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Binding of Analogues of the Antibiotics Distamycin A and Netropsin to Native DNA

Effect of Chromophore Systems and Basic Residues of the Oligopeptides on Thermal Stability, Conformation and Template Activity of the DNA Complexes

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Binding effects of DNA with two distamycin A analogues containing four and five 1-methylpyrrole residues (distamycin A contains three of these residues) and with a degradation product of netropsin have been investigated by ultraviolet absorbance, melting and circular dichroism measurements.

Increasing numbers of the methylpyrrole groups (3 to 5) enhance the thermal stability of the oligopeptide complex. On the other hand elimination of the basic groups of the netropsin molecule decreases the melting temperature of the oligopeptide · DNA complex enormously. The circular dichroism spectrum of DNA is also less affected by the degradation product compared to netropsin. Circular dichroism spectra of the complexes exhibit a pronounced band at the longer wavelength region and indicate an increasing chirality in the complex with increasing number of chromophores of the oligopeptides.

From the results it is concluded that electrostatic attraction of the oligopeptides by interaction of the propionamidino and guanidino groups with the negative phosphate sites of DNA is important for the formation of the complex. However, non-ionic forces, which have been discussed earlier, seem to be involved and may be attributed to the chromophore residues oriented in the helical grooves.

The different binding tendency of the oligopeptides with DNA is discussed together with the biological activity of the oligopeptides.

The antibiotics distamycin A and netropsin are antiviral [1–3] and to some extent antibacterial compounds [4] which show strong binding effects to DNA *in vitro* [4–8]. It has been demonstrated that interaction of these basic oligopeptides with DNA is accompanied by an (A + T) specificity and induces drastic changes in the double-helical conformation while the RNA conformation is relatively unaffected. There is little evidence for the role of the chromophores and basic groups of the antibiotics in their binding tendency to DNA.

Very recently a series of compounds structurally related to distamycin A have been synthesized by Arcamone *et al.* [9,10]. Analogues of distamycin A with increasing numbers of methylpyrrole residues [10] show enhanced antiviral activity compared to distamycin A [11]. In respect to the affinity of these oligopeptides to DNA their complex formation with DNA was of great interest. As a topographical investigation of nucleic acid helices in solution the binding effects of distamycin analogues and of a netropsin degradation product were compared with that of distamycin A and netropsin. The chemical structures of distamycin A, netropsin and their derivatives distamycin A_4, distamycin A_5 and netropsin derivative are shown in Fig. 1. Distamycin A_4 and distamycin A_5 represent analogues of distamycin A with one and two additional 1-methylpyrrole-2-carboxamide systems respectively, while in the netropsin derivative the basic guanidino and propionamidino groups have been eliminated.

In the present work the behaviour of the oligopeptide · DNA complexes with distamycin A and its analogues together with netropsin and its derivative has been investigated by ultraviolet absorbance, melting and circular dichroism measurements. The previously reported data on the melting be-

Part of these results have been presented at the *V. Jenaer Molekularbiologisches Symposium* on *Interaktionen bei Biopolymeren* (28. 8.–1. 9. 1970) [12].

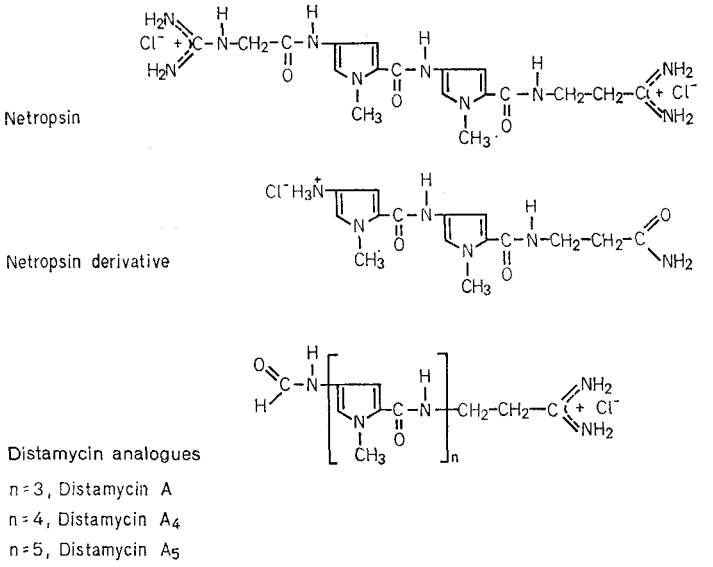

Fig. 1. *Chemical structures of netropsin, distamycin and their derivatives*

haviour of DNA complexes with distamycin A and netropsin is included for comparison. Increasing numbers of methylpyrrole residues up to five in the distamycin molecule increases the binding effects to DNA considerably. Elimination of the basic groups of the netropsin molecule reduces the binding to DNA. This observation is supported by the pronounced differences in the inhibition of the template activity of DNA in the RNA polymerase system with netropsin and its derivative.

