# INHIBITION OF RNA AND DNA POLYMERASE REACTIONS BY PLURAMYCIN A.

Nobuo Tanaka, Kazuo Nagai, Hideyo Yamaguchi and Hamao Umezawa
Institute of Applied Microbiology, University of Tokyo

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Pluramycin A, isolated from the culture of Streptomyces pluricolorescens by Maeda et al (1956), is a basic antibiotic of orange needle crystals. The antitumor and antibacterial activity was reported by Takeuchi et al (1957). Lein et al (1962) observed phage induction of lysogenic bacteria by pluramycin.

We investigated the activity of pluramycin A on macromolecular syntheses and observed that the antibiotic inhibits both protein and nucleic acids syntheses in the intact cells of bacteria. The effects were further investigated, using cell-free systems. The results are described in this communication. Protein synthesis was not significantly affected in bacterial cell-free systems but RNA and DNA polymerase reactions were markedly inhibited by the antibiotic, which was suggested to bind with DNA by the thermal transition curve.

Effects on protein synthesis in cell-free systems from E. coli: Pluramycin A was observed to exhibit no significant effects on $^{14}$C-leucine incorporation into protein and polyuridylate-directed polyphenylalanine synthesis in cell-free systems obtained from exponentially growing cells of E. coli B. The method employed principally followed the one developed by Nirenberg and Matthaei (1961). The results are summarized in Tables 1 and 2.

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Table 1. Effects of pluramycin A on $^{14}$C-leucine incorporation into protein in an E. coli cell-free system.

| Series | Leu incorporation | |
|---|---|---|
| | cpm/μg* | % inhibition |
| Control | 11.6 | |
| Pluramycin A | | |
| 1 μg/ml | 10.8 | 6.7 |
| 5 | 10.4 | 10. |
| 25 | 10.8 | 6.7 |

*cpm/μg tyrosine equivalent.

Table 2. Effects of pluramycin A on polyphenylalanine synthesis in an E. coli poly U system.

| Series | Phe incorporation | |
|---|---|---|
| | cpm/μg* | % inhibition |
| Control | 19.5 | |
| Pluramycin A | | |
| 1 μg/ml | 19.9 | - |
| 5 | 18.3 | 6.5 |
| 25 | 18.1 | 7.0 |

*cpm/μg tyrosine equivalent.

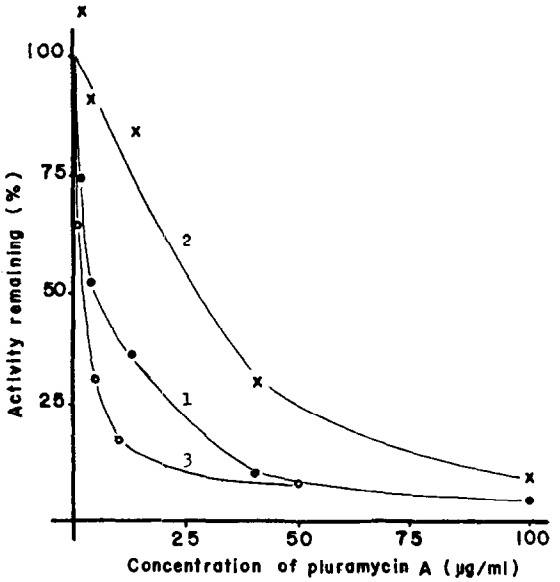


Fig. 1. Inhibition by pluramycin A of RNA polymerase reaction.

The same reaction mixture (0.25 ml) and assay method as described by Chamberlin and Berg (1962) was employed, except 3.5 μg tyrosine equivalent of enzyme, 25 μg of salmon sperm DNA, and 200 cpm/mμmole of $^{3}$H-ATP or $^{14}$C-CTP. $^{3}$H-ATP and native DNA was used for curve 1 (•); $^{3}$H-ATP and heat-denatured DNA for curve 2 (x); and $^{14}$C-CTP and native DNA for curve 3 (o).

Inhibition by pluramycin A of RNA polymerase reaction: RNA polymerase was partially purified from the extracts of E. coli B by the method of Chamberlin and Berg (1962). The cells were grown in a nutrient broth and harvested during the logarithmic phase. The purification procedure included: disruption of the cells by sonication, removal of nucleic acids by streptomycin, protamine precipitation and DEAE-cellulose chromatography. The partially purified enzyme showed a specific activity of approximately 5,200 mμ moles of $^{14}$C-CTP incorporated/hour/mg tyrosine equivalent. The RNA polymerase reaction was strictly dependent on the presence of added DNA. The reaction mixture for the assay was given in the legend to Fig. 1.

