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SHORT COMMUNICATIONS

Interactions of the Antibiotic Distamycin A with Homopolymeric Single-Stranded Polydeoxyribonucleotides and with ΦX 174 Deoxyribonucleic Acid

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SUMMARY

KREY, ANNE K., OLENICK, JOHN G. & HAHN, FRED E. (1976) Interactions of the antibiotic distamycin A with homopolymeric single-stranded polydeoxyribonucleotides and with ΦX 174 deoxyribonucleic acid. *Mol. Pharmacol.*, 12, 000-000.

The absorption spectrum of distamycin A was changed by poly dA, poly dT, poly dG, and poly dC and also by ΦX 174 DNA. Poly dA and, to a lesser extent, poly dG induced a Cotton effect in the optical rotatory dispersion spectrum of distamycin A. The complex of distamycin A and ΦX 174 DNA showed a reverisble cooperative transition upon heating and cooling. Distamycin A inhibited the template activities of poly dA and poly dT in an RNA polymerase reaction *in vitro*.

INTRODUCTION

The antibiotic distamycin A inhibits the replication of DNA-containing viruses (1-7), the induction of bacterial enzyme syntheses (8), and the DNA- and RNA-dependent biosyntheses of nucleic acids *in vitro* (9-14). Inhibition of DNA-dependent nucleic acid biosyntheses has been attributed to a preferential interaction of DMC[1] with A-T-rich DNA templates (13) or A-T-rich initiation sites in template DNA (14). A preference of DMC for A-T-rich regions of duplex DNA was inferred from biophysical indications of the binding of the antibiotic (15-18) and has been attributed to a high affinity of DMC for conformations regionally imposed on DNA by an abundance of A-T (17, 18).

We have investigated the alternative possibility, viz. an actual base specificity of DMC binding to DNA-like polymers, with a resulting template toxicity, and report here that DMC binds to poly dA and poly dT and inhibits the RNA polymerase reaction utilizing these two homopolymers as templates.

Distamycin A was purchased from Calbiochem; calf thymus DNA, from Worthington; poly dA and poly dT, from P-L Biochemicals; poly dC and poly dG, from General Biochemicals; single-stranded ΦX 174 DNA and *Micrococcus lysodeikticus* RNA polymerase, from Miles; [^{14}C]GTP and [^{14}C]UTP, from Amersham/Searle; and [^{3}H]CTP, from New England Nuclear; ATP and GTP were supplied by Calbiochem, and CTP and UTP, by P-L Biochemicals.

RNA polymerase activities were determined by following the incorporation of radioactive ribonucleoside triphosphates into acid-insoluble polynucleotides. The polymerase reactions were started with the addition of enzyme and, after 20 min of incubation at 30°, terminated by addition of an equal volume of cold 10% trichloroa-

[1] The abbreviation used is: DMC, distamycin A.

cetic acid containing 0.5% ATP. Bovine serum albumin was added to serve as carrier; the precipitates formed were collected on membrane filters (Millipore) and washed extensively with cold 5% trichloracetic acid containing 0.5% ATP. Filters were dissolved in a dioxane-based scintillation fluid, and radioactivity was determined in a Nuclear-Chicago liquid scintillation counter.

Absorption spectra were recorded in Cary model 14 spectrophotometer; optical rotatory dispersion spectra, in a JASCO ORD/UV-5 spectropolarimeter; and melting experiments, in a Gilford model 2000 spectrophotometer programmed for the automatic recording of temperatures and absorbance values.

The absorption maximum of DMC at 303 nm was reduced in intensity and shifted to longer wavelengths by poly dA, poly dG, poly dT, and poly dC (Fig. 1). The magnitude of the bathochromic shift decreased for the sequence poly dA > poly dT > poly dG > poly dC. Single-stranded ΦX 174 DNA caused a bathochromic shift larger than poly dA and increased the absorption intensity of the antibiotic (Fig. 2), in contrast to the four homopolymers, which decreased the absorbance of DMC. We conclude that DMC became bound to all five single-stranded polydeoxyribonucleotides but that the individual binding reactions were qualitatively different. It is possible that the affinities and stoichiometries of DMC binding to the studied polymers also are different.