MATERIALS AND METHODS

DNA samples used for measurements were described by Sarfert and Venner [13,14]: *Streptomyces chrysomallus* (A + T 28 mol-%) A_{260} (P) 6200; *Escherichia coli* B (A + T 47 mol-%) A_{260} (P) 6500; calf thymus (A + T 58 mol-%) A_{260} (P) 6600; T2 phage (A + T 62 mol-%) A_{260} (P) $\approx$ 6500; *Sarcina maxima* (A + T 71 mol-%) A_{260} (P) 6500.

Netropsin and distamycin were products from the culture of *Streptomyces netropsis* JA 2814 as previously mentioned [7]. The distamycin analogues were kindly donated by M. Ghione and F. Arcamone (Farmitalia, Milano).

The derivative was prepared by degradation of netropsin with 0.2 N NaOH as described by Finlay *et al.* [15]. After a second alkali treatment the product was recrystallized from ethanol-water mixture. The material obtained was chromatographically pure and showed no antibacterial activity in contrast to netropsin.

DNA · antibiotic complexes were prepared by mixing equal volumes of DNA and antibiotic solutions with desired concentrations as previously described [7]. In each case the antibiotic solutions and complex formation were made before starting the measurements. Stored solutions were never used since the analogues of distamycin in particular are more or less unstable in aqueous medium. The pH of the solutions was in the range 6.3 to 7 if not otherwise mentioned in the text.

Melting curves were made with a Uvispek spectrophotometer Model H-700 (Hilger & Watts Ltd., London) [16]. The pH was controlled by a pH meter M4 (Radiometer, Copenhagen). DNA · antibiotic complexes were measured against blanks containing the antibiotic solution of a corresponding concentration.

Circular dichroism spectra were repeatedly measured with a Roussel-Jouan dichrograph Model CD-185 using cells of 5-mm light path. Ellipticities were calculated on the basis of $A_{1\,cm}^{1\%}$ values known for the DNA samples [13,14]: $A_{260\,1\,cm}^{1\%} = 180$ (for most of bacterial and phage DNAs) and 200 (for calf thymus DNA).

The RNA polymerase system was employed using the enzyme fraction IV according to the method of Chamberlain and Berg [17]. The final volume of the assay (0.25 ml) contained: 25 µmol Tris-HCl pH 8, 1.5 µmol $MgCl_2$, 0.5 µmol $MnCl_2$, 1.5 µmol 2-mercaptoethanol, 0.05 µmol each of [^{32}P]UTP, ATP, GTP, CTP, 11 µg calf thymus DNA and approximately 20 µg protein of the enzyme preparation. The incubation proceeded for 20 min at 37 °C and was stopped by precipitation with $HClO_4$. After several washings on GC filter (Whatman) the radioactivity was counted.

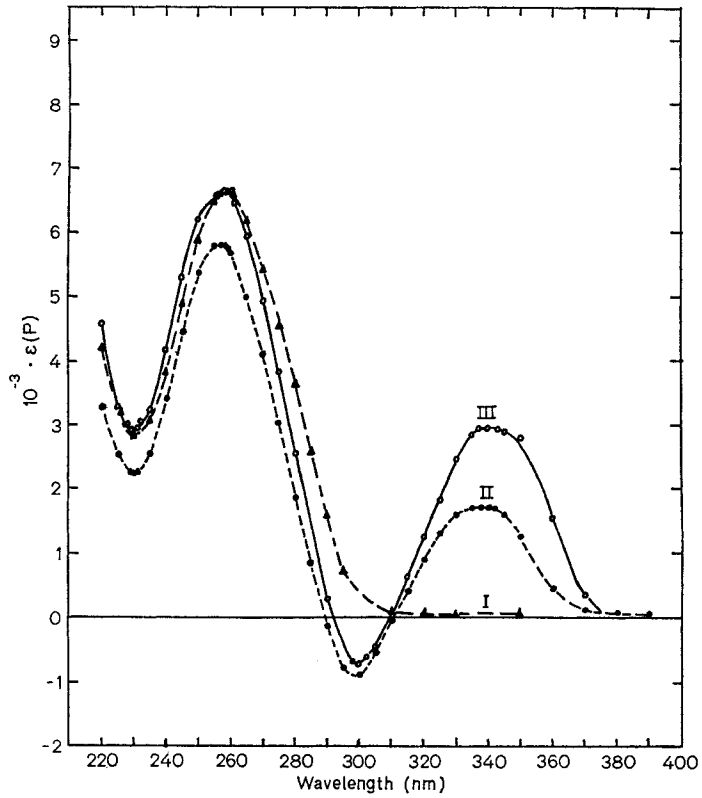

Fig. 2. *Ultraviolet-absorption spectra of the DNA · distamycin complexes in 20 mM $NaClO_4$ at 0.2 mol oligopeptide/DNA-P; (I) DNA; (II) DNA + distamycin A; (III) DNA + distamycin A_4*

The triphosphates ATP, GTP, CTP were obtained from Boehringer Mannheim GmbH (Mannheim, Germany); [^{32}P]UTP (300 counts $\times$ min^{-1} $\times$ nmol^{-1}) was a gift of Dr R. Lindigkeit (Institut für Zellphysiologie, Berlin).

