DIFFERENTIAL EFFECTS OF ANTIBIOTICS ON PEPTIDYL TRANSFERASE REACTIONS

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Received May 3, 1972

Summary: Some of peptidyl transferase inhibitors were found to exhibit different effects on the first and second peptide bond formation on the ribosome. Mikamycin B stimulated fMet-puromycin reaction, but inhibited fMet-Ala-puromycin reaction. The accumulation of fMet-Ala was observed in the presence of mikamycin B, while f2 RNA-directed synthesis of polypeptide was suppressed by the antibiotic. The same tendency was demonstrated with erythromycin. Mikamycin A, spiramycin, and blasticidin S inhibited both peptidyl transferase reactions. The data implied that the first and second peptide bond formation may be somewhat different.

It has been reported that oligopeptides are formed when polypeptide synthesis is blocked by some of peptidyl transferase inhibitor i.e. chloramphenicol (1), erythromycin (2), and mikamycin B (3). Erythromycin inhibits polylysine synthesis but causes the accumulation of di- and tri-lysines (2). In the course of further studying this problem, it has been observed that mikamycin B exhibits different effects on the first and second peptide bond syntheses. The former is stimulated but the latter is inhibited by the antibiotic. The different sensitivity of the peptidyl transferase reactions implied somewhat different mechanism in both reactions. The results are presented in this communication. Mikamycin belongs to the group of antibiotics: vernamycin, streptogramin, synergistin, ostreogrycin and staphylomycin.

Materials and Methods: The ribosomes and S-100 fraction were prepared from *E. coli* Q13 by the method of Bretscher (4). The S-100 fraction was treated with ribonuclease and passed through

phosphocellulose chromatography. The puromycin reaction was performed according to the procedure of Leder and Burstyn (5). The peptide assay principally followed the method of Kuechler and Rich (6). Methionine and methionylalanine were formylated by the method of Sheehan and Young (7). The other procedures were the same as described previously (8).

Results: The effects of several antibiotics, which were demonstrated to affect the 50S ribosomal subunit and interfere with peptidyl transferase reaction (9-11), on the formation of formylmethionyl-puromycin (fMet-PM) and formylmethionyl-alanyl-puromycin (fMet-Ala-PM) on the E. coli ribosomes with f2 RNA were studied. The results are summarized in Table 1. Mikamycins A and B, spiramycin, erythromycin, and blasticidin S were found to inhibit fMet-Ala-PM formation. The grade of inhibition was in accordance with that of f2 phage RNA-directed protein synthesis (The data are not presented). Mikamycin B slightly stimulated fMet-PM reaction, while it suppressed the synthesis of fMet-Ala-PM. The enhancement by mikamycin B of fMet-PM formation was also demonstrated, using AUG as a messenger (The data are not presented). The formation of acetyl-Phe-puromycin with poly U was markedly stimulated by mikamycin B. The same tendency was observed with erythromycin. Mikamycin A and blasticidin S inhibited the synthesis of both fMet-PM and fMet-Ala-PM. Spiramycin inhibited the fMet-Ala-PM reaction, but did not apparently affect the formation of fMet-PM in 10 min. reaction (Table 1). However, the fMet-PM synthesis was suppressed by spiramycin at the early period of reaction (Fig. 1). The effect of spiramycin was found to depend upon the rate of reaction. The formation of fMet-PM was observed to occur more rapidly than that of fMet-Ala-PM by the method employed. The grade of inhibition by spiramycin and blasticidin S of fMet-PM reaction was more marked

Table 1. Effects of antibiotics on puromycin reactions.

Additions		fMet-Ala-PM	fMet-PM	Ac-Phe-PM
Mikamycin B	1.1×10^{-5} M	67	103	181
	4.5×10^{-5}	50	118	196
Mikamycin A	1.7×10^{-5}	36	76	2
	6.7×10^{-5}	23	55	
Spiramycin	1.2×10^{-5}	29	98	16
	2.4×10^{-4}	15	93	13
Erythromycin	1.4×10^{-4}	80	102	138
Blasticidin S	2.3×10^{-6}	28		43
	2.3×10^{-5}	18	76	17

The number represents % incorporation. PM: puromycin.

