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Structural and Functional Analysis of Ribosomal Subunits from Vegetative Mycelium and Spores of *Streptomyces antibioticus*

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The structure and function of ribosomes from spores and vegetative mycelium of *Streptomyces antibioticus* were compared. Differences were observed in the sedimentation coefficient of ribosomes from spores (56·86S) and vegetative mycelium (69·77S). Reverse-phase high-performance liquid chromatography of ribosomal proteins of the 30S and 50S subunits revealed differences which included several polypeptides present in the vegetative ribosomes but absent from spore ribosomes. The latter were also defective in their ability to promote polyphenylalanine synthesis, the functional activity of both ribosomal subunits being affected. The soluble fraction of spores also showed decreased protein-synthesizing activity, compared to that of the vegetative mycelium. Recovery of normal ribosomal subunits and soluble fraction activity occurred early in the germination process, reaching activity values approaching those of the vegetative state during initiation of germination. It is suggested that regulation of cellular metabolism at the level of translation may be involved in the establishment of spore dormancy.

INTRODUCTION

The processes of differentiation associated with bacterial sporulation and spore germination are controlled by poorly understood mechanisms. Understanding the differentiation process requires a knowledge of the machinery of macromolecular biosynthesis, since the central problem is control of the transcription of particular genes and consequent synthesis of specific proteins. In bacterial endospores, such control may be exerted during differentiation at the level of transcription (Klier *et al.*, 1973; Losick *et al.*, 1979) or at the level of translation (Guha & Szulmajster, 1977; Setlow, 1981). In *Streptomyces*, there have been several studies on the pattern of macromolecular biosynthesis during spore germination (Hardisson *et al.*, 1978, 1980; Hirsch & Ensign, 1975; Mikulik *et al.*, 1977). Protein and RNA degradation (Guijarro *et al.*, 1982, 1983), the existence of a stable mRNA fraction in spores (Hardisson *et al.*, 1980; Quirós *et al.*, 1985) and some differences between the ribosomal proteins from spores and vegetative mycelium (Mikulik *et al.*, 1984) have been reported.

In order to obtain a better understanding of the germination process, we have analysed the protein biosynthetic machinery of *S. antibioticus* spores in an attempt to discover whether regulation at the level of translation could be involved in the sporulation and spore germination processes. This paper compares the ribosomes of dormant spores and vegetative mycelium of *S. antibioticus* and shows structural and functional differences.

METHODS

Culture conditions. The micro-organism used in this study was *Streptomyces antibioticus* ATCC 11891. The conditions used for vegetative growth, sporulation and germination have been described previously (Hardisson *et al.*, 1978). Spores were scraped from the agar surface with a spatula and resuspended in ice-cold deionized

Abbreviation: RP-HPLC, reverse-phase high-performance liquid chromatography.

(Milli-Q) water. The suspension was pulsed three times (30 s each) in a 150 W ultrasonic disintegrator to eliminate residual fragments of mycelium, a treatment which had no effect on spores. The spores were washed twice with ice-cold deionized water and once with buffer A (see below). Germination was observed by phase-contrast microscopy, which allowed identification of the different stages of germination (Hardisson *et al.*, 1978).

Buffers. Buffer A: 10 mM-HEPES/KOH, pH 7·5 at 20 °C; 10 mM-$MgCl_2$; 50 mM-NH_4Cl; 3 mM-2-mercaptoethanol. Buffer B: 10 mM-HEPES/KOH, pH 7·5 at 20 °C; 30 mM-$MgCl_2$; 1 M-NH_4Cl; 3 mM-2-mercaptoethanol. Buffer C: 10 mM-HEPES/KOH, pH 7·5 at 20 °C; 1 mM-$MgCl_2$; 100 mM-KCl; 3 mM-2-mercaptoethanol. Buffer D: 10 mM-Tris/HCl, pH 8·0 at 20 °C; 6 M-urea; 150 mM-LiCl; 3 mM-2-mercaptoethanol.

