EFFECTS OF VIOMYCIN AND POLYMYXIN B ON PROTEIN SYNTHESIS *IN VITRO*

Sir :

Of aminoglycosidic antibiotics, streptomycin, kanamycin, neomycin, paromomycin, gentamicin and hygromycin cause codon misreading in the bacterial ribosomal system[1,2], but kasugamycin[3,4] and spectinomycin[5] do not.

For the purpose of elucidating the structural basis of miscoding activity of aminoglycosides, the degradation products of aminoglycosidic antibiotics were investigated. Paromamine, neamine, deoxystreptamine, streptamine and N-methyl-deoxystreptamine exhibited miscoding activity, although the grade of miscoding was less than that caused by kanamycin. But actinamine, 3-amino-3-deoxyglucose, and 6-amino-6-deoxyglucose failed to exert miscoding activity by the method employed. It was indicated that the deoxystreptamine or streptamine moiety of aminoglycosidic antibiotics seems to play an important role in the miscoding activity[6,7].

Viomycin, a basic peptide antibiotic, was also reported to cause codon misreading[2]. It suggests that the basic property of antibiotics might be enough to cause codon misreading, and seems to conflict with our assumption that the deoxystreptamine or streptamine moiety of antibiotics is essential for the miscoding activity.

Table 1. Effect of viomycin and polymyxin B on protein synthesis with endogenous mRNA in *E. coli* ribosomal system

	Relative incorporation of ^{14}C-leucine
Complete	100
Polymyxin B	
1,000 units/ml	158
100 units/ml	97
10 units/ml	105
Viomycin	
100 μg/ml	16.5
10 μg/ml	11.3
1 μg/ml	12.3

Incorporation of ^{14}C-leucine (100) = 5.78 $\mu\mu$moles/mg, protein.

The complete reaction mixture contained : ATP 2 μmoles/ml, 3-phosphoglycerate (3PG) 5 μmoles/ml, ribosome of *E. coli* B 4 mg/ml, S-100 fraction of *E. coli* B 1.5 mg/ml, ^{14}C-leucine 0.2 μc/ml, KCl 100 μmoles/ml, Tris 50 μmoles/ml (pH 7.4) and $MgCl_2$ 10 μmoles/ml.

Incubation : at 37°C for 15 minutes.

In order to clarify the discrepancy, the effects of viomycin and polymyxin B, basic peptide antibiotics, on protein synthesis were studied in the ribosomal system obtained from *E. coli* B. The results are presented in this communication.

Viomycin was observed to inhibit protein synthesis with endogenous mRNA, polyphenylalanine synthesis with poly U and polylysine synthesis with poly A, but polymyxin B did not significantly inhibit amino acid incorporation into polypeptide in all the systems examined. By the method employed, both basic peptide antibiotics did not increase significantly the incorporation of lysine, arginine, serine and threonine with poly A and that of phenylalanine, leucine, serine and threonine with poly U. The results are summarized in Tables 1 and 2. Polymyxin B was observed to increase the amino acid incorporation at a concentration of 1,000 units/ml (100 mcg/ml). However, the concentration was too high to attribute to it any significance in the mechanism of action of polymyxin B, because the minimal growth-inhibitory concentration for the organism, *E. coli* B, was 0.02 units/ml.

In the present experiment, viomycin inhibited polypeptide synthesis, but failed to cause miscoding. It appeared to be different from the results obtained by DAVIES *et al.*[2] However, the miscoding activity of viomycin seemed to be insignificant even in the paper of DAVIES *et al.*[2]

In summary, viomycin was observed to inhibit protein synthesis, but not to cause miscoding in an *E. coli* ribosomal system. Polymyxin B did not significantly affect protein synthesis in the same system. The results suggested that the basic property of antibiotics alone seems not to be enough to cause miscoding.

Bottromycin A_2, another basic antibiotic, was observed to inhibit protein synthesis but not to cause codon misreading in a bacterial system[8,9]. It also seems to support the conclusion presented above.

Table 2. Effect of polymyxin B and viomycin on the amino acid incorporation with poly U or poly A in an *E. coli* ribosomal system

		Poly U				Poly A			
		Phe	Leu	Ser	Thr	Lys	Agr	Ser	Thr
Complete		100	6.0	2.0	1.5	100	7.5	0.14	0.32
-mRNA		2.7	2.4	1.9	2.2	16.4	8.6	0.12	0.35
Polymyxin B	1,000 units/ml	149	15.2	4.1	6.5	138	8.4	0.18	0.40
	100 units/ml	93	7.1	2.7	2.7	105	7.5	0.14	0.36
	10 units/ml	98	6.4	2.5	2.9	122	6.8	0.12	0.34
Viomycin	100 μg/ml	4.6	2.9	0.56	0.24	7.3	0.7	0.05	0.02
	10 μg/ml	9.2	2.1	0.76	0.20	24.9	2.8	0.06	0.09
	1 μg/ml	17.4	2.0	1.10	0.56	32.7	4.9	0.09	0.26
Kanamycin	10 μg/ml	24.1	8.4	3.7	2.8	156	21.3	0.68	1.68

The number represents relative incorporation of ^{14}C-amino acid. Incorporation of phenylalanine (100)=89 $\mu\mu$moles/mg protein. Incorporation of lysine (100)=540 $\mu\mu$moles/mg protein.

In the poly U system, the complete reaction mixture contained : ATP 2 μmoles/ml, 3PG 5 μmoles/ml, GTP 0.03 μmoles/ml, S-30 fraction of *E. coli* B 4 mg/ml, sRNA of *E. coli* B 500 μg/ml, poly U 50 μg/ml, ^{14}C-amino acid 0.2 μc/ml, Tris 50 μmoles/ml, NH_4Cl 160 μmoles/ml and $MgCl_2$ 10 μmoles/ml.

In the poly A system, the complete reaction mixture contained : ATP 2 μmoles/ml, 3PG 5 μmoles/ml, GTP 0.03 μmoles/ml, ribosomal fraction of *E. coli* B 1 mg/ml, S-100 fraction of *E. coli* B 0.8 mg/ml, sRNA of *E. coli* B 500 μg/ml, poly A 50 μg/ml, ^{14}C-amino acid 0.2 μc/ml, Tris 50 μmoles/ml, NH_4Cl 160 μmoles/ml and $MgCl_2$ 10 μmoles/ml.

Incubation : at 37°C for 15 minutes.

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