EFFECTS OF ETHANOL, METHANOL AND DIFFERENT ANTIBIOTICS ON THE ATPASE AND GTPASE ACTIVITIES ASSOCIATED WITH *B. STEAROTHERMOPHILUS* 5 S RNS- PROTEIN COMPLEX*

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1. Introduction

Recently we have been able to detect ATPase and GTPase activities with specific 5 S RNS–protein complexes isolated from *Bacillus stearothermophilus* [1, 2] and rat liver [3]. Further it was shown that GTP is a noncompetitive inhibitor of the ATPase and that ATP is a noncompetitive inhibitor of the GTPase [1–3] indicating that two sites are involved. Since the prokaryotic and eukaryotic 5 S RNA–protein complexes behave in a similar way it may be concluded that these enzymatic activities are of universal nature. The only GTPase activity so far detected in connection with ribosomes is the one associated with the elongation factors EF-G and EF-Tu [4], and it seems therefore possible that the GTPase activity found with 5 S RNA–protein complexes is the one normally associated with the elongation factors. More obscure seems, at the time, the role of the ATPase activity observed, since there is no evidence in the literature for an ATPase requirement during protein synthesis by prokaryotic ribosomes. There is one report concerning eukaryotic ribosomes which shows that ATP is required in initiation of protein synthesis [5]. In attempting to elucidate the significance of the ATPase and GTPase activity observed with specific 5 S RNA–protein complexes we have studied the effect of different antibiotics on both enzymatic activities. The results reported here will show that those antibiotics which bind directly to either the ribosomal A-site or P-site do not influence the enzymatic activities. The only antibiotics which so far have shown inhibitory effects are those associated with chain elongation.

2. Experimental

2.1. 70 S *B. stearothermophilus* 799 ribosomes and ribosomal subunits were isolated as previously described [6]. *B. stearothermophilus* 5S RNA was prepared as published [7]. Reconstitution and isolation of specific 5 S RNS–protein complexes was carried out as earlier described [8]. ATPase and GTPase activity assays were determined as reported [1].

2.2. *Sources of antibiotics*

Virginiamycin M+S were a gift of C. Cocito. Fusidic acid was a gift of K. Nierhaus, thiostrepton was a gift of M. Schweiger and blasticidin S was provided by B. Weisblum and it is the same charge which has been used for experiments described in ref. [9]. All following antibiotics were also a generous gift of B. Weisblum who has obtained them from the companies indicated: amicethin, althiomycin and clindamycin from Upjohn Co., Kalamazoo, Michigan, U.S.A.; Streptogramin A and Streptogramin B from Squibb Co., New Brunswick, New Jersey, U.S.A.; and Tylosin from Eli Lilly, Indianapolis, Indiana, U.S.A. Puromycin and chloramphenicol were purchased from Serva, Germany, and

* Paper No. 81 on 'Ribosomal Proteins' and No. 7 on 'Structure and Function of 5 S RNA'. Preceding paper is by V. A. Erdmann, M. Sprinzl and O. Pongs (1973) Biochem. Biophys. Res. Commun. 54, 942.

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Erythromycin from Mann Research Laboratories, U.S.A.

3. Results and discussion

The specific *B. stearothermophilus* 5 S RNS-protein complex contains, besides 5 S RNA, two ribosomal proteins (BL-5 and BL-22) as major components and four ribosomal proteins as minor components (BL-6, BL-10, BL-26 and BL-27) [8]. Using the two-dimensional gel electrophoresis developed by Kaltschmidt and Wittmann [10] we were unable to detect any non-ribosomal proteins in association with the 5 S RNA–protein complex [8]. Based on the mobility of the *B. stearothermophilus* [8] and E. coli [10] proteins in the two-dimensional electrophoresis and on the 5 S RNA binding results [8] it was concluded that *B. stearothermophilus* protein BL-5 corresponds to *E. coli* protein EL-5 and that BL-22 corresponds to EL-18. The identification of the protein(s) responsible for the ATPase and GTPase activities is presently under investigation.

Since some of the antibiotics studied require organic solvents in order to be solubilized we first studied the effect of methanol, ethanol and dimethyl sulfoxide (DMSO) on the enzymatic GTP and ATP hydrolysis by the specific 5 S RNA–protein complex of *B. stearothermophilus*. As can be seen from fig. 1A and B. DMSO did not influence the ATPase and GTPase activities at the concentration range studied. Methanol up to a concentration of 20% stimulated both hydrolytic activities and at higher concentrations significant inhibitions were obtained (fig. 1A and B). A similar effect was observed with ethanol on ATPase and GTPase activity, i.e. stimulation to a concentration of 20% followed by inhibition at higher levels of the alcohol (fig. 1A and B). The stimulatory effects of the alcohols studied are not entirely surprising for it has previously been observed that the fragment reaction [11] and EFG-dependent GTP hydrolysis with ribosomes lacking proteins L7/L12 are also stimulated by alcohol [12, 13].

