INHIBITION OF RNA POLYMERASE REACTION BY CHROMOMYCIN A_3

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Received March 22, 1965

Chromomycin A_3 is an antibiotic and antitumor substance produced by Streptomyces griseus No. 7 (ATCC 13273). It was isolated as yellow crystals by Tatsuoka et al. (1960) and its structure has recently been determined (Tatsuoka et al., 1964).

The mode of action of this new antibiotic has been studied by several groups of investigators. Wakisaka et al. (1963) observed an inhibition of C^{14}-adenine incorporation into RNA fraction of rabbit bone marrow cells, and Yano et al. (1963) showed a marked decrease in incorporation of C^{14}-orotic acid into RNA with rat liver ascites tumor cells. With Bacillus subtilis cells, the incorporation of P^{32} into RNA fraction (Omura, E., personal communications) and that of C^{14}-adenine into DNA as well as RNA fractions were found to be greatly reduced (Kamiyama and Kaziro, unpublished observation).

In this communication, we describe the inhibition of DNA-dependent RNA polymerase reaction by chromomycin A_3. The inhibition was more prominent when native rather than heat-denatured DNA was used as primer. The synthesis of poly A homopolymer catalyzed by RNA polymerase was affected to a much smaller extent. A substantial increase in Tm of calf thymus DNA was observed in the presence of chromomycin A_3, which suggests the binding of the antibiotic to the DNA.

During preparation of this manuscript, the inhibition of RNA-, and DNA polymerase reactions has independently been reported by Hartmann et al.

(1964). These authors also demonstrated that there is a marked shift of the absorption maximum of chromomycin A_3 in the presence of DNA.

RNA polymerase was partially purified from the extracts of Pseudomonas aeruginosa K. The bacteria were grown to the logarithmic phase in a glucose-glutamate medium (Kageyama and Egami, 1962). The purification steps included: disruption of the cells by sonic oscillation, removal of nucleic acids by streptomycin, protamine precipitation, and DEAE-cellulose column chromatography. At this step, the enzyme preparation was completely free of DNA, and the reaction was strictly dependent on the presence of added DNA. The partially purified enzyme had a specific activity of

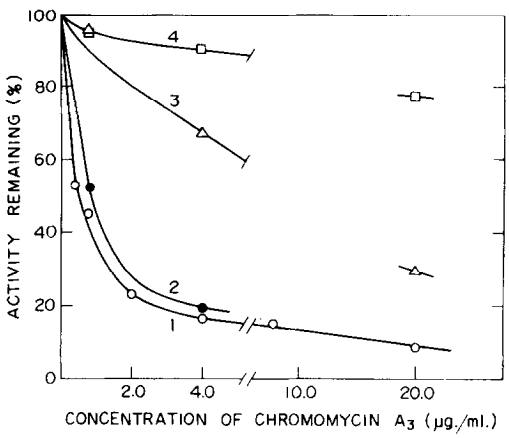

Fig. 1. Inhibition of RNA polymerase reaction by chromomycin A_3

The reaction mixture (0.25 ml) contained in µmoles; Tris-HCl, pH 8.1, 20; potassium phosphate, 2; mercaptoethanol, 2.5; $MgSO_4$, 5.0; three nonlabelled nucleoside triphosphates, each 0.1; phosphoenolpyruvate, 0.5; pyruvate kinase (5.6 units/mg), 45 µg; ATP-C^{14} or CTP-C^{14} (0.5 µc/µmole), 0.04, for curves 2(●) and 3(Δ), or for curve 1(O), respectively; native calf thymus DNA for curves 1 and 2, or heat-denatured calf thymus DNA for curve 3, 26 µg; chromomycin A_3 as indicated; and enzyme, 28 µg for curves 1 and 2, and 56 µg for curve 3. The incubation was carried out for 10 minutes at 37°. For the synthesis of poly A homopolymer (curve 4(□)), the assay system is similar to that of curve 3 except that it contained 0.5 µmole of $MnSO_4$ and 2.0 µmole of $MgSO_4$ instead of 5.0 µmoles of $MgSO_4$, and only C^{14}-ATP as nucleoside triphosphate. The incubation was done for 20 minutes at 37° in the presence of 28 µg of enzyme.

about 700 to 1000 mμmoles of C^{14}-ATP incorporated/hour/mg of protein. The reaction mixture for the assay system was given in the legend to Fig. 1.