Preparation of ribosome and soluble fractions. Mycelia or spores were washed twice in buffer A and resuspended in the same buffer. Approximately 1 g of glass beads (0·10–0·11 mm diameter) were added per ml of suspension and the mixture pulsed in a Braun model MSK mechanical homogenizer (five pulses of 1 min with intermittent cooling with dry-ice). More than 95% of the cells were broken by this treatment. The glass beads were removed and the extracts centrifuged at 30000 ***g*** for 30 min at 4 °C and the upper two-thirds of the supernatant retained (fraction S30). The S30 fraction was layered over an equal volume of buffer B containing 40% (w/v) sucrose and centrifuged in a Beckman rotor 42.1 at 30000 r.p.m. (100000 ***g***) overnight at 4 °C. The supernatant (designated as S100*) was dialysed at 4 °C against two changes of buffer A (500 ml each) and stored in aliquots at −70 °C. The ribosomes were resuspended in buffer A and centrifuged in an Eppendorf minifuge for 30 s to remove contaminating membranes. The ribosomes were then centrifuged through buffer B plus sucrose and, after resuspension in buffer A, stored in aliquots at −70 °C.

Preparation of ribosome subunits. Ribosomes (300 A_{260} units) were diluted into 7 ml buffer C, dialysed against buffer C for 2–3 h at 4 °C and then layered over a sucrose density gradient (10–30%, w/v, linear concentration gradient) made up in buffer C and centrifuged in a Beckman zonal rotor Z-60 at 45000 r.p.m. for 3 h at 4 °C. To recover the ribosomal subunits, buffer C containing 40% sucrose was pumped into the rotor edge with a peristaltic pump and fractions containing the 50S and 30S subunits monitored by measuring absorbance at 260 nm with a UV-1 (Pharmacia) single path monitor. The Mg^{2+} concentration in these fractions was increased to 10 mM and the 30S and 50S subunits collected by sedimentation at 30000 r.p.m. (100000 ***g***) overnight at 4 °C. The subunits were then purified by passage through a second gradient after which they were again recovered by centrifugation, resuspended in buffer A and stored in aliquots at −70 °C.

Polyuridylic-acid [poly (U)]dependent protein synthesis. In vitro protein synthesis was measured as the synthesis of polyphenylalanine directed by poly(U). The reaction mixture (total volume 100 µl) contained the following: 20 mM-HEPES/KOH, pH 7·5 at 20 °C; 10 mM-$MgCl_2$; 50 mM-NH_4Cl; 50 mM-KCl; 2·5 mM-ATP; 0·35 mM-GTP; 0·8 µg pyruvate kinase; 7·5 mM-phosphoenolpyruvate; 2·6 µCi (96·2 kBq) [^{3}H]phenylalanine (0·26 µM); 1·25 µg phe-specific tRNA; 50 µg poly(U); 25–50 pmol ribosomes; variable volume (10–30 µl) of S100*. Reaction mixtures were incubated at 30 °C and samples (10 µl), removed at various time intervals, were added to 1 ml 5% (w/v) trichloroacetic acid (TCA). After heating at 90 °C for 15 min, samples were filtered through Whatman GF/C discs (2·5 cm diameter), dried and the radioactivity estimated in a liquid-scintillation counter.

Preparation and analysis of ribosomal proteins. Proteins were extracted from ribosomes as described previously (Fierro *et al.*, 1987). Samples of ribosomal subunit proteins from dormant spores or vegetative mycelium, containing 100 µg protein in buffer D, were fractionated by reverse-phase high-performance liquid chromatography (RP-HPLC) using the method of Kerlavage *et al.* (1983) with some minor modifications (Fierro *et al.*, 1987). SDS-PAGE was done using 8–20% (w/v, acrylamide) gradient gel. The method used was based on that described by Laemmli (1970), with modifications (Fierro *et al.*, 1987). The following marker proteins (Pharmacia low molecular mass kit) were used: phosphorylase b (molecular mass 94000 kDa), bovine serum albumin (67000 kDa), ovalbumin (43000 kDa), carbonic anhydrase (30000 kDa), soybean trypsin inhibitor (20100 kDa) and β-lactalbumin (14400 kDa).

