Distamycin A Complexation with a Nucleic Acid Triple Helix

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ABSTRACT: The interaction of the minor groove binding drug distamycin with the T-A-T triple helix and the A-T double helix was studied using circular dichroism spectroscopy and thermal denaturation. The triple helix was made by the oligonucleotide $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$, where x is a hexaethylene glycol chain bridged between the 3′-phosphate of one strand and the 5′-phosphate of the following strand. This oligonucleotide is able to fold back on itself to form a very stable triplex. Changing the conditions allows the same oligonucleotide to be in a duplex form with a dangling arm. Circular dichroism spectroscopy demonstrates that the distamycin A molecule can bind to the triple-stranded form of this oligonucleotide. Spectral analysis shows that the bound distamycin exhibits a conformation and an environment slightly different from those which are observed when the drug is bound to the corresponding double-stranded structure. Furthermore, a second type of complex which is observed in the double-strand binding (two stacked distamycins in the minor groove) is not observed with the triple-stranded host. When distamycin is added to the triplex made of unbridged chains $(dA)_{12} + 2(dT)_{12}$, the triplex dissociates to give a double-stranded structure. Thermal denaturation experiments demonstrate that distamycin binding destabilizes the triplex whereas it stabilizes the duplex. These results are compared with those obtained by the same experimental approaches on other minor groove binding drugs.

There has been a considerable renewed interest on nucleic acid triple helices in the past few years. This is in large part due to the fact that triplex formation presents the possibility to design oligonucleotides or modified oligonucleotides that recognize their duplex target with a very high specificity, opening potential biomedical applications [reviewed in Hélène (1991a,b)]. These oligonucleotides may artificially participate in the control of gene expression by competing for protein binding sites on the DNA in the so-called anti-gene strategy (Cooney *et al*., 1988; Maher *et al*., 1989; Francois *et al.*, 1989; Birg *et al*., 1990; Hanvey *et al*., 1990; Orson *et al*., 1991; Young *et al*., 1991; Postel *et al*., 1991; Duval-Valentin *et al*., 1992; Grigoriev *et al*., 1992; Giovannangeli *et al*., 1993). These oligonucleotides may also be used as tools in molecular biology, for example, in chromosome mapping or as artificial nuclease (Moser & Dervan, 1987; Francois *et al*., 1989; Perrouault *et al*., 1990).

One of the problems encountered in the strategies using triplex formation is that the triple helices are generally less stable than the corresponding duplex. One way to modulate the triple helix formation may be to use nucleic acid binding ligands. However, compared to the extensive database available on the interactions between small ligands and double-stranded structures, relatively few studies have been concerned with the interaction of triple-stranded structures with these compounds. The binding of the prototype intercalator ethidium bromide was the first studied (Waring, 1974; Lehrman & Crothers, 1977; Lee *et al*., 1979; Scaria & Shafer, 1991; Mergny *et al*., 1991; Pilch & Breslauer, 1994). It was shown to destabilize the triplex structure. More recently, Hélène and co-workers described other heterocyclic intercalator derivatives of the benzopyridoindole family which can stabilize the triplex structure by intercalation (Mergny *et al*., 1992; Pilch *et al*., 1993a,b). Studies on quinoline derivatives were also recently reported, and these compounds were shown to intercalate in triplexes (Wilson *et al*., 1993).

Another group of drugs, minor groove binding drugs, has been investigated: a distamycin analog, Dist2 (Umemoto *et al*., 1990); netropsin (Park & Breslauer, 1992; Durand *et al*., 1992a); Hoechst 33258 (Durand *et al*., 1994a); berenil (Durand *et al*, 1994b; Pilch & Breslauer, 1994); and DAPI (4′,6-diaminophenylindole) (Pilch & Breslauer, 1994).

In this paper, using circular dichroism spectroscopy and thermal denaturation, we analyze the binding of one of these minor groove binding ligands, distamycin A, and we compare its binding properties to those obtained with netropsin, Hoechst 33258, and berenil using the same experimental methods (Durand *et al*., 1992a, 1994a,b) .

