Oligopeptide antibiotic distamycin A and its interactions with duplex DNA*

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Native calf thymus DNA and double-helical poly d(A–T) produced electric dichroic effects for distamycin A which were of larger amplitude than the effects observed with duplex poly dG·dC. Titration of distamycin with calf thymus DNA, monitored spectrophotometrically, indicated the existence of more than one form of the bound antibiotic. Such titration, monitored by electric dichroism, yielded a binding of 1 distamycin per 6 base pairs of A–T rich regions of DNA, i.e., it yielded a stoichiometry which was likewise derived from bathochromic shifts of the antibiotic by equimolar mixtures of A and T containing oligodeoxynucleotides and from biphasic melting profiles of poly d(A–T) with oligopeptide distamycin A.

INTRODUCTION

The oligopeptide antibiotic distamycin A, DMC (*Figure 1*), inhibits the replication of DNA-containing viruses[1–7], the induction of bacterial enzyme syntheses[8], and the *in vitro* DNA- and RNA-directed biosyntheses of nucleic acids[9–14]. Inhibition of DNA-directed nucleic acid biosyntheses has been attributed to a preferential interaction of DMC with A–T rich DNA templates[13] or with initiation sites in template DNA which are rich in A–T[14]. A preference of the antibiotic for A–T rich duplex DNA has been inferred from optical indications for the binding of distamycin to native DNAs and to synthetic duplex polydeoxynucleotides of different base composition and is likewise suggested by the results, presented here, of electrooptical and spectrophotometric studies of DMC with calf thymus DNA and with the double helical polymers poly d(A–T) and poly dG.dC.

Orientation of DNA by an electric field tends to align the long axis of the double helix in the direction of the applied field so that the base pairs of the nucleic acid assume a roughly perpendicular direction. This produces an increase in the 260 nm absorbancy of the bases for incident light polarized

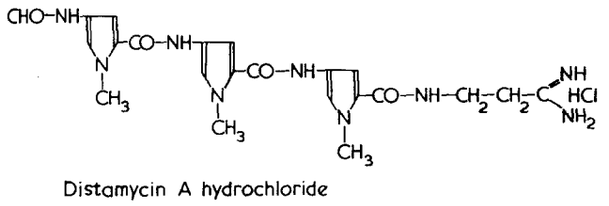

Figure 1 Structure of distamycin A hydrochloride

perpendicular to the field and an absorbancy decrease for light of parallel polarization (*Figure 2*). Absorbancy changes of equal magnitude but opposite sign from those obtained for the base pairs of DNA were observed at 315 nm for bound distamycin. These dichroic effects suggest for the composite transition moment of the *N*-methylpyrroles of DNA-bound distamycin an orientation, calculated according to established principles[19], of 39 degrees *versus* the helical axis when base pairs are assumed perpendicular to the helix axis in the B-form of DNA.

Distamycin's binding to poly d(A–T) resulted in dichroic effects similar to those observed for the antibiotic's attachment to DNA, suggesting approximately the same orientation of its transition moment in poly d(A–T) as was obtained for its complex with calf thymus DNA (*Figure 3*). In contrast, interaction of distamycin with poly dG.dC, a duplex which (X-ray diffracton studies indicate) coexists – unlike calf thymus DNA or double helical poly d(A–T) – in the B and A conformation of DNA[20], produced effects of smaller magnitude and opposite sign. Although no exact orientation can be derived from these effects because of an uncertainty of the relative amounts of the B and A forms for poly dG.dC in the present dichroism experiments in solution, the results nevertheless most importantly indicate that the antibiotic's transition moment inclination *versus* the helical axis is different in double helical poly dG.dC than it is in poly d(A–T) and in calf thymus DNA.

The present electro-optical results are in agreement with the earlier optical indications of a differential binding of distamycin to DNA or duplex polydeoxynucleotides according to A–T base composition[17]. In view of this heterogeneity of the binding and the finding that DMC binds to duplex DNA in a bimodal manner[18,21], it appeared of interest to investigate the suggestion[22] of regional differences in the antibiotic's attachment to DNA, determined by A–T. Such heterogeneity in the binding of distamycin may be indicated by a spectrophotometric titration of DMC with calf thymus DNA; this titration is described below.