Formation of fMet-Ala-PM: The initiation complex was prepared in an E. coli Q13 system, using f2 phage RNA and formyl-^{14}C-Met-tRNA, following the method of Kuechler and Rich (6). The reaction mixture for fMet-Ala-tRNA-ribosome complex formation contained (per ml): 3 mg initiation complex, 1 mg S-100, 0.5 mg ^{3}H-Ala-tRNA and 0.23 mM GTP, in a buffer (10 mM Mg acetate, 50 mM KCl, 10 mM NH_4Cl, 100 mM Tris, pH 7.4 and 6 mM 2-mercaptoethanol). It was incubated for a minute at 15°C and immediately layered on 6 ml of the buffer containing 1 M sucrose; and then centrifuged for 5 hrs. at 50,000 rpm. The pellet was suspended in the above buffer. More than 70 % of tritium counts was demonstrated by paper electrophoresis to represent fMet-Ala. The reaction mixture for fMet-Ala-PM formation contained 0.6 mg of formyl-^{14}C-Met-^{3}H-Ala-tRNA-ribosome complex and 10^{-4} M puromycin in 0.1 ml of the buffer, in which the concentration of Mg acetate was reduced to 6 mM. It was incubated for 10 min. at 37°C. The release of fMet-Ala-PM and fMet-PM was determined by the ethylacetate extraction method (5).

Formation of fMet-PM: The assay procedure was the same as above. The reaction mixture contained 0.3 mg ^{3}H-fMet-tRNA-ribosome complex and 10^{-4} M PM in 0.1 ml of the buffer.

Formation of Ac-Phe-PM: The following mixture was incubated at 30°C for 10 min.: 0.6 mg N-acetyl-^{14}C-phenylalanyl-tRNA, 0.1 mg poly U and 4 mg ribosomes in 1 ml buffer. It was layered on 1 M sucrose-containing buffer and centrifuged as above. The puromycin reaction was performed as described above, except that the concentration of Mg acetate was 11 mM.

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in 3 min. incubation than 15 min. (Fig. 1). The formation of acetyl-Phe-PM was inhibited by blasticidin S and spiramycin (Table 1).

Mikamycin B caused the accumulation of fMet-Ala but not that of fMet in a f2 RNA-directed tripeptide (fMet-Ala-Ser) synthesizing system (Fig. 2). Blasticidin S, mikamycin A, and spiramycin were

observed to inhibit the formation of fMet-Ala as well as that of fMet-Ala-Ser (The data are not presented).

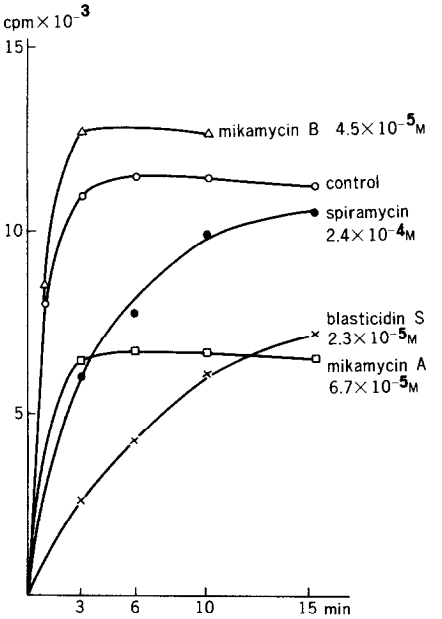

Fig. 1. Time course of formylmethionyl-puromycin formation.

The assay procedure was the same as described in the legend of Table 1.

Discussion: This investigation indicates that mikamycin B stimulates the first peptide bond formation but inhibits the second bond formation in f2 phage RNA-directed polypeptide synthesis. Both peptide bond syntheses are inhibited by mikamycin A, spiramycin, and blasticidin S. Erythromycin has been reported to enhance acetyl Phe-PM formation and not to affect fMet-PM synthesis (9,14).

