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STUDIES ON THE INTERACTION OF DISTAMYCIN A AND ITS DERIVATIVES WITH DNA

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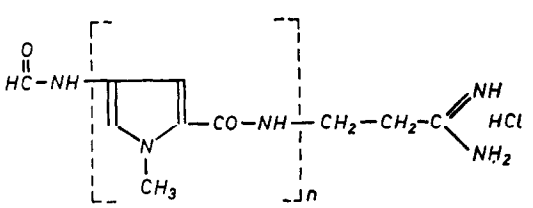
Abstract—The interaction of distamycin A with DNA is found to involve at least two classes of sites. A spectrophotometric study indicated a specific interaction of distamycin A and two derivatives, containing 4 and 5 pyrrole residues respectively, with DNA. The results of comparative studies are explained in terms of the contribution of the chromophore system of the antibiotics to the binding process. Results of the displacement of methyl green from its DNA complex by distamycin A and its derivatives suggested an increase in the extent of binding of displacing compounds to DNA by increasing the number of pyrrole residues. The biological implications of the interaction of the oligopeptide antibiotics with DNA are discussed.

DISTAMYCIN A (DST), a basic oligopeptide antibiotic (Fig. 1) isolated from "Streptomyces distallicus",[1] exhibits antiviral activity.[2–6] The inhibitory effect of this compound on the multiplication of viruses has been observed on animal[3,5] and bacterial[7,8] DNA



Distamycin A (DST); n = 3; M_W = 517·97
DST derivative with n = 4 (DST/4); M_W = 640·1
DST derivative with n = 5 (DST/5); M_W = 762·2

FIG. 1. Chemical structure of distamycin A and its derivatives.

viruses. In addition it has remarkably specific effects on the synthesis of enzymes inducible (such as β-galactosidase) in entero-bacteria.[9] Distamycin A has been reported to bind strongly to DNA *in vitro*.[10–12] The biological activity of distamycin A could be related to this strong and specific interaction with DNA and its interference in the DNA and RNA polymerase systems.[13–15] Recently Chandra *et al.*[16] and Casazza *et al.*[17] have studied the relationship between the chemical structure of distamycin A and related compounds and their biological activities. The antiviral activity of DST derivatives appeared to be dependent on the number of pyrrole residues. It was suggested[17] that the different biological activity of distamycin derivatives could be a

consequence of their different interaction with DNA. We present here a study on the interaction of distamycin A and its derivatives (Fig. 1) with DNA.

MATERIALS AND METHODS

Distamycin A and its derivatives were supplied by Farmitalia, Milano.

Calf thymus DNA was prepared according to the procedure of Zamenhof.[18] Yeast RNA was purchased from Sigma Chemical Co. and further purified by phenol extraction.

Spectrophotometric measurements were carried out in a Zeiss PMQ II Spectrophotometer.

The displacement of methyl green was measured as described by Kurnick and Radcliffe.[19]

The DNA-cellulose column chromatography was carried out as described by Inagaki and Kageyama.[20] Fractions of 4·5 ml were collected at 7·5-min intervals.

RESULTS AND DISCUSSION

Figure 2 shows the chromatographic behaviour of DST on DNA-cellulose column. Using the gradient elution technique, the elution pattern of DST showed two peaks centered at about 0·25–1 M in NaCl gradient and at about 3–4·5 M in urea gradient respectively. Similar behaviour was found, when the elution was carried out stepwise.