Determination of the number of ribosomes. At different stages of germination (as observed by phase-contrast microscopy) and in the exponential phase of vegetative growth, samples of the cultures were harvested by centrifugation, washed twice with deionized water and resuspended in 0·5 ml deionized water. Mycelia and spores were broken using glass beads as described by Guijarro *et al.* (1983). More than 95% of the cells were broken and the samples were centrifuged at 5000 r.p.m. for 15 min at 4 °C to remove cell walls and debris. The supernatant was then centrifuged at 40000 r.p.m. for 3 h at 4 °C. Protein and RNA concentrations in the supernatant were determined by the Lowry method and the orcinol method (Schneider, 1957), respectively. The ribosomal pellet was suspended in a small volume of deionized water and the protein and RNA content of the samples determined. The number of ribosomes per mg of cellular protein was estimated by the procedure described by Orlowski & Sypherd (1978). Certain calculations and assumptions had to be made: (i) because the combined molecular masses of *Streptomyces* rRNAs are unknown; those of *E. coli* were used (i.e. $1·55 \times 10^6$); (ii) the weight of rRNA per 70S ribosome was calculated by dividing the molecular mass of rRNA by Avogadro's number, giving a value of $2·57 \times 10^{-18}$ g per ribosome. Using these values and those calculated by RNA and protein determinations, the approximate number of ribosomes per mg of protein was calculated.

Chemicals. Pyruvate kinase, HEPES, GTP, 2-mercaptoethanol, poly(U) and phosphoenolpyruvate were from Sigma; ATP and phe-specific tRNA were from Boehringer Mannheim; KCl, NH_4Cl and $MgCl_2$ were from Merck; L-[2,3,4,5,6-^{3}H]phenylalanine [specific activity 101 Ci mmol^{-1} (3·73 TBq mmol^{-1})] was from Amersham. All other reagents were of analytical grade.

RESULTS

Isolation of ribosomes

Ribosomes isolated from the vegetative mycelium of *S. antibioticus* appeared typically as a transparent sediment after ultracentrifugation. However, spore ribosomes obtained in the same way (i.e. after washing in a high-salt buffer plus sucrose) bound a brown pigment. The pigment could not be removed from ribosomes by either successive additional washings in high-salt buffer (buffer B) or after extensive dialysis. However, pigment-free spore ribosomes could be obtained as follows: after high-salt washing the ribosomes were centrifuged in a 10–40% sucrose density gradient in the same buffer for 60 min at 45000 r.p.m. in a Beckman zonal rotor. A peak of clean and transparent ribosomes was detected while some of the ribosomes sedimented to the bottom of the tube together with the pigment, probably due to the formation of ribosomal aggregates. Interestingly, freezing at −70 °C somehow prevented removal of the pigment by this method. The identity of the pigment is not known but it absorbed in the visible range, was not soluble in organic solvents, bound more tightly to ribosomal RNA than to ribosomal proteins (i.e. was extracted together with RNA by glacial acetic acid) and did not give a positive reaction with the Folin phenol reagent.

Differences were also observed in the sedimentation coefficients of ribosomes from spores (pigment-free) and vegetative mycelium. Thus, when both types of ribosomes were centrifuged through a linear sucrose gradient, two different peaks were detected, with the one corresponding to spore ribosomes moving more slowly. Estimations of the sedimentation coefficients gave values of 69·77S (approx.) and 56·86S (approximately) for mycelial and spore ribosomes, respectively.

Changes in the absolute ribosome content during spore germination

We made some estimations of the number of ribosomes per mg of cellular protein at different stages of germination (dormant spores, dark spores and swollen spores) and in the vegetative mycelium (Table 1). Early in the germination process (dark spores), the number of ribosomes increased twofold compared to the number in dormant spores. Swollen spores gave the highest value for the number of ribosomes (about 3·5 times the value for dormant spores), which was higher than that found in the vegetative mycelium. This result is in accordance with increased biosynthetic activity during spore swelling (Hardisson *et al.*, 1978, 1980).

