INTERACTIONS OF THE ANTIBIOTIC, DISTAMYCIN A, WITH NATIVE DNA AND WITH SYNTHETIC DUPLEX POLYDEOXYRIBONUCLEOTIDES

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1. Introduction

The complex of distamycin A (DMC) with DNA is very stable: neither dialysis against 1% sodium lauryl sulfate [1] nor treatment with 6 M urea or with physiological concentrations of inorganic ions [2] change the absorption spectrum of bound DMC. The spectrum of the free antibiotic reappeared when $NaClO_4$, at 7.2 M, was supplied to the DNA–DMC complex [3]. It is not known whether the effect of ClO_4^- on DNA physically dissociated DMC from DNA or merely altered the conformation of the complex in such a manner that the spectrum changed. We report here a partial restoration of the free antibiotic's spectrum by enzymatic digestions of the DNA–DMC complex and the complete chemical extraction of the antibiotic from this complex with a biphasic phenol–water system.

Biophysical indicators of DMC's binding to DNA increase in magnitude with increasing A–T contents of compositionally different DNA's [3]; it has been suggested that DMC binds preferentially to A–T rich "domains" of DNA [3]. We report here binding studies of DMC to poly d(A–T), poly dA·dT, poly dI·dC and poly dG·dC which show that the presence of guanine, but not that of hypoxanthine, reduced the magnitudes of biophysical indicators of antibiotic binding.

DNA–ligand complexes can be broadly categorized into intercalative and non-intercalative structures. We have tested for intercalation of DMC into closed circular DNA viscometrically [4] and conclude that the antibiotic is not intercalated.

2. Materials and methods

DMC was purchased from Calbiochem, calf thymus DNA, deoxyribonuclease I and snake venom phosphodiesterase from Worthington Biochemical Corporation, poly d(A–T) and poly dG·dC from Miles Laboratories Inc. and poly dI·dC and poly dA·dT from General Biochemicals. Closed circular DNA was isolated from phage PM2, growing in *Pseudomonas* BAL-31. Inocula of these organisms were kindly supplied by Dr. W.R. Bauer who also advised us on technical details of the production of this DNA. The methods for its isolation have been described [5–7].

Absorption spectra were recorded in a Cary Model 14 spectrophotometer, optical rotatory dispersion spectra in a JASCO/UV-5 spectropolarimeter and thermal denaturation profiles in a Gilford 2000 spectrophotometer programmed for the automatic recording of temperatures and absorbancies. The composite absorption spectrum of the partly hydrolyzed DNA–DMC complex was resolved using an analog computer, the Dupont 310 Curve Resolver, and viscometric titrations of the PM2 DNA were performed in a flow viscometer [4]. The aqueous phenol system used to extract DMC from its complex with DNA was obtained by saturation of liquid phenol with 5×10^{-3} M Tris-HCl at pH 7.5.

3. Results

Deoxyribonuclease I hydrolyzes DNA predominantly to a variety of oligonucleotides. When the DNA–

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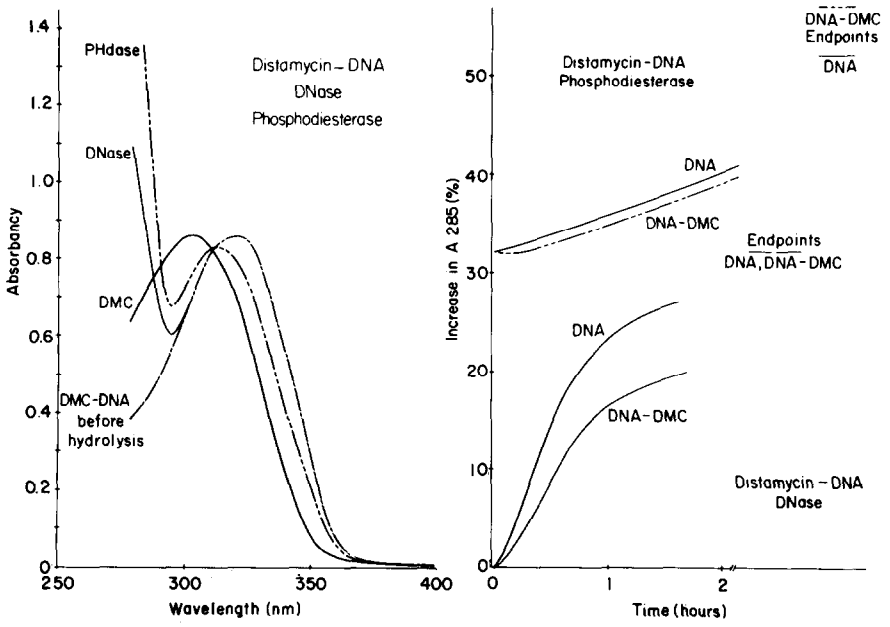

