DIFFERENTIAL EFFECTS OF AMINOGLYCOSIDES ON CISTRON-SPECIFIC INITIATION OF PROTEIN SYNTHESIS

Akira Okuyama and Nobuo Tanaka
Institute of Applied Microbiology, University of Tokyo, Tokyo

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Summary

The 30S initiation complex formation on f2 RNA is inhibited by kasugamycin, kanamycin and gentamicin; but not by streptomycin. The fMet-tRNA-stimulated binding of f2 RNA to the 30S ribosomal subunit is blocked by kasugamycin; but not by kanamycin and gentamicin. Kasugamycin inhibits initiation of translation of maturation protein cistron more markedly than that of coat protein cistron. Kanamycin and gentamicin block initiation of coat protein synthesis, but do not significantly affect initiation of maturation protein synthesis. Streptomycin inhibits both protein syntheses at the same level.

The effects of aminoglycoside antibiotics on initiation of protein synthesis were studied in an E. coli ribosomal system (1,2). Using poly AUG as a messenger, the 30S initiation complex formation was inhibited by kasugamycin; but not by kanamycin, gentamicin and streptomycin (1). Helser and Davies reported that kasugamycin blocked the binding of fMet-tRNA to mRNA-ribosome initiation complex in the presence of GMPPCP (3). The results seemed to support the conclusion that kasugamycin selectively affects initiation of protein synthesis. Lelong et al. (4) and Modolell and Davis (5) observed that streptomycin causes release of fMet-tRNA from the 70S initiation complex. The 70S initiation complex formation on f2 RNA is inhibited by streptomycin, kanamycin, gentamicin and kasugamycin (2).

In our further studies, it has been observed that kanamycin and gentamicin block the 30S initiation complex formation on f2 RNA, but do not significantly affect the one on poly AUG. The results

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suggest that the effects of aminoglycoside antibiotics may depend upon the structure or conformation of mRNA. For the purpose of confirming the assumption, the effects of kasugamycin, kanamycin, gentamicin and streptomycin on initiation of translation of coat and maturation protein cistrons have been comparatively investigated with f2 phage RNA. The results are presented in this communication. The aminoglycosides have been demonstrated to exhibit differential effects on initiation of translation of both cistrons. It indicates that the structure or conformation of mRNA around the initiation codon seems to bear an important relation to the activity of aminoglycoside antibiotics.

Results

The S-30 fraction, ribosomes, initiation factors, elongation factor EF T, aminoacyl-tRNA and f2 RNA were prepared from E. coli Q13 by the methods as described previously (1,2).

The f2 RNA-directed incorporation of histidine into protein (presumably noncoat protein) was markedly inhibited by kasugamycin and streptomycin, but not significantly by kanamycin and gentamicin. The incorporation of alanine and valine (presumably coat and noncoat protein syntheses) was less affected by kasugamycin than that of histidine. The former was more markedly blocked by kanamycin and gentamicin. The grade of inhibition by streptomycin was observed at the same level with both incorporations (Table 1). The results suggested that the synthesis of noncoat protein may be preferentially inhibited by kasugamycin, and that of coat protein by kanamycin and gentamicin; and streptomycin may affect both syntheses. In Table 1 are shown the effects of antibiotics at the concentrations, where the differential inhibition was clearly demonstrated. The difference of the inhibition grades in coat

Table 1. Differential inhibition by aminoglycosides of incorporation of histidine, alanine and valine into protein on f2 RNA.

Antibiotics	Incorporation of		
	histidine	alanine	valine
- (10 min. inc.)	0.53 pmoles	28.9 pmoles	
kasugamycin 50 μg/ml	0.10 (81)	16.7 (42)	
streptomycin 0.5	0.37 (30)	19.9 (31)	
kanamycin 1.0	0.42 (20)	12.7 (56)	
gentamicin 1.0	0.50 (5)	8.7 (70)	
10	0.50 (5)	5.8 (80)	
- (40 min. inc.)	0.86		273 pmoles
kasugamycin 50 μg/ml	0.34 (60)		175 (36)
100	0.18 (79)		101 (63)
streptomycin 0.5	0.62 (28)		202 (26)
10	0.14 (84)		27.3 (90)
kanamycin 1.0	0.83 (4)		88.1 (68)
gentamicin 1.0	0.85 (1)		73.7 (73)