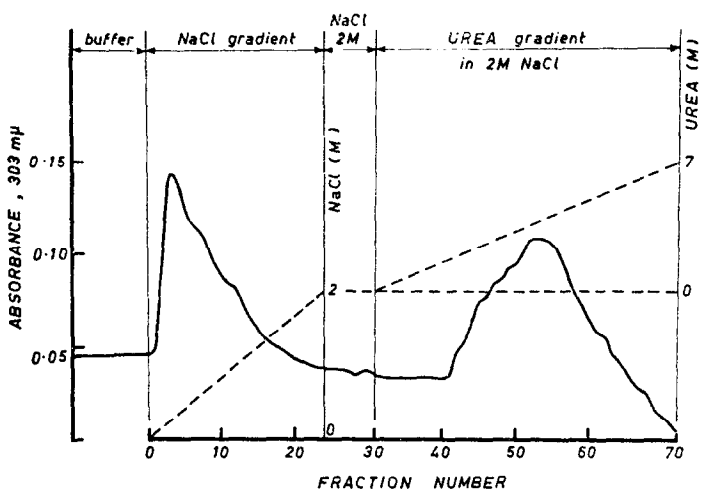


FIG. 2. DNA-cellulose chromatography of distamycin A. An excess amount of drug was loaded in a 0·6 × 4 cm DNA-cellulose column. After washing the column with the buffer, elution was carried out with a linear gradient (0–2 M) of NaCl in 100 ml of 0·01 M tris–0·001 M EDTA, pH 7·0, followed by 30 ml of 2 M NaCl, and successively with a linear gradient (0–7 M) of urea in 200 ml of former eluent. This experiment was carried out at room temperature. Flow rate 36 ml/hr. Recovery was 96 per cent.

The recovery was almost quantitative. The purity of distamycin A was checked by thin-layer chromatography. It is, therefore, unlikely that the elution pattern can be attributed to a mixture of two different molecular species.

This finding suggests the occurrence of at least two modes of interaction between antibiotic molecules and DNA; an electrostatic binding mode involving probably the

DNA phosphate groups and a stronger binding mode requiring urea for the dissociation. This type of interaction may involve some other kind of forces, such as hydrogen bonds and hydrophobic associations.

No conclusions may be drawn from the chromatographic behaviour of DST/4 and DST/5, as a remarkable amount of these derivatives was not eluted from the DNA-free cellulose column (control) by 0·01 M tris buffer.

In contrast to the behaviour of these derivatives, DST is slightly retarded and only a small amount of the antibiotic (about 15 per cent of the total amount dissociated from a DNA-cellulose column by 7 M urea) was retained by a DNA-free cellulose column. Recent studies[21] on the nature and specificity of the interaction of oligopeptide antibiotics netropsin and distamycin A with nucleic acids indicated two main classes of binding sites for netropsin on DNA; but no evidence was presented which indicated the existence of different binding sites for DST on DNA. In attempting to obtain further information about the mode of interaction of these antibiotics with DNA, a spectrophotometric study on binding of DST derivatives with DNA was undertaken.

Figure 3 shows the ultraviolet absorption spectra of the DST–DNA systems beyond 300 nm at ionic strength 0·01, pH 7·0. When increasing amounts of DNA were added to the DST solution, the absorption maximum was shifted from 303 nm to longer wavelengths. The red-shifts depended on DNA concentration. At a DST/DNA-P

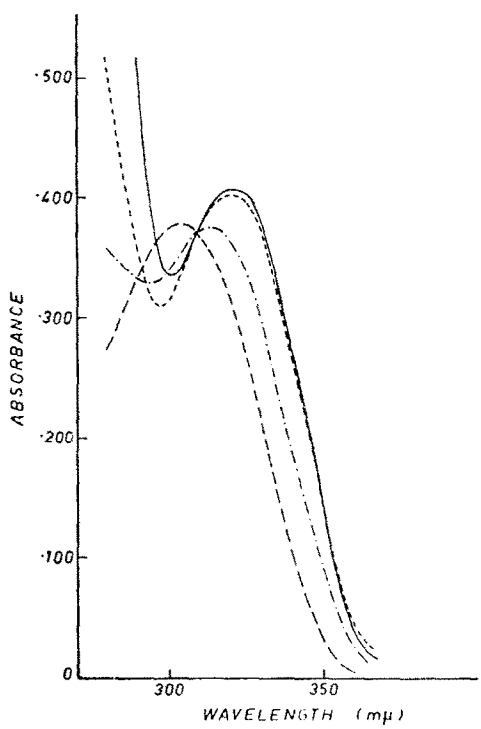


FIG. 3. Effect of calf thymus DNA on the ultra-violet absorption spectrum of DST beyond 290 nm. Samples contained $1{\cdot}25 \times 10^{-5}$ M of DST, 0·01 M tris–HCl (pH 7·0) and DNA at $0{\cdot}5 \times 10^{-4}$ (— · —), $1{\cdot}25 \times 10^{-4}$ M (– – –) and $2{\cdot}5 \times 10^{-4}$ M (————). No DNA was added to sample (— —).

molar ratio close to 0·1, native DNA caused a red-shift of the absorption maximum of about 18 nm. The above spectral changes were not inhibited by 10^{-1} M magnesium ion.