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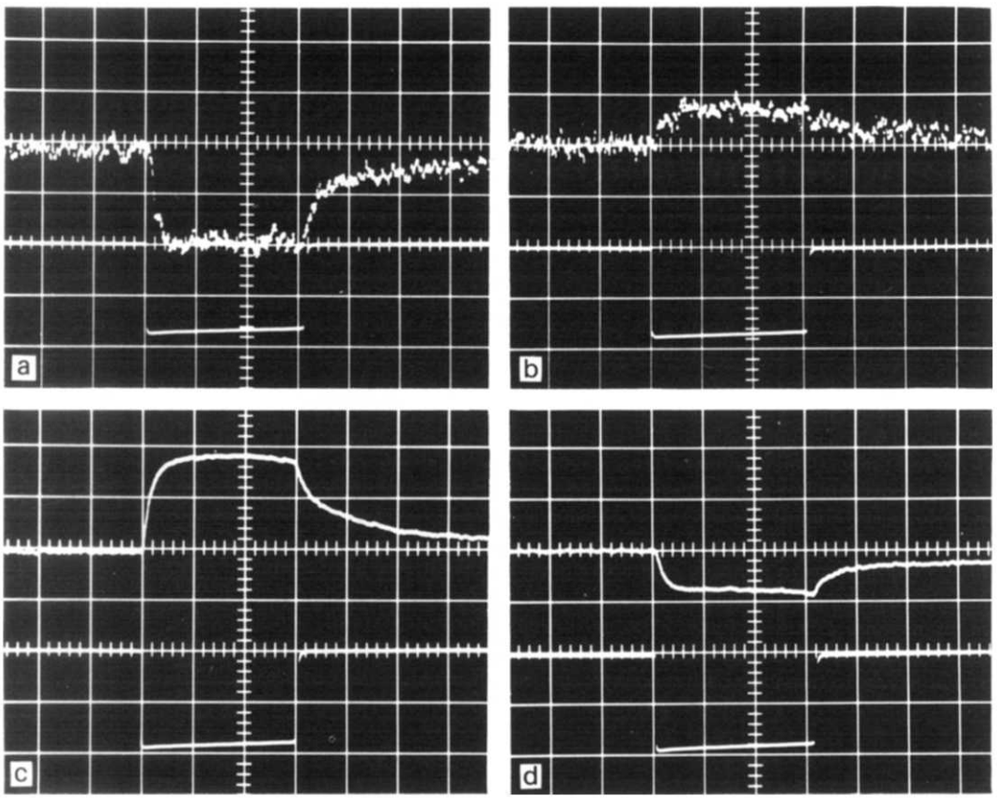

Figure 2 Electric dichroism of the bases of DNA and of the chromophores of bound distamycin. Oscilloscope upper beams (sensitivity 0.5 V per division) show changes in intensities of transmitted light obtained for incident light polarized parallel (0°) or perpendicular (90°) to the direction of the applied electric field (9 kV/cm). The observed intensity changes were converted to changes in absorbancies and to dichroic values as described elsewhere[24]. Lower oscilloscope beams (sensitivity 1 kV per division) display the applied voltage pulse of about 150μsec duration. Concentrations: 1.2×10^{-4} M DNA without DMC and 3.2×10^{-5} M distamycin with an 8-fold molar excess of nucleic acid. Buffer (used for all experiments of this investigation): 5 mM Tris-HCl at pH 7.5. (a) DNA, 0°; (b) DNA, 90°; (c) DNA–DMC, 0°; (d) DNA–DMC, 90°