Translation of poly(U) by ribosomes

The ability of the ribosomes of dormant spores and vegetative mycelium of *S. antibioticus* to synthesize protein was compared by assaying their ability to incorporate [^{3}H]phenylalanine into polypeptides as directed by the synthetic messenger RNA poly(U). Early experiments (data not given) showed that pigment-bound ribosomes of dormant spores had almost negligible activity. However, when we used pigment-free ribosomes from dormant spores, we could detect protein-synthesizing activity which represented about 24% of that observed with vegetative mycelium ribosomes (Tables 2). The protein-synthesizing activity of the ribosomes of both dormant spores and vegetative mycelium was proportional to the ribosome concentration in the range 10 to 50 pmols of ribosomes. The low activity detected with spore ribosomes was only observed using the soluble fraction (S100*) of the mycelium, with virtually no activity when the spore S100* was used. Similarly, mycelial 70S ribosomes were only active when combined with homologous S100* and were unable to synthesize polyphenylalanine in the presence of spore S100* (Table 2). These results suggest that dormant spores of *S. antibioticus* have both ribosome and S100* fractions that are defective in their ability to translate poly(U).

To determine whether or not the low ribosomal activity was subunit-mediated, the activity of

Table 1. *Number of ribosomes present during spore germination and vegetative growth*

The values were obtained as described in Methods. Each value in the Table is the mean of six independent determinations.

Stage	10^{-13} × No. of ribosomes per mg of protein
Dormant spores	8·14
Dark spores	15·12
Swollen spores	29·09
Mycelium	22·59

Table 2. *Poly(U)-dependent protein-synthesis activity of the soluble fractions of spores and mycelium*

Each assay contained 50 pmol of ribosomes. Values are the mean of three independent determinations ± SD for the incubation for incorporation of [^{3}H]phenylalanine into TCA-insoluble material after incubation for 45 min at 30 °C.

Source of: S100*	Source of: 70S	10^3 × [^{3}H]Phenylalanine incorporation (pmol)
Mycelium	Mycelium	142 ± 70
Mycelium	Spores	34 ± 20
Spores	Mycelium	5 ± 1
Spores	Spores	2 ± 1

the system was tested using purified ribosomal subunits of dormant spores and mycelium in homologous and heterologous combinations. Subunits were separated in a sucrose-density gradient with a low Mg^{2+} concentration and repurified through a second gradient. In either case (30S and 50S subunits), no contamination by the other subunit was detected and this was confirmed by the absence of activity of each separated subunit preparation (data not shown). Homologous re-association experiments were done with these subunits and it was observed that, while the total initial 70S ribosomal activity was recovered when mycelial ribosomal subunits were mixed, only about 50% recovery was obtained with spore ribosomal subunits (Table 3). When re-association was done in a heterologous manner, the results showed (Table 3) that both ribosomal subunits of dormant spores contributed to the decreased activity of spore ribosomes. However, it was the 50S subunit which gave the lowest values of activity in heterologous combinations.

We tested the possibility that during spore germination, the normal activity of the protein-synthesizing system was recovered. The S100* fraction and ribosomes were obtained from dormant and germinating spores (15 and 60 min incubation) and their ability to synthesize polyphenylalanine determined. After 15 min of germination, the activity of the translation system clearly increased, and much of the vegetative activity (approximately 70%) was recovered after about 60 min of germination (Table 4).

In order to determine if the recovery in activity was due to the soluble fraction or to the ribosomes (or both), experiments were done in which heterologous combinations were made between the soluble fractions and ribosomes. The results showed that approximately 90% of the normal activity of the ribosomes and of the soluble fraction was recovered after 60 min of germination (Table 4).

Analysis of the ribosomal proteins of dormant spores and vegetative mycelium

In an attempt to explain the functional changes accompanying the transition from dormant spores into vegetative cells, we investigated the protein composition of both types of ribosomes to detect possible structural changes. The simple analysis by SDS-PAGE of purified 50S and 30S ribosomal subunits clearly showed some of the major differences between subunits (Fig. 2, lanes

Table 3. *Poly(U)-dependent protein synthesis activity of reconstituted ribosomes*

The assays contained 50 pmol of 70S ribosomes or of each ribosomal subunit and the soluble fraction (S100*) was that from vegetative mycelium. Values represent the mean of three independent determinations ± SD for the incorporation of [^{3}H]phenylalanine into TCA-insoluble material after incubation for 45 min at 30 °C.

