INHIBITION BY THIOPEPTIN OF RIBOSOMAL FUNCTIONS ASSOCIATED WITH T AND G FACTORS

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Summary. Thiopeptin, a new antibiotic, inhibits T factor-dependent binding of phe-tRNA to ribosomes, without appreciable inhibition of GTP-Tu-phe-tRNA complex formation. GTP hydrolysis coupled with the phe-tRNA binding is affected by the antibiotic. It also interferes with fusidic acid-sensitive hydrolysis of GTP caused by interaction with G factor and ribosomes. Siomycin acts similarly.

Thiopeptin is a new antibiotic which is chemically related to siomycin and thiostrepton (1). Siomycin was reported to inhibit G factor-associated function by acting on 50 S ribosomal subunit (2,3). Thiostrepton is known to have the similar effect (4,5).

The studies presented here demonstrated that thiopeptin, as well as siomycin, interferes with functions of ribosome associated both with T factor (binding of aminoacyl-tRNA (6-8) and GTP hydrolysis (9,10)) and with G factor (GTP hydrolysis (11) which is sensitive to fusidic acid (12)). The grade of inhibition of the former was observed to be higher than the latter.

Materials. Ribosomes, T and G factors, phe-tRNA were prepared from E. coli Q13 as previously described (12). γ-^{32}P-GTP was a product of International Chemical and Nuclear Co.. Thiopeptin was supplied by Fujisawa Pharmaceutal Co., and siomycin by Dr. K. Tanaka, Research Laboratory, Sionogi & Co..

Results and Discussion. Table 1 shows that binding of phe-tRNA to ribosomes was inhibited by thiopeptin and also by siomycin and

Series	pmoles ^{14}C-phe-tRNA bound + T factor	- T factor
Complete	6.6 (100)	2.1 (100)
+ thiopeptin 5 x 10^{-7} M	3.7 (55)	
5 x 10^{-6}	2.0 (31)	2.0 (95)
5 x 10^{-5}	2.0 (31)	2.0 (95)
+ siomycin 6 x 10^{-7}	3.0 (46)	
6 x 10^{-6}	2.4 (36)	
+ tetracycline 4.5 x 10^{-5}	3.3 (50)	
Complete (hot TCA-insoluble)	0.2 (3)	

Table 1. Effects of antibiotics on T factor-dependent binding of ^{14}C-phe-tRNA to ribosomes with poly U. The reaction mixture, in a total volume of 0.1 ml, contained: T factor 2 μg, ribosomes 200 μg, poly U 4 μg, and ^{14}C-phe-tRNA 50 μg (31 pmoles ^{14}C-phe) in a medium consisting of 50 mM Tris-HCl, pH 7.4, 7 mM magnesium acetate, 160 mM NH_4Cl, and 2 mM DTT. It was incubated at 30° for 10 min. ^{14}C-phe-tRNA bound to ribosomes was collected on Millipore filter, and the radioactivity was determined by a liquid scintillation counter, using a toluene base scintillator.

tetracycline in the presence of T factor. In the absence of the factor binding of phe-tRNA was not significantly affected by thiopeptin. The product bound to ribosomes in the presence of T factor was analyzed by the use of BD-cellulose column (13), and it proved that without antibiotics, 80.3 % of the radioactivity was in the form of monophe, 16.3 % diphe and 3.4 % oligophe.

GTP hydrolysis caused by interactions of T factor, ribosomes, poly U, phe-tRNA and GTP was inhibited by thiopeptin and also by siomycin, as shown in Table 2. The extent of the inhibition of the reaction by the antibiotics corresponded to that of the T factor-dependent binding of phe-tRNA to ribosomes. The observed GTP hydrolysis was highly dependent on poly U and phe-tRNA, indicating that the reaction was coupled with phe-tRNA binding. Ineffectiveness of fusidic acid shows that the GTP hydrolysis was independent of G factor.

