MISCODING ACTIVITY OF AMINO SUGARS

Sir:

The primary site of action of aminoglycosidic antibiotics seems to be located in the bacterial ribosomal system. They inhibit protein synthesis in the cell-free system. Of this class, streptomycin, kanamycin, neomycin, paromomycin, gentamicin and hygromycin are believed to cause codon misreading, because they increase the incorporation of certain kinds of amino acids into polypeptide in the ribosome-polyribonucleotide system[1]. On the contrary, kasugamycin and spectinomycin fail to increase the amino acid incorporation, although they inhibit polypeptide synthesis[2,3].

For the purpose of elucidating the structural basis of the diverse activities of aminoglycosides, the degradation products of kanamycin were investigated. It was demonstrated that deoxystreptamine, although the activity is low, stimulates both polyribonucleotide- and DNA-directed incorporation of amino acid into polypeptide; 3-amino-3-deoxyglucose and 6-amino-6-deoxyglucose do not exhibit any significant activity[4].

The miscoding activity of other degradation products of aminoglycosidic antibiotics has been further investigated and the results are presented in this communication.

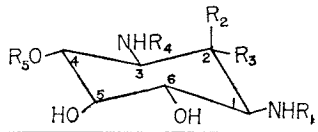
Preparation of extracts, sRNA and ribosomes of *E. coli* B, heat denaturation of salmon sperm DNA, and incorporation of amino acid into polypeptide were performed as described in the previous papers[4,5]. The specific activities of ^{14}C-amino acids used were serine 107 mc/mM, leucine 214 mc/mM, isoleucine 79.2 mc/mM, phenylalanine 222 mc/mM, lysine 214 mc/mM, arginine 222 mc/mM, valine 4.8 mc/mM, threonine 143 mc/mM, proline 179 mc/mM and amino acids mixture of *Chlorella* hydrolysate 9.4 mc/mmC.

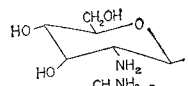
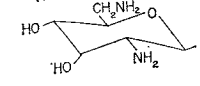
The effects of the degradation products of aminoglycosidic antibiotics on amino acid incorporation into polypeptide with poly U, poly A, poly C and heat-denatured salmon sperm DNA were studied in an *E. coli* cell-free system. They included deoxystreptamine, streptamine, N-methyl-deoxystreptamine, actinamine, paromamine and neamine (Table 1).

All of them except actinamine was obtained from Dr. K. MAEDA, National Institute of Health, Tokyo. Actinamine was kindly supplied by Dr. G.B. WHITFIELD, the Upjohn Company, Kalamazoo, Michigan. Some of them were further purified in our laboratory. All the samples used in this experiment were pure and no contamination with the aminoglycosidic antibiotics could be demonstrated by chemical and biological methods.

The effect on amino acid incorporation with polyribonucleotide and that on DNA-directed protein synthesis were simultaneously studied, because both seemed to be due to a common mechanism[5].

Table 1. Chemical structure of deoxystreptamine and its related compounds



	R_1	R_2	R_3	R_4	R_5
Deoxystreptamine	H	H	H	H	H
Streptamine	H	OH	H	H	H
N-Methyl-deoxystreptamine	CH_3	H	H	H	H
Actinamine	CH_3	H	OH	CH_3	H
Paromamine	H	H	H	H	
Neamine	H	H	H	H	

The incorporation of serine, leucine, isoleucine, phenylalanine and amino acid mixture with DNA was significantly increased by deoxystreptamine and streptamine at the concentration of 10^{-2} M and 10^{-3} M. Less increase of amino acid incorporation was observed with N-methyl-deoxystreptamine. Actinamine exhibited no significant activity. Paromamine and neamine increased the incorporation at the lower concentration (10^{-4} M). However the activity of all the above substances was much lower than that of kanamycin. The results are presented in Table 2.

The incorporation of phenylalanine

Table 2. Amino acid incorporation in the presence of the degradation products of aminoglycosidic antibiotics

Template		DNA					Poly U			
^{14}C-amino acid		Ser	Leu	Ileu	Phe	AAs	Phe	Leu	Ileu	Ser
Control		100	100	100	100	100	100	11	1	1
Deoxystreptamine	10^{-2} M	305	180	128	296	172	186	35	4	5
	10^{-3} M	213	143	119	250	155	146	23	2	3
Streptamine	10^{-2} M	272	252	174	268	259	163	20	1	3
	10^{-3} M	134	125	109	213	130	106	14	—	1
N-methyl-deoxystreptamine	10^{-2} M	116	121	140	196	152	132	22	—	2
	10^{-3} M	105	99	88	150	106	110	10	—	—
Actinamine	10^{-2} M	94	95	80	103	75	91	8	—	—
	10^{-3} M	125	108	112	154	115	97	15	1	2
Paromamine	10^{-4} M	297	280	203	384	240	90	65	7	5
Neamine	10^{-4} M	360	246	189	450	277	78	41	8	8
Kanamycin	10^{-5} M	1,430	970	820	1,390	428	73	87	8	11

The incorporation of phenylalanine with poly U (100) was 194.2 $\mu\mu$moles/mg tyrosine equivalent. The incorporation of amino acid with DNA (100) was 8.28 $\mu\mu$moles/mg tyrosine eq. for serine, 5.42 for leucine, 2.48 for isoleucine, 3.10 for phenylalanine, 9.50 for amino acid mixture.

