INHIBITION BY KASUGAMYCIN OF PROTEIN SYNTHESIS IN *PIRICULARIA ORYZAE*

Sir:

Kasugamycin is a new aminoglycosidic antibiotic, effective against a variety of microorganisms, including *Piricularia oryzae* and *Pseudomonas aeruginosa*[1~3].

The mechanism of action of the antibiotic has been investigated with *Escherichia coli* and *Pseudomonas aeruginosa*[4~6]. It has been observed to inhibit protein synthesis and binding of aminoacyl-sRNA to the ribosomes in bacterial systems. Contrary to the other aminoglycosidic antibiotics, such as streptomycin, kanamycin, neomycin, paromomycin, gentamicin and hygromycin[5,7,8], it fails to cause miscoding: stimulation of polyribonucleotide- or DNA-directed incorporation of amino acids into polypeptide[6,9,10]. Failure of miscoding activity of kasugamycin seems to be due to the fact that it is lacking the deoxystreptamine moiety in the molecule[11]. Deoxystreptamine possesses a weak miscoding activity and is contained in the molecules of aminoglycosidic antibiotics with miscoding ability[12,13].

The mechanism of action of kasugamycin was further investigated with *Piricularia oryzae*, a causative fungus of rice plant disease. The results are presented in this communication.

Piricularia oryzae NIAS was grown in the medium containing (per/L) Difco malt extract 20 g, glucose 20 g and peptone 1 g. The cells were collected on a Buchner filter in the logarithmic phase of growth (48 hour culture). The preparation of cell extracts and microsomes followed principally the method described by HUANG *et al.*[14] The extracts were adjusted to pH 8.0 with 0.1 N KOH immediately after disruption by French pressure cells. The ribosomes were prepared by treatment of the microsomes with 0.5 % sodium deoxycholate, and were repeatedly washed by ultracentrifugation.

The effect of antibiotics on the amino acid incorporation into polypeptide was studied in the microsomal system with poly U or poly A. In the poly U system, the incorporation of phenylalanine was slightly inhibited and that of leucine and serine were slightly increased by the presence of streptomycin, kanamycin and neomycin. In the poly A system, the incorporation of arginine and threonine was slightly increased by the antibiotics. By the method employed, polylysine formation with poly A was slightly increased by kanamycin but slightly inhibited by neomycin. Streptomycin, kanamycin and neomycin seemed to cause miscoding in the fungal system, but the grade of miscoding activity was observed to be much lower than that in the bacterial system. Kasugamycin and blasticidin S were observed to inhibit the amino acid incorporation into polypeptide with poly U or with poly A, and did not cause codon misreading or increase of amino acid incorporation in the fungal microsomal system. The grade of inhibition by kasugamycin was comparable to that in the bacterial system. The

Table 1. Relative incorporation of ^{14}C-amino acid in a microsomal system obtained from *Piricularia oryzae* NIAS.

Template		Poly U			Poly A		
^{14}C-Amino acid		Phe	Leu	Ser	Lys	Arg	Thr
None		100	6	9	100	10	1
SM	0.04 mM	83	19	16	114	17	4
KM	0.04 mM	75	28	19	151	32	7
NM	0.04 mM	61	40	22	81	23	4
KSM	1.54 mM	37	—	1	65	—	—
BS	0.12 mM	16	—	—	66	—	—

SM : Streptomycin, KM : Kanamycin, NM : Neomycin, KSM : Ksugamycin, BS : Blasticidin S

The reaction mixture contained: *Piricularia oryzae* NIAS microsomal fraction 800 μg, S-100 supernatant 200 μg, poly U (or poly A) 20 μg, ATP 0.4 μmoles, creatine phosphate 2 μmoles, creatine phosphokinase 40 μg and ^{14}C-amino acid 0.2 μc in a volume of 0.4 ml.

The buffer employed consists of KCl 30 mM, $MgCl_2$ 5 mM and Tris 10 mM, pH 7.6.

It was incubated at 35°C for 30 minutes. ^{14}C-Amino acid possessed the following specific activities: phenylalanine 393 mc/mM, leucine 214 mc/mM, serine 107 mc/mM, lysine 237 mc/mM, arginine 214 mc/mM and threonine 143 mc/mM.

Incorporation of phenylalanine (100) was 113 $\mu\mu$moles/mg tyrosine equivalent. Incorporation of lysine (100) was 597 $\mu\mu$moles/mg tyrosine equivalent.

Table 2. Binding of ^{14}C-aminoacyl-sRNA to the ribosomes of *Piricularia oryzae* in the presence of kasugamycin

	Control	KSM 1.4 mcg/ml	No poly U
Poly U-phe-sRNA	350 c.p.m.	201 c.p.m.	175 c.p.m.
% Inhibition		43 %	
Endogenous-aa-sRNA	957 c.p.m.	622 c.p.m.	
% Inhibition		35 %	
Endogenous-leu-sRNA	338 c.p.m.	298 c.p.m.	
% Inhibition		12 %	

The binding was performed by the procedure of NIRENBERG and LEDER[15].

The reaction mixture, in 0.5 ml, contained *Piricularia oryzae* NIAS ribosomes 600 μg, poly U 40 μg and ^{14}C-phenylalanyl-sRNA (4.7×10^4 c.p.m./mg) 330 μg.

In the case of ribosomes with endogenous messenger, it contained ribosomes 1.8 mg and ^{14}C-leucyl-sRNA (1.8×10^4 c.p.m./mg) 212 μg or aminoacyl-sRNA of ^{14}C-amino acids mixture (1.2×10^5 c.p.m./mg) 282 μg. The buffer employed was the same as in Table 1.

The reaction mixture was incubated at 15°C for 10 minutes and then diluted with 3 ml of cold buffer. The ribosomes were adsorbed on a Millipore filter (HA 0.45 μ Millipore Filter Corp) and washed with 2 additional 3 ml portions of cold buffer. Then the filter was dried and radioactivity was determined by a liquid scintillation counter.

results are summarized in Table 1.

The effect of kasugamycin on the binding of ^{14}C-aminoacyl-sRNA to the fungal ribosomes was studied by the Millipore filter method[15]. By this method, the binding of ^{14}C-phenylalanyl-sRNA to the ribosomes with poly U was inhibited 43 % in the presence of kasugamycin. That of sRNA labelled with ^{14}C-amino acid mixture (*Chlorella* protein hydrolysate) to the ribosomes with endogenous mRNA was inhibited 35 % and that of ^{14}C-leucyl-sRNA 12 %. The results are presented in Table 2.

It was demonstrated in this experiment that kasugamycin, a new aminoglycosidic antibiotic, inhibits protein synthesis and binding of aminoacyl-sRNA to the ribosomes in the fungal systems. The antibiotic does not cause codon misreading. It is therefore consistent with the effects seen in bacterial systems.

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