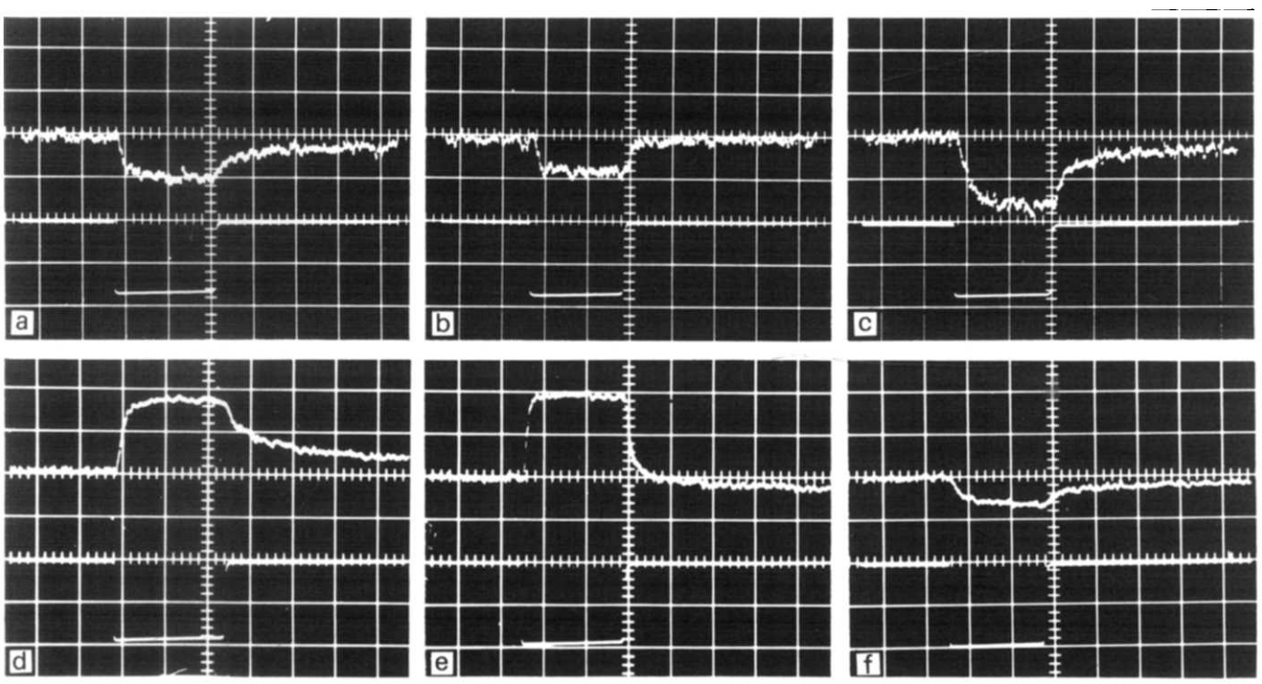

Figure 3 Dichroic effects of DNA (a), poly d(A–T) (b) and poly dG.dC (c) and of their complexes with distamycin; (d) DNA–DMC; (e) poly d(A–T)–DMC; (f) poly dG.dC–DMC. Intensity changes were recorded for parallel polarization (0°). Concentrations: 9×10^{-5} M polymers in the absence of the antibiotic and 3.2×10^{-5} M DMC with a ten-fold molar excess of the polymers. Oscilloscope upper beam sensitivity for polymers without distamycin was 1 V and that for the complexes with DMC 0.5 V per division; lower beam sensitivity: 1 V per division. Duration of pulse 120 μsec