RNA polymerase reaction, using salmon sperm DNA as a primer, was markedly inhibited by pluramycin A. Approximately 50 % inhibition was observed at the concentration of 2 μg/ml of the antibiotic with native DNA and at 20 μg/ml with heat-denatured DNA. The former reaction was more sensitive to pluramycin A than the latter was. The results are illustrated in Fig. 1.

Inhibition by pluramycin A of DNA polymerase reaction: DNA polymerase was partially purified from the extracts of E. coli B, following the method of Richardson et al (1964). The cells were grown in a nutirent broth and harvested while in the logarithmic phase. The purification procedure included: disruption of the cells by sonication, streptomycin precipitation, autolysis and ammonium sulfate fractionation.

DNA polymerase reaction, using salmon aperm DNA as a primer, was significantly inhibited by pluramycin A. About 50 % inhibition was demonstrated at the concentration of 2 μg/ml of the antibiotic with native DNA and at 7 μg/ml with heat-denatured DNA. The former reaction was more sensitive than the latter.

The results are presented in Table 3.

Table 3. Inhibition by pluramycin A of DNA polymerase reaction.

| Series | Primer DNA | | | |
|---|---|---|---|---|
| | native | | heat-denatured | |
| | Incorp. of dATP | | Incorp. of dATP | |
| | cpm | % | cpm | % |
| Control | 885 | 100. | 315 | 100. |
| 0 time | 22 | 0 | 27 | 0 |
| -DNA | 81 | 6.8 | 93 | 22. |
| +Pluramycin A 50 μg/ml | 25 | 0.3 | 40 | 4.3 |
| 25 | 39 | 2.0 | 46 | 6.4 |
| 10 | 142 | 14. | 133 | 36. |
| 5 | 289 | 31. | 207 | 60. |
| 2 | 398 | 44. | 234 | 73. |
| 1 | 619 | 69. | 267 | 84. |

The same reaction mixture (0.3 ml) and assay method as described by Richardson et al (1964) was used, except 5 μg of salmon sperm DNA, 0.3 unit of enzyme, and 0.02 μc of $^{14}$C-dATP.

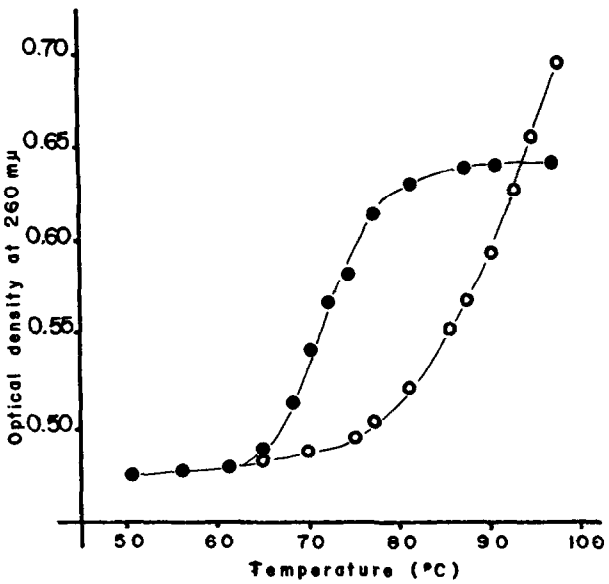


Fig. 2. Transition of melting temperature of salmon sperm DNA in the presence of pluramycin A.
The reaction mixture contained 25 μg/ml of salmon sperm DNA with (-○-) and without (-●-) 10 μg/ml of pluramycin A in 0.01 M NaCl, 0.01 M Tris-HCl pH 7.4.

Transition of Tm of DNA by pluramycin A: As illustrated in Fig. 2, the thermal transition curve of salmon sperm DNA was markedly shifted in the presence of pluramycin A (10 μg/ml). There observed a slow increase of the optical density at 260 mμ which reached the peak at higher temperature. The difference in Tm in the absence and presence of the antibiotic was approximately 15°. The results indicated that pluramycin A binds with and stabilizes the double strand structure of DNA to the denaturing effect of heat.

The results, presented in this communication, indicated that pluramycin A may be coupled with DNA and consequently inhibit DNA and RNA polymerase reactions.

## References

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