OPTICAL ROTATORY DISPERSION PROPERTIES OF NUCLEIC ACID COMPLEXES WITH THE OLIGOPEPTIDE ANTIBIOTICS DISTAMYCIN A AND NETROPSIN

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ORD measurements of nucleic acids in the presence of the oligopeptides netropsin and distamycin A have indicated association of the antibiotics with DNA and strong conformational changes of the DNA structure with specificity to AT-rich helical regions. The RNA conformation is relatively unaffected by these antibiotics. The results are explained in terms of a perturbation of the DNA secondary structure as well as of the chromophore system of the oligopeptides.

1. Introduction

The antibiotics distamycin A * and Netropsin isolated from *Streptomyces distallicus* and *Streptomyces netropsis* [1, 2] exhibit antiviral [3, 4] and to some extent antibacterial as well as antifungal properties. As compounds with unusual structures [1, 5, 6] of a basic oligopeptide type, studies on their influence on the structure of nucleic acids [7–9] may provide important information for model interactions besides their interference with DNA in the DNA- and RNA-polymerase system [10, 11]. Therefore the specific characterization of these binding effects are of special biochemical and also of biological interest. Our present data demonstrate pronounced conformational changes of the DNA induced by strong binding of Nt and Dst to AT-rich regions of DNA.

2. Materials and methods

DNA samples used were those described elsewhere [12]; tRNA was a commercial product of Serva Feinbiochemica GmbH & Co. (Heidelberg).

Spectrophotometric melting was determined as previously described [13]. ORD spectra were recorded with a Jasco spectropolarimeter Model ORD/UV–5 as employed in earlier studies [14, 15]. DNA concentrations ranging between 10^{-4} to 7×10^{-4} M DNA phosphorus for ORD measurements.

3. Results and discussions

In fig. 1A and B the variation of the ORD of complexes of DNAs from *E. coli* and calf thymus with increasing Nt-concentration is presented. The optical rotatory dispersion spectrum of the complexes is very different from that of DNA alone (curve 1). The maximum rotation of DNA at 290 nm becomes negative forming a trough in the vicinity of 300 nm whereas the trough at 255 nm and the cross-over (around 242 nm) shift to lower wavelengths. At the same time, the rotation of DNA at 255 nm decreases. The most interesting changes, however, are those observed at longer wavelengths: a new peak appears in the vicinity of 340 nm (fig. 1, curve 4) indicating a Cotton effect around 315 nm.

* *Abbreviations:*
Dst: distamycin A; Nt: netropsin

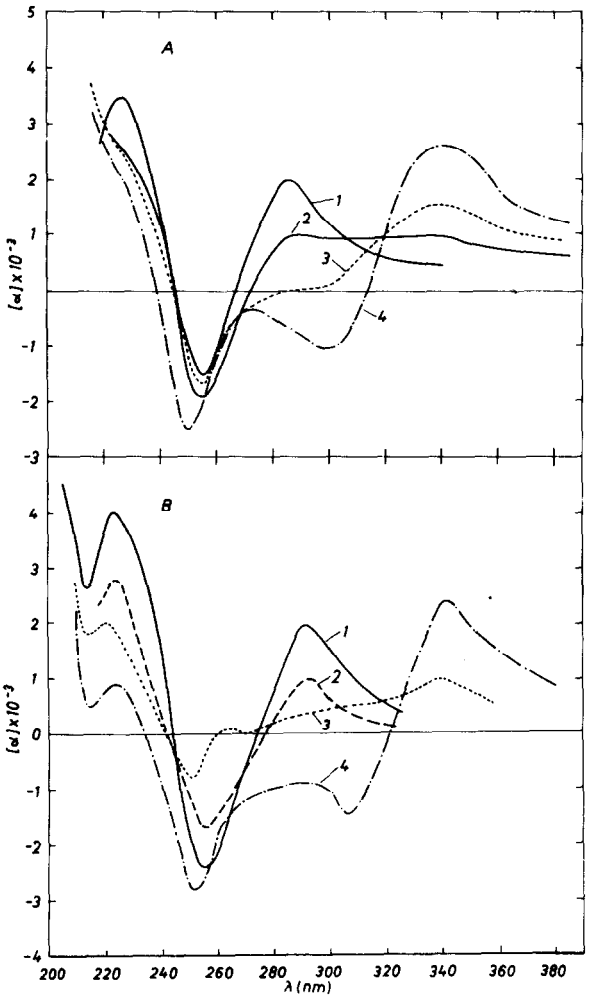

Fig. 1. ORD of the DNA-Nt complex at various Nt-concentrations in 0.02 M KCl, pH 7. A) *E. coli* DNA; 1) DNA without Nt; 2) 0.025 Nt/DNA-P; 3) 0.05 Nt/DNA-P; 4) 0.1 Nt/DNA-P. B) Calf thymus DNA; 1) without Nt; 2) 0.0012 Nt/DNA-P; 3) 0.012 Nt/DNA-P; 4) 0.12 Nt/DNA-P.

