NOTES

ON THE SITE OF ACTION OF BOTTROMYCIN A_2

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Bottromycin A_2, a peptide antibiotic, inhibits protein synthesis of bacteria[1]. It was observed previously that the inhibition of cell-free polypeptide synthesis by the antibiotic was decreased with increasing amounts of ribosomes[2]. From this it was suggested that bottromycin A_2 acts on ribosome. The present study is concerned with determining the subunit of the ribosome on which the antibiotic acts.

First, it was studied which subunit is responsible for the effect against the inhibition by bottromycin A_2, by examining the inhibition in a protein synthesizing system containing excess of 30S or 50S ribosomal subunit. As Table 1 shows, the presence of excess of 50S over 30S subunit decreased the inhibition by bottromycin A_2 of poly A-directed synthesis of polylysine. In contrast, no such effect was seen with the reversed combination of subunits. Similar effects were observed with inhibition by erythromycin which was used as a control, since it is known to act on the 50S subunit[3,4]. It follows from these results that bottromycin A_2 acts on 50S subunit.

The effect of pretreatment of separate subunits with bottromycin A_2 was also studied. As illustrated in Fig. 1, pretreatment of 50S subunit with the antibiotic caused more profound inhibition of poly A-directed synthesis of poly-lysine than pre-

Table 1. Inhibition by bottromycin A_2 and erythromycin of poly A-directed synthesis of polylysine in the presence of 30S or 50S ribosomal subunit in excess

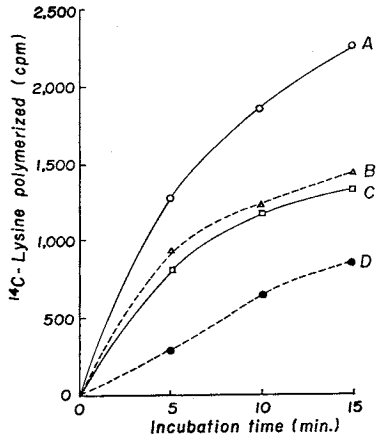
	Ribosomal subunits (A_{260})		^{14}C-Lysine polymerized (cpm)		Inhibition (%)
	30S	50S	Without antibiotic	With antibiotic	
Exp. 1 Bottromycin A_2 1.25×10^{-5} M	0.7	1.4	2,867	705	75
	1.4	1.4	2,813	718	75
	2.1	1.4	2,848	695	76
	0.7	2.8	2,948	1,042	65
	0.7	4.2	3,032	1,410	53
	0.7	—	153		
	—	1.4	301		
Exp. 2 Bottromycin A_2 2.5×10^{-5} M	0.7	1.4	4,408	695	84
	1.4	1.4	4,358	653	85
	2.8	1.4	4,288	640	85
	0.7	2.8	4,631	985	79
	0.7	5.6	4,752	1,633	66
Exp. 3 Erythromycin 5×10^{-7} M	0.7	1.4	3,759	675	81
	2.1	1.4	3,673	659	82
	0.7	4.2	3,965	1,345	66

The reaction mixture contained in 0.1 ml: 50 mM Tris-HCl, pH 7.4, 10 mM Mg acetate, 100 mM NH_4Cl, 2 mM dithiothreitol, 10 μg poly A, 0.2 mg protein of S-100, 40 mμmoles GTP, 4 A_{260} ^{14}C-lys-tRNA (40,000 cpm), and ribosomal subunits and antibiotic as indicated. The reaction was allowed to proceed at 37℃ for 15 minutes, and stopped by the addition of 10% TCA containing 0.1% phosphotungstic acid. After treatment at 90℃ for 20 minutes the precipitates were collected on glass fiber paper, and the radioactivity was determined with a liquid scintillation counter. Ribosomes, S-100, and tRNA were prepared from *E. coli* B. Ribosomal subunits were separated by dialysis of ribosomes against 10 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM Mg acetate, 100 mM NH_4Cl, and 2 mM dithiothreitol at 4℃ for 7 hours, followed by centrifugation on 8~30% sucrose gradient in a Beckman SW-25 rotor at 22,500 rpm for 13 hours. ^{14}C-Lys-tRNA was prepared as described in a previous paper[2]. Poly A was a product of Miles Laboratories, Inc., and ^{14}C-lysine (248 mCi/mmole) was purchased from Daiichi Pure Chemicals Co.

Fig. 1. Effect of pretreatment of ribosomal subunits with bottromycin A_2 on poly A-directed synthesis of polylysine.

30 S (0.7 A_{260}) or 50 S (1.4 A_{260}) subunit alone was preincubated in 0.1 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM Mg acetate, 100 mM NH_4Cl, and 2 mM dithiothreitol with 1.25×10^{-5} M bottromycin A_2 at 35℃ for 30 minutes. Then the complementary subunit (0.7 A_{260} 30 S or 1.4 A_{260} 50 S) and other components were added to start polylysine synthesis. Final composition of the reaction mixture, except for ribosomal subunits and antibiotic, and the procedures for the assay were the same as in Table 1.

A : No bottromycin A ; B : 30 S subunit preincubated ; C : Bottromycin A_2 added at the start of the reaction ; D : 50 S subunit preincubated. Final concentration of the antibiotic in B, C, and D was the same.



treatment of the 30S subunit or addition of the antibiotic at the start of polylysine synthesis. This result also indicates that bottromycin A_2 interacts preferentially with 50S subunit.

References

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