ASSOCIATION OF FUSIDIC ACID SENSITIVITY WITH G FACTOR IN A PROTEIN-SYNTHESIZING SYSTEM

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It was observed that fusidic acid and related steroidal antibiotics inhibit protein synthesis in the in vivo and in vitro bacterial systems (Yamaki, 1965; Harvey et al., 1966; Tanaka et al., 1967). In an E. coli system, they inhibit ribosome-dependent GTPase reaction with G factor of transfer enzymes, and the grade of inhibition is parallel to that of polypeptide synthesis. It was further suggested by the experiments, using puromycin reaction, that the antibiotics inhibit translocation of peptidyl-tRNA on the ribosomes (Tanaka et al., 1968).

Fusidic acid inhibits GTPase activity, observed in combination of the ribosomes and G factor. The localization of fusidic acid sensitivity was further studied with respect to the ribosomes and G factor of drug-sensitive E. coli cells and those of resistant cells. The results are presented in this communication. It was demonstrated that the fusidic acid sensitivity is associated with G factor, but not with the ribosomes.

Results

Fusidic acid-resistant mutants of E. coli B were obtained by using N-methyl-N'-nitro-N-nitrosoguanidine, following the method of

Adelberg et al. (1965). The other materials and methods were principally the same as described in the previous paper (Tanaka et al., 1968). The minimal inhibitory concentration of fusidic acid for growth of the parent strain of E. coli was approximately 100 μg/ml, and that for the antibiotic-resistant mutant was higher than 1,000 μg/ml.

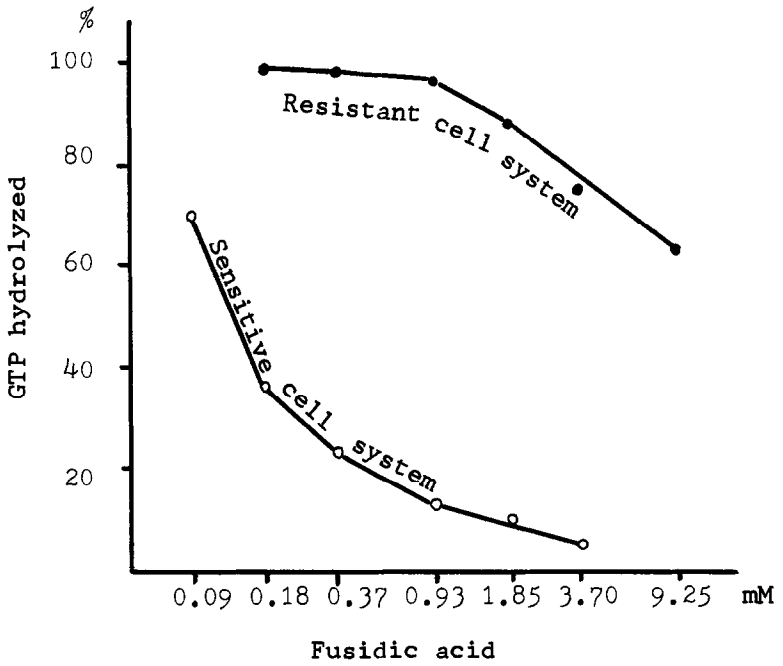

Fig. 1. Inhibition of Fusidic Acid on GTPase Reactions of G Factor and Ribosomes from Sensitive E. coli and of Those from Resistant Mutant.

The reaction mixture contained, in 0.2 ml, 0.05 M Tris-HCl, pH 7.4, 0.01 M Mg acetate, 0.16 M NH_4Cl, 0.002 M DTT, 100 μg ribosomes, 200 mμg G factor, fusidic acid, and 20 mμmoles ^{32}P-γ-GTP (2,500 cpm/mμmole). The mixture was preincubated without GTP for 10 min. at 37°, and further incubated with radioactive GTP for 10 min. at 37°.

It was found that the ribosome-dependent GTPase activity of G factor derived from the fusidic acid-resistant mutant was resistant to the antibiotic, whereas that from the sensitive strain was sensitive to fusidic acid (Fig. 1). It was parallel to the sensi-

tivity pattern of the intact cells. A similar inhibition pattern of fusidic acid was observed for polypeptide synthesis with poly U in systems obtained from the sensitive and resistant cells.

For the purpose of determining whether the sensitivity to fusidic acid lies in the ribosomes or in the G factor, ribosomes from the sensitive strain were combined with G factor from the resistant mutant and vice versa, and the inhibition of GTPase reaction and polyphenylalanine synthesis by fusidic acid was investigated. The results are summarized in Tables 1 and 2. They indicate that G factor is the site of action of fusidic acid, because GTP split reaction and polyphenylalanine synthesis were inhibited only when G factor from the sensitive cells was involved in the assay system.