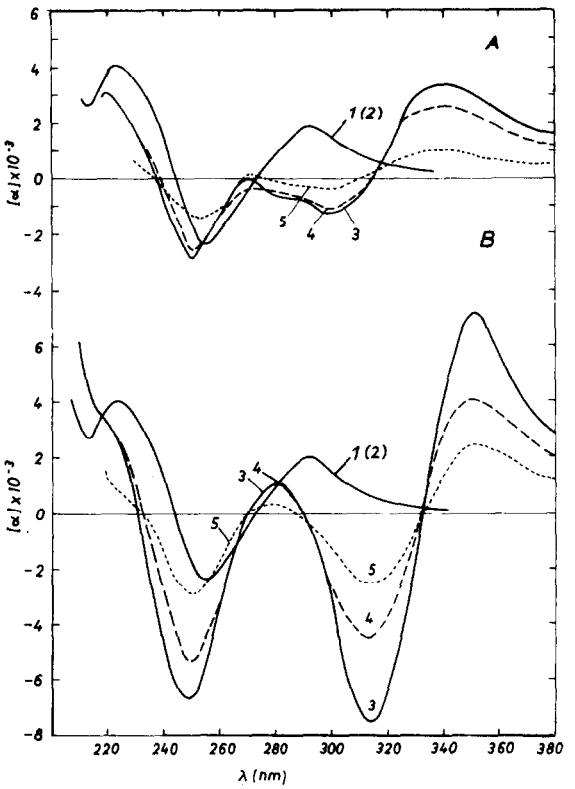

Fig. 2. Variation of ORD of the DNA-Nt and DNA-Dst complexes with base composition of DNA in 0.02 M KCl, pH 7. A) DNA-Nt complex; 1 and 2) *Str. chrysomallus* and calf thymus, respectively; 3) calf thymus (0.15 Nt/P); 4) *E. coli* (0.1 Nt/P); 5) *Str. chrysomallus* (0.1 Nt/P). B) DNA-Dst complex; curves a) and 2) *Str. chrysomallus* and calf thymus, resp.; 3) calf thymus (0.15 Dst/P); 4) *E. coli* (0.15 Dst/P); 5) *Str. chrysomallus* (0.1 Dst/P).

Nt and Dst show no absorption peak beyond 330 nm [5, 8]; the absorption maximum in the long wavelength range is located at 295 nm and 303 nm, respectively. On the other hand, difference spectra show a peak around 340 nm. This demonstrates that the exceptionally strong association of Nt to DNA [8] gives rise to an additional optically active transition. It is important to mention that no turbidity has occurred in any case at the concentrations employed. As further shown by fig. 1, the first deformation in the shape of the ORD is observed at 1 to 2 Nt/1000 DNA-P for calf thymus DNA (curve 2). The formation of the characteristic peak at 340 nm appears at approx. 1 to 2 Nt/100 DNA-P (fig. 1A, curve 2; fig. 1B, curve 3). This is consistent with the high affinity of Nt to DNA already found at very low concentrations [8].

A similar behavior is found in the ORD of the DNA-Dst complex but with some variations (fig. 2, curves 1 and 3 and the table). The new peak with positive rotation appears at 350 nm one trough around 315 nm.

Table

Variation of some characteristic optical and melting properties of DNA-netropsin (Nt) and DNA-distamycin A (Dst) complexes with DNA base composition.

DNA	AT (mole-%)	Antibiotic (moles/DNA-P)	λ peak (nm)	[α] ($\times 10^{-3}$)	λ, cross-over (nm)	λ trough (nm)	[α] ($\times 10^{-3}$)	ΔT_m * (°C)	Δh * (%)
Str. chrysomallus	28	0.1 Nt	340	1.0	310	298	−0.4	7.5	8
E. coli B	47	0.1 Nt	340	2.6	315	300	−1.1	17	10
calf thymus	58	0.15 Nt	342	3.5	315	300	−1.3	2.3	13
Str. chrysomallus	28	0.1 Dst	351	2.4	333	315	−2.5	4	14
E. coli B	47	0.15 Dst	351	4.1	333	315	−4.5	12	17.5
calf thymus	58	0.15 Dst	352	7.2	333	315	−7.5	18	18

Conditions: 0.02 M KCl (cf. legend of fig. 2).

* The increases in T_m and hyperchromicity are measured at 0.5 moles antibiotic per DNA-P.

