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Studies on the Role of Polyamines Associated with the Ribosomes from *Bacillus stearothermophilus*

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1. Spermine and spermidine were the main polyamines detectable in *Bacillus stearothermophilus*. 2. When grown at 65° *B. stearothermophilus* contained lower concentrations of polyamines per mg. of RNA than when grown at 45° or at 55°. 3. Ribosomes isolated from *B. stearothermophilus* in 0·01 M-tris–hydrochloric acid buffer (pH 7·4)–0·01 M-magnesium chloride contained sufficient polyamines to neutralize between 4% and 9% of their RNA phosphorus. 4. Removal of polyamines from the ribosomes by dialysis against M-potassium chloride did not appreciably alter the hypochromicity or thermal denaturation profiles of the ribosomes when measured in 0·01 M-tris–hydrochloric acid buffer (pH 7·4)–0·01 M-magnesium chloride, though it did cause a loss of ribosome particles sedimenting at greater than 78 S. 5. When ribosomes were dialysed against acridine orange solutions acridine orange bound to the ribosomes and did not displace spermine, but when a mixture of ribosomal RNA and spermine was dialysed against acridine orange the acridine orange displaced the spermine. It is concluded that polyamines in the ribosomes are less accessible for displacement by acridine orange than when polyamines are bound to ribosomal RNA.

Several studies have been made on the effects of polyamines on the physical properties of ribosomes. From these it is clear that polyamines can act in a similar fashion to Mg^{2+} in causing the aggregation of isolated ribosome sub-units and that they can partly replace Mg^{2+} in 70 S ribosomes (Cohen & Lichtenstein, 1960; Silman, Artman & Engelberg, 1965; Moller & Kim, 1965), and also that they can alter their thermal denaturation profiles (Mangiantini, Tecce, Toschi & Trentalance, 1965; Friedman, Axel & Weinstein, 1967). Whether polyamines have an essential role in maintaining the structure of ribosomes *in vivo*, or whether they simply act in substituting for Mg^{2+} *in vitro*, has not been ascertained. Hurwitz & Rosano (1967) have shown that the amounts of polyamines bound to ribosomes of *Escherichia coli* vary inversely with the concentration of Mg^{2+} in the growth medium, which suggests that Mg^{2+} and polyamines may have similar functions. The presence of endogenous polyamines in preparations of purified ribosomes has often been overlooked during studies of the effects of adding polyamines to such preparations. Therefore ribosomes from *Bacillus stearothermophilus* were isolated and conditions were found under which the endogenous polyamines may be removed. Then a comparison was made between the properties of these ribosomes and (*a*) those from which polyamines had not been removed and (*b*) those from which polyamines had been removed and had been subsequently replaced.

Polyamines are known to stabilize nucleic acids against thermal denaturation (Tabor, 1962; Friedman *et al.* 1967) and thus they could conceivably have a special role in thermophilic organisms. *B. stearothermophilus* can grow at temperatures between 37° and 65° and is therefore a convenient organism in which to study whether polyamines can influence the thermal stability of ribosomes. When *B. stearothermophilus* is grown at 65° its ribosomes have a higher thermal stability than those from mesophilic organisms. It appears that this difference cannot be accounted for by the presence of either different ribosomal proteins or of RNA having a much higher guanine plus cytosine content (Saunders & Campbell, 1966; Pace & Campbell, 1967), and it seemed possible that polyamines (Friedman *et al.* 1967) might be involved. The polyamine content of *B. stearothermophilus* grown at different temperatures was measured, and the thermal denaturation profiles of their ribosomes were examined.

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MATERIALS AND METHODS

Chemicals. Spermine tetrahydrochloride, spermidine trihydrochloride and tris were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Acridine orange, purchased from British Drug Houses Ltd. (Poole, Dorset), was purified by the method of Lamm & Neville (1965). Other chemicals were A.R. grade where available.