A similar effect of DNA on the absorption spectrum of DST was observed by Zimmer *et al.*[21] and by Krey *et al.*[12] The spectral changes are interpreted in terms of a contribution of the system of the chromophore to the binding process with DNA. In addition, we observed an isosbestic point and an increase in intensity of the absorption maximum of DST, when the antibiotic complexed with DNA in the region of DST/DNA-P ratio less than 0·25. We observed similar effects of DNA on absorption spectra of the derivatives containing four (DST/4) and five (DST/5) pyrrole rings. DST/4 (Fig. 4b, curve 1) exhibits a maximum at 308 nm and DST/5 (Fig. 4c, curve 1) at 310 nm; native DNA caused a red shift of about 20 nm in the region of antibiotic/DNA-P close to 0·1.

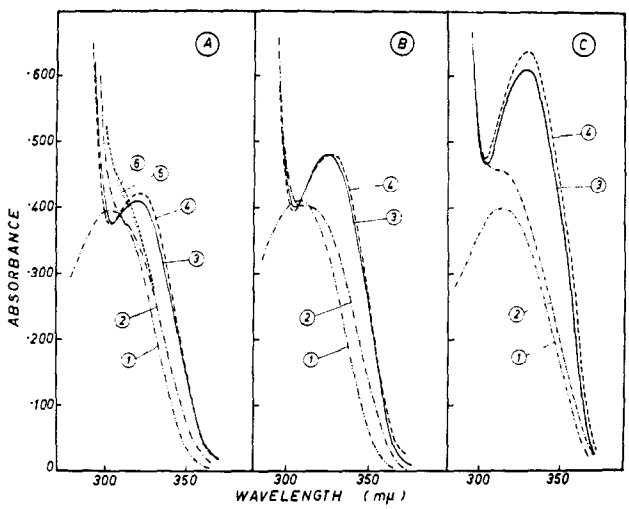


FIG. 4. Effects of native DNA, denatured DNA and yeast RNA on the ultraviolet absorption spectra of DST (A), DST/4 (B) and DST/5 (C), in 0·01 M tris–HCl (pH 7·0).
Curve 1 is the spectrum of free antibiotic. Other curves are the spectra of the antibiotics in the presence of yeast RNA (curve 2), denatured DNA (curve 3), native DNA (curve 4), apurinic DNA (curve 5) and apyrimidinic DNA (curve 6) at a molar antibiotic/nucleic acid-P ratio of 0·025.

In Fig. 4 a comparison of the effects of native and denatured DNA and of RNA on the ultraviolet spectra of the oligopeptide antibiotics containing 3, 4 and 5 pyrrole rings is presented. Three general observations contained in this figure need emphasis.

First, yeast RNA and (in the case of DST) apurinic and apyrimidinic DNA at $r = 0{\cdot}025$ did not change the characteristic absorption band of the free compounds. It is worth noting that, in presence of RNA, the absorption spectrum of DST/5 was enhanced.

Second, heat-denatured DNA at pH 7·0 bound all compounds tested, and, at corresponding values of r, the spectra of the bound antibiotic were similar to those found with native DNA.

Finally, in all cases, the red-shift of the characteristic absorption band was accompanied by an increase in the intensity of the absorption maximum.

This hyperchromic effect depended on DNA concentration (Fig. 3) and was more pronounced by increasing the number of pyrrole residues. Under identical condition of r and ionic strength, the denatured DNA-induced spectral changes were somewhat weaker.

It is unlikely that the different hyperchromic effect can be attributed to a different stability of tested compounds, as the spectra of the compound both in the absence and in the presence of DNA were read immediately after preparation.