Incorporation of ^{14}C-amino acid into polypeptide was performed following the method of NIRENBERG and MATTHAEI[6].

The reaction mixture contained: *E. coli* B S-30 fraction 700 μg, poly U (A, C) 10 μg (heat-denatured salmon sperm DNA 60 μg), ATP 0.2 μmoles, creatine phosphate 1 μmole, creatine phosphokinase 20 μg, *E. coli* B sRNA 30 μg and ^{14}C-amino acid 0.1 μc in a volume of 0.2 ml

The buffer employed consists of NH_4Cl 50 mM, Mg acetate 16 mM, 2-mercaptoethanol 6 mM, Tris 20 mM, pH 7.8. It was incubated at 35°C for 30 minutes.

the reaction mixture consisting of 30,000×g supernatant of *E. coli* B extract.

The radioactivity of the hot TCA-insoluble fraction was determined by a windowless gas flow counter, and protein content by the method of LOWRY.

Table 3. Amino acid incorporation In the presence of the degradation products of aminoglycosidic antibiotics

Template		Poly A					Poly C				
^{14}C-amino acid		Lys	Arg	Val	Ser	Thr	Pro	Ser	Thr	Arg	Leu
Control		100	9	2	1	1	100	4	4	10	3
Deoxystreptamine	10^{-2} M	135	16	12	7	4	211	26	20	26	22
	10^{-3} M	112	14	10	4	3	165	15	12	21	8
Streptamine	10^{-2} M	169	15	11	9	6	242	17	15	29	13
	10^{-3} M	130	9	5	4	1	179	6	8	16	10
N-methyl-deoxystreptamine	10^{-2} M	130	11	10	4	3	164	10	15	—	17
	10^{-3} M	100	7	4	1	—	93	6	10	13	8
Actinamine	10^{-2} M	98	4	3	1	—	82	1	4	—	—
	10^{-3} M	110	8	8	3	2	98	4	7	6	7
Paromamine	10^{-4} M	104	20	17	12	6	225	30	29	47	32
Neamine	10^{-4} M	95	26	22	10	9	151	29	18	36	26
Kanamycin	10^{-5} M	81	34	30	19	15	177	37	41	84	34

The incorporation of lysine with poly A (100) was 1,086 $\mu\mu$moles/mg tyrosine equivalent, and that of proline with poly C (100) was 52.8 $\mu\mu$moles/mg tyrosine equivalent.

with poly U was increased by deoxystreptamine, streptamine and N-methyl-deoxystreptamine. By the method employed, actinamine and paromamine exhibited no significant effects, but neamine and kanamycin inhibited its incorporation. The incorporation of leucine, serine and isoleucine with poly U was stimulated by deoxystreptamine, streptamine and N-methyl-deoxystreptamine, but not by actinamine. The degree of stimulation by deoxystreptamine was greater than with streptamine and N-methyl-

streptamine. Paromamine and neamine increased the incorporation, The activity was higher than deoxystreptamine but lower than kanamycin. The results are summarized in Table 2.

The incorporation of lysine, arginine, valine, serine and threonine with poly A was stimulated by deoxystreptamine, streptamine and N-methyl-deoxystreptamine, but not by actinamine. The grade of activity of deoxystreptamine and streptamine was higher than that of N-methyl-deoxystreptamine. The incorporation except that of lysine was increased by paromamine, neamine and kanamycin. The incorporation of proline, serine, threonine, arginine and leucine with poly C was stimulated by deoxystreptamine, streptamine and N-methyl-deoxystreptamine, but not by actinamine. The grade of stimulation was higher with deoxystreptamine and streptamine than that with N-methyl-deoxystreptamine. The incorporation was also stimulated by paromamine, neamine and kanamycin. The results are presented in Table 3.

In summary, the miscoding activity of paromamine and neamine was lower than that of kanamycin but higher than that of deoxystreptamine. The activity of streptamine was at the same level as or slightly less than deoxystreptamine. N-methyl-deoxystreptamine exhibited less activity than deoxystreptamine. Actinamine seemed to lack miscoding activity. The results indicated that the stereochemical structure of C_2 position and free amino groups at C_1 and C_3 of deoxystreptamine may play an important rôle in the miscoding activity of the amino glycosidic antibiotics (Table 1).

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