RESULTS AND DISCUSSION

Ultraviolet Absorption and Thermal Melting

Some characteristic absorbance changes of the DNA complexes with the analogues are listed in Table 1. In accordance with the previously reported ultraviolet absorption spectra of the netropsin and distamycin complexes [8] the intensity of the absorption at 259 nm and 280 nm is hypochromic relative to native DNA in the absence of oligopeptides. This effect is most pronounced in the case of netropsin and distamycin A (Table 1). However, the appearance of the absorption maximum around 325 nm and 340 nm is more characteristic for the behaviour of the oligopeptide upon interaction with DNA since DNA does not absorb in this region [8]. As indicated by Fig. 3 and in Table 1, DNA shows no maximum around 325 nm in the presence of netropsin derivative compared to the netropsin

Table 1. *Comparison of ultraviolet absorption of DNA · antibiotic complexes of calf-thymus DNA*
Results are given on a molar phosphorus basis. Solutions contained 0.2 mol antibiotic per DNA-P and were measured in 0.02 M $NaClO_4$ solution, pH $\approx$ 6.5

DNA · antibiotic complex	10^{-3} × Absorption coefficient			
	ε_{259}(P)	ε_{280}(P)	ε_{325}(P)	ε_{340}(P)
Native DNA	6.6	3.6	—	—
DNA · netropsin	6.0	2.75	1.05	—
DNA · netropsin derivative	6.2	3.3	0.3	—
DNA · distamycin A	5.8	1.85	—	1.75
DNA · distamycin A_4	6.4	2.5	—	2.96

complex although lower absorbancies are found at 259 nm and 280 nm. On the other hand, the absorption at 340 nm of the distamycin complexes increases considerably by going from distamycin A to distamycin A_4 (Table 1 and Fig. 2). The hyperchromicity observed at the longer wavelengths can be ascribed to changes of the chromophores of the oligopeptides due to an orientation upon interaction with DNA [8, 18]. In agreement with this interpretation the result of Table 1 also implies the importance of

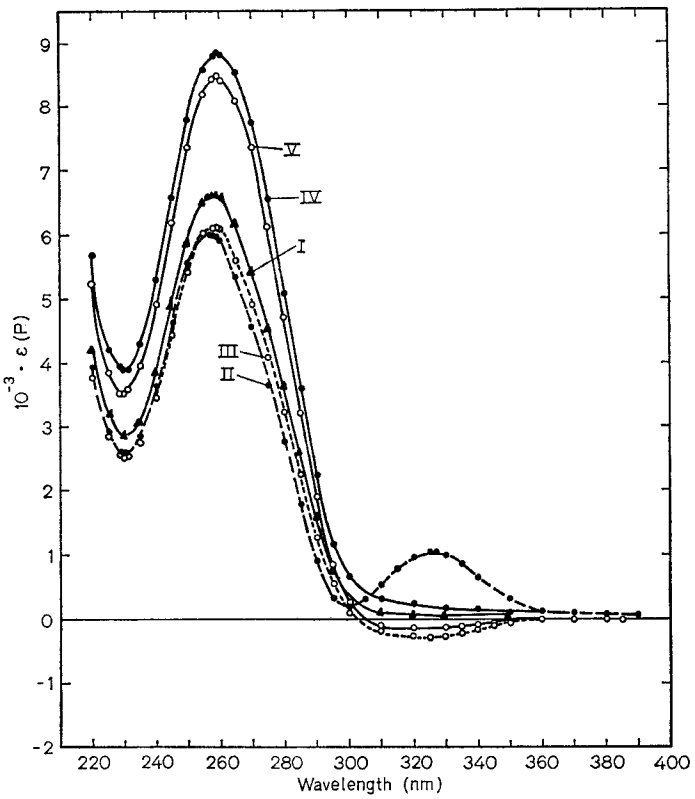

Fig. 3. *Ultraviolet-absorption spectra of the DNA · netropsin-derivative complex in 20 mM $NaClO_4$ at 0.2 mol oligopeptide/DNA-P; (I) DNA; (II) DNA + netropsin; (III) DNA + netropsin-derivative; (IV, V) after heating of (I) and (II) to 95 °C*

the aliphatic basic groups and the methylpyrrole system for the binding process.