Source of subunits:			10^3 × [^{3}H]phenylalanine
30S	50S	70S	incorporation (pmol)
–	–	Mycelium	1350 ± 60
–	–	Spores	270 ± 10
Mycelium	Mycelium	–	1510 ± 60
Mycelium	Spores	–	270 ± 20
Spores	Mycelium	–	780 ± 40
Spores	Spores	–	140 ± 8

Table 4. *Protein-synthesis activity of the soluble fraction and 70S ribosomes during spore germination and vegetative growth*

Each assay contained 50 pmol of ribosomes. Values represent the mean of three independent determinations ± SD for the incorporation of [^{3}H]phenylalanine into TCA-insoluble material after incubation for 45 min at 30 °C. Abbreviations: DS, dormant spores; VM, vegetative mycelium; GS (15), germinating spores, 15 min of germination; GS (60), germinating spores, 60 min of germination.

S100*	Ribosomes	10^3 × [^{3}H]Phenylalanine incorporation (pmol)
DS	DS	2 ± 0·06
VM	DS	310 ± 10
DS	VM	2 ± 0·02
GS (15)	GS (15)	100 ± 7
VM	GS (15)	630 ± 20
GS (15)	VM	550 ± 30
GS (60)	GS (60)	1010 ± 50
VM	GS (60)	1210 ± 40
GS (60)	VM	1280 ± 40
VM	VM	1420 ± 80

1 and 2; Fig. 4, lanes 1 and 2). Several protein bands observed in the ribosome subunits from mycelium were absent in the homologous subunit from spores. In addition, some changes in band intensity were observed and proteins occasionally detected in spores were absent in vegetative mycelium. The RP-HPLC analysis of purified ribosome subunits from spores and mycelium gave a clearer picture of the differences. When the protein pattern of the 50S ribosome subunits from spore and mycelial ribosomes was compared (Fig. 1*a* and 1*b*, respectively), at least nine different peaks which were only present or were larger in the mycelial 50S subunit were detected (Fig. 1*a*, peaks A to I). In contrast, only one peak present in the 50S subunit from spores was absent from the homologous mycelial subunit (Fig. 1*b*, peak J) and found to be a single protein species (Fig. 2, lane J). The differential peaks were analysed by SDS-PAGE and showed a single protein band (Fig. 2, lanes A, C, D, E, H, I and J), or two different proteins (Fig. 2, lanes B, F and G). It must be emphasized that similar amounts of protein (100 μg) were applied to the RP-HPLC column and the SDS-PAGE gels and differences in peak height can therefore not be attributed to variations in the amount of protein analysed.

The interpretation of results obtained by RP-HPLC analysis of spore and mycelial 30S subunits (Fig. 3*a* and 3*b*, respectively) is more complex. Three peaks present in the mycelial 30S subunits (Fig. 3*a*, peaks L, M and Q) were absent from the homologous spore subunit and, when analysed by SDS-PAGE, were each found to contain a unique protein species (Fig. 4, lanes L, M and Q). In contrast, only one peak present in the 30S subunit from spores (Fig. 3, peak V) was