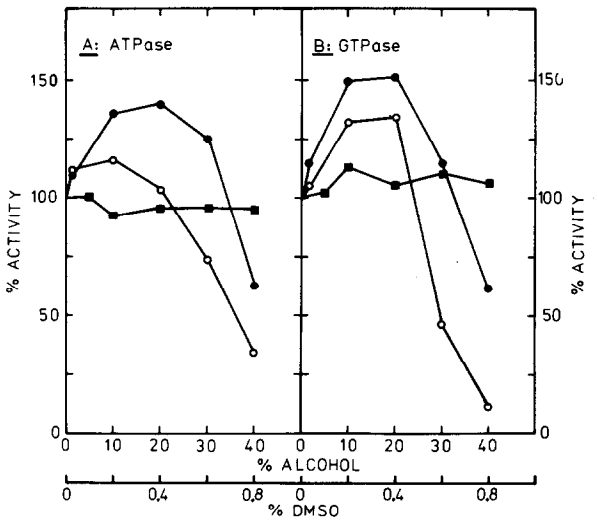

Fig. 1. Effect of methanol (•——•), ethanol (o——o) and DMSO (▪——▪) on ATPase and GTPase activity of *B. stearothermophilus* 5 S RNA–protein complex. For details of assay see Legend to table 1 and ref. [1].

Recently we reported that fusidic acid and thiostrepton which are known inhibitors of GTPase activity associated with translocation [4], also inhibit the GTPase and ATPase activities described here [1, 2]. It therefore seemed necessary to extend this study to other antibiotics. As can be seen from table 1 the following 50 S ribosomal subunit antibiotics did not influence either ATPase or GTPase activities: amicetin, althiomycin, blasticidin S, clindamycin, chloramphenicol, puromycin, streptogramin B, tylosine, virginiamycin M, virginiamycin S and the combination of both M and S virginiamycin components. Table 1 shows further that the 30 S subunit antibiotic tested, tetracycline, was also without any influence on the enzymatic reactions studied.

The results obtained with erythromycin, which is a macrolide known to effect the large ribosomal subunit, did show a significant inhibitory effect upon GTPase hydrolysis, whilst ATP activity was not influenced (fig. 2). We interpret this specific inhibitory effect of erythromycin as further evidence that ATP and GTP are hydrolysed at two distrinct sites [1, 2]. Why erythromycin inhibits the GTPase activity is not clear. Erythromycin and tylosine, both macrolides, show differences in behavior with respect to the hydrolytic activities of the 5 S RNA–protein complex. Evidence from other sources (see Reviews [14–17]) confirms our finding that the mechanism of action of these two macrolides is not identical.

From the data presented it seems to emerge that

Table 1
Effect of antibiotics on ATPase and GTPase activity

Antibiotic	Concentration range (μg/ml)	Solvent	% Solvent in assay	Effect on	
				ATPase activity	GTPase activity
Amicetin	20–1000	H_2O		None	None
Althiomycin	20–1000	DMSO	0.4	None	None
Blasticidin S	20–1000	H_2O		None	None
Clindamycin	20–1000	H_2O		None	None
Chloramphenicol	20–1000	Methanol	20	None	None
Puromycin	20–1000	Methanol	20	None	None
Streptogramin A	20–1000	Ethanol	20	None	None
Streptogramin B	20–1000	Ethanol	20	None	None
Tetracycline	20–1000				
Tylosin	20–1000	Ethanol	20	None	None
Virginiamycin M	0.2–10	Ethanol	10	None	None
Virginiamycin S	0.2–10	Ethanol	10	None	None
Virginiamycin M+S	0.2–10	Ethanol	10	None	None
Fusidic Acid*	20–500	Methanol	20	Inhibition	Inhibition
Thiostrepton*	50–400	DMSO	0.8	Inhibition	Inhibition
Erythromycin	20–2000	Methanol	20	None	Inhibition

* Data taken from reference [1].

Assay conditions for ATPase and GTPase were those earlier described [1]. 0.07 A_{260} units of *B. stearothermophilus* 5 S RNA–protein complex were incubated in 100 μl volumes in the presence of 0.12 mM [γ-^{32}P]GTP or ATP, 0.1 mM $MgCl_2$, 3 mM NH_4Cl, 6 mM 2-mercaptoethanol, and 0.1 M Tris–HCl. ATPase was measured at pH 7.8 and 60°C for 30 min. GTPase activity was determined at pH 7.4 and 45°C for 30 min. Organic phosphate determination was carried out as previously described [1]. 100% activity for ATPase corresponds to 3 nmoles ATP hydrolysed and for GTP to 0.8 nmoles.

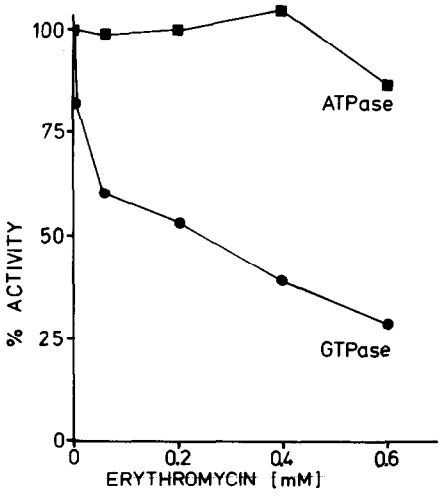

Fig. 2. Effect of erythromycin on *B. stearothermophilus* 5 S RNA–protein complex associated ATPase (■——■) and GTPase (●——●) activity. Details of assay conditions are described in legend to fig. 1 and ref. [1].

only those antibiotics which interfere with translocation or elongation factors-dependent GTPase [4, 14–17] are effecting the enzymatic activities associated with the 5 S RNA–protein complex. This suggests, that the GTPase activity described is the one which is involved with EF-G and EF-Tu during protein synthesis. Antibiotics which can be assigned with a fair amount of certainty to either the ribosomal A- or P-site [14–17] have so far not shown any effect.

In connection with the results obtained here it seems noteworthy to point out that 5 S RNA is directly involved in the enzymatic binding of aminoacyl-tRNAs to ribosomes [2, 18, 19].

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