EFFECT OF DISTAMYCIN A ON THE STRUCTURE AND TEMPLATE ACTIVITY OF DNA IN RNA – POLYMERASE SYSTEM

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It is shown that native and denatured DNA form complexes with the oligopeptide-antibiotic distamycin A. The pronounced inhibition of the incorporation of AMP into RNA in the DNA directed RNA-polymerase system is due to the interference of the antibiotic with DNA.

1. Introduction

Distamycin A is an antibiotic with antitumor [1] and antiviral [2–5] activity isolated from cultures of *Streptomyces distallicus*. The antiviral activity of this compound has been predominantly observed with DNA viruses [2–5]. Distamycin has been shown to interfere with the morphogenesis of T_1 and T_2 bacteriophages. The antibiotic is a basic oligopeptide [6] the structure of which is shown in fig. 1. The present studies indicate that the biological damage by distamycin A is mediated through its interaction with DNA. Some physio-chemical interactions between DNA and distamycin *in vitro*, and its effect on the template activity of DNA in the RNA-polymerase system are reported here.

2. Materials and methods

DNA samples used in physico-chemical studies were those described by Sarfert and Venner [7]. ^{14}C-ATP was obtained as tetralithium salt from NEN Chemicals GmbH, Germany (Catalogue No. NEC 417); other triphosphates were obtained from Zellstofffabrik Waldhof, Mannheim. For experiments with the RNA-polymerase system, calf thymus DNA was supplied by Worthington, Freehold, N.J., USA. All other chemicals were analytical grade reagents from Merck, Darmstadt. RNA-polymerase was isolated from *E. coli* cells according to the procedure of Zillig et al. [8]. We used fraction V of the procedure reported above [8] preserved in buffer containing 20% glycerol at −20°C. The reaction mixture contained in 0.2 ml: 0.03 M tris acetate, pH 7.9; 0.13 M ammonium chloride; 0.03 M magnesium acetate; 0.001 M each of GTP, CTP, UTP and ^{14}C-ATP; 0.01 M phosphoenol pyruvate; 10 μg pyruvate kinase and 50 μg of calf thymus DNA. The reaction was started with 30–50 μg enzyme protein and incubations were carried out for 20 min at 37°C. The reaction was stopped by adding 7% perchloric acid and serum albumin was used as carrier. The precipitate was washed twice with perchloric acid and once with methanol. The residue was dissolved in 0.1 N NaOH and the radioactivity was determined using dioxane scintillator in a Packard scintillation spectrometer model 3375.

Protein was estimated by the method of Lowry et al. [9].

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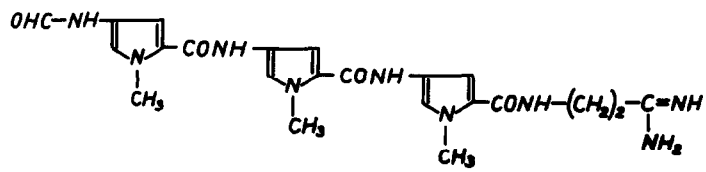

Fig. 1. Chemical structure of distamycin A.

Distamycin A was isolated from cultures of *Streptomyces netropsis* J-A 2814 [10].

3. Results and discussion

The influence of distamycin A on the UV-absorption of DNA is shown in fig. 2. The absorbance of DNA decreases in the presence of distamycin A. This effect is dependent on the antibiotic/DNA-P ratio (r).

