, was an immediate accumulation of ppGpp (Fig. 2). The peak of the pool

Fig. 4

Fig. 5

Fig. 4. Effect of SHX treatment on accumulation of protein ($\square$, control samples; $\blacksquare$, SHX-treated samples) and on intensity of scattered light as measure of biomass (Δ , control samples; $\blacktriangle$, SHX-treated samples) and on accumulation of RNA ($\circ$, control samples; $\bullet$, SHX-treated samples). Experimental details are described in Methods.

Fig. 5. Effect of α MG treatment on accumulation of protein, RNA and intensity of scattered light as measure of biomass. Symbols are as for Fig. 4.

size was reached around 15 to 20 min after the onset of downshift. Thereafter, the ppGpp pool decreased, becoming constant after about 40 min of the downshift. The equilibrated post-shift level of ppGpp was very low, i.e. 10 pmol (mg dry wt) $^{-1}$. pppGpp was detected only in the first 15 min after shift down.

The pool sizes of ribonucleoside triphosphates ATP, GTP and CTP fell after addition of α MG, but, in contrast to SHX treatment, the ATP level decreased only slightly, i.e. to 80% of the control value 10 min after the shift. Thereafter, ATP returned to the control level (Fig. 2). The GTP pool, however, decreased continuously. The post-shift level was 50% of the control level after 15 min and reached the final level of 30% of control after 60 min. The size of CTP also decreased after imposing the carbon source downshift. It reached a minimum (about 40% of control after 10 min), then increased again until it became constant after about 50 min at 70% of the control (data not shown).

Accumulation of RNA and protein ceased immediately after addition of α MG (Fig. 5). After 20 min, accumulation of protein recommenced, but at a much lower rate than before the shift. The values for intensity of scattered light indicated that growth continued after a lag period of 20 min, but at a very reduced specific growth rate.

DISCUSSION

Occurrence of nucleoside polyphosphates in S. hygroscopicus

Streptomyces hygroscopicus accumulated ppGpp and pppGpp, in common with other streptomycetes (An & Vining, 1978; Simuth *et al.*, 1979; Hamagishi *et al.*, 1980; Hamagishi *et al.*, 1981; Nishino & Murao, 1981; Stastna & Mikulik, 1981). On the other hand, we could not find adenosine polyphosphates, which have been described for some other *Streptomyces* strains (Simuth *et al.*, 1979; Hamagishi *et al.*, 1981). This discrepancy could be due either to the

organism or to different cultural conditions. We cannot exclude the possibility that adenosine polyphosphates are present in *S. hygroscopicus* at different stages of growth.

The concentration of (p)ppGpp in *S. hygroscopicus* during exponential growth was considerably lower than in *S. griseus* (An & Vining, 1978), in *S. aureofaciens* (Simuth *et al.*, 1979) and in *S. galileus* (Hamagishi *et al.*, 1981). We could see only faint (p)ppGpp spots on the autoradiographic films which were not distinguishable from the background by measuring radioactivity. This difference in the basal level of (p)ppGpp between *S. hygroscopicus* and other *Streptomyces* strains might be due to strain peculiarities, to the entirely different cultivation conditions or to different stages of growth. In the case of *S. hygroscopicus* we used young non-differentiated mycelia (see preceding paper). We suppose that the mycelia of *S. galileus*, *S. aureofaciens* and *S. griseus* contained already aged differentiated mycelia as well as young non-differentiated cells. Perhaps these aged differentiated regions were restricted in growth and hence could accumulate (p)ppGpp, unlike the present study where pool sizes were determined under balanced growth.

*Effect of serine hydroxamate treatment on nucleoside polyphosphate pools,
RNA and protein accumulation*

SHX, an analogue of serine (Tosa & Pizer, 1971), induces a stringent response in a variety of micro-organisms. Among these are *E. coli* (Tosa & Pizer, 1971), *K. pneumoniae* (Riesenberg & Kari, 1981), *R. meliloti* (Belitsky & Kari, 1982) and *B. subtilis* (Belitsky & Shakulov, 1980). SHX was also active in *S. hygroscopicus*. Its action resulted in an immediate cessation of accumulation of protein and RNA (Fig. 4), linked with rapid accumulation of (p)ppGpp (Figs 2 and 3). This is the first case where stringency in streptomycetes has been induced and shown to be connected with accumulation of (p)ppGpp. We conclude that *S. hygroscopicus* controls RNA accumulation during amino acid starvation stringently, like most other bacterial species. *Caulobacter crescentus* seems to be an exception in this respect since during amino acid starvation it did not accumulate (p)ppGpp and RNA synthesis continued (Chiaverotti *et al.*, 1981). *Rhizobium meliloti* is another exception. It controlled RNA accumulation during amino acid starvation stringently but without concomitant accumulation of (p)ppGpp (Belitsky & Kari, 1982).

In *S. hygroscopicus* the inhibition of RNA accumulation during SHX treatment was not due to inhibition of protein synthesis or any side effect of SHX because the simultaneous addition of chloramphenicol relieved the SHX inhibition of RNA accumulation. Whether *S. hygroscopicus* shows the other characteristics of stringent response, known from *E. coli*, has yet to be demonstrated. The experimental system described in the preceding paper (Riesenberg & Bergter, 1984) is suitable for such investigations.