Organisms and growth media. Bacillus stearothermophilus strain 8923 was obtained from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen. It was grown with aeration on medium consisting of 10·0g. of tryptone, 5·0g. of yeast extract, 2·5g. of K_2HPO_4, 5·0mg. of $MgSO_4,7H_2O$, 5·0mg. of $CaCl_2,2H_2O$, 5·0mg. of $FeCl_3,5H_2O$ and 5·0g. of glucose/l. The temperature was 55° unless otherwise stated. *Escherichia coli* type B was grown at a temperature of 37° in a medium containing glucose, amino acids and salts (Monod, Cohen-Bazire & Cohn, 1951).

Isolation of ribosomes. Cells were harvested in the exponential phase, washed three times with 100vol. of TM buffer* and then suspended in 4vol. of the same buffer. The cells were treated ultrasonically for 3min. with a Dawes Soniprobe type 1130A (setting 5, 2·5A). The cell debris was removed by two 15min. centrifugations at 25000*g*. The ribosomes were pelleted by spinning at 105000*g* for 3hr. Ribosomal RNA was extracted from ribosomes with guanidinium chloride (Cox, 1966).

Determination of polyamines, RNA and phosphate. Polyamines were extracted in cold 0·5N-$HClO_4$, separated by paper electrophoresis and stained with ninhydrin (Raina & Cohen, 1966). RNA was extracted in 0·5N-$HClO_4$ for 10min. at 75° and determined as described by Ceriotti (1954). Phosphate was determined as described by Ames & Dubin (1960).

Hypochromicity of ribosomes. A 0·8ml. volume of 0·25N-KOH was added to 0·02ml. of ribosome suspension and incubated at 37° for 24hr. The sample was then neutralized with 0·2ml. of N-HCl and diluted to a final volume of 3ml. A 0·02ml. sample of ribosome suspension was diluted with 0·8ml. of 0·25M-KCl and then further diluted with water to a volume of 3ml. The E_{260} value of the KOH-treated ribosomes was compared with that of the control.

Spectrophotometric titrations with acridine orange. These were carried out as described by Furano, Bradley & Childers (1966). A 0·18ml. volume of mM-acridine orange in mM-sodium cacodylate buffer, pH 6·7, was diluted with 2·6ml. of mM-sodium cacodylate buffer, pH 6·7. The E_{504} value was measured in a Hilger Uvispek spectrophotometer. Samples of the ribosome suspension were added, the whole was thoroughly mixed and the E_{504} value was measured. The total volume of ribosome suspension added never exceeded 0·17ml., so that the acridine orange was not diluted by more than 7% during the titration. The change in the absorption spectrum during the titration was checked in a Unicam SP.800 spectrophotometer and was found to agree with that described by Furano *et al.* (1966).

Dialysis. All experiments involving dialysis were performed at 4° with 8/32in. Visking tubing that had been previously washed with EDTA as described by Tashiro & Siekevitz (1965). In experiments designed to study the effect of varying the ionic strength of the dialysing medium on the removal of polyamines from the ribosomes, 1ml. suspensions of ribosomes in TM buffer were dialysed against three changes (200ml. each) of the appropriate salt solutions for 24hr. To study whether acridine orange could displace polyamines attached to ribosomes or to ribosomal RNA, 1ml. samples of ribosomal suspensions or of ribosomal RNA solutions were dialysed against 200ml. of mM-sodium cacodylate buffer, pH 6·7, containing 15μmoles of acridine orange. The uptake of acridine orange into the dialysis sac was estimated by sampling the diffusate and measuring its E_{504} value. To correct for the adsorption of acridine orange on the dialysis tubing and on the glass walls of the containers, a series of dialysis sacs containing 1ml. of TM buffer were each dialysed against 200ml. of mM-sodium cacodylate containing 5–15μmoles of acridine orange. The diffusates were sampled in these controls at the same times as in the test samples. A correction was made by subtracting from each test sample the amount of acridine orange that attached to the tubing and walls at its final concentration of free acridine orange. This correction was about 5% of the amount of acridine orange that bound to the ribosomes or RNA.