Differences in the stabilizing effects of these oligopeptides are demonstrated by the melting behaviour of the DNA complexes as shown in Fig. 4 and 5. At comparable conditions and constant molar ratio antibiotic/DNA-P the stabilization of the DNA double helix by distamycin increases with increasing number of methylpyrrole residues from 3 to 5 indicating enhanced shielding of the helix destabilizing forces. The hyperchromicity observed for the DNA complexes increases relative to DNA alone. This can be explained by a contribution of the oriented chromophores of the complexed oligopeptides to the absorbance change on heating since the oligopeptides absorb in this region [8]. In the case of the DNA · distamycin A_5 complex (Fig. 4) there is still a hypochromic change on heating before the thermal transition. A reasonable explanation for this absorbance decrease could be given by the assumption that some of the chromophores of the distamycin A_5 molecules become first oriented when loosening of the DNA base-pair arrangements appears in the premelting range. This would mean that not all methylpyrrole systems are fixed to DNA in some regions at room temperature because of the greater length of distamycin A_5. As shown further by Fig. 4, the DNA · netropsin complex is extremely stable. Prolonged dialysis of this complex against 0.02 M salt does not decrease the melting temperature (see open squares). As an oligopeptide containing two methylpyrrole groups its melting region is near that of the distamycin A_4 complex with four methylpyrrole residues. However, the netropsin derivative has practically no stabilizing action on DNA at 0.2 mol oligopeptide/DNA-P as can be seen in Fig. 4. The plot of the increase of melting temperature *versus* increasing molar ratios of oligopeptides/DNA-P demonstrates more clearly the differences of the melting behaviour between the corresponding DNA complexes (Fig. 5). ΔT_m is about 21 °C at 0.5 netropsin/DNA-P while the derivative shows a very low ΔT_m of 2—3 °C (Fig. 5A). The netropsin complex exhibits a sharp increase of T_m up to about 15 °C within a small concentration range up to 0.05 netropsin/DNA-P; then the curve becomes flatter. Contrary to the first ascending part of the curve for netropsin a weak and smoothly increasing curvature is obtained in the case

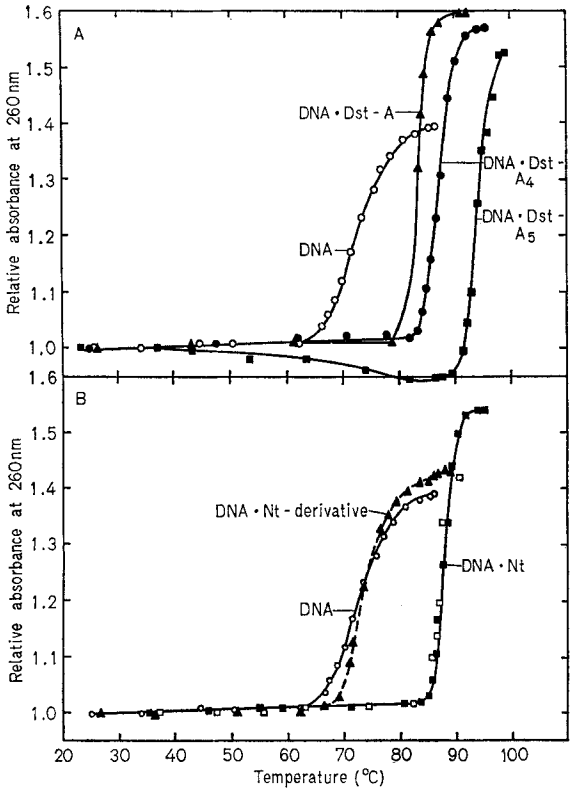

Fig. 4. *Melting curves of DNA · oligopeptide complexes in 20 mM $NaClO_4$, pH ≈ 6.5, 0.2 mol oligopeptide/DNA-P.* Open squares of (B) were measured after dialysis for 27 h against the solution medium. Dst = distamycin; Nt = netropsin

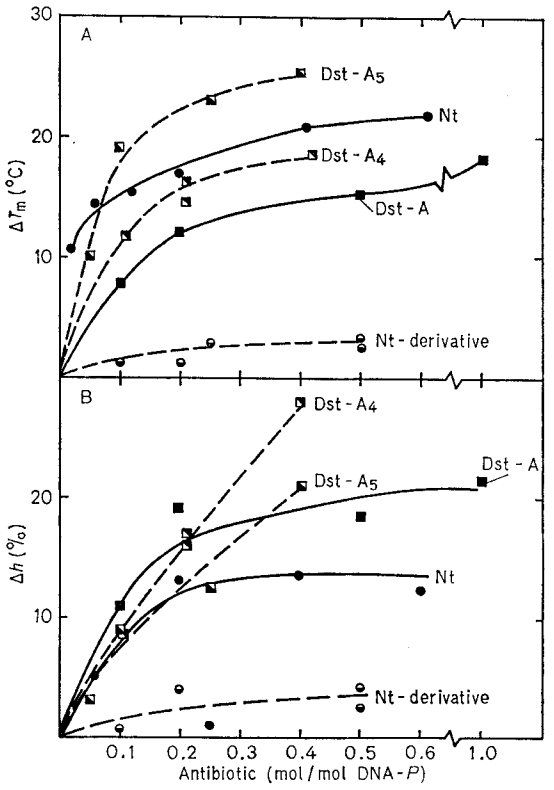

Fig. 5. *Variation of the increase in (A) the melting temperature and (B) hyperchromicity of calf-thymus DNA with increasing ratio of mol oligopeptide/DNA-P at 20 mM $NaClO_4$, pH ≈ 6.5.* Dst = distamycin; Nt = netropsin