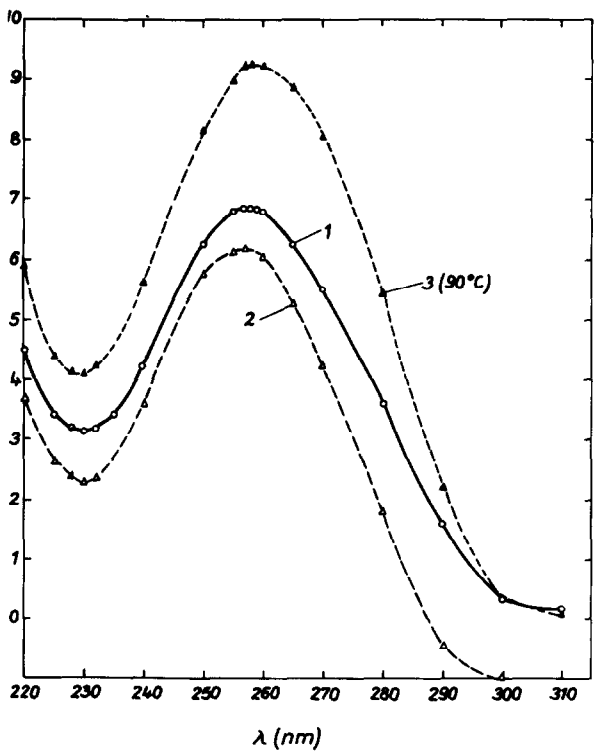

Fig. 2. UV-absorption spectra of *E. coli* DNA in 0.02 M KCl without (1) and with (2) distamycin A at 25°C and 90°C.

After thermal denaturation, the absorbance reaches a constant value.

We have further investigated the melting behaviour of calf thymus DNA in the presence of distamycin A. As demonstrated in fig. 3, the melting profile of native DNA shifts towards higher temperatures with increasing antibiotic concentration. The hyperchromicity also increases from 40 to about 60% when r is raised from r = 0 to r = 1. It is interesting to note that denatured DNA shows a cooperative transition and a hyperchromicity of 60% in the presence of distamycin A. From these results and some others [11], we conclude a strong binding of distamycin A to DNA while less interactions have been observed with RNA [11]. There are some indications [11, 12] that besides electrostatic interactions other binding types might be involved in the formation of the DNA-distamycin complex.

The pronounced interactions observed between DNA and distamycin A could explain the biological damage caused by this antibiotic. Thus, we have studied the effect of distamycin A on the template activity of DNA in the RNA-polymerase system. The effects of distamycin A on the template activities of both native and denatured DNA are shown in fig. 4. DNA was denatured by heating at 95°C for ten min and then chilled immediately in ice.

Distamycin A inhibits the template functions of native as well as of denatured DNA. In these experiments the antibiotic was added after DNA and triphosphate incubation, and the reaction was started immediately with the enzyme. As shown in fig. 4, the template activity of native DNA is more sensitive to the antibiotic effect as compared to that of denatured DNA. Puschendorf and Grunicke [13] have recently studied the action of distamycin A on the template functions of native and denatured DNAs in the DNA-polymerase reaction. They found that the

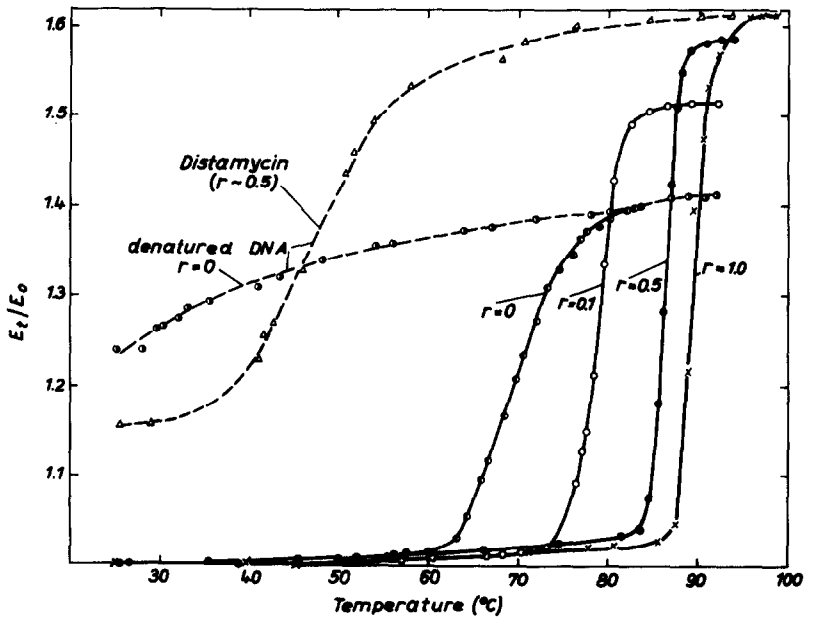

Fig. 3. Melting temperature of native and denatured calf thymus DNA in 0.02 M KCl in the presence of various distamycin A/DNA-P ratios (r).

denatured DNA is more sensitive to distamycin A in their reaction. These differences are perhaps due to the substrate specificity in these two reactions. It is known that DNA-polymerase prefers single stranded DNA as template whereas RNA-polymerase is more active on double stranded DNA [14].

