EFFECT OF DISTAMYCIN A ON THE TEMPLATE ACTIVITY OF DNA IN A DNA POLYMERASE SYSTEM

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1. Introduction

Distamycin A is an antibiotic produced by *Streptomyces distallicus* with antiviral [1–4] and antitumor [5] properties. The structure (fig. 1) has been identified and confirmed by total synthesis [6]. The mechanism of the antiviral and cytostatic activity of Distamycin A is still obscure. Recently Zimmer et al. [7] have demonstrated that Distamycin is bound to DNA. In order to investigate the biological consequences of this effect the influence of Distamycin A on the template activity of DNA in a DNA polymerase system was studied. It is demonstrated that Distamycin A strongly inhibits the template activity of DNA for DNA polymerase.

2. Materials and methods

Distamycin A was donated by Farmitalia, Milano, Italy. dTTP-^{3}H (10 c/mmole) was obtained from Schwarz Bioresearch, Inc., Orangeburg, N.Y. dATP, dGTP, dCTP and dTTP were purchased from Sigma Chemical Comp., St. Louis, Mo.

Erlich ascites tumor cells were grown and harvested as described previously [8]. Cells were suspended in an equal volume of water at 0°, kept in an icebath for 15 min and disrupted by 30 strokes with a loose and 30 strokes with a tight fitting pestle in a Dounce type homogenizer. The resulting homogenate was centrifuged for 2 hr at 100,000 × *g* and the clear supernatant used as source of enzyme in the DNA polymerase assay.

Assay of template activity: A modification of the assay described by Smellie et al. [9] was used. The

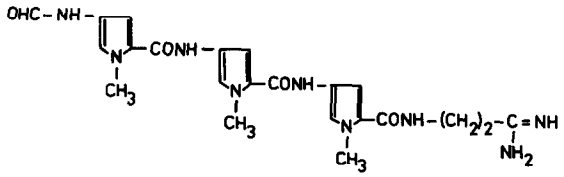

Fig. 1. Distacymin A.

system contained in a total volume of 1.0 ml in μmoles: Tris, pH 7.9, 40.0; $MgCl_2$ 5.0; mercaptoethanol 1.0; dATP, dGTP, dCTP, dTTP 0.005 each; dTTP-H^3 (10 c/mmole) 0.5 μc; ATP 5.0; various concentrations of calf thymus DNA as indicated, and about 20 mg of enzyme protein.

The reaction mixture was incubated for 30 min at 37°. Reaction was stopped by the addition of 4.0 ml of 0.7 N-$HClO_4$. The precipitate was washed three times with 5.0 ml of 0.2 N-$HClO_4$, dissolved in 0.5 ml of "Nuclear Chicago Solubilizer" (NCS). 10 ml scintillator (naphthalene 738 g; 2,5 diphenyloxazol 0.46 g; xylene 3500 ml; dioxane 3500 ml; ethanol 2100 ml) was added and the solution counted by liquid scintillation spectrophotometer. Incorporation of label from ^{3}H-TTP required the presence of either denatured or native DNA (table 1). All four deoxyribonucleotides are necessary for full activity. Radioactivity incorporated into the acid-insoluble product could be rendered acid-soluble by treatment with pancreatic deoxyribonuclease (table 1).

3. Results

Fig. 2 shows the effect of various concentrations of Distamycin A on the DNA polymerase system. An

Table 1
Requirements for ^{3}H-dTMP incorporation into DNA. The complete system is described under Methods. The assay plus DNase contained 0.8 mg deoxyribonuclease I of bovine pancreas.

	μμmoles dTMP incorporated	%
Complete system plus denatured DNA (100 μg)	206.1	100.0
Complete system plus native DNA (100 μg)	264.3	128.0
Minus DNA	2.4	1.2
Minus dATP	58.5	28.4
Minus dCTP	103.8	50.4
Minus dGTP	82.8	41.6
Minus dATP, dCTP, dGTP	28.8	14.0
Complete system plus denatured DNA (100 μg) plus DNase	<0.1	<0.2

