MECHANISM OF PROTEIN SYNTHESIS INHIBITION BY FUSIDIC ACID AND RELATED ANTIBIOTICS

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Fusidic and helvolinic acids, steroidal antibiotics of protolanostane skeletone, were demonstrated to inhibit protein synthesis in the in vivo and in vitro bacterial systems (Yamaki, 1965; Harvey et al., 1966; Tanaka et al., 1967). They neither affect synthesis of aminoacyl-sRNA nor formation of aminoacyl-sRNA-messenger-ribosome complex; but they inhibit amino acid transfer from aminoacyl-sRNA to protein on the ribosomes.

The transfer enzyme was purified from E. coli and separated into two complementary factors, T and G (Nishizuka and Lipmann, 1966). The latter factor exhibited ribosome-dependent GTPase activity. The detailed site of action of steroidal antibiotics was studied in a system with purified transfer enzymes of E. coli, and the results are presented in this communication.

Fusidic acid and related antibiotics were observed to inhibit ribosome-dependent GTPase activity of G factor. They inhibited polypeptide synthesis as well, and the grade of inhibition of both reactions was parallel. Puromycin-dependent release of peptide from the ribosomes was not significantly affected by fusidic acid in the absence of GTP and G factor; but the puromycin reaction, enhanced by GTP and G factor, was inhibited by fusidic acid. The results indicate that fusidic acid and

related antibiotics affect polypeptide synthesis by inhibiting GTPase activity of G factor and inhibiting translocation of aminoacyl-sRNA on the ribosomes. It seems to support the assumption of Nishizuka and Lipmann (1966) that GTPase reaction, linked to the ribosomes and G factor, is essential for translocation of aminoacyl-sRNA on the ribosomes.

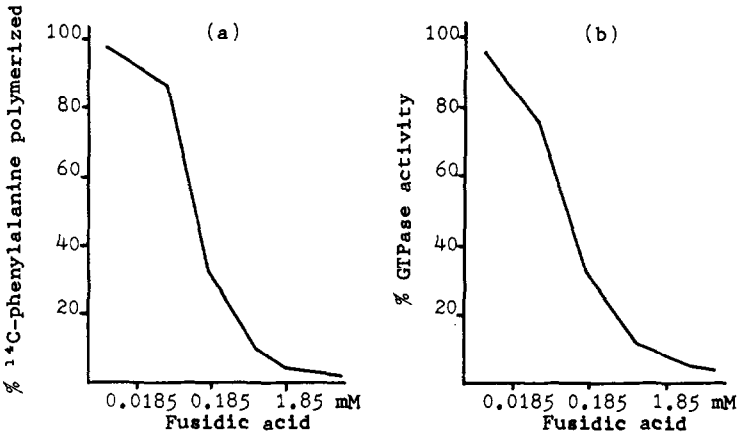
Results

Washed ribosomes, T and G factors were prepared from the extracts of E. coli B by the method of Nishizuka and Lipmann (1966). The sRNA of E. coli B was labelled with ^{14}C-amino acid, following the method of von Ehrenstein and Lipmann (1962). ^{32}P-γ-GTP was prepared by the method of Conway and Lipmann (1964), using photophosphorylation of GDP by spinach chloroplasts and purification by Dowex-1 chromatography. The steroidal antibiotics were kindly supplied by Prof. S. Okuda, Institute of Applied Microbiology, University of Tokyo, and Dr. Y. Nakayama, Nihon Kayaku Co., Ltd.

The effects of fusidic acid on poly U-directed polyphenylalanine synthesis and ribosome-dependent GTPase activity of G factor were comparatively studied. Fusidic acid was observed to inhibit both reactions. By the method employed, similar dose-inhibition curves were obtained for both reactions. The results are illustrated in Fig. 1. Approximately 65 % inhibition was demonstrated at the concentration of 0.185 mM of fusidic acid for polypeptide synthesis and for GTPase activity.

The effects of various steroidal antibiotics on poly A-directed polylysine synthesis and ribosome-dependent GTPase

Fig. 1. Effects of Fusidic Acid on Polyphenylalanine Synthesis and on Ribosome-dependent GTPase Activity of G Factor.