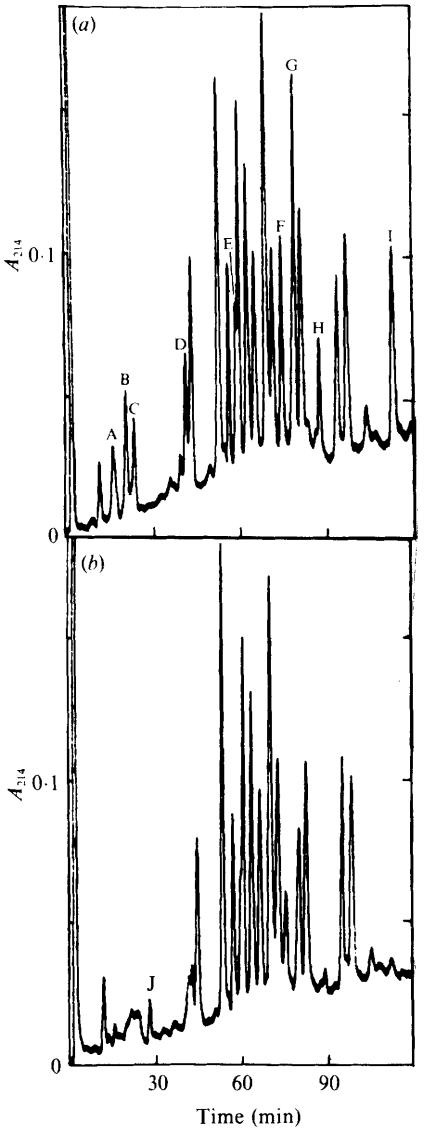

Fig. 1

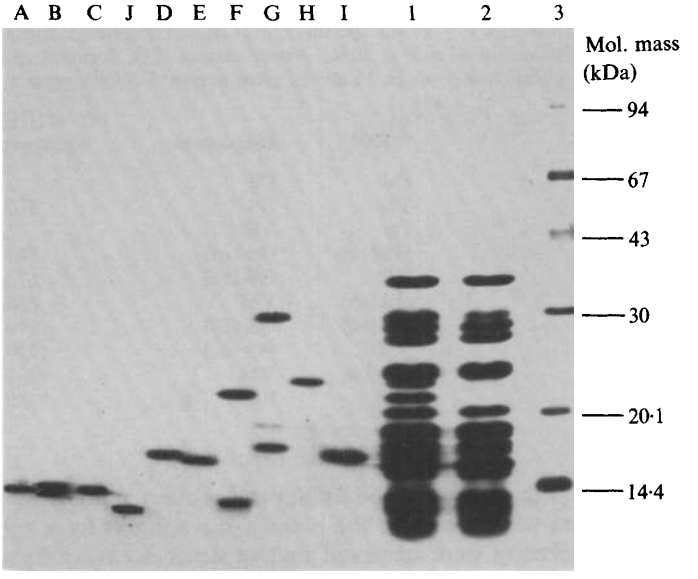

Fig. 2

Fig. 1. RP-HPLC chromatograms of 50S ribosomal subunit proteins. (*a*) Vegetative mycelium; (*b*) spores. Peaks labelled A to I were only present or larger in the vegetative 50S subunits whereas peak J was only detected in the spore subunits. The chromatographic conditions are described in Methods.

Fig. 2. SDS-PAGE of differential 50S ribosomal subunit proteins from Fig. 1. Freeze-dried RP-HPLC fractions or total 50S proteins were analysed in an 8–20% (w/v) polyacrylamide gradient as described in Methods. Lanes A to I in the gel correspond to the same peaks in Fig. 1. Lanes 1 and 2, total 50S ribosomal proteins from vegetative mycelium and spores, respectively; lane 3, molecular mass markers (see Methods).

not detected in vegetative ribosomes and only one single protein species was present (Fig. 4, lane V). The remaining differences in the RP-HPLC patterns were changes in peak height, which can be mostly explained by the presence of extra proteins in the mycelial subunits under the same chromatographic peak (Fig. 4, lanes O and P) or differences in the concentration of individual proteins (Fig. 4, lanes K and S).