A possible interpretation for the adverse effects of mikamycin B is that it might interfere with translocation of peptidyl-tRNA and mRNA on the ribosome. However, the assumption is unable to explain the stimulatory effect on the first peptide bond formation. Moreover no evidence has been so far obtained for the inhibition by

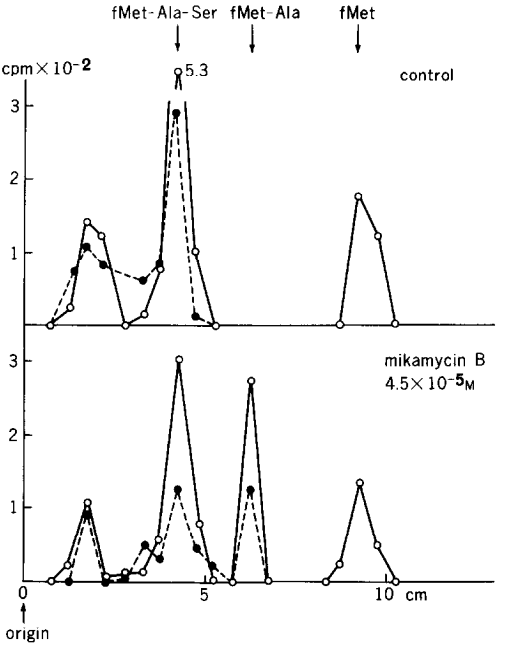

Fig. 2. The effect of mikamycin B on f2 RNA-directed peptide synthesis.

The reaction mixture (0.2 ml) for peptide synthesis contained per ml: 50 mM Tris (pH 7.4), 60 mM NH_4Cl, 7.9 mM Mg acetate, 10 mM 2-mercaptoethanol, 3 mM ATP, 0.5 mM GTP, 5 mM PEP, 20 μg pyruvate kinase, 1 mg S-100, 500 μg ^{3}H-fMet-tRNA (1,540 cpm/μg) treated with 10 mM $CuSO_4$ at pH 5.5 (12), 1 mg f2 RNA, 50 nmole serine, 0.2 μCi ^{14}C-alanine (99 mCi/mmole), 3 mg ribosomes, and antibiotics. It was incubated for 10 min. at 37°C, layered on 6 ml of 1 M sucrose-containing buffer, and centrifuged for 5 hours at 50,000 rpm (Table 1). The pellet was treated with 1 M NH_4OH for 25 min. at 37°C. Then it was lyophilized, dissolved in 0.2 ml H_2O with addition of 10 μl of formic acid, and centrifuged for 10 min. at 3,000 rpm. It was applied to a 0.6 x 2.5 cm Dowex 50 column and eluted with 1.8 ml of H_2O (13). The samples were lyophilized, dissolved in 20 μl H_2O and applied to filter paper in 0.3 x 1.5 cm strip. Electrophoresis was performed for 2 hours at 47 volts/cm (6). The buffer used for electrophoresis contained (per liter) 14 ml pyridine and 12.5 ml acetic acid, pH 4.8.
—o— ^{3}H, --•-- ^{14}C.

mikamycin B of translocation. It does not affect the G factor-dependent GTPase activity of ribosomes (15).

It has been established by the previous works (3,16) and the present one that mikamycin B affects the peptidyl transferase reaction. It inhibits polylysyl-puromycin reactions (16) and the

formation of fMet-Ala-PM (Table 1). Mikamycin B binds to the 50S ribosomal subunit (The data are not presented), and may change the activity of peptidyl transferase; and thus stimulates the first peptide bond formation but inhibits the second bond formation.

A few hypotheses can be proposed for the differential inhibition by mikamycin B of peptidyl transferase reactions. It may be due to different donor substrates (peptidyl-tRNA), or different sites or states of donors. The difference of peptidyl transferase might be also considered.

Since fMet-tRNA, an initiator, binds to ribosomes in a way different from the subsequent aminoacyl-tRNA (17), the binding site or state of fMet-tRNA may be different from that of the other peptidyl-tRNA. The activity of erythromycin on peptidyl transferase reaction has been reported to depend upon the property of donor peptidyl-tRNA (10). The adverse effects of mikamycin B may be due to the difference of donor substrates: fMet-tRNA and fMet-Ala-tRNA. It implies the interaction or competition of mikamycin B with the donors. However, we have so far failed to find any evidence of supporting this assumption. Mikamycin B has been found to accumulate oligopeptide in other systems, such as that of polylysine synthesis, in which the donors are different from the present ones (3).

The initiation factor, F_3, has been reported to stimulate fMet-PM formation with T4 phage mRNA by changing the configuration of fMet-tRNA (18). The participation of F_3 in the activity of mikamycin B may be excluded, because it also stimulates fMet-PM reaction with AUG.

Finally it is possible that different transferases may be involved in the first and second peptide bond formations. The hypothesis is supported by the differential effects of mikamycin B and the possible difference of binding sites or states of donors.

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