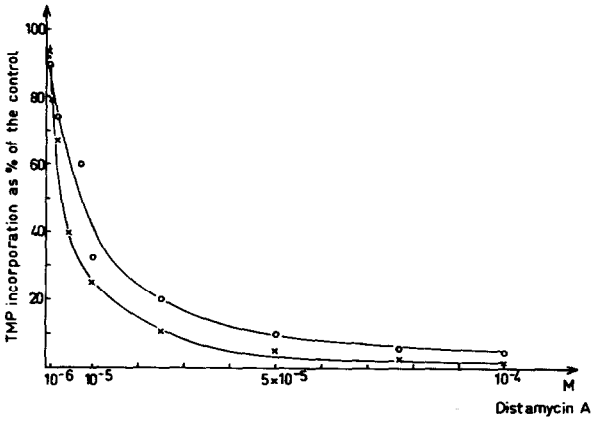

Fig. 2. Effect of various concentrations of Distacymin A on the incorporation of TMP into DNA. Details are described under Methods. All assays were performed in the presence of 100 μg either native or denatured DNA. x–x–x template: denatured DNA; o–o–o template: native DNA.

inhibition of DNA synthesis can be observed even at the lowest concentration of Distamycin used (5×10^{-7} M). 50% inhibition is observed at 3×10^{-6} M Distamycin using denatured DNA or at 6×10^{-6} M using native DNA as a template. The effect of a constant dose of Distamycin A in the presence of increasing concentrations of DNA is pictured in fig. 3. Compared to untreated controls the percent inhibition of TMP incorporation is about the same at all DNA concentrations tested.

In order to investigate if the observed inhibition of the DNA-polymerase reaction is caused by an impairment of the enzyme or due to an interaction of Distamycin A with DNA, DNA was preincubated with Distamycin, extensively dialysed to remove any free drug and finally tested for template activity. Table 2 demonstrates that the percent inhibition observed after preincubation of DNA with 10^{-4} M Distamycin is very close to the percent inhibition which is observed after direct addition of the drug to the polymerase assay.

It can be concluded, therefore, that the inhibition of DNA synthesis observed after addition of Distamycin A to a DNA-polymerase system is due to a decrease in template activity caused by an interaction of Distamycin with DNA.

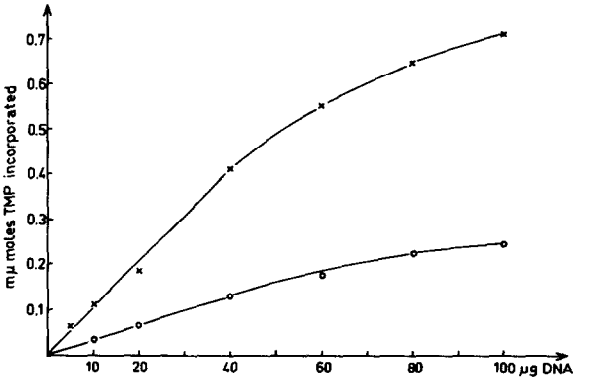

Fig. 3. Effect of Distamycin A (7.7×10^{-6} M) on the incorporation of TMP into DNA at various concentrations of template DNA. Details are described under Methods.
x–x–x control; o–o–o plus Distamycin A.

4. Discussion

Zimmer et al. [7] have shown that Distamycin A is bound to DNA. Our experiments demonstrate that

Table 2
Effect of a preincubation of DNA with Distamycin A on the template activity of DNA in the DNA polymerase system. 5 ml samples of denatured calf thymus DNA in H_2O (400 μg/ml) were preincubated with various concentrations of Distamycin A at 37°. After 1 hr the samples were dialysed against 2 X 15 l of H_2O at +4°C for 18 hr. 0.02 ml samples of the dialysed DNA solution were assayed for template activity. All assays were done in triplicate. Absolute values are given as means ± s.d. Percent values are calculated from the means.

	$\mu\mu$moles TMP incorporated	%
DNA preincubated without drug	319.2 ± 7.5	100.0
DNA preincubated with Distamycin A		
10^{-4} M	29.4 ± 3.4	9.5
2.5×10^{-4} M	10.8 ± 1.9	3.5
5×10^{-4} M	4.3 ± 2.1	1.3

a binding of Distamycin to DNA results in a decrease of the template activity of DNA for DNA synthesis.

The concentrations of Distamycin A needed to produce a significant inhibition of DNA template activity are in the same range as those required for an antiviral or antitumor effect. It is possible therefore that the antiviral and cytostatic activity of Distamycin A is due to its interaction with DNA.

Besides its effect on DNA synthesis, the binding of Distamycin to DNA may also affect the template activity of DNA for RNA synthesis. Observations that Distamycin A inhibits the induction of several enzymes [10,11] may in part be explained by a decreased template activity of DNA in the RNA polymerase reaction.

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