of its derivative. A very similar different behaviour has been found for the increase of the DNA viscosity induced by netropsin and its derivative [19]. The hyperchromic changes support the differences of the melting temperature : DNA in the presence of netropsin shows very low Δh values compared to the DNA · netropsin complex (Fig. 5B). Consequently we may conclude from these differences that the strong binding of netropsin to DNA depends on the presence of the two basic centers, the guanidino and propionamidino groups. Fig. 5A further indicates that the increasing stabilizing action of the distamycin analogues with increasing 1-methylpyrrole-2-carboxamide systems is relevant for the whole concentration range examined. This again reflects the contribution of the methylpyrrole groups to the binding effect of the molecule. The main differences in the interaction process between the distamycin compounds and netropsin may be seen for the ratio below 0.07 mol oligopeptide/DNA-P (Fig. 5B). In this region ΔT_m of the netropsin complex is always higher than that of the distamycin complexes while at higher ratios the distamycin A_5 complex shows greater stability. This initial rise of ΔT_m is more steep compared to distamycin A and its analogues. The differences are consistent with differences in their binding mechanism concluded from viscosity measurements [7, 19]. Reinert [19] has shown that netropsin is bound to DNA without aggregation while the distamycin analogues may cause intra- and intermolecular cross linking. Comparison with our melting data of Fig. 4 and 5 suggests that netropsin is more tightly bound to DNA than the distamycin compounds. This may be explained by the presence of the two basic residues in the netropsin molecule, the guanidino and propionamidino groups, while distamycin A and its analogues contain only the latter.

The effect of base composition on the melting of DNA · oligopeptide complexes is shown in Fig. 6. It may be seen that the stabilizing action of distamycin A_5 on DNA considerably increases with increasing (A + T) content similar to distamycin A and netropsin as previously [7] demonstrated. The very different curves obtained for the (A + T) dependence of T_m and of the hyperchromic changes (Fig. 6,

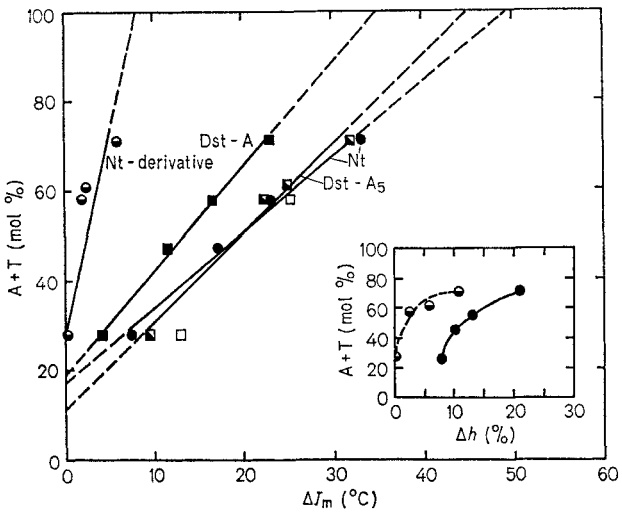

Fig. 6. *Dependence of increase in melting temperature on base composition of DNA · oligopeptide complexes in 20 mM $NaClO_4$, pH ≈ 6.5.* ●, netropsin at 0.2 mol/DNA-P; ◒, netropsin-derivative at 0.25 mol/DNA-P; ■, distamycin A at 0.5 mol/DNA-P; □, distamycin A_5 and ◪, at 0.25 mol/DNA-P. Inserted figure: Plot of (A + T) content of DNA *versus* Δh in the presence of netropsin and its derivative; conditions are those given above

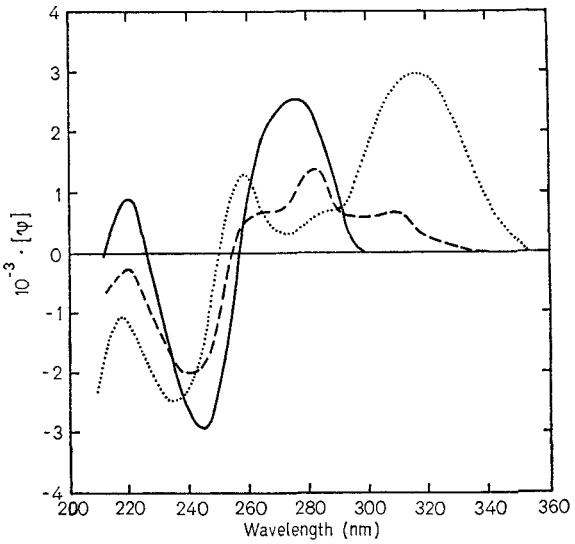

Fig. 7. *Circular dichroism spectra of calf-thymus DNA on interaction with netropsin and its derivative in 0.02 M $NaClO_4$, pH ≈ 6.5, at 0.1 mol oligopeptide/DNA-P.* ——, DNA; ·····, DNA · netropsin; – – –, DNA · netropsin-derivative

insert) between the complexes of netropsin and its derivative agree with the interpretation of the role of basic groups for the strong binding of this oligopeptide to DNA. An (A + T)-specific small binding effect of netropsin derivative is still present. This could reflect a weak interaction of the methylpyrrole rings due to a specific affinity to (A + T)-rich domains of the DNA molecule. The observed ΔT_m of 9.5 °C measured for poly[d(A — T)] · poly[d(A — T)] at 0.34 netropsin/DNA-P supports this interpretation (Fig. 6).