Fig. 1. Enzymatic hydrolysis of the distamycin–DNA complex. At the left are absorption spectra of distamycin alone and of the complex before hydrolysis, after hydrolysis by DNAase I and subsequent hydrolysis by snake venom phosphodiesterase. At right are shown the relative increases in absorbancies at 285 nm observed during the digestion of DNA and of its complex with distamycin A. All absorbancy increases were calculated in respect to the absorbancy of DNA alone at 285 nm. Concentrations: 3.1×10^{-5} M distamycin A and 5×10^{-4} M DNA phosphorus; 0.063 μg/ml DNAase I, 10^{-3} M Mg^{2+} ions and 5×10^{-3} M Tris-HCl at pH 7.5 for hydrolysis by DNAase I, and 3.3 μg/ml phosphodiesterase, 7.3×10^{-4} M glycine at pH 9.0 for hydrolysis by snake venom phosphodiesterase.

DMC complex was digested exhaustively with this enzyme, the rate of hydrolysis was less than that of non-complexed DNA but identical endpoints (absorbancies at 285 nm) were attained (fig. 1). The absorption spectrum of the hydrolyzate of the DNA–DMC complex (fig. 1) exhibited the same absorption maximum at 321 nm which is shown by DMC bound to intact calf thymus DNA [2]. When the DNAase hydrolyzate was subsequently digested with snake venom phosphodiesterase, a new absorption peak of DMC appeared at 311 nm (fig. 1). This was resolved using an analog computer into two spectrophotometric components, one the free antibiotic with an absorption maximum at 303 nm and the other bound DMC with the maximum at 321 nm. The spectrophotometric endpoints of the snake venom phosphodiesterase digestions of the hydrolyzates of drug-free and drug-complexed DNA (fig. 1) differed as a result of an increased absorbancy of DMC at 285 nm upon its liberation. Phosphodiesterase hydrolyzes DNA to its component mononucleotides. However, when we tested for effects of the individual deoxyribonucleotides on the absorption spectrum of DMC, none were detected. It is inferred that the phosphodiesterase digestion did not go to completion and that part of DMC remained attached to unhydrolyzed oligonucleotides. The drug is known to bind to single-stranded or denatured DNA [2, 8].

DMC was quantitatively extracted from its complex with calf-thymus DNA using a biphasic phenol–water system [9]. DNA was found in the phenol-poor phase while DMC was extracted into the phenol-rich phase of the extraction system (fig. 2). To our knowledge, this is the first complete physical separation of DMC from DNA which has been attained.

Poly d(A–T), poly dA·dT and poly dI·dC caused red shifts in the absorption maximum of DMC (fig. 3) comparable to the shift which results from the binding of the antibiotic to native DNA [2]. In contrast, poly dG·dC produced a lesser red shift and decreased the intensity of DMC's absorption band.

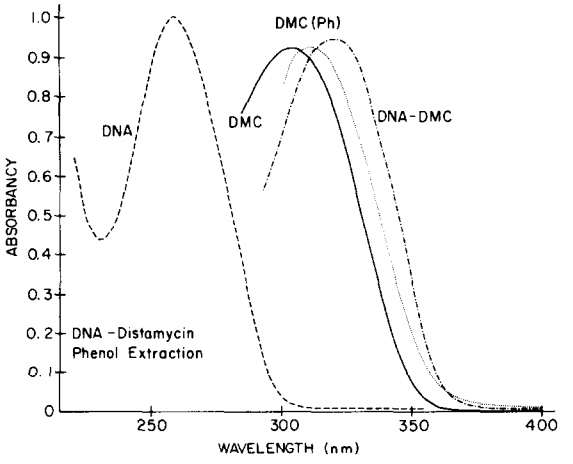

Fig. 2. Dissociation of the distamycin–DNA complex by extraction with a biphasic phenol–water system [9]. Absorption spectra of 3.3×10^{-5} M distamycin A (DMC) and of its complex with 5.1×10^{-4} DNA (DNA–DMC) in 5×10^{-3} M Tris-HCl at pH 7.5 before phenol extraction. Spectra of distamycin A in the phenol-rich phase, DMC(Ph), and of DNA (diluted) in the phenol-poor phase of the extraction system.