Recent studies with rifamycin revealed that this compound is a specific inhibitor of DNA-directed

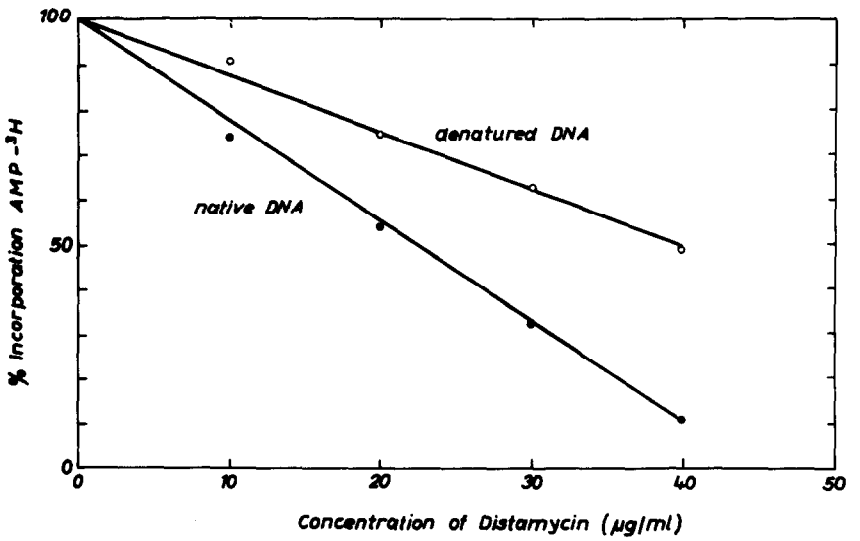

Fig. 4. Effect of distamycin A on the template activities of native and denatured DNA in the RNA-polymerase reaction.

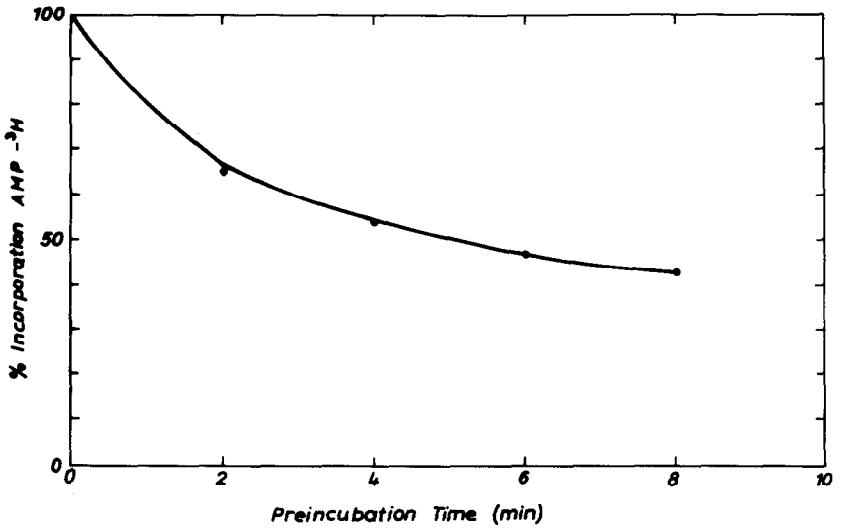

Fig. 5. Template activity of native DNA preincubated with distamycin A. Native DNA was incubated with distamycin (25 μg/ml) at 37°C. The incubated samples were immediately chilled and used in the next five minutes. Controls were treated in the same manner but without distamycin. The reaction was started by adding the enzyme. The reaction was terminated after 20 min.