Circular Dichroism

Considerable information on the interaction specificity of netropsin and distamycin with DNA and RNA has been obtained by optical rotatory dispersion measurements [6]. The circular dichroism spectra of the DNA · oligopeptide complexes present direct indications for the conformational changes of the DNA helical structure and for additional chiral effects induced on reaction with netropsin, distamycin A and their analogues. The results are shown in Fig. 7. The Cotton effect of native DNA around 278 nm is reduced in the DNA · netropsin complex. At the same time a circular dichroism maximum appears at 258 nm together with pronounced changes of the negative and second positive circular dichroism band around 245 nm and 220 nm, respectively. Netropsin, distamycin A and the analogues are optically inactive. However, an optically active transition is induced upon binding of these oligopeptide structures to DNA. The new circular dichroism band is observed for the netropsin complex in the vicinity of the 316 nm (Fig. 7) in agreement with our previously reported optical rotatory dispersion data [6]. In this region the netropsin molecule absorbs but DNA does not [8]. This optically active transition is consistent with an absorption maximum found in the difference spectrum of the DNA · netropsin complex measured against netropsin between 320 nm and 325 nm [8], as may be seen in Fig. 3. Comparison with the effect of netropsin derivative on the circular dichroism spectrum of DNA is important in interpreting the spectra of the netropsin and distamycin complexes. As shown in Fig. 3 there is no ultraviolet absorption maximum at 325 nm in the presence of netropsin derivative. Apparently circular dichroism is more sensitive than the ultraviolet absorption measurement. Fig. 7 shows that the circular dichroism spectrum of the DNA-netropsin-derivative complex is intermediate between that of DNA and the netropsin complex. The circular dichroism band around 316 nm is considerably deformed. Little complex formation occurs due to a weak interaction in the case of netropsin derivative which is in line with our melting measurements (Fig. 4 and 5) and the viscosity data of Reinert [19]. The induced circular dichroism in the absorption region of the oligopeptide may be interpreted in terms of the appearance of a chirality in the bound netropsin molecules with oriented chromophores [chirality is used for dissymmetry as a more common and relevant term in stereochemistry; the term chirality being derived from the Greek (hand) is equivalent to handedness and char-

Table 2. *Effect of the solvent on thermal stability of the DNA · antibiotic complexes*
Glycol solution (98%) contained 20 mM $NaClO_4$ + 1 mM EDTA; measurements were made at 0.25 mol oligopeptide/DNA-P

Oligopeptide in the DNA complex	ΔT_m in		
	20 mM $NaClO_4$	7.2 M $NaClO_4$	Glycol (98%)
	°C	°C	°C
Netropsin	18.5	0.8[a]	24.8
Netropsin-derivative	3.3	—	—
Distamycin A_5	24.5	0.9	17.6

[a] Concn netropsin = 0.5 mol/DNA-P.

acterizes a molecule with a given conformation which shows no identity with its mirror image]. Consequently orientation of the chromophores should be suspected in the vicinity of the bases within the grooves of the helix. Since both the molecules netropsin and its derivative have the same two chromophores but differ in the nature of the terminal basic groups (*cf.* Fig. 1) it is very likely that strong electrostatic interaction between the basic residues of the oligopeptide and negatively charged phosphate sites fixes the molecule in such a way that the arrangement of the chromophores is maintained. Netropsin derivative contains only a weak basic amino group. Thus the slight interaction with DNA could be attributed to the chromophore system. The interaction of the chromophores involves non-electrostatic forces [7, 18]. Some indications are presented in Table 2 by comparison of ΔT_m of the oligopeptide complexes with different environments. At 20 mM $NaClO_4$ distamycin A_5 shows the largest ΔT_m compared to netropsin. Elimination of the electrostatically active basic groups reduces the binding effect. At 7.2 M $NaClO_4$, where both electrostatic and hydrophobic as well as some other types of interactions are destroyed, ΔT_m is practically zero for netropsin and distamycin A_5. However in glycol medium containing 20 mM $NaClO_4$ netropsin shows a high ΔT_m opposite to a much smaller stabilizing action of distamycin A_5. Since glycol is ineffective in destroying electrostatic forces this difference in ΔT_m suggests that binding of the chromophore system involves other than electrostatic interactions. The contribution of the oligopeptide chromophores to the induced circular dichroism is also supported by the spectra of the distamycin analogues (Fig. 8). In the region of 290 to 360 nm distamycin A, distamycin A_4 and distamycin A_5 show a negative and positive circular dichroism band when bound to DNA. The ultraviolet absorption spectrum shown in Fig. 2 reveals a maximum in the long wavelength region in agreement with the circular dichroism band around 360 nm. At a comparable ratio of 0.25 mol oligopeptide/DNA-P these circular dichroism bands

