# STRUCTURAL BASIS OF KANAMYCIN FOR MISCODING ACTIVITY.

Nobuo Tanaka, Hiroshi Masukawa and Hamao Umezawa
Institute of Applied Microbiology, University of Tokyo, Tokyo

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For the purpose of elucidating the structural basis of the kanamycin action, the three moieties of the antibiotic have been studied for the activity of causing codon misreading and the results are presented in this communication. The stimulation of amino acid incorporation directed by polyribonucleotide or DNA is observed with deoxystreptamine, but not with 3-amino-3-deoxyglucose and 6-amino-6-deoxyglucose in E. coli cell-free systems.

Davies et al (1964, 1965) presented a hypothesis that aminoglycosidic antibiotics, such as streptomycin, kanamycin, paromomycin, neomycin, gentamicin, and hygromycin B, cause codon misreading, which results in a disturbance of protein synthesis. This hypothesis is based on the finding that the aminoglycosides increase the incorporation of certain kinds of amino acids into polypeptide in the bacterial ribosome-polyribonucleotide systems. Contrary to the other aminoglycosidic antibiotics, spectinomycin was reported to cause no detectable misreading of polyribonucleotides (Davies et al, 1965). On the other hand, McCarthy and Holland (1965) revealed that single stranded DNA is able to serve as a direct template for protein synthesis in the presence of aminoglycosidic antibiotics.

Tanaka et al (1966) observed that kasugamycin, a new aminoglycosidic antibiotic, inhibited protein synthesis and binding of aminoacyl-sRNA to the ribosomes in bacterial systems. It

failed to stimulate polyribonucleotide- or DNA-directed incorporation of amino acids into polypeptide in the ribosomal systems. Moreover, neomycin- or kanamycin-stimulated, DNA-directed protein synthesis was inhibited by kasugamycin. In this respect, the activity of kasugamycin seemed to differ from those of kanamycin, neomycin, paromomycin, streptomycin, gentamicin, and hygromycin B, although they all belong to the group of aminoglycosides and interfere with bacterial protein synthesis.

For the purpose of elucidating the structural basis of the diverse activities of aminoglycosidic antibiotics, the degradation products of kanamycin was investigated in the present experiments. The antibiotic consists of three moieties: deoxystreptamine, 3-amino-3-deoxyglucose and 6-amino-6-deoxyglucose. It was demonstrated that deoxystreptamine definitely stimulated both polyribonucleotide- and DNA-directed incorporation of amino acids into polypeptide; but no significant effects were observed with 3-amino-3-deoxyglucose and 6-amino-6-deoxyglucose. Deoxystreptamine or streptamine is contained in the molecule of kanamycin, neomycin, paromomycin, gentamicin, hygromycin B and streptomycin; but not in kasugamycin and spectinomycin. Spectinomycin has N,N'-methylactinamine. Actinamine is a stereoisomer with streptamine. It is suggested by the present experiments that deoxystreptamine, although lacking in antimicrobial activity, may play an important role in the codon misreading caused by aminoglycosidic antibiotics: i.e. the stimulation of polyribonucleotide- or DNA-directed incorporation of amino acids into polypeptide in the bacterial ribosomal systems.

Deoxystreptamine, 6-amino-6-deoxyglucose and 3-amino-3-deoxyglucose were prepared by hydrolysis of kanamycin in 6 N HCl and purified by repeated chromatography (Maeda et al, 1958).

Table 1. Amino acid incorporation with poly U in the presence of kanamycin and its degradation products.

| Addition | | Relative incorporation of amino acid | | | |
|---|---|---|---|---|---|
| | | Phe | Leu | Ileu | Ser |
| None | μmole/ml | 100 | 13 | - | 10 |
| Deoxystreptamine | 0.04 | 164 | 18 | 2 | 19 |
| | 0.4 | 199 | 20 | 6 | 34 |
| | 4. | 244 | 36 | 8 | 79 |
| 3-Aminoglucose | 0.4 | 86 | 13 | - | 12 |
| | 4. | 95 | 10 | - | 10 |
| 6-Aminoglucose | 0.4 | 83 | 17 | - | 11 |
| | 4. | 85 | 8 | - | 9 |
| Kanamycin | 0.04 | 58 | 12 | 3 | 74 |

The reaction mixture contained: E. coli B S30 fr. 600 μg, poly U 10 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 15 mM, 2-mercaptoethanol 6 mM and Tris 20 mM, pH 7.8. It was incubated at 35° for 30 min. Incorporation of phenylalanine (100) was 103 μμmoles/mg tyrosine equivalent.