Thermal denaturation profiles of ribosomes. These were measured in a Unicam SP.800 spectrophotometer as described by Stevens (1967). Each sample was equilibrated for 30min. at 54° or 60° and then the temperature was raised by 2° increments to 86°.

Sedimentation velocities. Sedimentation velocities were performed in a Spinco model E analytical ultracentrifuge run at 39460rev./min. at 20±2°. All runs were in a 12mm. standard cell fitted with a Kel-F centrepiece. The boundaries were followed with a schlieren optical system, photographs being taken at 4min. intervals.

RESULTS

B. stearothermophilus was grown at 45°, 55° and 65° to test whether the organism adapts itself to growing at higher temperatures by producing higher concentrations of polyamines, and whether the ribosomes in cells grown at the higher temperatures show greater thermal stability. The polyamine concentrations in whole cells and in isolated ribosomes are shown in Table 1 and the thermal denaturation profiles of ribosomes are shown in Fig. 1. Results for *E. coli* are included for comparison. There is a substantial difference in the growth rates of *B. stearothermophilus* grown at 45° (generation time 165min.) and 65° (generation time 30min.). The polyamine concentration is slightly lower for cells grown at 65° than at either 45° or 55°. The ribosomes from cells grown at 45° appear slightly less heat-stable than those from cells grown at 55° or 65°, though more stable than ribosomes from *E. coli*. Though the ribosomes of *B. stearothermophilus* are slightly more heat-stable when

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* Abbreviations: TM buffer, 0·01M-tris–HCl buffer (pH 7·4)–0·01M-$MgCl_2$; $d\epsilon_{504}/d(P/D)$, the change in ϵ_{504} of acridine orange when bound to polymer (P represents molar RNA concentration expressed in terms of nucleotide residues and D the molar concentration of acridine orange, i.e. the same nomenclature as adopted by Furano *et al.* 1966).

Table 1. *Polyamine concentrations in* B. stearothermophilus *and* E. coli

Cells were harvested in the exponential phase. Polyamines and RNA were determined as described in the Materials and Methods section. Ribosomes were isolated as described in the Materials and Methods section. Putrescine was not detectable in *B. stearothermophilus* and spermine was not detectable in *E. coli*; they would have been detectable if present to the extent of 2% or more of the total polyamines.

	Polyamines in whole cells (μmoles/m-mole of RNA phosphate)			Polyamines in ribosomes (μmoles/m-mole of RNA phosphate)		
Growth temp.	Putrescine	Spermidine	Spermine	Putrescine	Spermidine	Spermine
B. stearothermophilus						
45°	—	14·0	22·0	—	3·8	6·0
		18·0	29·0			
		22·0	29·0			
55	—	12·0	30·0	—	8·2	12·1
		22·0	31·0			
65	—	9·0	11·0	—	2·2	21·0
		4·0	18·0			
		8·6	23·0			
E. coli						
37°	54·0	1·1	—	1·3	1·0	—
	53·0	1·3				

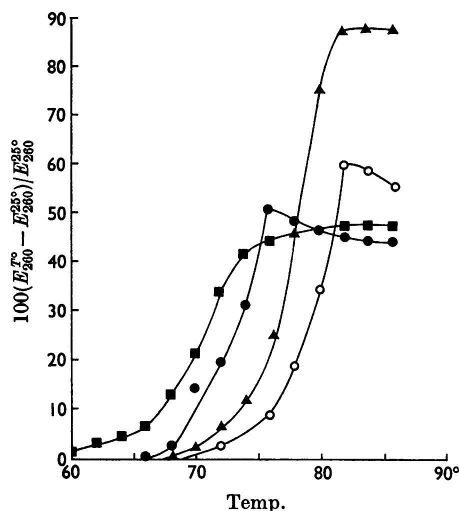

Fig. 1. Thermal denaturation profiles of ribosomes from *B. stearothermophilus* and *E. coli*. *B. stearothermophilus* ribosomes: ●, grown at 45°; ○, grown at 55°; ▲, grown at 65°. *E. coli* ribosomes: ■, grown at 37°. All ribosomes were undialysed preparations suspended in TM buffer.

synthesized at higher temperatures, it does not appear necessary for cells to produce higher concentrations of polyamines whether for stabilizing ribosomes or for other purposes.