The number in the bracket represents % inhibition.
The reaction mixture contained: 50 mM Tris-HCl, pH 7.5, 30 mM KCl, 9 mM $Mg(AcO)_2$, 6 mM 2-mercaptoethanol, 3 mg protein/ml S-30 fraction, 0.5 mg/ml f2 RNA, 0.15 mg/ml tRNA, 0.2 μCi/ml ^{14}C-valine (280 mCi/mmole) or ^{14}C-alanine (173 mCi/mmole), or 2 μCi/ml ^{3}H-histidine (51 Ci/mmole), 0.025 mM amino acids except labelled one, 2 mM ATP, 5 mM PEP, 20 μg/ml pyruvate kinase, and 0.1 mM GTP; 0.2 ml in each tube. It was incubated at 37°C for 10 or 40 min. The TCA-insoluble radioactivity was determined with correction for the values obtained in parallel mixtures without messenger.

and noncoat protein syntheses was less pronounced at higher concentrations of kasugamycin. The inhibition by streptomycin of both syntheses was observed at the same level at high and low concentrations.

The formation of 70S initiation complex was investigated by binding ^{14}C-fMet-tRNA to the 70S ribosome with f2 RNA. It was inhibited by kasugamycin, streptomycin, kanamycin and gentamicin. The binding of Ala-tRNA, which was dependent on EF T, f2 RNA and fMet-tRNA (2), was markedly inhibited by streptomycin, kanamycin and gentamicin, but less affected by kasugamycin. The binding of Arg-tRNA was profoundly blocked by kasugamycin and streptomycin, but not by kanamycin and gentamicin (Table 2). The results indicated that initiation of translation of coat protein cistron and that of

Table 2. Differential inhibition by aminoglycosides of translation of coat and maturation protein cistrons.

I. Coat protein cistron.

Antibiotics		^{14}C-fMet-tRNA bound	^{3}H-Ala-tRNA bound
-		1.35 pmoles	1.02 pmoles
kasugamycin	10 μg/ml	0.66 (51)	0.71 (30)
streptomycin	10	0.04 (97)	0.09 (91)
kanamycin	10	0.12 (92)	0.11 (89)
gentamicin	10	0.07 (95)	0 (100)

II. Maturation protein cistron.

Antibiotics		^{14}C-fMet-tRNA bound	^{3}H-Arg-tRNA bound
-		1.37 pmoles	0.11 pmoles
kasugamycin	10 μg/ml	0.73 (47)	0.02 (81)
streptomycin	10	0.07 (95)	0.01 (89)
kanamycin	10	0.15 (89)	0.11 (-)
gentamicin	10	0.04 (97)	0.13 (-)

The number in the bracket represents % inhibition.
The reaction mixture contained: 50 mM Tris-HCl, pH 7.5, 60 mM NH_4Cl, 6 mM $Mg(AcO)_2$, 10 mM 2-mercaptoethanol, 2 mg/ml 1 M NH_4Cl-washed 70S ribosomes, 0.6 mg/ml initiation factors, 160 μg/ml ^{14}C-fMet-tRNA (^{14}C-Met 222 mCi/mmole), 200 μg/ml ^{3}H-Ala-tRNA (^{3}H-Ala 34 Ci/mmole) or ^{3}H-Arg-tRNA (^{3}H-Arg 8.9 Ci/mmole), 0.2 mM GTP, 100 μg/ml EF T, 1 mg/ml f2 RNA and antibiotics; 0.1 ml in each tube. It was incubated at 37°C for 10 min. The radioactivity, collected on Millipore filter, was assayed with correction for values without messenger.

maturation protein cistron (6) are differentially affected by aminoglycoside antibiotics. The former is preferentially inhibited by kanamycin and gentamicin, and the latter by kasugamycin. Streptomycin blocks both initiations at the same level.

The binding of f2 RNA to the native 30S ribosomal subunit was stimulated by fMet-tRNA. The formation of f2 RNA-30S ribosome complex was not significantly affected by kasugamycin, streptomycin, kanamycin and gentamicin in the absence of fMet-tRNA. However, the fMet-tRNA-stimulated binding of f2 RNA to the 30S ribosomal subunit was inhibited by kasugamycin, but not by streptomycin, kanamycin and gentamicin. The binding of fMet-tRNA to the 30S ribosome-f2 RNA complex was significantly suppressed by kasugamycin, kanamycin and gentamicin, but not by streptomycin (Table 3).