In addition, a second small positive peak is observed at 280 nm. The profile of the curves implies that an optically active transition appears in the longer wavelength region which is centered around 330 nm for the Dst-complex and 315 nm for the Nt-complex. DNA alone does not exhibit a Cotton effect beyond 310 nm and the oligopeptides Nt and Dst are optically inactive. Thus it is likely that the binding of these antibiotics to DNA induces the optically active transition due to perturbation of the chromophores of Nt and Dst. Specific complex formation between DNA and oligopeptides is further demonstrated by the dependence of the ORD changes on AT content of DNA. In both cases, the corresponding changes in rotatory properties increase with increasing AT content of the DNA. This can be explained in terms of a high specificity of the attachment to AT-rich regions, which is in close agreement with the observed AT-specificity in the melting and viscosity behavior of the complexes [8]. The most important optical and melting properties are compared in the table.

The deformation of the ORD spectra of DNA on addition of the oligopeptides also suggests that these interactions are accompanied by a perturbation of the DNA B-conformation. Pronounced deformations of the ORD and CD of DNA have been previously reported in ethanol [16], glycol [17], in the presence of high salt concentrations [18] and of certain metal ions [15, 19]. Polylysine also affects the optical rotatory properties of DNA [20, 21]. The changes found in our ORD spectra below 320 nm resemble those caused by ethanol [16] or high salt concentrations [18]. This has been attributed to the tilting of the stacked bases relative to the helix axis [22]. Therefore the perturbation of the DNA secondary structure by Nt- and Dst-binding could be associated with a base tilting. The AT-specificity suggests that AT-rich regions are more sensitive to such deformations than GC-rich domains. This could be a result of possible differences between the properties of an AT-rich (or AT-cluster) and GC-rich (or GC-cluster) region due to variations in geometry. At present clear evidence is lacking,

The sensitivity of the DNA conformation to changes caused by the oligopeptide binding can also be deduced from fig. 3. RNA, which exists in an A-conformation [22], does not show deformation effects in its ORD profile on addition of Nt and Dst. On the other hand, complexes of denatured DNA show specific conformational changes. This can be explained by conformational changes induced in unspecific base pair regions of the secondary structure of denatured DNA. The pronounced differences between DNA and RNA could explain the strong antiviral activity of Dst in the case of DNA viruses and its lack of effect against RNA viruses [3, 23]. It further supports the findings on inhibition of the DNA template activity in the DNA- and RNA-polymerase system [10, 11] at biological concentrations (10^{-6} to 10^{-7} M of Dst).

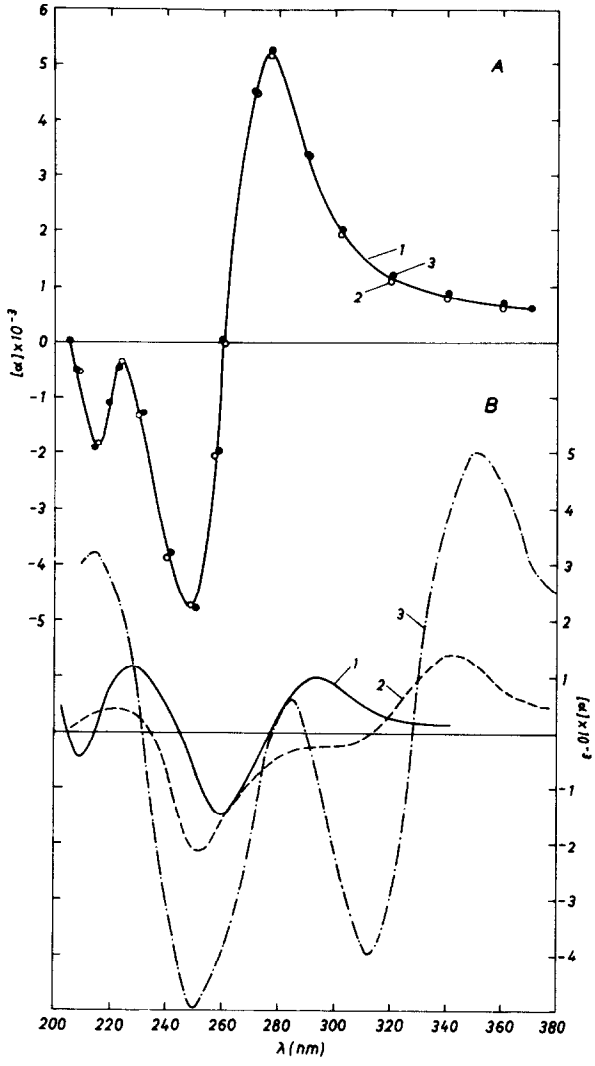

Fig. 3. Comparison of ORD spectra of denatured DNA and t-RNA in 0.02 M KCl, p.H 7. A) t-RNA; 1) without antibiotic; 2) 10^{-4} M Nt; 3) 10^{-4} M Dst. B) Heat denatured DNA from calf thymus; 1) DNA without antibiotic; 2) 0.1 Nt/P; 3) 0.15 Dst/P.

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