MODE OF ACTION OF KUJIMYCINS, NEUTRAL MACROLIDE ANTIBIOTICS

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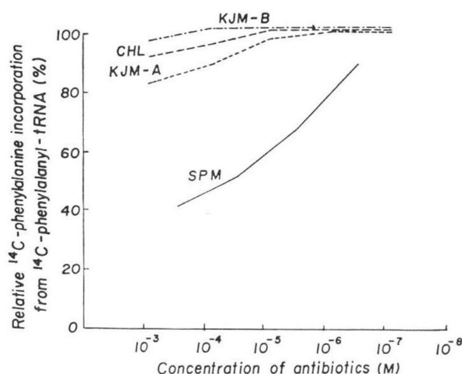
Many investigations have been reported[1] on the mode of action of basic macrolide antibiotics, which inhibit the peptide elongation step and do not inhibit the initiation, codon recognition and termination steps in protein synthesis of bacterial cells. Furthermore, a 16-membered basic macrolide, spiramycin (SPM)[2] inhibits the binding of aminoacyl-tRNA to ribosomes but a 14-membered one, erythromycin (EM)[3], does not.

MAO[4] and VAZQUEZ[5] reported that a 14-membered neutral macrolide, lankamycin and a 16-membered one, chalcomycin (CHL), inhibited the amino acids incorporation in *Escherichia coli* cell-free system and thereby these antibiotics are also assumed to inhibit the peptide elongation step. But lankamycin and CHL have never been compared on each step in protein synthesis such as the binding of aminoacyl-tRNA to ribosomes.

In the present study, we have examined the mode of action of kujimycin A(KJM-A) and kujimycin B(KJM-B; syn. lankamycin[6]), compared their inhibition type and looked for some difference in the mode of action between 14-membered neutral macrolides, KJMs and a 16-membered one, CHL.

Fig. 1. Relative inhibition of polyphenylalanine synthesis from ^{14}C-phenylalanyl-tRNA by kujimycin A, B, chalcomycin and spiramycin as a function of antibiotics concentration.



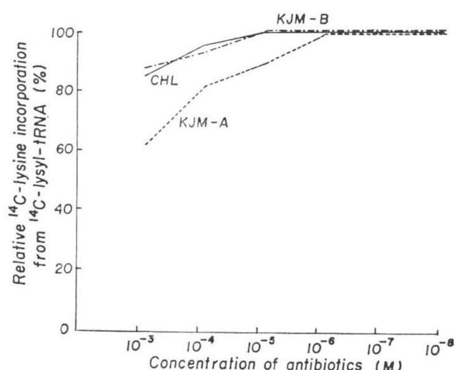
The reaction mixture contained, in a final volume of 0.25 ml: 23 μ moles of tris-HCl (pH 7.4), 47 μ moles of NH_4Cl, 3.7 μ moles of $MgCl_2$, 250 mμ moles of GTP, 2.9 μ moles of DTT, 1 μ mole of ^{12}C-phenylalanine, 50 μg of poly U, 0.103 mg protein of dialyzed 100S fraction, 0.123 mg protein of ribosomes and ^{14}C-phenylalanyl-tRNA (20,896 c.p.m.) with or without antibiotics.
Incubation time was 5 minutes at 37°C.

Table 1 shows that the effect of KJM-A and KJM-B on the incorporation of ^{14}C-phenylalanine, ^{3}H-uridine and ^{3}H-thymidine into protein and nucleic acid in EDTA-treated and untreated cell of *E. coli* Q_{13}. As it was predicted, the incorporation of both ^{3}H-uridine and ^{3}H-thymidine into nucleic acid were not blocked by KJMs in EDTA-treated cells during the time of incubation. Protein synthesis was

Table 1. Effect of kujimycins on the incorporation of ^{14}C-phenylalanine, ^{3}H-uridine and ^{3}H-thymidine into protein and nucleic acids in untreated and EDTA-treated cells of *E. coli* Q_{13}

		c.p.m.		
		^{14}C-phenylalanine	^{3}H-uridine	^{3}H-thymidine
(a) Untreated *E. coli* cells	KJM-A 10^{-3} M	1.79×10^4	6.9×10^4	6.9×10^4
	KJM-B 10^{-3} M	1.84×10^4	6.9×10^4	6.8×10^4
	Control	1.90×10^4	6.8×10^4	6.6×10^4
(b) Treated *E. coli* cells	KJM-A 10^{-3} M	0.72×10^4	6.7×10^4	7.0×10^4
	KJM-B 10^{-3} M	1.21×10^4	6.6×10^4	6.8×10^4
	Control	1.92×10^4	6.6×10^4	6.8×10^4

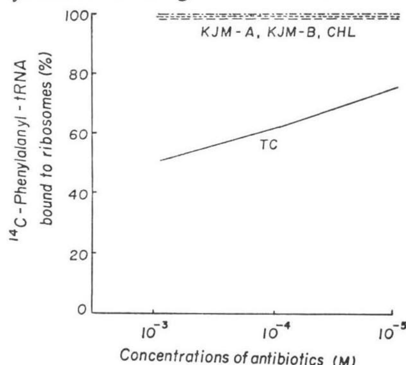
Fig. 2. Relative inhibition of polylysine synthesis from ^{14}C-lysyl-tRNA by kujimycin A, B and chalcomycin as a function of antibiotics concentration.