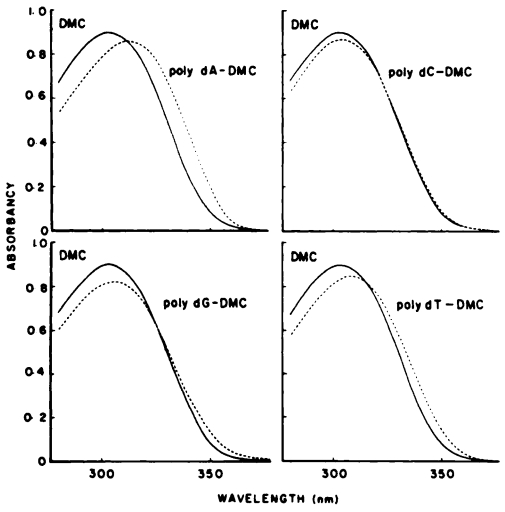

FIG. 1. *Effect of synthetic polydeoxynucleotides poly dA, poly dC, poly dG, and poly dT on absorption spectrum of distamycin A*

Concentrations: 0.032 mM DMC, 0.272 mM polymers, 5 mM Tris-HCl, pH 7.5.

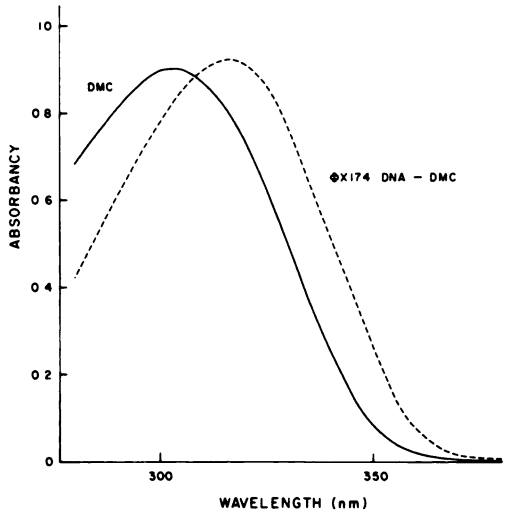

FIG. 2. *Influence on absorption spectrum of DMC by single-stranded bacteriophage ΦX 174 DNA*

For concentrations, see Fig. 1.

Poly dA, poly dC, poly dG, and poly dT exhibited multiple Cotton effects in the wavelength region between 200 and 300 nm (Fig. 3). Poly dG showed the largest molecular amplitudes in its ORD spectrum, and the rotatory dispersion of poly dA was unique in that a more prominent effect was recorded at the shorter wavelength end of the spectrum. We have reported a similar observation for single-stranded DNA (19).

Single-stranded DNA induces a large Cotton effect in the near ultraviolet absorption band of DMC (20). A similar effect was induced by poly dA (Fig. 3). In contrast, poly dG induced a much smaller Cotton effect, and poly dT, as well as poly dC, failed to render the absorption bands of DMC optically active. The ORD spectra in Fig. 3 suggest the formation of an ordered complex of DMC with poly dA.

The complex of DMC with denatured DNA shows, with increasing temperature, a reversible cooperative transition (10).

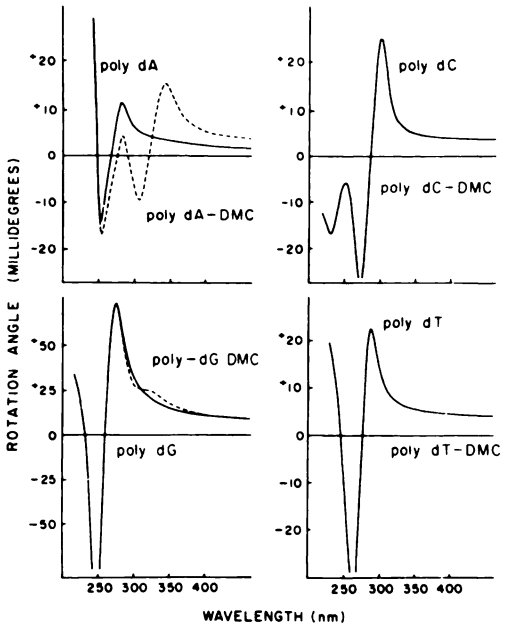

FIG. 3. *Optical rotatory dispersion spectra of synthetic polymer–distamycin complexes and of polymers alone*

Concentrations used were those of Fig. 1.

Figure 4 shows a similar phenomenon for the complex of DMC with ΦX 174 DNA; this DNA alone exhibited only a slight, noncooperative hyperchromic transition upon heating. We attribute the reversible cooperative transtition of the DMC–ΦX 174 DNA complex to the dissociation or reformation of an ordered complex of the antibiotic with a single-stranded DNA.

A large but noncooperative transition was observed for poly dA (Fig. 4); poly dT and poly dG did not change their absorbances at 260 nm when heated through an interval from room temperature to above 90°, and poly dC showed an unexpected hypochromic effect upon heating. DMC had no influence on the absorbance of poly dA, poly dT, or poly dC in these heating experiments, but the complex of DMC with poly dG showed a small hyperchromic change (Fig. 4).