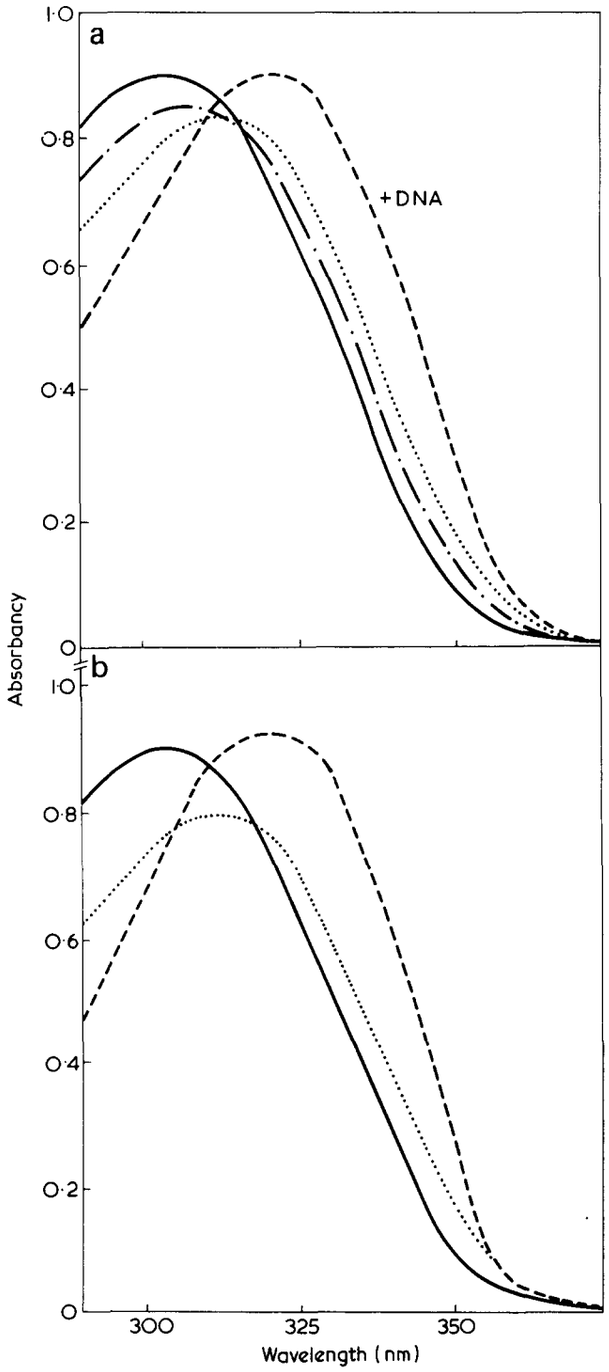

Figure 4 (a) Spectrophotometric titration of distamycin with calf thymus DNA. Shown is the spectrum of DMC, 3.2 X 10^{-5} M, in the absence (——) and presence of 1.1 (— — —), 2.1 (. . . . .) and 8.5 (— . — . — .) molar excess of nucleic acid. (b) Distamycin's spectrum without polymers and with an 8.5-fold concentration of poly d(A–T) (— — —) and poly dG.dC (. . . . .)

EXPERIMENTAL AND RESULTS

Increasing concentrations of calf thymus DNA progressively shifted the antibiotic's absorption maximum to longer wavelengths, reduced its intensity at low molar ratios of nucleotides over DMC, and subsequently, at higher DNA concentrations, increased again the absorption intensity of distamycin A (*Figure 4a*). The observed absorption spectra show, firstly, the absence of an isosbestic point, that is they indicate the existence of more than one mode of binding for the attachment of distamycin to calf thymus DNA. The spectra, secondly, reveal red-shifts in the presence of high concentrations of the nucleic acid which resemble the large red-shifts observed with the synthetic duplexes poly d(A–T) (*Figure 4b*), poly dA.dT[17], and poly dI.dC[17] but not the small shift observed with poly dG.dC (*Figure 4b*), i.e. they indicate for the mode of distamycin binding at the high DNA concentrations a preferential interaction of the antibiotic with nucleic acid regions rich in A–T while, in contrast, the spectral reductions in antibiotic absorbancies at the lower DNA concentrations indicate for the second mode of the distamycin attachment a weaker interaction of DMC with G–C abundant regions of DNA.