To perform this study, we take advantage of a system we have recently developed. We used a single oligonucleotide, $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$, where x is a hexaethylene glycol chain bridged between the 3′-phosphate of one strand and the 5′ phosphate of the following strand (Durand *et al*., 1992b). This oligonucleotide has been shown to fold back twice on itself to give a triple helix. Due to its intramolecular character, this triplex was found to be very stable and did not require $MgCl_2$ or spermine for formation and is therefore very suitable for the studies of interactions with small ligands.

MATERIALS AND METHODS

The synthesis of the 36-mer $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ was achieved on a DNA automatic synthesizer using the phosphoramidite procedure. Introduction of the hexaethylene glycol linker x was performed following the previously described method (Durand *et al*., 1990).

Oligonucleotide solutions were prepared with a buffer containing 10 mM sodium cacodylate, 0.2 mM Na_2-EDTA, pH 7.0. The concentrations of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$,

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$(dA)_{12}$, and $(dT)_{12}$ were determined by the UV absorption at 90 °C, i.e., with compounds in the denatured state, assuming as extinction coefficients 8500 and 15 000 M^{-1} cm^{-1} for T and A, respectively. The concentration used to calculate the CD amplitude was that of the nucleotide unit. Solutions of $(dA)_{12}$ and $(dT)_{12}$ were combined to give mixtures with a stoichiometric 1:1 [$(dA)_{12}$ to $(dT)_{12}$] molar ratio.

Distamycin A *N*-[5-[[(3-amino-3-iminopropyl)amino]carbonyl]-1-methyl-1*H*-pyrrol-3-yl]-4-[[[4-(formylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl]amino]-1-methyl-1*H*-pyrrole-2-carboxamide, was purchased from Sigma and used without further purification. Its concentration was determined optically using an extinction coefficient of 34 000 M^{-1} cm^{-1} at 303 nm. Due to its instability in aqueous solution, fresh samples were prepared for each experiment.

Circular Dichroism Spectroscopy. Circular dichroism measurements were carried out on a Jobin-Yvon Mark IV dichrograph. Data acquisition and analysis were performed on a microcomputer interfaced to the spectrometer. Optical cells with a path length of 1.0 cm were used. The temperature of the cell was adjusted with a circulating refrigerated water bath and held constant to ±0.5 °C. Each CD spectrum was run at least twice, and we checked for possible base line shifts.

UV Melting Curves. Absorbance versus temperature profiles were measured using an LKB spectrophotometer interfaced to a microcomputer. The temperature of the cell holder was regulated by circulating liquid using a Haake water bath controlled by the microcomputer and measured by a thermistance. The measurements were initiated approximately near 7 °C, and the temperature was increased by 2 °C increments with equilibration for 1 min after reaching each temperature. The absorbance was followed at 260 and 284 nm. Previous studies on the triple helix formation between adenylates and thymidylates compounds (Riley *et al*., 1966; Cassani & Bollum, 1969; Pilch *et al.*, 1990; Durand *et al*., 1992b) showed that the melting of the third strand from the underlying duplex is accompanied by a hyperchromic absorbance change at 284 nm, whereas denaturation of the duplex strands is not. Both of these events, however, are accompanied by hyperchromic absorbance changes at 260 nm. The absorbance data were transferred to disk for storage and analyzed later. For measurements at temperature below 20 °C, nitrogen was continuously flushed through the sample compartment to prevent condensate formation. Numerical differentiations were performed to obtain differential melting profiles from which melting temperatures (T_m) were deduced.

RESULTS

Binding of Distamycin to the Double-Stranded Form of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$. Considerable information on the binding of distamycin to nucleic acids has been obtained from circular dichroism measurements (Zimmer *et al*., 1972; Zimmer & Luck, 1972; Luck *et al*., 1974, 1977; Wahnert *et al*., 1975; Marck *et al*., 1982; Dasgupta *et al*., 1987, 1990; Rao *et al*., 1988). Free in solution, distamycin shows an intense absorption band at 303 nm. For symmetry reasons, free distamycin does not exhibit any optical activity. However, its binding to nucleic acids induces a CD signal which can be used to monitor the binding process.