(a) Polypeptide Synthesis
100% = 1,250 cpm/0.2 ml

(b) GTPase Activity
100% = 1,820 cpm/0.2 ml

The assay for polyphenylalanine synthesis was performed in the reaction mixture, containing, in a total volume of 0.2 ml, 50 mM Tris-HCl pH 7.4, 160 mM NH_4Cl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 500 µg/ml ribosomes, 40 µg/ml poly U, 500 µg/ml ^{14}C-phenylalanyl-sRNA (150,000 cpm/mg RNA), 80 µg/ml T factor, 2.5 µg/ml G factor, 100 µM GTP and the antibiotic.

The ribosome-dependent GTPase activity of G factor was assayed by measuring liberation of radioactive inorganic phosphate from ^{32}P-γ-GTP as described by Conway and Lipmann (1964). The reaction mixture contained, in a total volume of 0.2 ml, 50 mM Tris-HCl pH 7.4, 160 mM NH_4Cl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 500 µg/ml ribosomes, 2.5 µg/ml G factor, 100 µM ^{32}P-γ-GTP (120,000 cpm/mµmole), and the antibiotic.

Both reaction mixtures were preincubated in the absence of GTP for 10 min. at 30°, and then incubated with GTP for 10 min. at 30°. The radioactivity of ^{14}C was determined by a windowless gas flow counter, and that of ^{32}P by a GM counter.

activity of G factor were comparatively studied. They inhibited both reactions, although the grades of inhibition were diverse. As summarized in Table 1, a parallelism was observed for the inhibition of both reactions with the various derivatives of steroidal antibiotics.

The effects of fusidic acid on puromycin-dependent release of polyphenylalanine from the ribosome-poly U complex were studied in the absence and presence of GTP and G factor. As illustrat-

Table 1. Effects of Various Steroidal Antibiotics on Polylysine Synthesis and on Ribosome-dependent GTPase Activity of G Factor.

Steroidal antibiotics		GTP hydrolyzed	Polylysine synthesized
Control		100	100
Fusidic acid	0.37 mM	27	25
	1.85	9	8
Helvolinic acid	0.36	52	57
	1.80	22	40
Helvolic acid	0.36	20	32
	1.80	12	16
7-Propionyl-helvolinic acid	0.37	12	15
	1.85	8	10
24,25-Dibromo-helvolic acid	0.32	27	35
	1.60	13	22
3-Dihydrohelvolic acid	0.34	82	81
	1.70	52	48
Methylhelvolinate	0.37	101	97
	3.70	92	93

Hydrolysis of ^{32}P-γ-GTP was 100 = 1,820 cpm/0.2 ml, and incorporation of ^{14}C-lysine was 100 = 723 cpm/0.2 ml.

The assay conditions for GTPase activity were the same as indicated in Fig. 1. Polylysine synthesis was assayed by poly A-directed incorporation of ^{14}C-lysine into polypeptide. Poly U and ^{14}C-phenylalanyl-sRNA were replaced by 100 μg/ml poly A and 500 μg/ml ^{14}C-lysyl-sRNA (90,000 cpm/mg RNA) in the reaction mixture, indicated in Fig. 1. Na_2WO_4, containing TCA, was used instead of TCA.

