ENHANCEMENT OF RIBOSOME-DEPENDENT GTPase FROM *E. COLI* BY STREPTOMYCIN

Abraham NOVOGRODSKY
Department of Biophysics, The Weizmann Institute of Science, Rehovot Israel

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1. Introduction

Several antibiotics known to inhibit protein synthesis in *E. coli* were tested for their effect on ribosome-dependent GTPase activity. Chloramphenicol and tetracycline had no effect whereas streptomycin was found to enhance GTPase activity. Some characteristics of the streptomycin-induced stimulation of GTPase activity are discussed in the present communication.

2. Materials and methods

2.1. *Materials*

GTP γ-^{32}P was synthesized according to Jagendorf and Avron [1]. Streptomycin sulfate was obtained from Calbiochem.

2.2. *Bacterial strains*

E. coli B was used throughout the experiments reported here. A streptomycin-resistant mutant (*E. coli* B, SM^R) was obtained from Dr. H. Engelberg, Department of Bacteriology, Hebrew University, Hadassah Medical School, Jerusalem.

2.3. *Preparation of ribosomes*

Ribosomes were prepared according to Nishizuka and Lipmann [2]. Ribosomal subunits, 30 S and 50 S were prepared as follows. Ribosomes were dissociated by dialysis against a solution containing: 0.01 M Tris-HCl pH 7.4, 0.05 M NaCl and 0.001 M $MgCl_2$, and the ribosomal subunits were separated by sucrose gradient centrifugation. The 30 S and 50 S ribosomal subunits were analyzed in the analytical centrifuge, at concentrations of 3–5 mg/ml, and found to be homogeneous.

2.4. *G factor*

G factor was purified according to Nishizuka and Lipmann [2].

2.5. *Assay of GTPase activity*

Reaction mixtures contained the following (unless otherwise specified) in a total volume of 0.25 ml: 10 μmoles of Tris-HCl pH 7.4, 3 μmoles of 2-mercaptoethanol, 2.5 μmoles of $MgCl_2$, 40 μmoles of NH_4Cl, 3.0 units A_{260} of ribosomes and 0.3–0.8 units of G factor. This was incubated for 10 min at 30° prior to the addition of 5 nmoles of GTP γ-^{32}P (specific activity 35–93 cpm/pmole). Incubation was performed for 15 min at 30°. The reaction was terminated by the addition of 1.1 ml 20% perchloric acid. Inorganic ^{32}P released was determined as the phosphomolybolate complex according to Conway and Lipmann [3]. One unit of G factor activity is defined as that amount which hydrolyzes 1 nmole of GTP into GDP and inorganic phosphate under the assay conditions specified above.

3. Results and discussion

Ribosome-dependent GTPase from *E. coli* is stimulated by streptomycin. As is shown in table 1, streptomycin does not enhance GTPase activity of either

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Table 1
Enhancement by streptomycin of ribosome-dependent GTPase from *E. coli.*

Additions	GTPase activity (pmoles ^{32}Pi released) Streptomycin –	+
Ribosomes	< 2	< 2
G factor	8.7	8.6
Ribosomes + G factor	296	473

GTPase was assayed as described in sect. 2.5. Specific activity of GTP γ-^{32}P was 93 cpm/pmole. Where indicated the following were added: 0.3 units of G factor, 4 μg of streptomycin.

Table 2
Effect of streptomycin at different concentrations on GTPase activity.

Streptomycin (μg/ml)	GTPase activity (pmoles of ^{32}Pi released)
–	336
0.2	614
0.4	653
0.8	680
2.0	759
4.0	785

GTPase was assayed as described in sect. 2.5. Specific activity of GTP γ-^{32}P was 82 cpm/pmole. 0.3 units of G factor were added.

Table 3
The requirement of NH_4^+ for the stimulation of GTPase activity by streptomycin.