Binding of GTP to T factor and formation of GTP-T factor-phe-tRNA complex, examined by the Millipore filtration method (14,

Series	pmoles GTP hydrolyzed
Complete	4.2 (100)
- poly U, - phe-tRNA	1.2 (29)
- poly U, - phe-tRNA, - T factor	0.3
- poly U, - phe-tRNA, - ribosomes	0.4
+ thiopeptin 5 x 10^{-7} M	2.6 (62)
5 x 10^{-6}	1.1 (26)
+ siomycin 6 x 10^{-7}	2.8 (67)
6 x 10^{-6}	1.4 (33)
+ fusidic acid 10^{-4}	4.1 (97)

Table 2. Inhibition by antibiotics of GTP hydrolysis caused by interaction of T factor, ribosomes, poly U, phe-tRNA and GTP. The reaction mixture, in a total volume of 0.1 ml, contained: T factor 2 μg, ribosomes 200 μg, poly U 4 μg, ^{12}C-phe-tRNA 50 μg, and γ-^{32}P-GTP 50 pmoles (31,000 cpm) in the same medium as in Table 1. It was incubated at 30° for 10 min. ^{32}Pi liberated was extracted as phosphomolybdate by isobutanol-benzene (11), and the radioactivity was determined by a GM counter.

Additions	pmoles γ-^{32}P-GTP retained by Millipore filter
None	0.4
T factor	3.7
T factor, thiopeptin	3.6
T factor, phe-tRNA	1.6
T factor, phe-tRNA, thiopeptin	1.7

Table 3. Millipore filtration of γ-^{32}P-GTP after incubation with T factor in the presence and absence of phe-tRNA and thiopeptin. 50 pmoles γ-^{32}P-GTP (29.000 cpm) was incubated with the additions indicated in 0.1 ml of the same medium as in Table 1. Amounts of additions were: T factor 2 μg, ^{12}C-phe-tRNA 50 μg, and thiopeptin 5 x 10-6 M. Incubation was performed at 30° for 10 min. The radioactivity, collected on Millipore filter, was determined by a GM counter.

15), were not appreciably affected by thiopeptin (Table 3). It indicates that the antibiotic blocks the interaction of GTP-Tu-phe-tRNA complex and ribosomes.

Ribosome-catalyzed puromycin reaction, assayed by formation of N-acetylphe-puromycin from N-acetylphe-tRNA, was observed not to be inhibited by thiopeptin.

Series	nmoles GTP hydrolyzed
Complete	3.20 (100)
- ribosomes	0.08
- G factor	0.06
+ thiopeptin 5×10^{-7} M	3.03 (95)
5×10^{-6}	0.68 (21)
+ siomycin 6×10^{-7}	2.91 (91)
6×10^{-6}	0.80 (25)
+ fusidic acid 4×10^{-5}	0.46 (14)

Table 4. Effects of antibiotics on GTPase reaction of G factor with ribosomes. The reaction mixture, in a total volume of 0.1 ml, contained: G factor 0.5 μg, ribosomes 200 μg, and γ-^{32}P-GTP 10 nmoles (6,300 cpm). The reaction medium was the same as in Table 1 except for 10 mM magnesium acetate. Incubation was at 30° for 10 min.

Table 4 shows that thiopeptin inhibited GTPase reaction exhibited by combination of G factor and ribosomes which was sensitive to fusidic acid. Siomycin was also active. The inhibitory effect of both antibiotics on this reaction was observed to be weaker than on the T factor-dependent GTP hydrolysis as well as the phe-tRNA binding to ribosomes, i.e. at 5×10^{-7} M thiopeptin and 6×10^{-7} M siomycin, the latter reactions were significantly inhibited, whereas the former reaction was not. It is, however, still necessary to compare the effects precisely under different conditions.

Lucas-Lenard and Haenni (7) observed that T factor-dependent binding of aminoacyl-tRNA requires both 30 S and 50 S ribosomal subunits. According to a report by Brot et al. (16), GTP-Tu-aminoacyl-tRNA complex binds to 30 S ribosomal subunits, and in the presence of 50 S ribosomal subunits the rate of aminoacyl-tRNA binding is increased and GTP hydrolysis occurs. Bodley and Lin (17) and Modolell et al. (3) showed that G factor interacts primarily with 50 S ribosomal subunits. Siomycin was demonstrated to act on 50 S ribosomal subunits (2,3). It is , therefore, suggested

that thiopeptin, and also siomycin, act on a certain site on 50 S ribosomal subunits which participates in interactions both with T factor and with G factor. From the results presented above, it is proposed that effect of these antibiotics on T factor-deperdent function is more closely related to inhibition of protein synthesis

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