The polyamine content of the isolated ribosomes is probably significantly different from the concentration present in ribosomes *in vivo* as the former depends very much on the isolation procedure (Tabor & Kellog, 1967). It is noteworthy, however, that when ribosomes are isolated under the same conditions those from cells grown at 65° retain a higher proportion of the total polyamines. It should also be noted that *E. coli* retains a much lower proportion of its polyamines in the isolated ribosomes, presumably because of the weaker binding by putrescine as compared with spermidine and spermine. There are sufficient polyamine amino groups in the ribosomes isolated from *B. stearothermophilus* to neutralize between 4% and 9% of their ribosomal RNA phosphates.

To remove polyamines from the ribosomes they were dialysed against buffered sodium chloride solutions of different concentrations and the polyamines that remained non-diffusible were measured. Table 2 shows the loss of polyamines with increasing ionic strength, and it is seen that spermidine loss occurs at lower ionic strength than that of spermine. To remove polyamines completely M-sodium chloride was required. In later experiments sodium chloride was replaced by potassium chloride, since K^+ seems to be the predominant intracellular cation in bacteria (Lubin & Kessel, 1960; Lubin & Ennis, 1964). M-Potassium chloride was also found to remove all the polyamines. The polyamines were completely removed from the ribosomes in subsequent experiments by the following procedure. Ribosomes, suspended in TM buffer, were dialysed against three changes (300 vol. each) of M-potassium chloride–0·1 M-tris–hydrochloric acid buffer, pH 7·6, unless otherwise stated, and then dialysed against two changes (300 vol. each) of TM buffer. The

Table 2. *Loss of polyamines from the ribosomes of* B. stearothermophilus *by dialysis*

The undialysed ribosomes contained 6·5 μmoles of spermidine and 16·3 μmoles of spermine/m-mole of RNA phosphate. Dialysis was carried out at 4° with three changes (200 vol. each) of medium for 24 hr.

Dialysing medium	I	Loss of spermidine (%)	Loss of spermine (%)
mM-$MgCl_2$–mM-tris–HCl, pH 7·6	0·0032	68	20
0·01 M-NaCl–0·01 M-$MgCl_2$–0·01 M-tris–HCl, pH 7·6	0·042	69	26
0·1 M-NaCl–0·01 M-$MgCl_2$–0·01 M-tris–HCl, pH 7·6	0·132	100	85
1·0 M-NaCl–0·01 M-$MgCl_2$–0·01 M-tris–HCl, pH 7·6	1·032	100	100

Table 3. *Certain physical properties of ribosomes and ribosomal RNA from* B. stearothermophilus *and the effects of dialysis on these properties*

Undialysed ribosomes and ribosomal RNA were suspended in TM buffer. Dialysis was performed as described in the Results section. The S values (uncorrected) and the percentage hypochromicity were measured as described in the Materials and Methods section. Acridine orange titrations were performed as described in the Materials and Methods section. The S values underlined are the principal peaks, others being present only in trace amounts. For explanation of $d\epsilon_{504}/d(P/D)$ see Fig. 4 and the Results section. Hypochromicities and $d\epsilon_{504}/d(P/D)$ are given as means ± S.D. of the numbers of determinations in parentheses.

