INHIBITION BY KASUGAMYCIN OF INITIATION COMPLEX FORMATION ON 30S RIBOSOMES.

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Received February 24, 1971

Summary

The mechanism of action of kasugamycin, an aminoglycosidic antibiotic, has been comparatively studied with those of other aminoglycosides. The initiation complex formation on 30S ribosomes is inhibited by kasugamycin but not by streptomycin, kanamycin or gentamicin, although the binding of fMet-tRNA to 70S ribosomes is inhibited both by kasugamycin and by streptomycin. The 70S complex formation is inhibited by kasugamycin but not by streptomycin when GTP is replaced by GMPPCP. The results indicate that the 30S initiation complex formation is a primary site of kasugamycin action but the other aminoglycosides interfere with a certain process after forming the complex.

Kasugamycin, an aminoglycosidic antibiotic, inhibits protein synthesis by interacting with the 30S ribosomal subunit (1-3). The mechanism of inhibition of protein synthesis seems to be different from that of other aminoglycosides such as streptomycin, kanamycin, neomycin, gentamicin etc., because kasugamycin does not cause miscoding *in vitro* (1,2) and the resistance is located at a distance from the streptomycin region on *E. coli* chromosome (3). Streptomycin has been reported to interfere with the binding of aminoacyl-tRNA, to cause polysome breakdown, to inhibit initiation, or to induce breakdown of the completed 70S initiation complex (4,5).

The action of kasugamycin on initiation of protein synthesis has been investigated. The initiation complex formation on 30S ribosomes is inhibited by kasugamycin but not by streptomycin, kanamycin or gentamicin, although the binding of fMet-tRNA to 70S ribosomes is inhibited both by streptomycin and by kasugamycin. The results are presented in this communication.

The preparation of S-30 fraction, washed ribosomes and initiation factor from E. coli followed the method of Ohta et al. (6). The native 30S ribosomes were obtained from the S-30 fraction by sucrose density gradient centrifugation. RNA of f2 phage was prepared by the method of Nathans et al. (7). Poly AUG and AUG were products of Miles Laboratories. F-^{14}C-Met-tRNA was prepared by the method of Hershey and Thach (8) and T factor by Nishizuka and Lipmann (9).

The f2 RNA-directed incorporation of valine into protein was inhibited by kasugamycin. Approximately 50% inhibition was observed at the concentration of 10^{-5}M (Table 1). The grade of inhibition was higher than that of poly U-directed polyphenylalanine synthesis (1).

Table 1. Inhibition by kasugamycin of f2 phage RNA-directed protein synthesis.

Kasugamycin	^{14}C-Valine incorporated	% Inhibition
0	9.61 pmol/tube	
2×10^{-6} M	8.64	10
2×10^{-5}	3.83	61
2×10^{-4}	0.48	95

The reaction mixture contained: 50 mM Tris-HCl, pH 7.5, 160 mM NH_4Cl, 8 mM $Mg(AcO)_2$, 6 mM 2-mercaptoethanol, 3 mg protein/ml S-30 fraction of E. coli extracts, 500 µg/ml of f2 RNA, 150 µg/ml E. coli tRNA, 0.2 µCi/ml ^{14}C-valine (165 µCi/µmole), 0.025 mM amino acids except valine, 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° for 30 min. The TCA-insoluble radioactivity was determined with correction for the values obtained in parallel mixtures without messenger.

The effect of kasugamycin on the initiation process was compared with streptomycin and the results are summarized in Table 2. The binding of fMet-tRNA to 70S ribosomes with f2-RNA or AUG was inhibited by kasugamycin and by streptomycin. About 50% inhibition was observed at the concentration of 10^{-5}M of kasugamycin. The binding with GMPPCP was inhibited by kasugamycin but not by streptomycin. The binding of fMet-tRNA by 30S ribosomes with poly AUG was inhibited by kasugamycin but not by streptomycin, kanamycin or

gentamicin (Table 2). The same results were obtained with AUG and f2-RNA. The results indicate that kasugamycin affects the initiation complex formation on 30S ribosomes. It is different from the action of other aminoglycosides such as streptomycin, kanamycin, and gentamicin, which seem to interfere with a certain reaction(s) sequentially occurring on 70S ribosomes. The results with streptomycin was in accordance with those of Modollel and Davis (5), who concluded that streptomycin induces breakdown of the completed 70S initiation complex.

Table 2. Effects of kasugamycin on binding of fMet-tRNA to ribosomes.

Ribosomes	mRNA	Addition	fMet-tRNA bound (pmol/tube)	% Inhibition
70S	f2 RNA	-	4.44	
		ksg 2×10^{-5} M	2.01	55
		2×10^{-4}	0.09	98
		str 2×10^{-5}	0	100
70S	AUG	-	6.30	
		ksg 2×10^{-4} M	0	100
70S	f2 RNA	GMPPCP	1.21	
		+ ksg 2×10^{-4}M	0	100
		+ str 2×10^{-5}	1.11	8
30S	polyAUG	-	4.45	
		ksg 2×10^{-4} M	2.14	62
		str 2×10^{-5}	4.42	1
		kan 2×10^{-5}	4.48	-
		gen 2×10^{-5}	4.59	-

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 washed 70S ribosomes, 0.6 mg/ml initiation factor, 150 µg/ml f-^{14}C-Met-tRNA (300,000 cpm/mg), 1 mg/ml f2 RNA or 100 µg/ml AUG, 0.2 mM GTP, 0.1 ml in each tube. It was incubated at 37° for 15 min. The radioactivity, collected on Millipore filter, was assayed with corrections for values without messenger. In the 30S experiment, the ribosomes and initiation factor were replaced by 0.6 mg/ml native 30S ribosomes and mRNA by 200 µg/ml poly AUG.
ksg:kasugamycin, str:streptomycin, kan:kanamycin, gen:gentamycin.

The poly U-directed binding of Phe-tRNA to 70S ribosomes with or without T factor and GTP was inhibited by kasugamycin as well as by streptomycin (Table 3). However the grade of inhibition was less than that of the 30S initiation complex formation.

Table 3. Effects of kasugamycin on Phe-tRNA binding to ribosomes with poly U.

Antibiotics	^{14}C-Phe-tRNA bound (pmol/tube) with T and GTP		without T and GTP	
-	8.55		1.50	
ksg 2×10^{-5} M	8.03	(6)		
2×10^{-4}	6.05	(29)		
5×10^{-4}	5.00	(40)	1.12	(25)
str 2×10^{-5} M	5.52	(35)		
tet 4×10^{-4} M	2.10	(75)	1.05	(30)

The reaction mixture contained: 50 mM Tris-HCl, pH 7.4, 160 mM NH_4Cl, 8 mM $Mg(acO)_2$, 2 mM DTT, 0.6 mg/ml 70S ribosomes, 40 µg/ml T factor, 50 µg/ml poly U, 150 µg/ml ^{14}C-Phe-tRNA and 0.2 mM GTP. It was incubated at 37° for 20 min. and the binding was assayed by the Millipore filter method.
ksg: kasugamycin, str: streptomycin, tet: tetracycline.

The puromycin reaction by 70S or 50S ribosomes and translocation of peptidyl-tRNA were not significantly affected by kasugamycin (The data are not shown). It suggests that the primary site of action of kasugamycin is the initiation step of protein synthesis rather than the elongation process.

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