Table 2. Amino acid incorporation with poly A in the presence of kanamycin and its degradation products.

| Addition | | Relative incorporation of amino acid | | | | |
|---|---|---|---|---|---|---|
| | | Lys | Arg | Val | Ser | Thr |
| None | μmole/ml | 100 | 3 | 6 | 1 | 2 |
| Deoxystreptamine | 0.04 | 134 | 7 | 9 | 2 | 3 |
| | 0.4 | 173 | 9 | 20 | 8 | 4 |
| | 4. | 200 | 9 | 32 | 17 | 12 |
| 3-Aminoglucose | 0.4 | 134 | 3 | 5 | - | 3 |
| | 4. | 105 | 7 | 2 | 1 | 2 |
| 6-Aminoglucose | 0.4 | 97 | 4 | 7 | 1 | 2 |
| | 4. | 108 | 6 | 3 | - | - |
| Kanamycin | 0.04 | 88 | 27 | 38 | 11 | 29 |

The reaction mixture was the same as in Table 1, except that poly U was replaced by poly A. Incorporation of lysine (100) was 400 μμmoles/mg tyrosine equivalent.

Table 3. Amino acid incorporation with poly C in the presence of kanamycin and its degradation products.

| Addition | | Relative incorporation of amino acid | | | | |
|---|---|---|---|---|---|---|
| | | Pro | Ser | Thr | Arg | Leu |
| None | μmole/ml | 100 | 8 | 12 | 1 | 1 |
| Deoxystreptamine | 0.04 | 149 | 10 | 10 | 1 | 5 |
| | 0.4 | 189 | 38 | 27 | 3 | 6 |
| | 4. | 247 | 89 | 77 | 9 | 21 |
| 3-Aminoglucose | 0.4 | 106 | 7 | 12 | - | 1 |
| | 4. | 129 | 12 | 20 | 1 | 1 |
| 6-Aminoglucose | 0.4 | 90 | 7 | 8 | 1 | - |
| | 4. | 118 | 15 | 17 | 1 | 1 |
| Kanamycin | 0.04 | 186 | 47 | 105 | | 3 |

The reaction mixture was the same as in Table 1, except that poly U was replaced by poly C. Incorporation of proline (100) was 45.6 μμmoles/mg tyrosine equivalent.

Table 4. Amino acid incorporation with heat-denatured salmon sperm DNA in the presence of kanamycin and its degradation products.

| Addition | | Relative incorporation of amino acid | | | |
|---|---|---|---|---|---|
| | | Ser | Thr | Phe | Leu |
| None | μmole/ml | 100 | 100 | 100 | 100 |
| Deoxystreptamine | 0.04 | 265 | 126 | 140 | 182 |
| | 0.4 | 516 | 214 | 248 | 258 |
| | 4. | 983 | 399 | 585 | 464 |
| 3-Aminoglucose | 0.4 | 83 | 90 | 83 | 91 |
| | 4. | 104 | 98 | 87 | 100 |
| 6-Aminoglucose | 0.4 | 94 | 102 | 112 | 111 |
| | 4. | 92 | 100 | 104 | 107 |
| Kanamycin | 0.04 | 1,480 | 1,710 | 1,050 | 994 |

The reaction mixture was the same as in Table 1, except that poly U was replaced by heat-denatured salmon sperm DNA 60 μg. Incorporation of serine, threonine, phenylalanine, and leucine (100) was 8.32, 4.39, 2.30, and 1.32 μμmoles/mg tyrosine equivalent respectively.

The effects of the degradation products of kanamycin on the incorporation of phenylalanine, leucine, isoleucine, or serine into polypeptide with poly U, that of lysine, arginine, valine, serine, or threonine with poly A, that of proline, serine, threonine, arginine, or leucine with poly C, and that of serine, threonine, phenylalanine, or leucine with heat-denatured salmon sperm DNA were investigated in an E. coli cell-free system. The results are summarized in Tables 1, 2, 3 and 4. In the poly U system, kanamycin was observed to inhibit the incorporation of phenylalanine but to stimulate those of isoleucine and serine. Deoxystreptamine increased the incorporation of all the four amino acids tested; but 3- and 6-aminoglucoses did not significantly affect it. In the poly A and poly C systems, kanamycin and deoxystreptamine were found to stimulate the incorporation of various amino acids: both correct and incorrect ones. But 6- and 3-aminoglucoses failed to exhibit a significant influence on it. The same tendency was observed with the DNA-directed incorporation of amino acids into protein.

In the present experiments, deoxystreptamine was demonstrated to increase polyribonucleotide- or DNA-directed incorporation of amino acids into polypeptide, although higher concentration was needed than kanamycin. The results indicated that the kanamycin action of codon misreading may be dependent on the deoxystreptamine moiety of the molecule, which is common with neomycin, paromomycin, gentamicin, hygromycin B and streptomycin. The aminoglucose moieties of kanamycin may enhance or modify the activity of deoxystreptamine. Kasugamycin fails to cause codon misreading, probably because it lacks in a deoxystreptamine moiety in the molecule.

Deoxystreptamine failed to inhibit poly U-directed incorpora-

tion of phenylalanine into polypeptide, but increased it. The inhibition of poly U-directed polyphenylalanine synthesis by kanamycin seems to be not directly related to the deoxystreptamine moiety.

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