First dialysing medium	Second dialysing medium	S (s)	Hypochromicity (%)	Acridine orange titration [$d\epsilon_{504}/d(P/D)$]
Undialysed ribosomes				
—	—	$\underline{112}$ $\underline{95}$ $\underline{77}$ 56 26	40·3 ± 1·8 (5)	375 ± 16 (6)
Dialysed ribosomes				
0·01 M-KCl–mM-tris–HCl, pH 7·6	TM buffer	$\underline{112}$ $\underline{95}$ $\underline{74}$ 57 26	38·6 ± 1·5 (5)	450 ± 38 (6)
1·0 M-KCl–0·1 M-tris–HCl, pH 7·6	TM buffer	108 $\underline{78}$ $\underline{56}$ 36	40·4 ± 1·5 (5)	768 ± 35 (6)
1·0 M-KCl–0·1 M-tris–HCl, pH 7·6	TM buffer containing 10 μM-spermidine and 10 μM-spermine	93 $\underline{74}$ $\underline{56}$ 36	37·0 ± 0·7 (5)	650 ± 51 (6)
1·0 M-KCl–0·1 M-tris–HCl, pH 7·6	TM buffer containing 20 μM-spermidine and 20 μM-spermine	116 $\underline{74}$ $\underline{55}$ 34		735 ± 54 (6)
Ribosomal RNA				
—	—	$\underline{27}$ $\underline{18}$	47·0	1380

composition of the second dialysing medium was sometimes varied to include spermidine and spermine to study the effects of restoring polyamines to the ribosomes.

The effects of removal of polyamines from the ribosomes, and their subsequent replacement, on the S values, hypochromicity and acridine orange titrations are shown in Table 3, and the effects on the thermal denaturation profiles of the ribosomes are shown in Fig. 2. If M-potassium chloride is used as the first dialysing medium it causes the disappearance of ribosome particles sedimenting faster than 78 s, whereas if 0·01 M-potassium chloride is used, a treatment that does not remove polyamines, then particles sedimenting at 78 s or greater predominate. However, when polyamines are restored to the ribosomes in the second dialysing medium significant amounts of the larger ribosome particles do not reappear; on the other hand, it cannot be concluded that the disappearance of large ribosome particles is due to the specific removal of polyamines. It was found that if the concentrations of spermidine and spermine in the second dialysing medium were increased above 20 μM, or the magnesium chloride concentrations above 10 mM, this caused precipitation of the ribosomes, leaving only a small proportion in solution, and these sedimented at 73 s, 56 s and 39 s.

The hypochromicity is not significantly different in any of the preparations (Table 3), and the thermal denaturation profiles are not substantially altered, though there is sometimes variation in the hyperchromic effect on heating (Fig. 2). The 'melting' of the ribosome structure is not a reversible phenomenon and it is questionable what factors account for the hyperchromic effect. Elson (1959) suggests that the hyperchromic effect is due to the release of a latent ribonuclease, which then hydrolyses ribosomal RNA. On the other hand, it could be mainly due to the disruption of the linkages

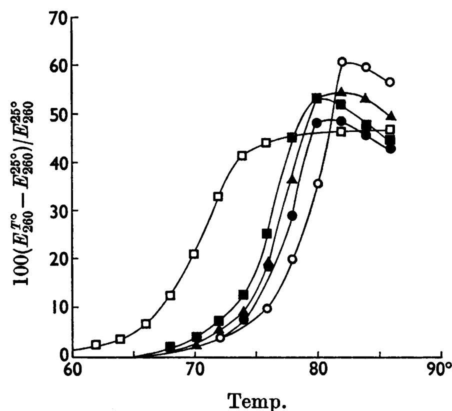

Fig. 2. Thermal denaturation profiles from *B. stearothermophilus* and *E. coli*. *B. stearothermophilus* ribosomes: ○, undialysed; ●, dialysed against 0·01 M-KCl–mM-tris–HCl, pH 7·6, and then into TM buffer; ▲, dialysed against 1·0 M-KCl–0·1 M-tris–HCl, pH 7·6, and then into TM buffer; ■, dialysed against 1·0 M-KCl–0·1 M-tris–HCl, pH 7·6, and then into TM buffer containing 10 μM-spermidine and 10 μM-spermine. *E. coli* ribosomes: □, undialysed.

between RNA and protein and subsequent denaturation of the protein with or without the complete 'melting' of the RNA. A combination of these could account for the effect, and the extent of each could vary with the species of ribosomes.