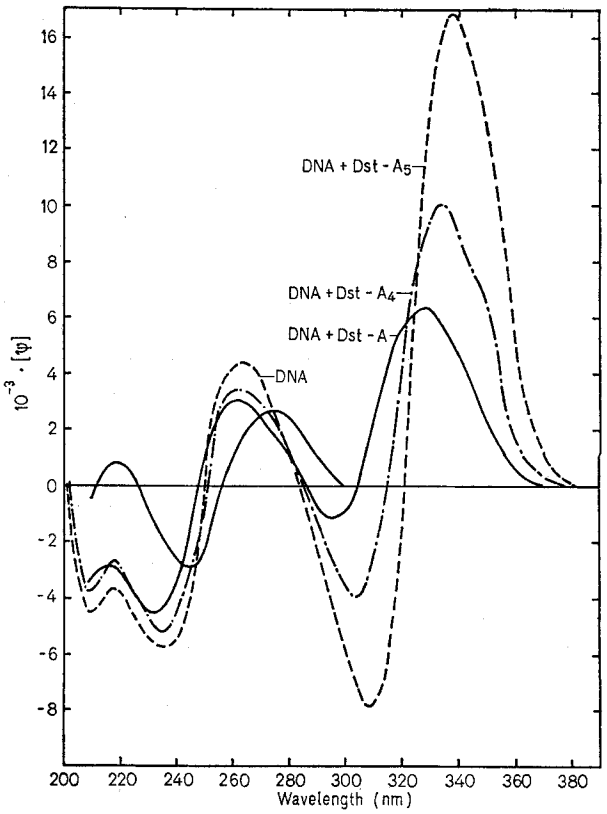

Fig. 8. *Circular-dichroism spectra of calf-thymus DNA on interaction with distamycin A and its analogues in 0.02 M $NaClO_4$, pH ≈ 6.5, at ≈ 0.1 mol oligopeptide/DNA-P*

Table 3. *Circular-dichroism bands of DNA complexes*

DNA · oligopeptide complex	λ_{max_1}	$10^{-3} \times [\Psi]$	λ_{max_2}	$10^{-3} \times [\Psi]$
	nm		nm	
Netropsin	316	3.0	none	—
Distamycin A	328	6.5	296	−1.2
Distamycin A_4	333	10.0	204	−4.0
Distamycin A_5	338	16.6	308	−7.9

shift towards longer wavelengths and the magnitude of the ellipticity increases with increasing number of chromophores. The most important values are summarized in Table 3. The results are consistent with increasing chirality caused in the bound state of the oligopeptide molecule. This chiral effect is influenced by the number of chromophores. The changes observed in the shape of the circular dichroism spectrum of DNA are similar below 280 nm for netropsin and distamycin complexes (Fig. 7 and 8). Some minor differences in this region may be explained by their different interaction mechanism as shown by the viscosity behaviour [7, 19].

It appears likely that the circular dichroism changes reflect a perturbation of the DNA B-conformation which associates the binding process as previously discussed [6]. As can be seen from the magnitude of the changes in the circular dichroism spectra below 280 nm the effect is enhanced with increasing number of chromophores (Fig. 7 and 8). An alternative explanation, that the circular dichroism spectrum of the DNA · oligopeptide complexes reflect changes below 280 nm simply to the arrangement of the oligopeptides, cannot be ruled out. On the basis of the present data an exact geometrical orientation of oligopeptide chromophores to the bases cannot by given. Further work on this subject is in progress.

Induced circular dichroism bands have been also reported for nitroaniline-labelled polyamines bound to DNA or RNA [20,21], known as "reporter molecules". The methylpyrrole oligopeptides, however, show exceptionally high specificity to the DNA double-helical structure but not to RNA as evidenced by the complete lacking of optical rotatory dispersion changes at the longer wavelengths region [6,7]. This difference agrees well with the absence of changes in the ultraviolet absorption and melting behaviour of RNA in the presence of netropsin [5,7]. In contrast to RNA, the DNA B-conformation is very sensitive for perturbation upon association with the oligopeptides as supported by the circular dichroism of the DNA complexes of the distamycin analogues.

DNA-Template Activity in the RNA-Polymerase System

It has been shown that the template activity of DNA in the RNA polymerase system is greatly inhibited by netropsin [8]. Since direct binding to the template is responsible for this inhibition *in vitro*, comparison with the action of netropsin derivative is of valuable interest. Fig. 9 indicates no or very slight inhibition of the template activity by netropsin derivative up to 100 μM while netropsin causes a decrease to about 60%; 50% inhibition is obtained at approximately 30 μM netropsin. At this molarity of netropsin derivative the template activity is 100%. The results are in good agreement with the decreased affinity of netropsin derivative to DNA as observed in our physico-chemical measurements. Consequently the correlation of the differences between the effects of netropsin and netropsin derivative, found in the template properties and binding studies, again suggests that the inhibition is due to the interaction with the DNA. On the other hand the physico-chemical results can be used for interpretation of the molecular mechanism of the RNA polymerase system *in vitro*.