In summation: spectrophotometry (Fig. 1) indicated interactions of DMC with poly dA, poly dG, poly dT, and poly dC and also (Fig. 2) with ΦX 174 DNA. Interactions

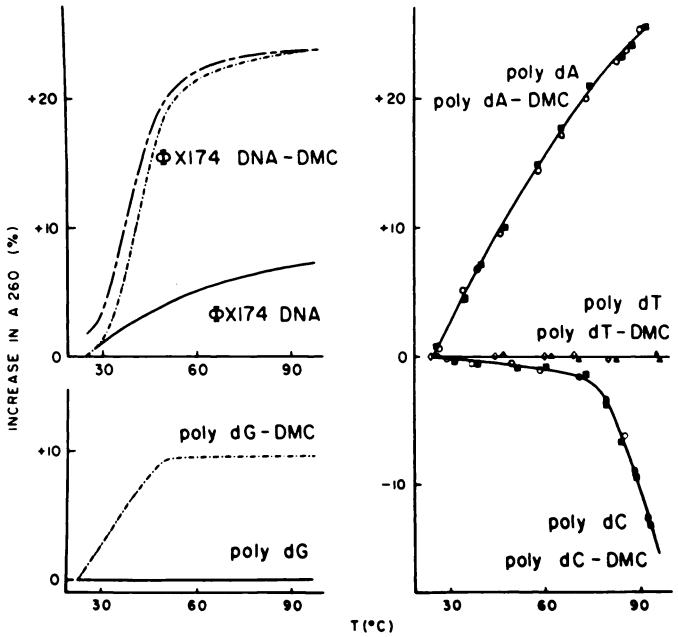

FIG. 4. *Melting profiles in 20 mM KCl of synthetic polydeoxynucleotides and of ΦX 174 DNA in the absence and presence of DMC*

Concentrations: 27.2 μM antibiotic and 54.4 μM polymer phosphorus. — - —, hyperchromicity upon heating; ·–·–, hypochromicity upon cooling of the ΦX 174 DNA–antibiotic complex. Open symbols at right refer to melting of copolymers alone, and solid symbols, to melting in the presence of distamycin.

with poly dA and poly dG were confirmed by spectropolarimetry (Fig. 3), and melting experiments indicated an interaction of DMC with ΦX 174 DNA and with poly dG (Fig. 4).

In decresing order of efficiency, poly dT, duplex (calf thymus) DNA, poly dA, and poly dC served as templates in the RNA polymerase reaction *in vitro* (Table 1). Poly dG had no template activity, as previously observed by others (21). DMC inhibited the template activity of double-stranded DNA, poly dA, and poly dT, producing typical log dosage-response curves (Fig. 5A), but the antibiotic did not interfere with the template activity of poly dC. A plot of the magnitude of the bathochromic shifts produced by duplex DNA, poly dA, and poly dT in the absorption spectrum of DMC as a function of the 50% inhibitory concentrations of the antibiotic in the RNA polymerase reactions with these templates is shown in Fig. 5B. This reveals a systematic correlation between the DMC-polymer dissociation constants (= ED_{50}) and one biophysical parameter of the binding of DMC to templates. Our observations collectively indicate that DMC acted as a template poison when the templates contained either A-T pairs or A or T singly.

The principal conclusion from our work is that DMC exhibited base specificities for

TABLE 1

Relative transcription efficiencies for various DNA templates

Template	RNA formation
	cpm
Poly dT	6800
Native DNA	1700
Poly dA	1000
Poly dC	500
Poly dG	0