Table 3. Effects of aminoglycosides on binding of ^{3}H-f2 RNA to the native 30S ribosomal subunit.

Series	^{3}H-f2 RNA bound		^{14}C-fMet-tRNA bound
	+ fMet-tRNA	- fMet-tRNA	
Complete	5.25 pmoles	2.14 pmoles	3.55 pmoles
- ^{14}C-fMet-tRNA	3.19		
- ^{3}H-f2 RNA			1.32
- ribosomes	0.29		0.17
+ kasugamycin 100 µg/ml	3.55 (83)	2.08	1.81 (49)
+ streptomycin 10	5.43 (-)	2.31	3.57 (-)
+ kanamycin 10	5.23 (1)	2.02	2.41 (32)
+ gentamicin 10	5.62 (-)	2.07	2.27 (36)

The number in the bracket represents % inhibition.
The reaction mixture contained: 50 mM Tris-HCl, pH 7.5, 60 mM NH_4Cl, 6 mM $Mg(AcO)_2$, 10 mM 2-mercaptoethanol, 400 µg/ml native 30S subunit, 0.2 mM GTP, 160 µg/ml ^{14}C-fMet-tRNA, 200 µg/ml ^{3}H-f2 RNA (1,500 cpm/µg) and antibiotics; 0.1 ml in each tube. It was incubated at 37°C for 10 min. The radioactivity, collected and carefully washed on Millipore filter, was determined.

Table 4. Effects of aminoglycosides on binding of ^{3}H-f2 RNA to the washed 70S ribosomes.

Series	^{3}H-f2 RNA bound	^{14}C-fMet-tRNA bound
Complete	1.99 pmoles	1.42 pmoles
- initiation factors	0.52	0.17
- ^{14}C-fMet-tRNA	0.98	
- ^{3}H-f2 RNA		0.78
+ kasugamycin 100 µg/ml	0.88	0.87 (86)
+ streptomycin 10	1.34	0.98 (68)
+ kanamycin 10	1.68	1.04 (60)
+ gentamicin 10	1.83	0.97 (71)

The number in the bracket represents % inhibition.
The reaction mixture was the same as described in the legend of Table 3, except that the native 30S ribosomal subunit was replaced by 2 mg/ml salt-washed 70S ribosomes and 0.6 mg/ml initiation factors.

The formation of 70S initiation complex (the binding of fMet-tRNA to 70S ribosome-f2 RNA) was markedly blocked by all the aminoglycosides examined. The binding of f2 RNA to the 70S ribo some in the presence of fMet-tRNA was inhibited by kasugamycin, but less affected by kanamycin, gentamicin and streptomycin (Table 4). The binding of f2 RNA, enhanced by fMet-tRNA, seemed to be completely affected by kasugamycin. The suppression by streptomycin might be caused by breakdown of 70S initiation complex formation (2,4,5).

Discussion

The initiation of *in vitro* f2 translation was demonstrated mainly to occur at coat and maturation protein cistrons, which result in syntheses of peptides: fMet-Ala--- and fMet-Arg--- (6).

It has been observed in the present experiment that aminoglycoside antibiotics exhibit differential effects on initiation of translation of these cistrons. The 30S initiation complex formation on f2 RNA is blocked by kasugamycin, kanamycin and gentamicin; but not by streptomycin.

Kanamycin and gentamicin inhibit initiation of translation of coat protein cistron, which forms a base-pairing hairpin loop near the initiation codon AUG (7,8) and requires initiation factor IF3 (9); but they do not significantly affect initiation of translation of maturation protein cistron, which do not form a hairpin loop near the initiation codon. This is in accordance with the results that kanamycin and gentamicin do not significantly affect the 30S initiation complex formation on poly AUG (1).

Kasugamycin preferentially blocks initiation of translation of maturation protein cistron, which lacks in a hairpin loop at the initiation site. It is also in accordance with the results that kasugamycin inhibits the 30S initiation complex formation on poly AUG (1).

Streptomycin inhibits translation of both cistrons. It does not significantly affect the 30S initiation complex formation; but causes breakdown of the 70S initiation complex (2,4,5). Garcia-Patrone et al. (10) reported that streptomycin and other aminoglycosides inhibit dissociation of 70S ribosomes caused by dissociation factor. The inhibition of ribosomal dissociation may be involved in the inhibition of 70S initiation complex formation, as demonstrated in this experiment.

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