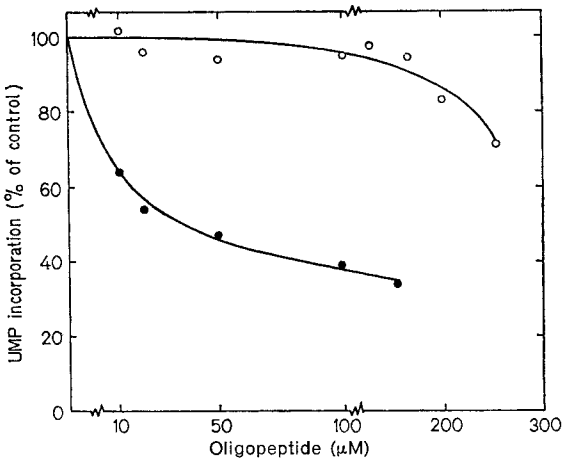

Fig. 9. *Comparison of the difference between the effect of netropsin and its derivative on the incorporation of UMP into RNA at various concentrations of the oligopeptides.* UMP incorporation without oligopeptide was set as 100%, corresponding to 1000 counts/min per incubation mixture. Details are described under Methods. ●, netropsin; ○, derivative

Table 4. *Comparison between the effect of DNA-complex formation on physical-chemical properties and biological activity* ΔT_m was determined at 0.2 mol antibiotic/DNA-P. Results with the *Vaccinia* virus were taken from [11]. Antibacterial activity was detected by the dilution test method using *Staphylococcus aureus* and *E. coli* as test strains. n.i. = no inhibition observed; n.d. = not determined

Antibiotic	ΔT_m	$10^{-3} \times [\Psi]_{max_1}$	Antiviral activity (*Vaccinia* virus)	Minimal inhibition concn of bacterial growth with	
				S. aureus	*E. coli*
	°C			μg/ml	
Distamycin A	12.0	6.5	100	5	>100
Distamycin A_4	15.5	10.1	400	n.d.	n.d.
Distamycin A_5	22.0	16.6	1000	12	>100
Netropsin	18.0	3.0	—	5	5
Netropsin derivative	1.5	0.5	—	n.i.	n.i.

Physico-Chemical Properties of the Complexes and Biological Activity of the Antibiotics

In Table 4 an attempt was made to compare some characteristic physico-chemical parameters of the DNA complexes with the biological effects of the antibiotics observed *in vivo*. According to the results of Verini, Casazza and Fioretti [11] the antiviral activity considerably increases with increasing number of methylpyrrole residues. The binding effects indicated by the melting temperature ΔT_m and the ellipticity (ψ) exhibit a similar increasing tendency in the distamycin series. In dilution tests using *S. aureus* (SG 511) we found a very similar behaviour. Netropsin is known to interfere with the growth of various bacteria [15,22]. Netropsin and distamycin A show

comparable antibacterial action against *Staphylococcus aureus* (Table 4). However, differences in the antibiotic behaviour between netropsin, distamycin A and distamycin A_5 have been found against gram-negative bacteria. While netropsin inhibits *E. coli* (SG 458) in concentrations of 5—10 μg/ml the distamycin antibiotics show, up to 100 μg/ml, no remarkable inhibition effect. This may be explained with different penetration through the microbial cell walls. On the other hand differences of growth inhibition of bacteria (Table 4) of netropsin and its derivative correlate well with the extremely reduced interaction tendency of netropsin-derivative compared to netropsin. From these observations one is tempted to conclude that there exists a correlation between the biological behaviour and the physico-chemical binding effects of the antibiotics. This could be an indication for the molecular mechanism in the mode of action observed *in vivo*. Studies *in vivo* with cultures of *E. coli* have indicated that nucleic acid synthesis is inhibited prior to the protein synthesis [4] which can be explained by direct interference of the antibiotics with the DNA replication. However, direct evidence for the interaction of netropsin and distamycin with DNA *in vivo* is still lacking.

CONCLUSIONS

As demonstrated by the present results the binding effects of the DNA · oligopeptide complexes of netropsin and distamycin A are strongly influenced by variation of the chemical nature of the antibiotic molecules.

With increasing number of 1-methylpyrrole-2-carboxamide residues in the distamycin series the stabilizing action on the helix and the induced conformational changes of the DNA B-form is increased.

The main electrostatic attraction of the oligopeptide antibiotics to DNA can be attributed to the presence of basic groups, such as propionamidino and guanidino residues in the netropsin molecule and the propionamidino group in distamycin.

From the results it is tempted to conclude that the specificity of interaction and the conformational changes are due to the chromophore systems linked to one or two ionic groups in the oligopeptide molecule. The interaction of chromophores involves non-ionic forces. It appeared reasonable to explain the induced circular dichroism bands at long wavelengths to chiral effects which could be caused by certain steric orientation of the oligopeptide chromophores in the vicinity of the bases. More definite conclusions need further detailed analysis of this subject.

This binding tendency of the antibiotics to DNA explains well the inhibition of the DNA template activity by these types of oligopeptides *in vitro*.

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