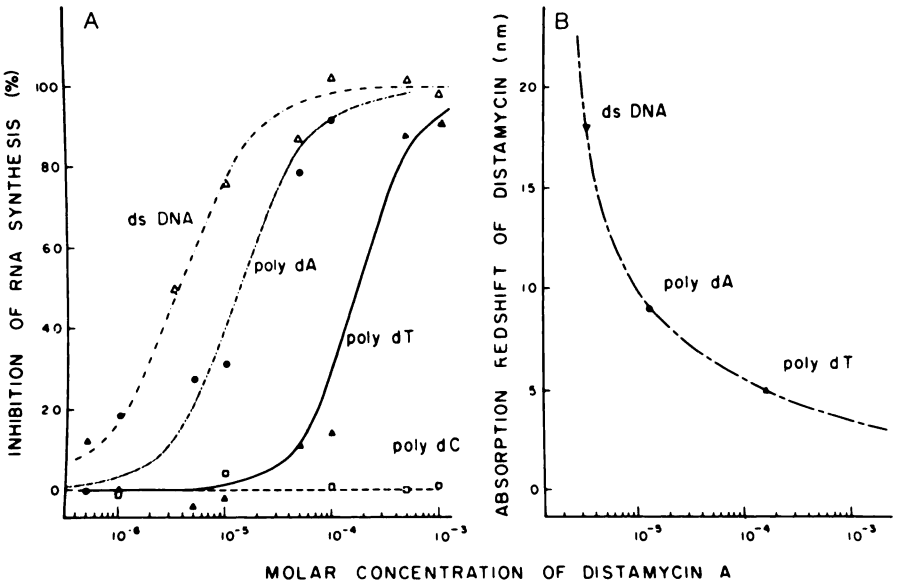

FIG. 5. *Inhibition of RNA polymerase reaction by distamycin A*

A. Inhibition by distamycin of the RNA polymerase reactions directed by poly dA, poly dC, poly dT, and native (double-stranded, ds) calf thymus DNA. Assays using poly dA, poly dC, poly dG, or poly dT as template contained [^{14}C]UTP, [^{14}C]GTP, [^{3}H]CTP, or [^{14}C]ATP, respectively, as substrate, while the reaction directed by native DNA contained ATP, CTP, GTP, and [^{14}C]UTP. Concentrations: 1.2 mM unlabeled triphosphates; 1.2 mM labeled triphosphates, each with a specific activity of 1 μCi/μmole; 0.0744 mM polymer phosphorus; 0.1 M Tris-HCl, pH 7.5; 4 mM $MnCl_2$; 36 μg (27 units) of enzyme per 0.5-ml reaction mixture.

B. Comparison of the inhibition by DMC of the RNA polymerase reaction mediated by the above polymers with the absorption red shift these polydeoxynucleotides produce for the antibiotic.

A or T when these bases were constituents of single-stranded DNA-like homopolymers. These conclusions rest on optical observations as well as on the demonstration of template toxicity of DMC in the RNA polymerase reaction *in vitro*. In contrast, the strong binding of DMC to duplex DNA (15, 16) or to duplex polymers containing deoxyadenylic and thymidylic acids (17, 18) can be attributed to a preference of the antibiotic for double-helical polymers which possess the B-conformation (22).

Optical indications of the binding of DMC to homopolydeoxynucleotides were strongest for poly dA. The Cotton effect induced by this polymer in the ORD spectrum of DMC (Fig. 3) may, like that induced by double-helical DNA (20), arise from an ordered alignment of the *N*-methylpyrrole chromophores of DMC with the polymer. Poly dA has been suggested to exist in near neutral solution as a single-stranded helix (23); this is in accord with our melting experiments, which showed (Fig. 4) evidence of an unstacking of bases in a noncooperative manner. We observed no contribution of liberated DMC to the hyperchromicity of heated poly dA and infer that the antibiotic remained bound to this polymer at elevated temperatures (Fig. 4). By analogy to poly rU (24), we assume that poly dT has no secondary structure at room temperature. Indeed, heating this polymer produced no hyperchromic response (Fig. 4), and poly dT induced no Cotton effects in the ORD spectrum of DMC (Fig. 3). Poly dG has been suggested to exist in an ordered "self-complexed" form (25, 26). The large Cotton effect of the polymer (Fig. 3) is in accord with such an idea, but we have not seen evidence of a melting out of secondary structure at temperatures up to at least 90° (Fig. 4). It is difficult to evaluate optical indications of binding of DMC to poly dG (Figs. 1, 3, and 4), since the polymer does not function as a template in the RNA polymerase reaction, and possible inhibition by DMC therefore could not be investigated. The optical indication (Fig. 1) of DMC binding of poly dC was weak; the antibiotic did not influence the atypical hypochromic response of this polymer to heat (Fig. 4), and no induced Cotton effect in the ORD spectrum of DMC was observed (Fig. 3). Finally, DMC had no template toxicity for poly dC in the RNA polymerase reaction (Fig. 5) up to an antibiotic concentration of 1 mM.

We conclude that the template toxicity of DMC was a function of the relative magnitude of biophysical indications of binding to the templates (Fig. 5B) and was, among single-stranded homopolymers, restricted to poly dA and poly dT. We propose that these observations are based upon actual base specificities of the binding of DMC rather than on conformational properties of the homopolymers.

The RNA polymerase reaction is known to be subject to product inhibition by the newly formed RNA strand, which *in vitro* remains attached to the DNA template (27). It is worthy of consideration that DMC may bind strongly to the poly dA–poly rU and poly dT–poly rA hybrids which are formed during the RNA polymerase reaction with homopolymeric templates and may thereby inhibit reinitiation of transcription in a manner different from that which has been proposed for the effects of DMC on A-T-rich duplex DNA templates (14, 28).

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