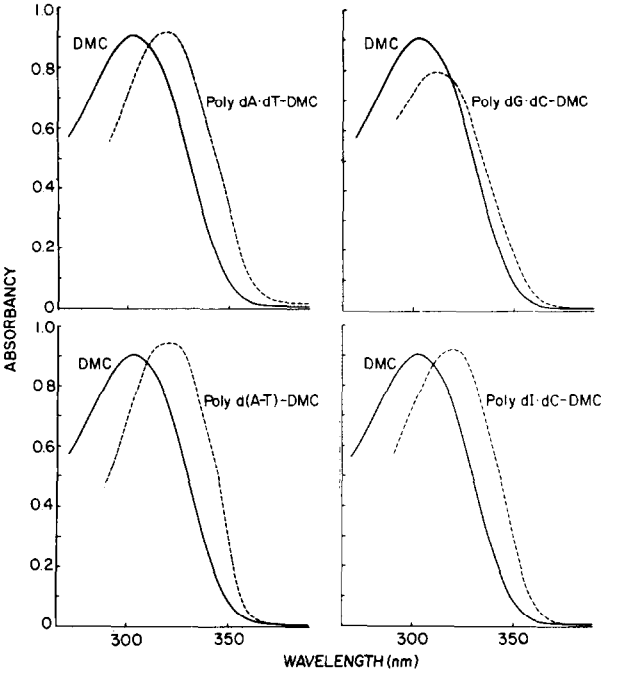

Fig. 3. Effect of the synthetic duplex polymers polymers poly d(A–T), poly dA·dT, poly dG·dC and poly dI·dC on the absorption spectrum of distamycin A. Concentrations: 3.2×10^{-5} M distamycin, 2.72×10^{-4} M polymers, 5×10^{-3} M Tris-HCl at pH 7.5.

All four polymers induced a Cotton effect in the 321 nm absorption band of DMC (fig. 4). The three double helices devoid of guanine, induced Cotton effects with large molecular amplitudes but this amplitude was less for the effect induced by poly dG·dC.

DMC shifted the thermal denaturation profiles ("melting curves") of all four polymers to higher temperatures (fig. 5); these shifts were large for poly d(A–T), poly dA·dT and poly dI·dC while the shift for poly dG·dC was much smaller.

These three biophysical experiments show that DMC became bound to all four duplex polydeoxyribonucleotides but that the binding to poly dG·dC was qualitatively and possibly stoichiometrically different from that to polymers not containing G.

Flow-dichroism experiments with the DNA–DMC complex [2] have shown that the orientation of the *N*-methylpyrrole chromophores of the antibiotic relative to that of the base pairs in DNA is highly regular but it could not be inferred whether these chromophores are coplanar or perpendicular to the base-pairs. Although intercalation binding of DMC appeared unlike since *N*-methylpyrrole does not possess the prerequisite minimal planar area of ~ 30 Å^2, we considered it desirable to test experimentally for intercalation binding of DMC to double-helical DNA. A viscometric titration of closed circular PM2 DNA with DMC revealed a similar decrease in viscosity as has been reported for linear DNA [3]. Subsequent titration of the DNA–DMC complex with ethidium bromide showed that no less ethidium was required to bring about the typical increase and decrease in the viscosity of PM2 DNA than to produce the identical effect with ethidium alone (fig. 6). We conclude that there was no contribution of DMC to the conformational transitions of closed circular DNA resulting from intercalation and, hence, that DMC was not bound in this manner.

4. Discussion

The DNA–DMC complex was partly dissociated by enzymatic hydrolyses and the antibiotic was

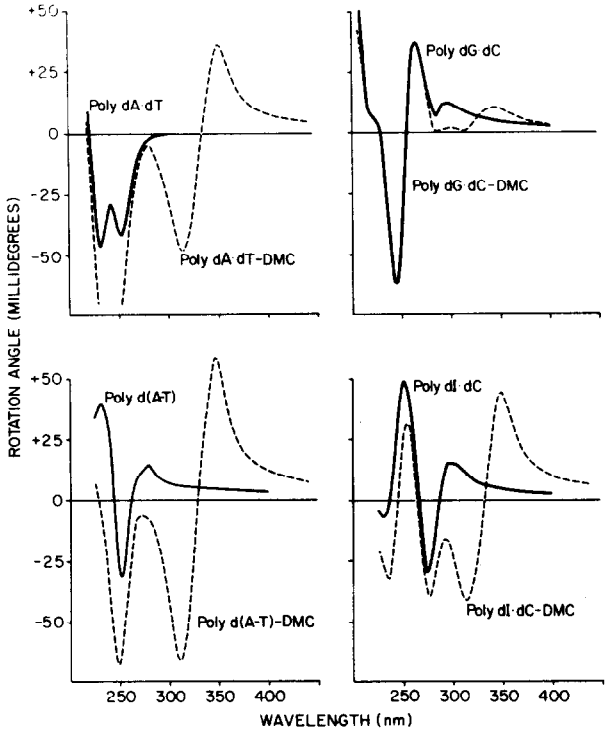

Fig. 4. Optical rotatory dispersion (ORD) spectra of the four distamycin–polymer complexes and of the polymers alone. For concentrations see fig. 3.