These observations provide strong support for contribution of the chromophore of the antibiotics to the binding mechanism.

Concerning the relationships between the above contribution and the number of pyrrole residues, an attempt was made to investigate whether the extent of binding and/or the affinity for DNA could be affected by the number of pyrrole residues. The results obtained by the displacement of methyl green from its DNA complex by DST, reported by Krey and Hahn,[12] suggested that methyl green and DST attach to the same binding site of DNA. We observed similar displacement reaction with DST/4 and DST/5. In addition, a comparison of the rates of displacement (Fig. 5) suggested an increase in relative affinity for DNA of the compound DST/5 with respect to DST.

Furthermore, an increase in the ability to displace methyl green by increasing the number of pyrrole residues was observed (Fig. 6), indicating an increase in the extent of binding of displacing compounds to DNA.

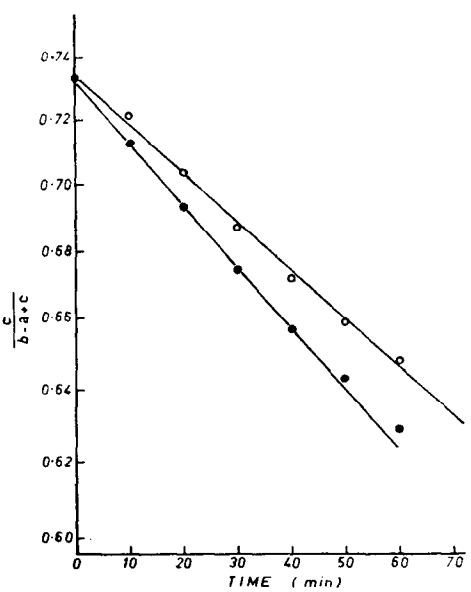


FIG. 5. Displacement of methyl green (MG) from its DNA complex by DST (○) and DST/5 (●): plot of log $c/[b - a + c]$ as a function of time. a = initial concentration of DNA bound MG($9{\cdot}26 \times 10^{-6}$ M); b = initial concentration of antibiotic ($1{\cdot}25 \times 10^{-5}$ M); c = amount of MG remaining bound to DNA determined by measuring the optical density at 642 nm.

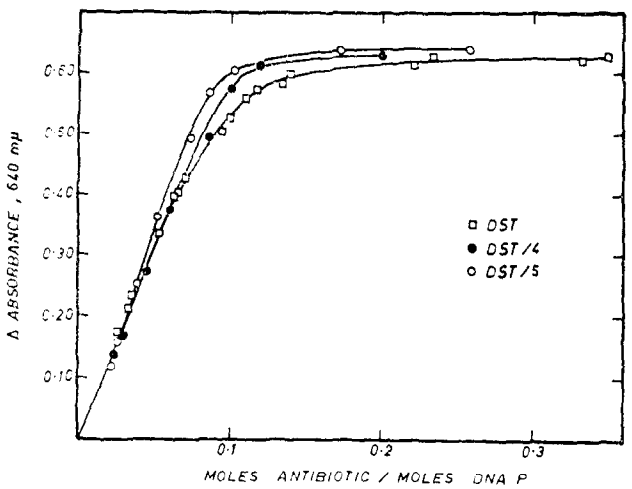


FIG. 6. Displacement of methyl green from its complex with calf thymus DNA by distamycin A and its derivatives. 0·5 ml of antibiotics solutions at various concentrations were added to 2 ml of MG–DNA complex. The final concentration of DNA is 0·12 mg/ml. The absorbance at 640 nm was read after storage in the dark overnight. The results are expressed as the difference of absorbance at 640 nm with and without antibiotic.

The present results are in accordance with those reported by Zimmer *et al.*,[22] who found that the stabilizing action of DST on DNA increased with increasing number of pyrrole residues. Chandra *et al.*,[16] reported that the inhibition of template activity of DNA, in DNA-dependent RNA polymerase system, is dependent on the number of pyrrole residues in the distamycin molecule.

These observations fit with the hypothesis[17] that the increase in biological activity of DST derivatives by increasing the number of pyrrole residues could be a consequence of their different interaction with DNA.

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