RNA-polymerase. However, this inhibition is not caused by any interaction between the antibiotic and DNA. The antibiotic inhibits by interacting directly with the enzyme. Whether the inhibitory action of distamycin is entirely due to its interaction with DNA was tested by preincubating DNA with the antibiotic for various periods of time. The samples of native DNA preincubated for various times were used in the RNA-polymerase reaction. The results are given in fig. 5.

The inhibition of RNA-polymerase activity continues to increase with the preincubation period.

Table 1
Effect of distamycin A on the RNA-polymerase reaction at various intervals.

Incubation time (min)	Without distamycin	With distamycin (20 μg/ml)	Inhibition (%)
2	3147*	617	80
4	3607	963	74
8	5274	1887	64

* AMP-^{3}H incorporated. The figures indicate cpm/incubation mixture.
The reaction was terminated at 2, 4 and 8 min after incubation with the enzyme at 37°C, then cooled in ice, and the reaction was stopped with 7% $HClO_4$ and continued as described in materials and methods.

Inhibition takes place after 2 min, and graudally increases up to 8 min. Thus the interaction between DNA and distamycin A is time dependent and is completed in the first 10 min. This is in good agreement with the results in table 1. In these experiments the RNA-polymerase reaction was terminated at various times.

The incubation experiments in table 1 with and without distamycin, were run parallel. Maximum inhibition is observed at 2 min, followed by a decrease at 4 and 8 min, indicating that inhibition is not due to an effect on the enzyme. This is in accordance with the results reported in fig. 5 and explains the kinetic experiments with preincubated DNA.

The experiments reported in table 1 and fig. 5 demonstrate that the inhibition of RNA-polymerase by distamycin A can be attributed to its binding to DNA. This is presumably the reason for its specific effect on DNA viruses [2–5]. Its differential behavior towards the template functions of native and denatured DNA indicates that the action of this antibiotic can be compared to actinomycin and chromomycin.

Our results obtained in the RNA-polymerase sytem are in good agreement with similar effects

observed very recently and independently by Puschendorf and Grunicke*.

* We wish to thank Dr. H.Grunicke for kindly sending the manuscript of their results (report presented at the meeting of the Gesellschaft für Biologische Chemie, Hannover, October 1969).

References

[1] A.DiMarco, M.Gaetani, P.Orezzi, P.Scotti and F.Arcamone, Cancer Chemotherapy Rep. 18 (1962) 15.
[2] A.M.Gasazza and M.Ghione, Chemotherapia 9 (1964) 80.
[3] M.A.Verini and M.Ghione, Chemotherapia 9 (1969) 144.
[4] G.H.Werner, P.Ganter and Y.de Ratuld, Chemotherapia 9 (1964) 65.
[5] J.Journel, P.Ganter, F.Koenig, Y. de Ratuld and G.H.Werner, Antimicrobial agents and chemotherapy (Am. Soc. Microbiol.) p. 599.
[6] F.Arcamone, S.Penco, V.Nicolella, P.Orezzi and A.M.Pirelli, Nature 203 (1964) 1064.
[7] E.Sarfert and H.Venner, Z. Physiol. Chem. 340 (1965) 157.
[8] W.Zillig, E.Fuchs and R.Millette in: Procedures in nucleic acid research, eds. G.L.Cantoni and D.R.Davies (Harper and Row, London, 1966) p. 323.
[9] O.H.Lowry, N.I.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[10] H.Thrum, Naturwissenschaften 46 (1959) 87.
[11] Ch.Zimmer, I.Haupt and H.Thrum, in: Wirkungsmechanismen von Fungiziden, Antiobiotica und Cytostatica (Akad. Verlag, Berlin, 1969) in press.
[12] Ch.Zimmer et al., in preparation.
[13] B.Puschendorf and H.Grunicke, FEBS Letters 4 (1969) 355.
[14] O.K.Honickel, A.Sippel and G.Hertmann, Vortrag Deutsche Gesellschaft für Biologische Chemie, Colloq. April 21–24 (Tecklenburg, 1968).