An attempt was made to ascertain whether any of the possibilities considered above was being measured in ribosomes of *B. stearothermophilus*. Comparison of the spectra of the native and denatured ribosomes (Fig. 3) shows that there is considerable light-scattering in the heated ribosomes as judged by the absorption at 320 mμ. Two methods were used to assess the extent of hydrolysis of RNA during the measurement of the hyperchromic effect. The first was to measure the proportion of material absorbing at 260 mμ that was not precipitated by addition of 25% perchloric acid and 0·75% uranyl acetate both before and after heating the ribosomes, and the second was to measure the loss of diffusible material absorbing at 260 mμ before and after heating (Table 4). There is good agreement between the results obtained by the two methods. When ribosomes are heated there is an increase of 0·37 E_{260} unit for material that is soluble in perchlorate–uranyl acetate. Complete hydrolysis of the ribosomal RNA to acid-soluble nucleotides would give rise to a much greater increase than 0·37 E_{260} unit, since most of the absorption at 260 mμ of native ribosomes is due to RNA, which has a hypochromicity of 47% (Table 3). Thus protein denaturation and partial hydrolysis of RNA contribute to the hyperchromic effect,

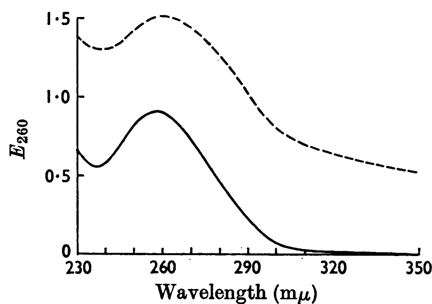

Fig. 3. Absorption spectrum of native (——) and heat-denatured (----) ribosomes from *B. stearothermophilus*. Ribosomes were suspended in TM buffer at a concentration of about 60 μg. of RNA/ml. Denatured ribosomes were heated to 86° as described in the Materials and Methods section, and their absorption spectrum at that temperature was plotted.

Table 4. *Measurement of degradation of ribosomal RNA on heating intact ribosomes from* B. stearothermophilus

Duplicate 3 ml. samples of ribosomes, diluted with TM buffer to give E_{260} 1·0, were heated to 86° under the same conditions as described for thermal denaturation profiles. The absorption of soluble nucleotides was determined in the first sample after removal of RNA and protein by precipitation with 0·5 ml. of 25% (v/v) $HClO_4$–0·75% uranyl acetate. The absorption of non-diffusible material was measured in the second sample after dialysis against 500 ml. of TM buffer for 18 hr.

Unheated ribosomes	E_{260}
Total absorbing material	1·0
$HClO_4$–uranyl acetate-soluble material	0·07
Non-diffusible material	0·93
Heated ribosomes	
Total absorbing material after heating to 86°	1·44
Total absorbing material after heating to 86° and cooling to 25°	1·35
$HClO_4$–uranyl acetate-soluble material	0·44
Non-diffusible material	0·84

though it is not possible to say if either is the initiating effect. It is noteworthy (Table 4) that there is little reversal of the hyperchromic effect on cooling denatured ribosomes.

Titrations with acridine orange are fully described by Furano *et al.* (1966). A typical titration curve obtained with undialysed ribosomes is shown in Fig. 4. The $\epsilon_{540\,(min.)}$ corresponds to the maximum uptake of acridine orange by ribosomes and the positive slope, $d\epsilon_{504}/d(P/D)$, is the region where there is excess of ribosome phosphate as compared with acridine orange. Furano *et al.* (1966) have suggested that this slope can be used as an indication

of polymer flexibility, though this has been questioned by Cotter, McPhie & Gratzer (1967). The results (Table 3) show that there is a change in $d\epsilon_{504}/d(P/D)$ when ribosomes are dialysed to remove polyamines, but the change is not reversed when polyamines are restored to the ribosomes.