As shown in the figure, RNA polymerase from P. aeruginosa was strongly inhibited by chromomycin A_3 (curve 1). About 50% inhibition was observed with concentrations of 0.4 to 0.8 μg/ml of the antibiotic. The inhibition was slightly less when C^{14}-ATP was used as labeled nucleotide (curve 2) instead of C^{14}-CTP. With heat-denatured DNA, the rate of the reaction in the absence of chromomycin A_3 was about one-fourth of that observed with native DNA, and this reaction was less sensitive to the inhibitory action of the antibiotic (curve 3). Chromomycin A_3 inhibited only slightly the synthesis of poly A, catalyzed by RNA polymerase with heat-denatured DNA as primer (curve 4).

The thermal transition curve of calf thymus DNA was markedly shifted to the right in the presence of chromomycin A_3 (Fig. 2). There was a

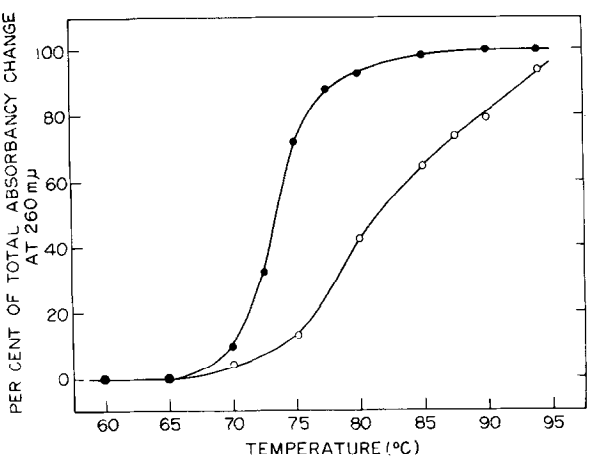

Fig. 2. Melting profile of calf thymus DNA in the presence and absence of chromomycin A_3

The tubes containing a solution of 13 μg/ml of calf thymus DNA in 0.015M NaCl, 0.0015M Na citrate and 0.01M Tris-HCl, pH 7.5, with (O) or without (●) 50 μg/ml of chromomycin A_3, were heated at desired temperatures for 20 minutes followed by rapid cooling in ice. The optical density at 260 mμ was measured by Hitachi Perkin-Elmer spectrophotometer, Model 139.

rather slow increase in the optical density at 260 mμ which reached the maximum value at higher temperatures. The difference in Tm in the presence and absence of chromomycin A_3 was about 10°. The results suggest that the antibiotic binds with and stabilizes the helical structure of the DNA to the denaturing effect of heat.

Discussions. From the foregoing results, it is evident that chromomycin A_3 binds with DNA, and thus inhibits DNA-dependent RNA polymerase reaction. Since the reaction primed with heat-denatured DNA was much less sensitive to the antibiotic, the latter may require a double-stranded helical configuration for its binding with DNA molecule. A preliminary experiment showed that inhibition of the incorporation of C^{14}-CTP was greater with DNA from P. aeruginosa (AT/GC=0.49), than with that from calf thymus (AT/GC=1.29). This result, together with the fact that poly A formation with denatured DNA as primer was little inhibited by chromomycin A_3, might suggest the requirement of the presence of guanine residues, as in the case of actinomycin D (Kersten, 1961; Goldberg et al., 1962; Kahn et al., 1963), for the binding of the antibiotic with DNA. It will be of interest to test this possibility using dAT copolymer as primer.

In collaboration with Drs. S. Hasegawa, H. Kobayashi, and Y. Miura, we have examined the effect of chromomycin A_3 on the multiplication of E. coli phages. Although E. coli cells are not well permeable to chromomycin A_3, higher concentrations of the antibiotic reduced the yield of phage MS2 by 90% under certain conditions, but did not affect that of phages T2 and ϕx174. The detailed mechanism of this inhibition is now under investigation, meanwhile it might be assumed that chromomycin A_3 could also inhibit the reaction catalyzed by the RNA-dependent RNA polymerase induced by the infection of an RNA phage.

Summary. Chromomycin A_3 was shown to inhibit DNA-dependent RNA polymerase reaction. The inhibition was more remarkable when native,

rather than heat-denatured DNA was used as primer. The binding of the antibiotic to the DNA was suggested from the shift of the thermal transition curve of calf thymus DNA.

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