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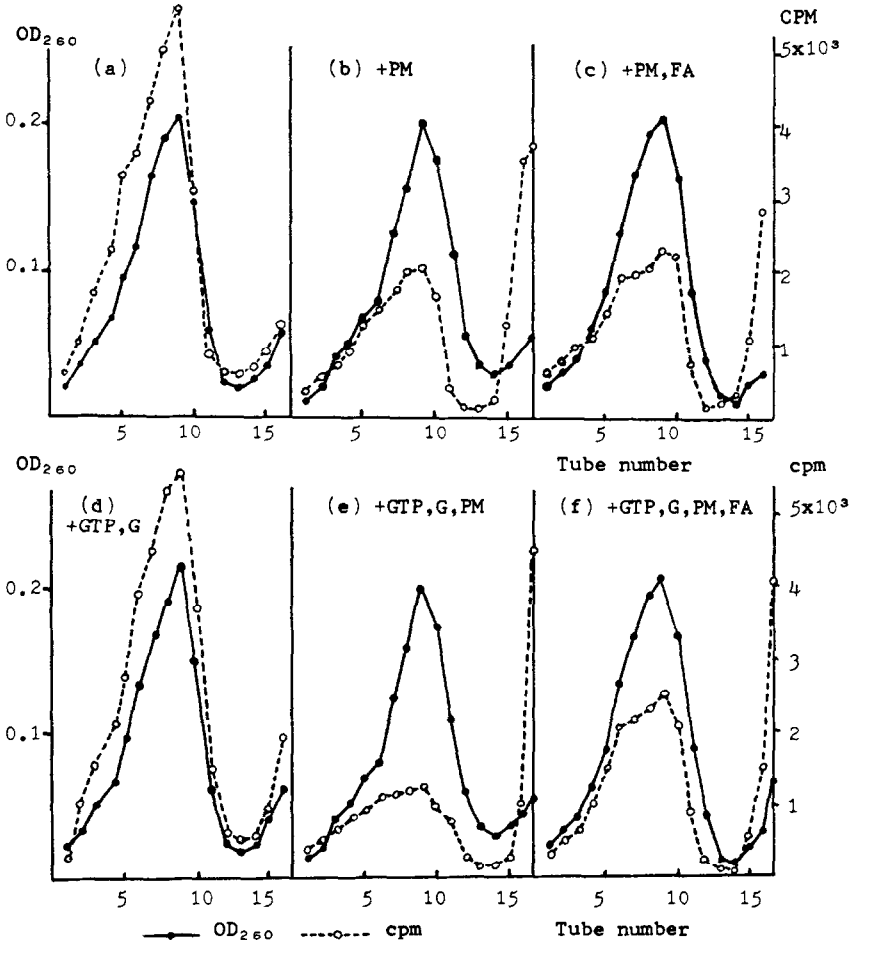
ed in Fig. 2, puromycin-dependent release of peptide was not significantly affected by fusidic acid in the absence of GTP and G factor. But the puromycin reaction, stimulated by GTP and G factor, was markedly inhibited by fusidic acid. Similar results were obtained in the experiments, using puromycin-dependent release of polylysine from the ribosomes.

Discussion

Fusidic and helvolinic acids inhibit amino acid transfer from aminoacyl-sRNA to polypeptide in the protein-synthesizing system. Fusidic acid and related antibiotics inhibit ribosome-dependent

Fig. 2. Effects of Fusidic Acid on Puromycin-dependent Release of Peptide from the Ribosomes in the Absence and Presence of GTP and G Factor.



The reaction mixture contained, in a total volume of 0.5 ml, ^{14}C-phenylalanyl-sRNA-charged ribosomes of E. coli B 4.0 mg/ml, poly U 50 µg/ml, NH_4Cl 150 mM, Mg acetate 12 mM, 2-mercapto-ethanol 6 mM, and Tris 5 mM, pH 7.2. It was incubated at 35° for 10 min. and sucrose density gradient, linear 3 to 20 %, centrifugation analysis was performed at 40,000 rpm for 60 min. at 0°. Each fraction of 25 drops was assayed for OD_{260} and for TCA-insoluble radioactivity. (a) Control. (b) With puromycin 0.1 mM. (c) With puromycin 0.1 mM and fusidic acid 0.5 mM. (d) With G factor 15 µg/ml and GTP 0.1 mM. (e) d + puromycin 0.1 mM. (f) d + puromycin 0.1 mM + fusidic acid 0.5 mM.

GTPase activity of G factor. The grade of inhibition is parallel to that of polypeptide synthesis. GTP split, linked

to the ribosomes and G factor, seems to be essential for translocation of peptidyl-sRNA on the ribosomes (Nishizuka and Lipmann, 1966); and it is inhibited by fusidic acid and related antibiotics. In the absence of GTP and G factor, peptidyl-sRNA attached to the "peptide site" of ribosomes may be released by puromycin, but peptidyl-sRNA attached to the "amino acid site" may not be released by puromycin. However, in the presence of GTP and G factor, peptidyl-sRNA attached to the "amino acid site" of ribosomes may be translocated to the "peptide site" and released by puromycin (Traut and Monro, 1964; a review by Schweet and Heintz, 1966). Fusidic acid does not inhibit the puromycin reaction, analogous to peptide bond formation; but it inhibits the translocation of peptidyl-sRNA from the "amino acid site" to the "peptide site" on the ribosomes. And it seems to be caused by the inhibition of GTPase activity of the ribosomes and G factor.

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