Table 1. Effects of Fusidic Acid on GTP Split Reaction by Ribosomes and G Factor from Sensitive and Resistant Cells in Various Combinations.

Ribosomes	G factor	GTP hydrolyzed (mμmoles) - FA	+ FA 0.185 mM	+ FA 1.85 mM
S	-	0.05		
S	S	4.97	2.09 (58)	0.60 (88)
S	R	3.11	3.05 (2)	2.83 (9)
-	S	<0.01		
R	-	0.01		
R	S	5.02	1.77 (65)	0.43 (91)
R	R	3.15	3.12 (1)	2.81 (11)
-	R	<0.01		

S:Obtained from the sensitive strain. R:From the resistant mutant. The numbers in parentheses represent % inhibition by fusidic acid (FA). The assay conditions for GTPase were the same as indicated in Fig. 1.

The above results could be interpreted by an assumption that the G factor prepared from the drug-resistant mutant might contain an enzyme or a factor, inactivating fusidic acid, and the G factor itself might be sensitive to the antibiotic. However, as presented in Table 3, the incubation of fusidic acid with the G factor from

Table 2. Effects of Fusidic Acid on Poly U-directed Phenylalanine Polymerization in Systems, Including Various Combinations of the Ribosomes and G Factor.

Ribosomes	G factor	Phenylalanine polymerized (μμmoles) - FA	+ FA 1.85 mM
S	-	0.3	
S	S 50 mμg	13.2	1.6 (88)
S	R 40	6.4	5.6 (13)
S	R 80	10.6	9.6 (10)
R	-	0.3	
R	S 50 mμg	9.8	0.7 (93)
R	R 40	4.1	3.8 (8)
R	R 80	7.2	6.1 (15)

The reaction mixture, 0.2 ml, contained 0.05 M Tris-HCl, pH 7.4, 0.01 M Mg acetate, 0.16 M NH_4Cl, 0.002 M DTT, 100 μg ribosomes, 8 μg poly U, 10 μg T factor, 100 μg ^{14}C-phenylalanyl-sRNA (52 μμmoles phenylalanine), G factor, fusidic acid, and 20 mμmoles GTP. All the ingredients except GTP were preincubated for 10 min. at 37°, and then incubated with GTP for 10 min. at 37°. The T factor employed was prepared from the sensitive parent strain.

Table 3. Incubation of Fusidic Acid with G Factor from the Resistant Mutant and the Inhibition of Ribosome-dependent GTPase Activity.

Time of incubation	Fusidic acid	G factor	GTP hydrolyzed mμmoles (% inhibition)
0 min.	0 mM	G(s) 100mμg + G(r) 160mμg	6.34
0	1.85	" "	3.23 (49)
10	1.85	" "	3.10 (51)
20	1.85	" "	3.24 (49)
30	1.85	" "	3.12 (51)
0	0	G(s) 200mμg	6.29
0	1.85	"	0.61 (90)
0	0	G(r) 320mμg	6.88
0	1.85	"	6.20 (10)

G(s): G factor obtained from the sensitive parent strain.
G(r): G factor obtained from the drug-resistant mutant.
Fusidic acid (3.7 mM) was incubated with G(r)(1.6 μg/ml) in a medium, containing 0.05 M Tris-HCl, pH 7.4, 0.01 M Mg acetate, 0.16 M NH_4Cl, and 0.002 M DTT at 37°. The incubated mixture of 0.1 ml was then added to an equal volume of reaction medium, containing 0.05 M Tris-HCl, pH 7.4, 0.01 M Mg acetate, 0.16 M NH_4Cl, 0.002 M DTT, 100 μg ribosomes, and 100mμg G(s). ^{32}P-γ-GTP 20 mμmoles was added to the mixture and incubated for 15 min. at 37°. The ribosomes employed were obtained from the sensitive parent strain.

the resistant mutant did not significantly influence the activity of the antibiotic, as demonstrated by the inhibition of ribosome-

dependent GTPase activity of G factor from the sensitive cells. It indicated that the G factor from the drug-resistant mutant did not contain a factor which inactivated fusidic acid under the conditions employed.

It was demonstrated in the present experiment that the fusidic acid sensitivity lies in the G factor but not in the ribosomes. The association of the antibiotic sensitivity with G factor was also suggested by the observation that excess amount of G factor reversed the activity of fusidic acid but excess amount of ribosomes did not significantly affect the activity in the systems from the sensitive cells (Tanaka et al.: J. Biochem., Tokyo, in press).

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