NH_4Cl (mM)	GTPase activity (pmoles ^{32}Pi released) Streptomycin –	+
–	859	723
80	927	1512
160	350	631

GTPase was assayed as described in sect. 2.5. Specific activity of GTP γ-^{32}P was 38 cpm/pmole. 0.35 units of G factor were added. Where indicated 4 μg of streptomycin were added.

ribosomes or G factor alone but that catalyzed by the combination of both.

The effect of streptomycin at different concentrations on GTPase activity is shown in table 2. Approximately 50% of the maximal stimulatory effect of streptomycin is attained at a concentration of 0.2 μg/ml. Similar concentrations of streptomycin are required for the inhibition of polyphenylalanine synthesis directed by poly U [4] and for misreading of poly U [5]. As shown in table 3, NH_4^+ is required for the stimulation of GTPase activity by streptomycin. NH_4^+ has been reported to affect ribosome-dependent GTPase activity [3] and to be required for the binding of phenylalanyl-tRNA to ribosomes [6] and for the polymerization of phenylalanine [7].

The enhancing effect of streptomycin on GTPase is probably not related primarily to its cationic properties. This conclusion is supported by the fact that the cationic agents, spermine and putrescine, at concentrations of 40 μg/ml, did not affect GTPase activity.

As shown in table 4 GTPase activity catalyzed by ribosomes from a streptomycin resistant strain is not stimulated by the drug, in contrast to the stimulation observed with ribosomes isolated from a sensitive strain.

Streptomycin sensitive sites were found to reside on 30 S ribosomal subunits [8, 9]. This was shown by testing the effect of streptomycin on protein synthesis catalyzed by hybrid 70 S ribosomes reconstructed from 30 S and 50 S ribosomal subunits isolated from bacterial strains resistant and sensitive to the antibiotic. A similar approach has been taken by us. Hybrid 70 S ribosomes, reconstructed from 30 S and 50 S ribosomal subunits isolated from strains sensitive and resistant to streptomycin were assayed for GTPase activity in the presence of streptomycin. As is shown in table 5 the GTPase activity of ribosomes reconstructed using 30 S subunits from a sensitive strain is markedly stimulated by streptomycin. The stimulatory effect of streptomycin on ribosomes reconstructed using 50 S subunits from a sensitive strain is less pronounced.

The mechanism by which streptomycin enhances ribosome-dependent GTPase is not known. It is plausible that streptomycin induces, upon interaction with a specific site on the 30 S ribosomal subunit, a

Table 4
Effect of streptomycin on GTPase activity catalyzed by ribosomes isolated from *E. coli* strains sensitive (SM^S) and resistant (SM^R) to streptomycin.

Additions	GTPase activity (pmoles ^{32}Pi released) Streptomycin	
	–	+
Ribosomes SM^S	708	1171
Ribosomes SM^R	760	714

GTPase was assayed as described in sect. 2.5. Specific activity of GTP γ-^{32}P was 35 cpm/pmole. 0.8 units of G factor were added. Where indicated 4 μg of streptomycin were added.

Table 5
Effect of streptomycin on 70 S ribosomes reconstructed from 30 S and 50 S ribosomal subunits isolated from *E. coli* strains, sensitive and resistant to streptomycin.

Additions	GTPase activity (pmoles ^{32}P released) Streptomycin		Stimulation index (+/ – streptomycin
	–	+	
30 S SM^S + 50 S SM^S	421	1196	2.84
30 S SM^R + 50 S SM^R	183	228	1.25
30 S SM^S + 50 S SM^R	217	581	2.68
30 S SM^R + 50 S SM^S	289	438	1.51

GTPase was assayed as described in sect. 2.5. Specific activity of GTP γ-^{32}P was 86 cpm/pmole. 0.5 units of G factor were added. Where indicated the following were added: 0.7 A_{260} of 30 S SM^S, 0.75 A_{260} of 30 S SM^R, 1.0 A_{260} of 50 S SM^S, 1.2 A_{260} of 50 S SM^R, 4 μg of streptomycin.

conformational change which affects GTPase activity. Since G factor and ribosome-dependent GTPase are involved in the translocation reaction in protein synthesis [10, 11], it is possible that streptomycin affects this reaction.

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