STUDIES ON THE COMPLEX OF DISTAMYCIN A WITH CALF THYMUS DNA

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1. Introduction

The antibiotic, distamycin A (DMC, fig. 1), has the unique property of inhibiting induced enzyme synthesis in bacteria [1]. This provides a potential tool in the study of regulatory processes at the gene. DMC alters the melting behavior of calf thymus DNA [2] and inhibits the DNA-dependent RNA and DNA polymerase reactions *in vitro* [2, 3]. These physical and biochemical effects can be attributed to the formation of a complex of DMC with DNA. We report here studies on the complex of DMC with calf thymus DNA and conclude that it has a highly ordered structure.

2. Materials and methods

DMC was obtained from Calbiochem, calf thymus DNA from Worthington and the DNA-methyl green compounds from Sigma. All experiments were carried out in 5 mM tris-HCl buffer at pH 7.5; solutions were made in water which had been deoxygenated by passing nitrogen through it.

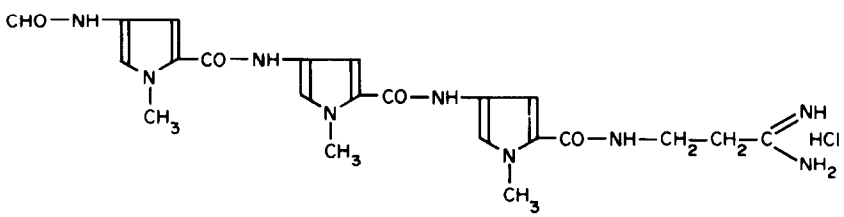
Light absorptions were recorded in a Cary model 14 spectrophotometer and optical rotatory dispersion (ORD) spectra in a Jasco ORD-UV-5 spectropolarimeter. Flow dichroism was measured as described previously [4]. During the determination of rates of methyl green displacement from DNA, reaction mixtures were kept in the dark and were only illuminated for periodic spectrophotometric measurements. The analysis of the displacement data was performed on an Olivetti Underwood programma 101 desk computor programmed to calculate $c/(b-a+c)$.

3. Results

The absorption maximum of DMC was shifted from 303 nm to 321 nm in the presence of double helical DNA (fig. 2). Progressive dilution of DMC alone in tris-HCl did not produce a red shift of the maximum at 303 nm. The absorption spectrum of DMC in acetonitrile was identical to that in buffer. Therefore, the hypsochromic shift produced by DNA can be neither the result of decreasing interactions between DMC molecules nor of placing the chromophores of the antibiotic into a less polar environment. We speculate that in the complex with DNA the *N*-methylpyrrole chromophores of DMC are arranged in a manner which reduces intramolecular interactions between these ring systems.

The intensity of the absorption maximum of DMC was not significantly altered when the antibiotic complexed with DNA. Minor changes in intensity were observed (fig. 2), but were difficult to reproduce because DMC is unstable in solution. The DNA-DMC complex was stable in 6 M urea, in 10^{-1} M NaCl and in 10^{-2} M Mg acetate; these chemicals did not readily reverse the DNA-induced hypsochromic shift in the antibiotic's absorption spectrum.

DMC displaced methyl green from its complex with DNA (fig. 3). The time course of displacement was followed spectrophotometrically as a decrease in the absorbance of the DNA-methyl green complex at 642 nm. Free methyl green is unstable at physiological pH and loses its color. When a is the initial concentration of DNA-bound methyl green, b that of DMC, and c the amount of methyl green bound to DNA at any time after the beginning of the displacement reaction,



DISTAMYCIN A HYDROCHLORIDE

Fig. 1. Structure of distamycin A hydrochloride.

the plot of log $c/[b-a+c]$ as a function of time (fig. 3) yielded a straight line, indicating that the displacement reaction was of second order with time. Presumably, the progress of the reaction depended upon the concentration of two molecular species, methyl green-DNA and DMC. This suggests that methyl green and DMC attach to the same binding sites of DNA.

The flow dichroism of the purines/pyrimidines and of DMC in the DNA-antibiotic complex was of the same magnitude but of opposite sign (fig. 4). This indicates that the *N*-methylpyrroles of DMC are placed in an orderly array relative to the planes of DNA's base pairs in the complex. If the direction of the transition moment for the 321 nm absorption band of complexed DMC were in the planes of the *N*-methylpyrroles, the result in fig. 4 would mean that these planes are arranged approximately perpendicular to the planes of the base pairs in DNA. Since this direction is not known, an unambiguous interpretation of the dichroic phenomenon in terms of the structure of the DNA-DMC complex cannot be given.

