EFFECT OF SIOMYCIN ON THE G FACTOR DEPENDENT GTP HYDROLYSIS BY *ESCHERICHIA COLI* RIBOSOMES

Sachihiko WATANABE and Kentaro TANAKA
Shionogi Research Laboratory, Shionogi and Co., Ltd., Fukushima-ku, Osaka, Japan

Received 4 February 1971

1. Introduction

Previously, we have shown that siomycin A and its water soluble derivative, monothiomalic acid siomycin A, inhibit bacterial protein biosynthesis but not protein synthesis in rabbit reticulocytes or their cell-free system [1]. We have also reported that siomycin (monothiomalic acid-siomycin A) inhibits polyphenylalanine synthesis in *E. coli* cell-free system, interacting with 50 S subunits of the ribosomes, and that this antibiotic does not inhibit the synthesis of *N*-acetylphenylalanyl-puromycin by the ribosomes, though the enhancement of this reaction induced by the addition of G factor and GTP, which has been believed to represent the participation of a translocation step of peptide synthesis [2], was markedly suppressed by the antibiotic [3]. In the translocation step, the peptidyl-tRNA-mRNA complex is believed to move across the ribosome from an acceptor site to a donor site and deacylated-tRNA at the donor site is released in a reaction in which GTP is hydrolyzed to GDP and Pi in the presence of G factor. The GTP hydrolysis catalyzed by G factor and ribosome is also observable in the conditions uncoupled from peptide synthesis [4].

In this paper, the effect of siomycin (monothiomalic acid-siomycin A) on this type of G factor dependent GTP hydrolysis by ribosomes was investigated. The results indicate that siomycin strongly inhibits this GTP hydrolysis and that the inhibition is reversed by the addition of 50 S ribosomal subunits.

2. Materials and methods

Ribosomes were prepared from *E. coli* Q 13 and washed with NH_4Cl solution according to Erbe and Leder [5]. Ribosomal subunits were separated by overnight dialysis against buffer containing low magnesium concentration and subsequent sucrose gradient centrifugation as described in a previous paper [3]. The G factor preparation was obtained from postribosomal supernatant of *E. coli* Q13 cells [5, 6]. This G factor preparation, purified by column chromatography on hydroxylapatite [6], was used throughout this study and it showed no significant activity for hydrolysis of GTP in the absence of ribosomes. The hydrolysis of ^{3}H-GTP was determined by paper chromatographic analysis using DEAE-cellulose paper [7]. To obtain a clear separation of GTP and GDP, the paper was spotted with the reaction mixture and prewashed with water, then developed with 0.7 M ammonium formate (pH 3.1) [7]. After the papers had dried, they were cut into small pieces and radioactivity was measured by liquid scintillation counter using conventional toluene scintillation mixture. Siomycin (monothiomalic acid–siomycin A) was kindly provided by Dr. M. Ebata and his collaborators in this laboratory. ^{3}H-GTP (1000 μCi/μmole) was obtained from Schwarz BioResearch Inc.

3. Results and discussion

As shown in fig. 1 and table 1, G factor dependent

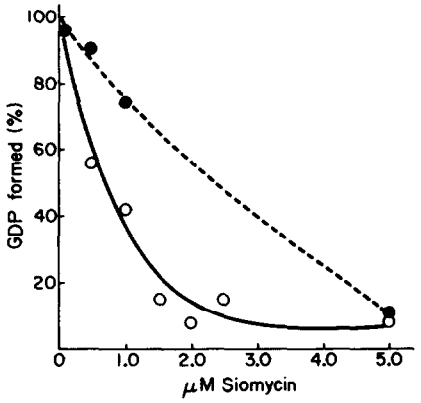

Fig. 1. Effect of siomycin on G factor dependent GTP hydrolysis by *E. coli* ribosomes. The reaction mixture contained the following components in a final volume of 50μl; 50 mM tris-HCL (pH 7.8), 10 mM magnesium acetate, 160 mM NH_4Cl, 5 mM dithiothreitol, and cited amounts of G factor, ribosomes or ribosomal subunits and siomycin. The mixture was preincubated at 37° for 5 min and then 55 nmoles of ^{3}H-GTP (specific activity 5.3 μCi/μmole) was added and the incubation was continued a further 10 min at the same temperature. A) (- - - -) NH_4Cl washed ribosome 0.31 A_{260} unit and 7.25 μg of G factor were used. B) (——) The mixture of 30 S ribosomal subunits (0.11 A_{260} unit) and 50 S ribosomal subunits (0.19 A_{260} unit) was employed in place of the NH_4Cl washed ribosomes in Exp. A.

GTP hydrolysis by ribosomes was strongly inhibited by siomycin.

As previously reported by Nishizuka and Lipmann [4], both of the 50 S and the 30 S ribosome subunits were needed for this G factor dependent GTP hydrolysis. A small amount of GDP formed by 50 S subunits may be due to the presence of a trace amount of 30 S subunits contaminating the 50 S subunit preparation.

As we found in the preceding study on the inhibitory effect of this antibiotic on polyphenylalanine synthesis, the inhibitory effect of siomycin on the G factor dependent GTP hydrolysis was also significantly reversed by an increment on the amount of 50 S ribosome subunits, but it is hardly reversed by a further addition of 30 S ribosome subunits (table 1, exp. 2).

These facts suggest that siomycin inhibits the G factor dependent GTP hydrolysis by ribosomes interacting with 50 S ribosomal subunits, and that this inhibitory action is a plausible cause of inhibition of the translocation step of peptide synthesis by this antibiotic.

Table 1.
Reversal by the addition of 50S ribosomal subunits of siomycin inhibition on GTP hydrolysis dependent on G factor and ribosomes.

	Additions	GDP formed (nmoles)
Exp. 1		
I	50S subunits (0.19 A_{260} unit)	3.13
II	30S subunits (0.11 A_{260} unit)	0.87
III	I + II	14.3
Exp. 2		
IV	the same as in Exp. 1 (III)	14.9
V	IV + siomycin	2.72
VI	IV + 50S subunits (0.57 A_{260} unit)	26.4
VII	V + 50 S subunits (0.57 A_{260} unit)	23.7
VIII	IV + 30 S subunits (0.33 A_{260} unit)	17.2
IX	V + 30 S subunits (0.33 A_{260} unit)	2.02

Reaction conditions were the same as described in the legend to fig. 1 Exp. B except that a fixed amount (2.0 μM) of siomycin was added when its effect was examined.

Acknowledgements

The authors wish to thank Mr. M. Tamaki for his capable technical assistance. Thanks are also due to Dr. M. Ebata and his collaborators for their kind gift of siomycin.

References

[1] K. Tanaka, S. Watanabe and M. Tanaki, J. Antibiotics 23 (1970) 13.
[2] H. Weissbach, B. Redfield and N. Brot, Arch. Biochem. Biophys. 127 (1968) 705.
[3] K. Tanaka, S. Watanabe, H. Teraoka and M. Tamaki, Biochem. Biophys. Res. Commun. 39 (1970) 1189.
[4] Y. Nishizuka and F. Lipmann, Arch. Biochem. Biophys. 116 (1966) 344.
[5] R.W. Erbe and P. Leder, J. Mol. Biol. 39 (1969) 441.
[6] Y. Kaziro and N. Inoue, J. Biochem. (Tokyo) 64 (1968) 423.
[7] J.F. Morrison, Anal. Biochem. 24 (1968) 106.