The reaction mixture contained, in a final volume of 0.25 ml: 23 μ moles of tris-HCl (pH 7.4), 47 μ moles of NH_4Cl, 3.7 μ moles of $MgCl_2$, 250 mμ moles of GTP, 2.9 μ moles of DTT, 50 μg of poly A (heated at 90°C for 5 minutes to destroy secondary structures), 152 μg protein of dialyzed 100S fraction, 108 μg protein of ribosome and ^{14}C-lysyl-tRNA (24,400 c.p.m.) with or without antibiotics.

Incubation time was 10 minutes at 37°C, 200 μg polylysine and 0.25 % (w/v) sodium tungstate in 5 % (w/v) trichloro-acetic acid (pH 2.0) was used to stop the reaction.

Fig. 3. Effect of antibiotics on the ^{14}C-phenylalanyl-tRNA binding to ribosomes.



The reaction mixture contained, in a final volume of 0.20 ml: 0.002 moles of tris-acetate (pH 7.2), 10 μ moles of KCl, 3 μ moles of Mg-acetate, 1 μ mole of ^{12}C-phenylalanine, 20 μg of poly U, 490 μg protein of ribosomes and ^{14}C-phenylalanyl-tRNA (38,900 c.p.m.).

The reaction mixture was incubated for 10 minutes at 30°C and was stopped by adding 3 ml of cold buffer.

The ^{14}C-phenylalanyl-tRNA bound to ribosomes was determined by the procedure of NIRENBERG and LEDER.

inhibited 62.5 % and 36.8 % by KJM-A and KJM-B at the concentration of 10^{-3} M respectively.

Fig. 1 shows the effect of KJM-A, KJM-B, CHL and SPM on the formation of polyphenylalanine from ^{14}C-phenylalanyl-tRNA. KJM-A and KJM-B showed about 17 % and 4 % inhibition of polyphenylalanine synthesis in the concentration of 8×10^{-4} M respectively.

In polylysine synthesis from ^{14}C-lysyl-tRNA, KJM-A exhibited about 40 % inhibition in the presence of 8×10^{-4} M, and inhibition ratio of KJM-B and CHL were 14 % and 15 % of control at the same concentration. From these results, it is apparent that KJM-A had more greater inhibitory effect than KJM-B in polypeptide synthesis.

From the above results, it is assumed that the difference in degree of inhibition is derived from a slight difference of chemical structure in which C-4 hydroxy group of arcanose in KJM-A is acetylated in KJM-B. It may suggest that the C-4 hydroxyl group is related with its affinity to biopolymers which participate in the polypeptide synthesis.

On the other hand, the inhibitory effect of KJM-A and B, CHL and tetracycline (TC) on the non-enzymatic binding of ^{14}C-phenylalanyl-tRNA to *E. coli* 70S-ribosome are shown in Fig. 3. KJM-A, KJM-B and CHL did not inhibit this step of protein synthesis under the condition which the presence of 8×10^{-4} M of TC resulted approximately 50 % inhibition.

Although the results are not presented here, KJM-A and KJM-B did not prevent the formation of aminoacyl-tRNA and the attachment of ^{3}H-poly U to ribosomes.

Thus, KJMs seems to inhibit only the incorporation of amino acids to polypeptide after the formation of aminoacyl-tRNA-ribosome-messenger-RNA complex. These characteristics are common to those of EM.

CHL also did not interfere with the binding of aminoacyl-tRNA to ribosomes as KJMs. Therefore, there is no difference in the inhibition of the binding of aminoacyl-tRNA to ribosomes between 14- and 16-membered neutral macrolides, KJMs and CHL, while between basic macrolides, SPM[2] interferes the binding but EM[3] does not.

These results suggest the similarity in the mode of action among KJMs, CHL and EM.

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References

1) PESTKA, S.: Inhibitors of ribosome functions. Annual Review of Microbiology. 488~562, 1971
2) AHMED, A.: Mechanism of inhibition of protein synthesis by spiramycin. Biochim. Biophys. Acta 166: 205~217, 1968
3) TANAKA, K.; H. TERAOKA, T. NAGIRA & M. TAMAKI: ^{14}C-Erythromycin-ribosome complex formation and non-enzymatic binding of aminoacyl transfer RNA to ribosome-messenger RNA complex. Biochim. Biophys. Acta 123: 435~437, 1966
4) MAO, J. C.-H. & R. G. WIEGAND: Mode of action of macrolides. Biochim. Biophys. Acta 157: 404~413, 1968
5) VAZQUEZ, D.: Antibiotics affecting chloramphenicol uptake by bacteria. Their effect on amino acid incorporation in a cell-free system. Biochim. Biophys. Acta 114: 289~295, 1966
6) OMURA, S.; S. NAMIKI, M. SHIBATA, T. MURO, H. NAKAYOSHI & J. SAWADA: Studies on the antibiotics from *Streptomyces spinichromogenes* var. *kujimyceticus*. II. Isolation and characterization of kujimycin A and B. J. Antibiotics 22: 500~505, 1969