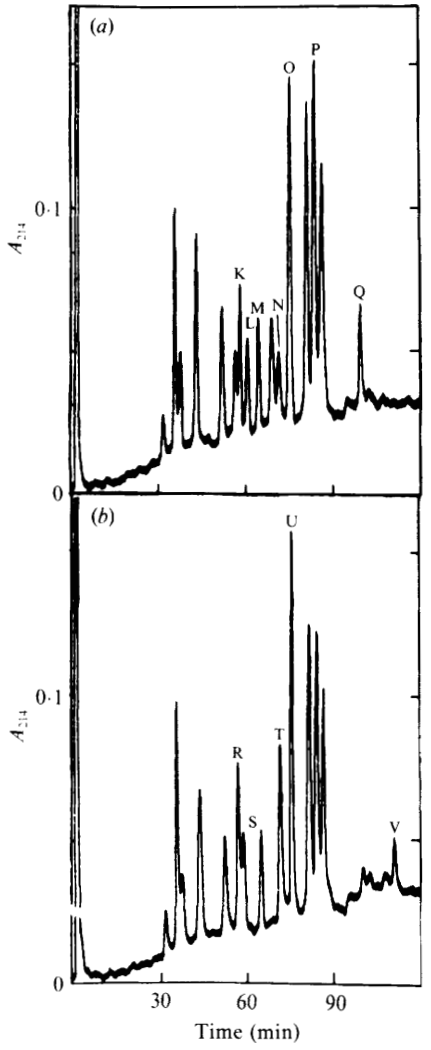

Fig. 3

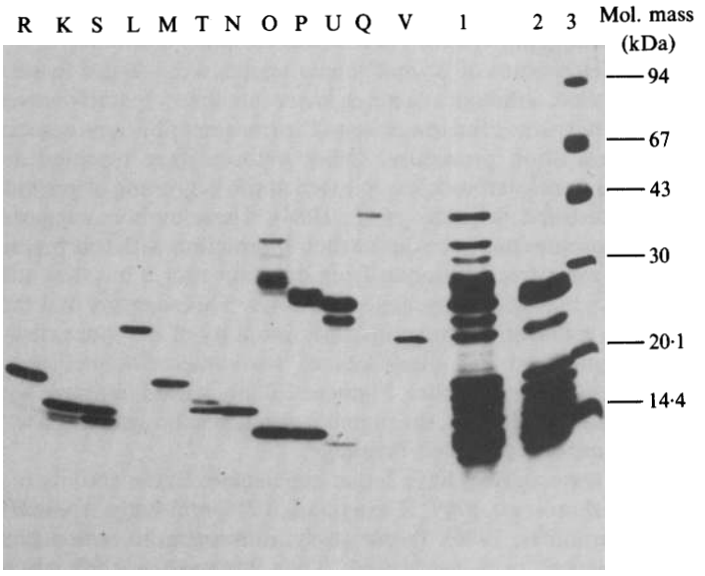

Fig. 4

Fig. 3. RP-HPLC chromatograms of 30S ribosomal subunit proteins. (*a*) Vegetative mycelium; (*b*) spores. Peaks labelled K to Q (vegetative subunits) and R to V (spore subunits) show the differences between the subunits.

Fig. 4. SDS-PAGE of differential 30S ribosomal subunit proteins from Fig. 3. Freeze-dried RP-HPLC fractions or total 30S proteins were analysed in an 8–20% polyacrylamide gradient as described in Methods. Lanes R to V in the gel correspond to the same peaks in Fig. 3. Lanes 1 and 2, total 30S ribosomal proteins from vegetative mycelium and spores, respectively; lane 3, molecular mass markers (see Methods).

In order to exclude the possibility that proteolysis could be responsible for these structural differences, the following experiments were done. Firstly, ribosomes from both developmental stages were isolated in the presence of protease inhibitors (*o*-phenanthroline, PMSF and EDTA, all at 1 mM final concentration) throughout the isolation procedure. These three compounds inhibit more than 95% of the spore protease activity (Guijarro *et al.*, 1983). No significant differences (data not shown) were detected in the presence or absence of these protease inhibitors. In the second control experiments, spores and vegetative mycelium were mixed at a 1 : 1 dry weight ratio and ribosomes isolated and analysed by RP-HPLC. If observed differences

between spores and vegetative mycelium were caused by proteolysis, it would be expected that this 'mixed ribosomal population' would give the RP-HPLC protein pattern of spore ribosomes due to spore proteases acting on the mycelial ribosomes proteins. Addition of both types of patterns was obtained, and similar results were obtained with purified ribosomal subunits (data not shown). Therefore, we can conclude that the structural differences detected between spore and mycelium ribosomes are not due to proteolysis during the isolation of ribosomes.

DISCUSSION

The initiation of protein biosynthesis during spore germination of different *Streptomyces* species differs with respect to the lag period before biosynthesis begins. In most of the species studied, the lag period was short (Ensign, 1978; Hardisson *et al.*, 1980; Mikulik *et al.*, 1977, 1984) but in one case there was a long delay before synthesis of macromolecules commenced (Nagatsu & Matsuyama, 1970). In *S. antibioticus*, protein biosynthesis was detected after 5 min of germination and the rate increased rapidly in the first hour of germination (Hardisson *et al.*, 1980). The study reported here aimed to determine whether some defects in the spore protein-synthesizing system contributed to spore dormancy.