The pool size of pppGpp increased earlier than that of ppGpp after SHX treatment (Fig. 3). This is in agreement with the general opinion that pppGpp is the precursor of ppGpp. We conclude that *S. hygroscopicus* most probably uses the same pathway for deriving ppGpp as *E. coli* (Kari *et al.*, 1977). The rapid decrease of the GTP pool after SHX treatment supported the opinion that GTP is a precursor of pppGpp in *S. hygroscopicus*, as has been found for *E. coli*. But GTP was converted to something other than only pppGpp, since the GTP level decreased by more than 200 pmol (mg dry wt)⁻¹, whereas the two guanosine polyphosphates together never increased by more than 50 pmol (mg dry wt)⁻¹.

Since SHX treatment led to a decrease of all four ribonucleoside triphosphate pools concomitant with a significant inhibition of macromolecular synthesis we conclude that amino acid starvation resulted in an inhibition of nucleotide synthesis in *S. hygroscopicus* as in *E. coli* (Cashel & Gallant, 1968). However, the decrease of ATP and GTP pool in *S. hygroscopicus* after amino acid starvation, by a factor of about four, is more severe than in *E. coli* or any other bacteria investigated so far. In *B. subtilis*, GTP was the only triphosphate which decreased, also by about a factor of four, during amino acid starvation (Gallant & Margason, 1972). Whether the behaviour of the ATP pool during the stringent response is a characteristic property of only *S. hygroscopicus*, or of the other streptomycetes as well, remains to be determined.

Effect of α MG on nucleoside polyphosphate pools, RNA and protein accumulation

α MG has been used successfully in *E. coli* for induction of carbon source downshift (Hansen *et al.*, 1975). It is an analogue of glucose which inhibits glucose uptake and accumulates as non-metabolizable α MG-6-phosphate (Kessler & Rickenberg, 1963). α MG has been found to be active also in *K. pneumoniae* (Riesenberg & Kari, 1981). It also works in *S. hygroscopicus* (Figs 2 and 5). The general characteristics of the α MG downshift seemed to be similar to that of *E. coli* (Hansen *et al.*, 1975) and of *K. pneumoniae* (Riesenberg & Kari, 1981), i.e. both RNA and protein accumulation were curtailed, pppGpp accumulated rapidly, and after a transient appearance diminished to the basal level, whereas the ppGpp pool increased significantly. Therefore in *Streptomyces*, ppGpp may play a similar regulatory role during downshift as in other bacteria.

*Comparison of pool sizes of nucleoside polyphosphates of *S. hygroscopicus* and *E. coli**

An important difference between *S. hygroscopicus* and *E. coli* became apparent by comparing the pool size of ppGpp after induction of the stringent response or after imposing carbon source downshift. The equilibrated pool size of ppGpp after the stringent response was about 0.5 to 1 mM in *E. coli* (Nierlich, 1978). For comparison, we converted these data into pmol (mg dry wt)⁻¹ and obtained 1500 to 3000 pmol. We assumed for *E. coli* growing in glucose/mineral salts medium a doubling time of 45 min, mean cell length, *L*, 2.62 μ m, mean cell diameter, 2*R*, 0.62 μ m (Grover *et al.*, 1977), mean cell mass, *M*, 0.264×10^{-6} μ g (Günther & Bergter, 1971). This means that the pool size of ppGpp in *S. hygroscopicus* [18 pmol (mg dry wt)⁻¹, see Fig. 2] was about 100-fold lower than in *E. coli*.

The comparison was also made for the pool sizes of ATP and GTP of exponentially growing *E. coli* and *S. hygroscopicus*. We converted (as described above) the intracellular concentrations of ATP (1 to 2 mM) and of GTP (0.3 to 1 mM) published by Nierlich (1978) for *E. coli* into 3000 to 6000 pmol ATP (mg dry wt)⁻¹, and 900 to 3000 pmol GTP (mg dry wt)⁻¹. The ATP pool and GTP pool of *S. hygroscopicus* with about 1000 and 300 pmol (mg dry wt)⁻¹ were about fivefold lower than in *E. coli*. With a bioluminescent assay, similar ATP pools were determined in exponentially growing mycelium of *S. aureofaciens* [300 pmol (mg dry wt)⁻¹ (Curdova *et al.*, 1976)], of *S. fradiae* [1500 pmol (mg dry wt)⁻¹ (Vu-Trong *et al.*, 1980)] and of *S. noursei* [330 pmol (mg dry wt)⁻¹, (F. Hänel, personal communication)]. Only streptomycetes derived from the above-mentioned strains with improved antibiotic production exhibited a significantly lower ATP pool during exponential growth (Curdova *et al.*, 1976; F. Hänel, personal communication).

This lower ATP pool size of exponentially growing mycelium of *S. hygroscopicus* was not due to an incomplete extraction procedure since extraction with 0.1 M-, 1 M- and 2.5 M-formic acid revealed no significantly different pool sizes. The lower content of nucleoside triphosphates in *S. hygroscopicus* compared to *E. coli* might be due to the diversity of these unrelated bacteria.

The most striking result was that during stringent response, the ppGpp pool in *S. hygroscopicus* was about 100-fold lower than in *E. coli*. The mycelia of *S. hygroscopicus* used in these studies were derived from spores and were not yet differentiated. This means that each hyphal part of the mycelia synthesized DNA, RNA and protein to the same extent. This has been shown autoradiographically for synthesis of DNA (C. Kummer, personal communication). Since all mycelial parts seemed to be metabolically active, one can assume that the analogues used acted everywhere in the mycelium. Therefore, the very low ppGpp pool during the stringent response cannot be explained by a restriction of parts of the mycelium (e.g. apices) capable of ppGpp synthesis. From this we conclude that if ppGpp is indeed involved in the stringent control in *S. hygroscopicus* as it is in many other bacteria, then the target molecule should be very sensitive towards ppGpp.

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