Cotter *et al.* (1967) have shown that when ribosomes are titrated with acridine orange ribosomal proteins are not displaced, and it appears that only the free phosphate groups on the RNA are titrated. By a similar experiment it should be possible to determine whether polyamines are displaced during titration with acridine orange. Initially, attempts were made to test whether the removal of polyamines from ribosomes by dialysis enabled more RNA phosphate groups to become titratable. However, since the polyamines could mask only a small proportion of the RNA phosphates, and since it is difficult to obtain a sharp end point as the slope in the region of excess of dye is curved (see Fig. 4 and Cotter *et al.* 1967), it was not possible to obtain conclusive results by this method.

Instead, ribosomes were dialysed against acridine orange solution, and the binding of the dye to ribosomes, together with the amounts of polyamines remaining non-diffusible after uptake of the dye, were measured. The rate of uptake of acridine orange is shown in Fig. 5 and the amounts of polyamines remaining attached to the ribosomes at

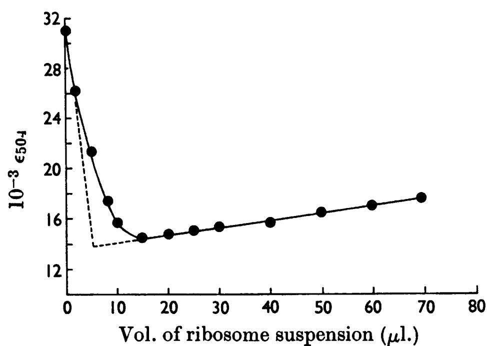

Fig. 4. Spectrophotometric titration of acridine orange against ribosomes from *B. stearothermophilus*. Acridine orange (0·18 μmole in 3 ml. of mM-sodium cacodylate buffer, pH 6·7) was titrated against an undialysed suspension of ribosomes containing 20 μmoles of RNA phosphate/ml.

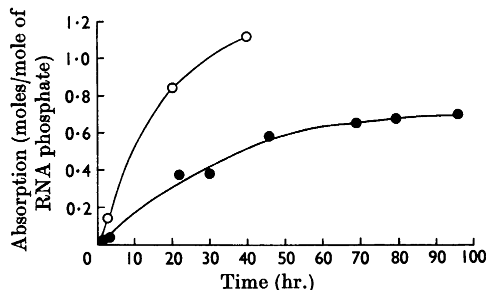

Fig. 5. Absorption of acridine orange by ribosomes and ribosomal RNA from *B. stearothermophilus*. Samples (1 ml.) of either ribosomes or of ribosomal RNA and spermine, each at a concentration of 5 mM-RNA phosphate, were dialysed at 4° against 200 ml. of mM-sodium cacodylate buffer, pH 6·7, containing 15 μmoles of acridine orange. ○, Absorption of acridine orange by ribosomal RNA; ●, absorption of acridine orange by ribosomes.

Table 5. *Displacement of polyamines bound to ribosomes and ribosomal RNA by dialysis against acridine orange solutions*

Samples (1 ml.) of ribosomes or ribosomal RNA and spermine, each at a concentration of 5 mM-RNA phosphate, were dialysed at 4° against 200 ml. of mM-sodium cacodylate buffer, pH 6·7, containing 15 μmoles of acridine orange for the times stated. In the controls no acridine orange was added.

Time of dialysis (hr.)	Dialysing medium	Spermine present in dialysis sac (μmoles/m-mole of RNA phosphate)	Spermidine present in dialysis sac (μmoles/m-mole of RNA phosphate)
Ribosomes			
0	Acridine orange in cacodylate buffer	9·25	3·24
48	Acridine orange in cacodylate buffer	10·9	Undetectable
96	Acridine orange in cacodylate buffer	6·3	Undetectable
48	Cacodylate buffer	10·0	Undetectable
Ribosomal RNA and spermine			
0	Acridine orange in cacodylate buffer	20·4	
3	Acridine orange in cacodylate buffer	17·6	
20	Acridine orange in cacodylate buffer	6·7	
42	Acridine orange in cacodylate buffer	0·0	
42	Cacodylate buffer	26·0	