The stoichiometry for one of the two modes of distamycin's attachment to antive calf thymus DNA was estimated from three different biophysical experiments. The first experiment was an electro-optical titration of DMC with the nucleic acid (*Figure 5*). Graded concentrations of calf thymus DNA produced increasing amounts of bound distamycin and therefore also progressively dichroic effects. The observed effects approached saturation in the presence of high concentrations of DNA, i.e. in the presence of those concentrations of the nucleic acid which caused in the spectrophotometric titration the large antibiotic red-shifts without intensity reductions, determined by distamycin's attachment to DNA regions rich in A–T, which suggests a stoichiometry for DMC's interaction with the A–T rich DNA regions of one molecule of distamycin bound per approximately six nucleotide pairs.

In the second biophysical experiment, the amount of DMC bound to the A–T rich regions of DNA was estimated from the spectral shifts oligomers produced in the absorption spectrum of distamycin A (*Figure 6*). Equimolar mixtures

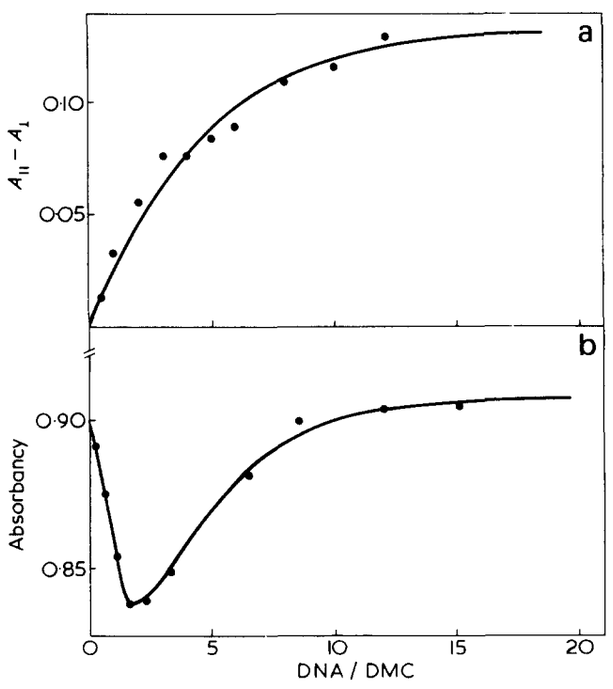

Figure 5 Electro-optical titration of distamycin with calf thymus DNA. (a) Panel gives the dichroic effects produced for 3.2 X M^{-5} M DMC by increasing concentrations of DNA while (b) shows for comparison the corresponding changes in the absorption intensity of distamycin

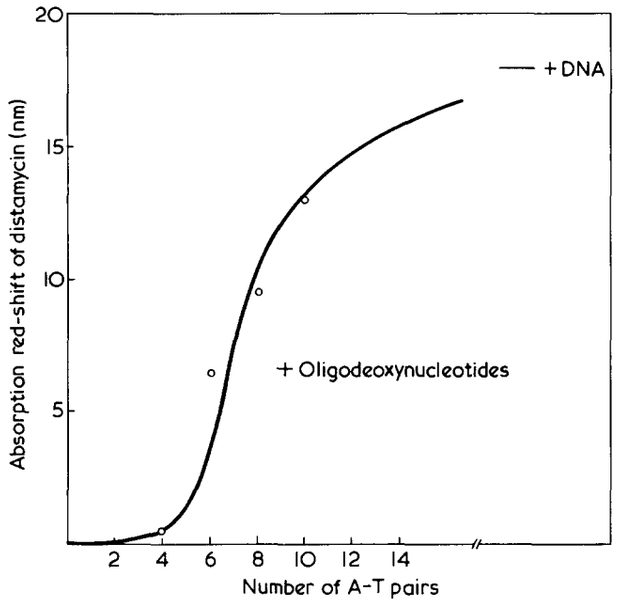

Figure 6 Absorption red-shifts of distamycin produced by equimolar mixtures of tetramers, hexamers, octamers, and decamers of deoxyadenylic and thymidylic acids. Concentrations: 3.2 X 10^{-5} M DMC, 2.72 X 10^{-4} M oligomers

of oligodeoxynucleotides containing adenine and thymine produced, with increasing chain length, progressively larger absorption red-shifts for the antibiotic. These absorption shifts yield again the stoichiometry of one distamycin molecule per approximately six A–T pairs of DNA.