Ribosomes of *S. antibioticus* spores were found to be capable of synthesizing polyphenylalanine, although at a much lower rate than vegetative mycelium ribosomes. The spore ribosome activity was even lower when the ribosomes became associated with a brown pigment during the purification procedure. Other authors have reported a similar dark pigment bound to *S. granaticolor* ribosomes, released at the beginning of germination when normal ribosome activity is restored (Mikulik *et al.*, 1984). These authors suggested that the low activity of the spore ribosomes might be due to their interaction with this pigment. In *S. antibioticus*, we could obtain pigment-free ribosomes from dormant spores but they still showed low activity in comparison with that of the mycelial ribosomes. This suggests that the low ribosomal activity is not due to the pigment but to an intrinsic property of the spore ribosomes. We have also observed bound pigment in the ribosomes of two other *Streptomyces* species (*S. griseus* and *S. scabies*) (unpublished results). Pigment colours varied but were similar to the colour of harvested intact spores. Therefore, the pigment appears to be associated with the spore integuments and binds to ribosomes after cell breakage.

Some authors have found deficiencies in the activity of the ribosomes of *Bacillus* endospores (Bishop *et al.*, 1969; Kieras *et al.*, 1978) and fungal spores (Horikoshi & Ikeda, 1968; Jaworski & Stumhofer, 1984). In our study, differences in both structure and function of ribosomes were observed in *S. antibioticus*. Thus, high-salt washed ribosomes of dormant spores exhibited a different sedimentation coefficient than vegetative ribosomes, probably explained by the detection by RP-HPLC and SDS-PAGE of several differences in the ribosomal protein patterns. The involvement of rRNA breakdown in the low spore ribosomal activity can not be completely excluded but because we were able to isolate undegraded rRNA from both developmental stages (data not shown), gross degradation of rRNA cannot be main cause of ribosome inactivity. The differences observed in protein patterns were mainly caused by the presence of polypeptides in the vegetative ribosomes which were absent in the spore ribosomes. The results of control experiments excluded the possibility that such differences are due to proteolysis. Interestingly, when spore ribosome subunits were reconstituted, recovery of protein-synthesizing activity was only approximately 50%, whereas all of the mycelial ribosome activity could be recovered in this way. This suggests that some of the proteins absent from spore subunits are located in regions of interaction between the subunits.

Heterologous cross-over experiments between spore and mycelium ribosome subunits also showed differences in the activity of the 30S and 50S spore ribosomal subunits. However, the contribution of the large subunit to the low activity was greater, suggesting that the ribosomal functions present in this subunit are more affected. Specific alteration of the 30S ribosomal subunit has also been reported during sporulation of *Bacillus subtilis* (Guha & Szulmajster, 1977). Two different hypotheses may explain the low activity of spore ribosomes. (i) A fraction of the spore ribosomes could be totally inactive with the remainder being completely functional. (ii)

The entire spore ribosomal population could be defective to a similar extent. We believe that the latter possibility is more plausible, since if only a fraction of the spore ribosomes was defective, a complete set of ribosomal proteins would have been detected in the RP-HPLC and SDS-PAGE analysis. Furthermore, this would imply that only quantitative differences would be observed while our data showed both qualitative and quantitative variations in the protein patterns of the 30S and 50S subunits between both developmental stages.

We have also shown that the soluble fraction (S100*) of *S. antibioticus* spores possessed a decreased protein-synthesizing activity when combined with ribosomes either from spores or from vegetative mycelium. Whether this defect is due to aminoacyl-tRNA synthetases or to elongation factors will require further studies.

Recovery of the normal activity of the ribosomal subunits and the soluble fraction occurs early in the germination process. We do not know if this recovery in the ribosomal activity is due to the presence of newly synthesized ribosomes or to the reactivation of pre-existing ribosomes, through biosynthesis of the missing proteins and their incorporation into the defective ribosomes. The structural and functional differences in ribosomes suggest that transition from the dormant to the vegetative state is a very complex process.

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