DMC is optically inactive [5]. DNA induced Cotton effects in the ORD spectrum of DMC (fig. 5A). These effects were of larger molecular amplitudes than the intrinsic Cotton effects of DNA (fig. 5B). By subtracting the ORD spectrum of DNA alone from that of

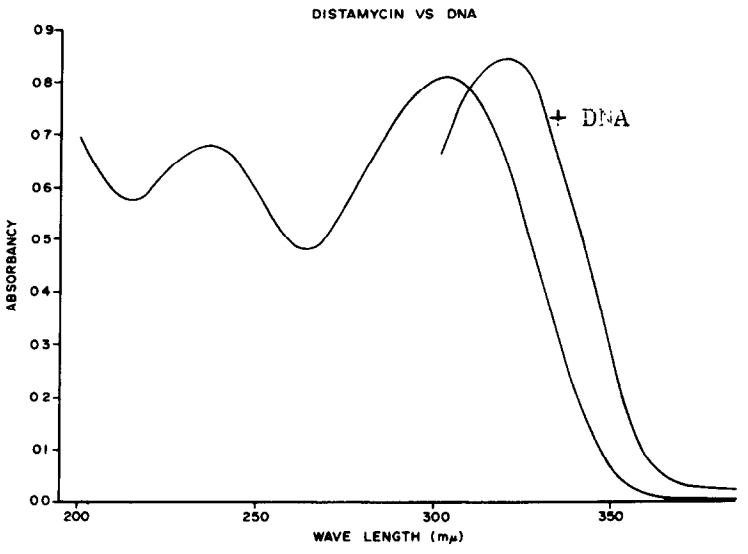


Fig. 2. Effect of DNA (5×10^{-4} M phosphorus) on the absorption spectrum of distamycin A (2.9×10^{-5} M).

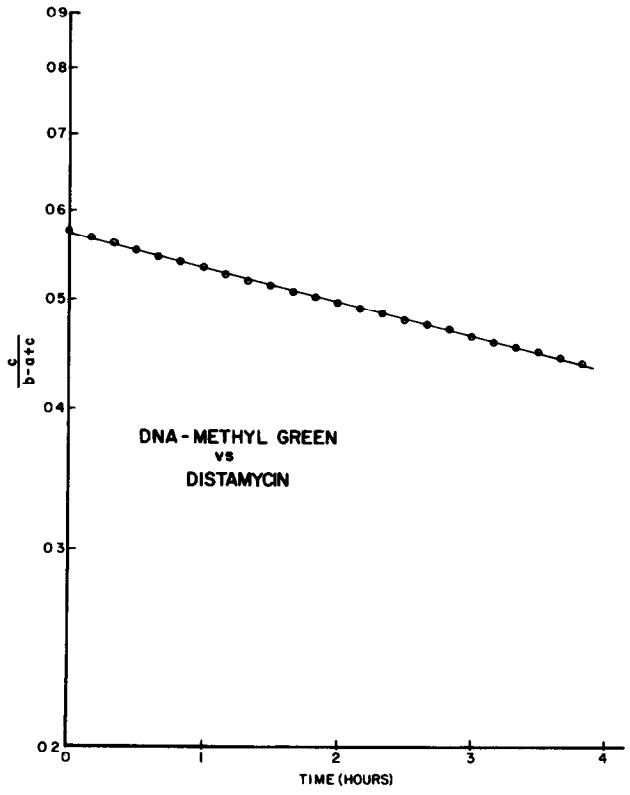


Fig. 3. Displacement of methyl green from its DNA complex by distamycin A; a = initial concentration of DNA-bound methyl green (2.09×10^{-5} M); b = initial concentration of distamycin A (3.2×10^{-5} M); c = the amount of methyl green remaining bound to DNA determined by measuring absorbance at 642 nm.

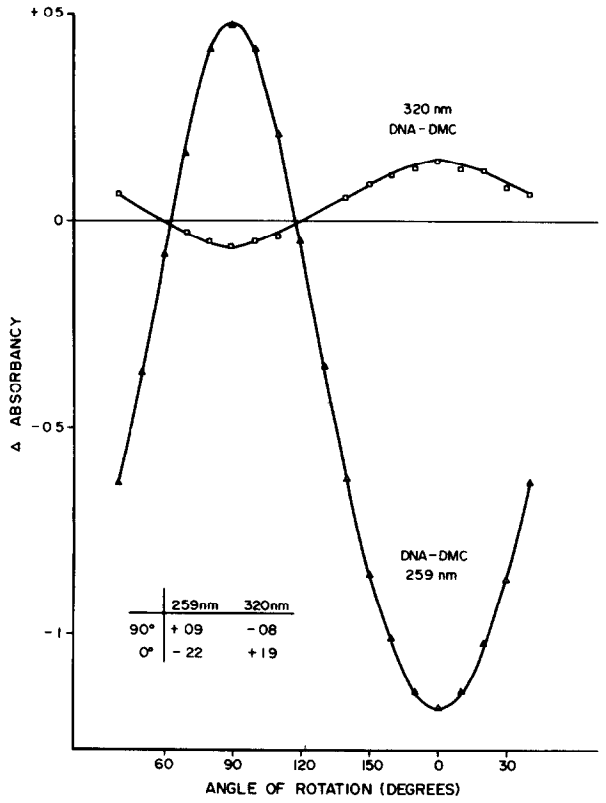


Fig. 4. Flow-dichroism of the DNA-distamycin A complex measured at 259 nm for component bases of DNA and at 320 nm for distamycin A. The concentration of DNA was 4×10^{-3} M phosphorus and of distamycin A 1.1×10^{-4} M. The insert shows the fractional change in absorbance when the complex was flow oriented [4].