The final experimental estimation of this stoichiometry was obtained from the thermal denaturation profiles of poly d(A–T) with distamycin A. The melting curves of poly d(A–T) were biphasic for polymer mixtures with limiting amounts of DMC (*Figure 7a*), indicating, in analogy to the melting of certain model polypeptide complexes with DNA[23], a thermal denaturation (at lower temperatures) of polydeoxynucleotide segments free of distamycin, independently from thermal denaturations (at higher temperatures) of poly d(A–T) in the complex reduced the hyperchromicity of free polymer segments until zero hyperchromicity was obtained when poly d(A–T) was completely occupied by distamycin (*Figure 7b*). This extrapolation arrives at the same stoichiometry which has been obtained from the electro-optical titration and from the oligomer spectral shifts and therefore likewise indicates the binding of one distamycin per six A–T pairs of DNA.

CONCLUSIONS

In summation: Distamycin binds differentially to double helical polydeoxynucleotides of different base composition and it attaches in a bimodal manner to calf thymus DNA, with a (1:6) preference for A–T rich regions of the nucleic acid and a second mode of binding to G–C abundant regions in which the antibiotic probably assumes a different orientation than in the DNA regions which are rich in A–T.

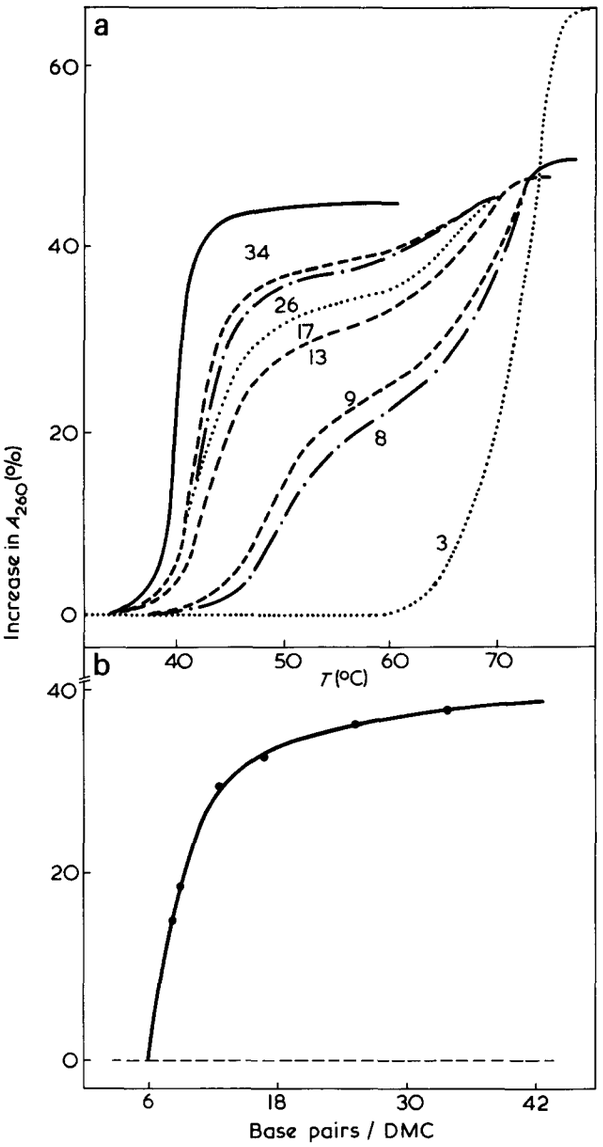

Figure 7 (a) Thermal denaturation of poly d(A–T), 5.44 X 10^{-5} M, in the absence and in the presence of graded concentrations of DMC. The numbers above the melting curves indicate the ratio of polymer base pairs over antibiotic. (b) Relation of hyperchromicity of the lower temperature melting transition to ratio of poly d(A–T) base pairs over distamycin

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