different stages of the dialysis are given in Table 5. Though the uptake of acridine orange never reached 1 mole of acridine orange/mole of RNA phosphate, when the ratio became 0·7 mole of acridine orange/mole of RNA phosphate (Fig. 5), which occurred after 96hr. dialysis, there was little loss of polyamines from the ribosomes when compared with ribosomes dialysed against mM-sodium cacodylate buffer alone (Table 5). This suggests either that the acridine orange binds preferentially to RNA phosphates that have no polyamines attached to them, or that polyamines are attached in regions inaccessible to acridine orange. In a comparable experiment in which the ribosomes were replaced by ribosomal RNA and spermine there was a more rapid uptake of acridine orange (Fig. 5) accompanied by complete loss of spermine (Table 5). This suggests that in ribosomes the spermine is inaccessible to acridine orange, but that in ribosomal RNA spermine is accessible and therefore displaced.

DISCUSSION

It is noteworthy that *B. stearothermophilus* was found to synthesize spermidine and spermine, unlike most bacteria, e.g. *Bacillus subtilis* (Herbst, Weaver & Keister, 1958; Bachrach & Cohen, 1961), which make putrescine and spermidine. This is in agreement with the findings of Toschi (see Mangiantini *et al.* 1965), but unlike the preliminary observations of Friedman *et al.* (1967) in which the presence of putrescine and spermidine is reported. We could find no trace of putrescine in whole extracts of the bacteria, and if it is present, which is likely since it is the precursor of spermidine and spermine (Tabor, Rosenthal & Tabor, 1958), it accounts for less than about 2% of the cell's polyamines. From the work of Tabor (1962) it is clear that the combination spermine and spermidine would have a greater stabilizing effect on DNA than would an equivalent concentration of putrescine and spermidine. The results of Friedman *et al.* (1967) on the stabilization of ribosomal RNA suggest that the same relationship holds as with DNA, namely that spermidine stabilizes to a greater extent than putrescine, and in our work spermidine and spermine have been shown to bind more strongly to the ribosomes than would putrescine and spermidine. Thus, under physiological conditions, more polyamines are likely to be bound to ribosomes in *B. stearothermophilus* than in *E. coli*, and this might be a factor enabling the ribosomes from *B. stearothermophilus* to function at higher temperatures.

It appears, however, that removal of the polyamines from the ribosomes of *B. stearothermophilus* does not decrease their thermal stability in conditions where there is an adequate concentration of Mg^{2+}. The ribosomes of *B. stearothermophilus* from which the polyamines have been removed are more stable than those of *E. coli* that still contain their polyamines. The change in the slope $d\epsilon_{504}/d(P/D)$ on titration with acridine orange after ribosomes have been dialysed against M-potassium chloride and TM buffer is probably due, not to the removal of the polyamines alone, but also to the removal of some other factor or to some irreversible change induced by exposing the ribosomes to a medium of high ionic strength. The exact interpretation of the slope may be complex (Cotter *et al.* 1967), but it seems that at least it is an indication of whether acridine orange molecules are still able to stack parallel to one another when they are attached to only a fraction of the RNA phosphate groups and is thus probably a function of conformation of the macromolecule.

The loss of particles sedimenting at greater than 78s on dialysis against M-potassium chloride could be due to degradation, during dialysis, of messenger RNA by ribosomal ribonuclease. If this were the case, then the release of ribosomal ribonuclease must depend on the salt concentration, since it does not occur to an appreciable extent in 10mM-potassium chloride.

Cotter *et al.* (1967) suggest that the phosphate groups which are titrated by acridine orange are the double-helical regions of the RNA on the outside of the ribosome. The finding that acridine orange will displace spermine bound to ribosomal RNA, but not spermine bound to ribosomes, suggests that spermine may be located in less-accessible positions on the ribosome and probably not on the outside.

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