the DNA-DMC complex, we obtained an ORD difference spectrum (not shown) which could be resolved on an analog computor (Dupont 310 Curve Resolver) into two Cotton effects associated with the two electronic transitions of DMC (fig. 2). Certain basic ligands such as irehdiamine cause slight changes in the molecular amplitudes of DNA's intrinsic Cotton effects [6]; since it cannot be experimentally excluded that DMC also produces such changes, the ORD difference spectrum of the DNA-DMC complex could conceivably contain small errors. For this reason we present the uncorrected ORD spectrum of the complex (fig. 5A). Single-stranded DNA, prepared as described previously [7], also induced Cotton effects in the ORD spectrum of DMC but these were of lesser molecular amplitudes than those shown in fig. 5A.

We have confirmed the observation [2] that DMC stabilizes DNA to thermal denaturation. The melting profile of DNA (30 μg/ml) in the presence of 3.4 μM DMC was displaced by +6° from that of DNA alone. At this DMC/mononucleotide quotient of ~ 0.03, the shapes of the two melting curves were similar; when this ratio is increased to 0.1 or more, the DNA-DMC complex melts in a more cooperative manner than DNA alone [2].

4. Discussion

DMC forms an ordered complex with DNA which

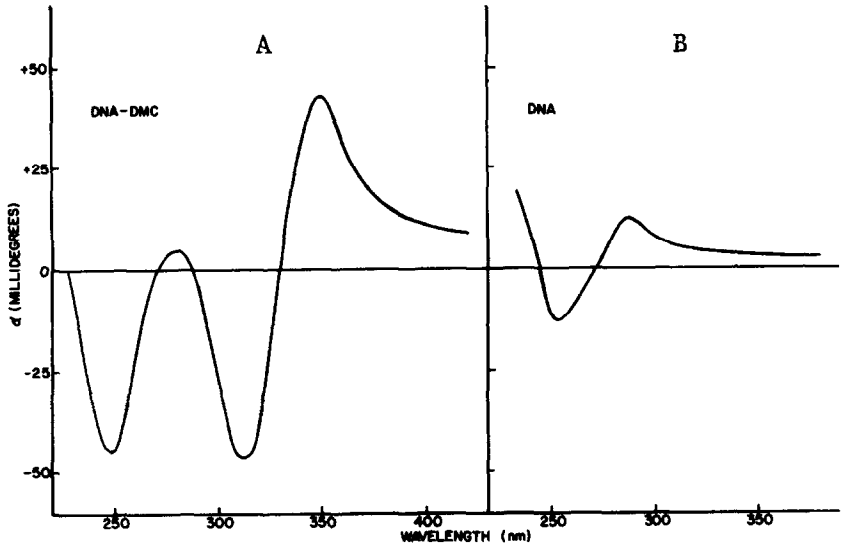


Fig. 5(A). Optical rotatory dispersion spectrum of the complex of distamycin A (3.1×10^{-5} M) with double-stranded DNA (2×10^{-4} M phosphorus). (B) Optical rotatory dispersion spectrum of DNA (2×10^{-4} M phosphorus).

is more stable to urea and inorganic ions than certain other DNA-ligand complexes [8, 9]. The structure of DMC (fig. 1) suggests that the propionamidine residue of the molecule is electrostatically attracted to phosphates of DNA. This alone, however, does not explain the properties of the DNA-DMC complex.

The positive DNA-induced Cotton effect in DMC's ORD spectrum is possibly conformational in origin, arising from an alignment of the *N*-methylpyrroles with the helix. That Cotton effects are also induced by single-stranded DNA is in accord with the observation that DMC forms a complex with denatured DNA which melts cooperatively like a highly ordered structure [2]. The flow dichroism of DMC in its complex with DNA indicates that a high degree of order also exists in the complex of DMC with double-helical DNA.

We have no knowledge of the conformation of DMC in aqueous solution, nor do our results permit us to express preference for any of the conceivable structural models of a DNA-DMC complex. It is unlikely that the ability of DMC to inhibit the RNA polymerase reaction [2] explains the specific inhibition of induced enzyme synthesis by DMC [1]: an inhibition of messenger RNA biosynthesis, as by actinomycin [10], precludes *all* protein synthesis after preexisting mRNA is dissimilated. Efforts to elucidate the biological action of DMC will have to take into account that the antibiotic is a DNA-complexing agent.

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