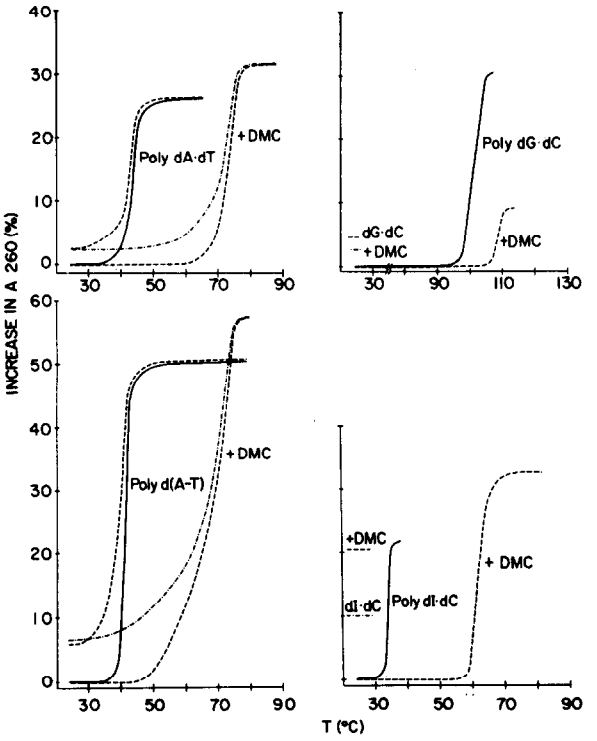

Fig. 5. Thermal denaturation of the four polymers (fig. 3), 5.44×10^{-5} M, in the absence and in the presence of 6.4×10^{-6} M distamycin A. Absorbancy increases were calculated in respect to the absorbancies of the polymers alone at 260 nm. The experiments with poly dG·dC were carried out in sealed cuvettes.

removed quantitatively from the complex by phenol extraction. It has been speculated that DMC forms covalent bonds with DNA [1], especially with adenine [10]. The present result suggests that such is not the case.

The indications of DMC's binding to DNA-like duplex polymers are stronger for those containing adenine, thymine, cytosine or hypoxanthine than for one polymer which contained guanine. These observations are consistent with two alternate explanations. i) Guanine as such, or one peculiar conformation which an abundance of guanine may impose locally on certain regions of DNA [11], exclude strong binding of DMC. ii) Adenine, thymine, single A–T pairs or a peculiar conformation which an abundance of these pairs may impose on certain regions of DNA [12], enable DMC to interact strongly with DNA or DNA-like duplex polymers. In view of suggestions that base-dependent peculiar local conformations exist in DNA [11, 12], we give preference to conformational hypotheses of greater or lesser binding of DMC rather than to the idea of singular base specificity for binding to, or partial exclusion from, DNA. As long as research on base-dependent regional conformations of DNA remains in a state of flux, it will be difficult to propose structures of complexes of DMC with such regions.

Inhibitions by DMC of enzyme induction [13] and of the initiation of the RNA polymerase reaction, attributed to preferential occupancy of A–T rich initiation sites in DNA [14], suggest a biological role of the antibiotic which may resemble that of a catholic repressor. The biological significance of our present results lies in the proof that the base composition of duplex polydeoxyribonucleotides determines their interactions with DMC and that in phenol extraction the DNA–DMC complex behaves like a

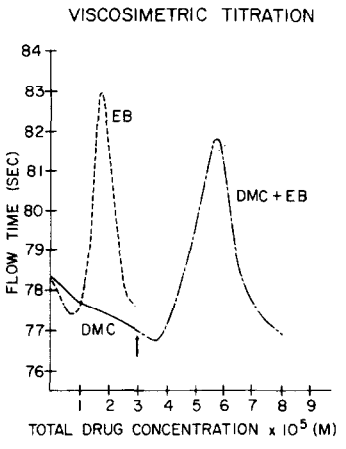

Fig. 6. Viscosimetric titration of closed circular PM2 DNA with ethidium bromide (EB) and with distamycin A followed by ethidium bromide (DMC+EB). EB: Ethidium bromide (8.1×10^{-4} M) was added in 5 μl aliquots to 1.0 ml PM2 DNA (102 μg/ml). DMC+EB: Distamycin A (3.5×10^{-4} M) was added in 10 μl aliquots to 1.0 ml PM2 DNA (102 μg/ml). After addition of a total of 100 μl distamycin (marked by an arrow) ethidium bromide was added stepwise to the 1.1 ml distamycin–PM2 DNA mixture. Titrations were performed at 20° in 0.2 M Tris-HCl, 0.2 M NaCl, 0.01 M EDTA at pH 7.4.

nucleic acid–protein complex. The complex of DNA with DMC, an oligopeptide, may be considered a simple model of DNA–protein interactions.

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