Figure 1A shows the CD spectra obtained upon addition of distamycin to the $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ oligonucleotide

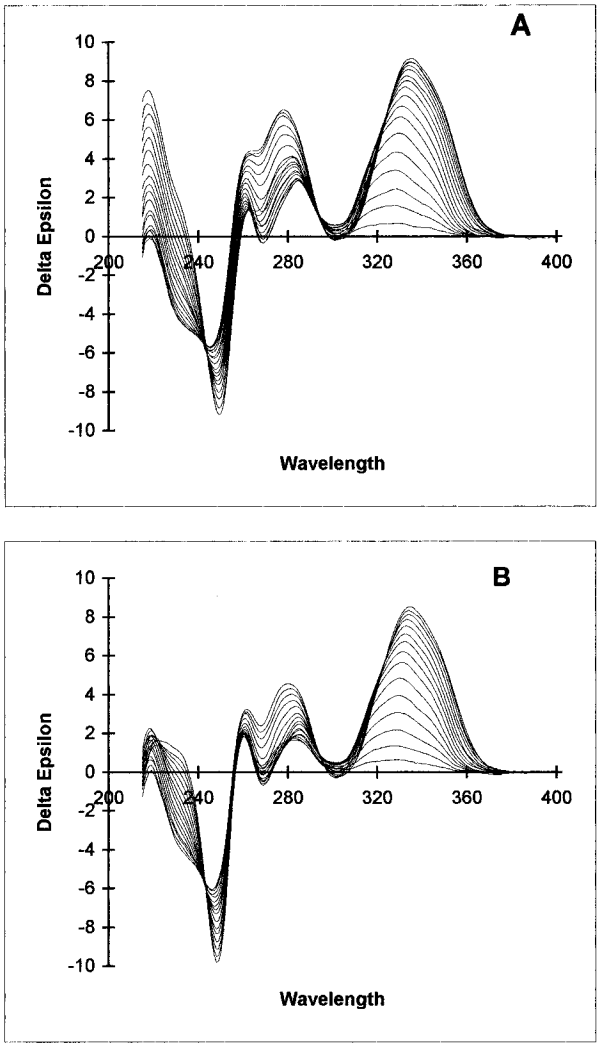

FIGURE 1: CD spectra of the oligonucleotides in the presence of increasing concentrations of distamycin. The drug to oligonucleotide ratio varied in order of increasing CD intensity signal in the 300−370 nm region from 0 to 6.8. (A) The duplex form of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ at 11 °C in the absence of NaCl. The nucleotide unit concentration was 8.6×10^{-5} M. (B) The mixture $(dA)_{12}$ + $(dT)_{12}$ + $(dT)_{12}$ in the presence of 1 M NaCl. The nucleotide concentration unit was 1×10^{-4} M. Buffer contained 10 mM sodium cacodylate and 2×10^{-4} M Na_2-EDTA, pH 7.0.

in the absence of NaCl at 11 °C. Under these conditions, the oligonucleotide adopts a double-stranded structure with a dangling $(dT)_{12}$ arm (Durand *et al*., 1992b). The presence of this dangling $(dT)_{12}$ arm does not perturb this binding experiment since it is known that distamycin A does not exhibit significant binding to single-stranded polynucleotides and particularly to poly(dT) as compared to double-stranded structure (Zimmer & Wähnert, 1986). In a control experiment using the oligonucleotide $(dT)_{12}$ free in solution, we did not observe any significant induced CD signal under the conditions of this experiment. Therefore, the appearance of a strong CD band, centered at a wavelength longer than 300 nm, upon addition of distamycin A to the $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ oligonucleotide under these conditions, indicates the complexation of the dye to the double-stranded part of the oligonucleotide.

Careful examination of the CD spectra shows that the binding process occurs in at least in two steps. For ratios of distamycin to oligonucleotide between 0 and 3, the wavelength of the induced CD maximum is located at about

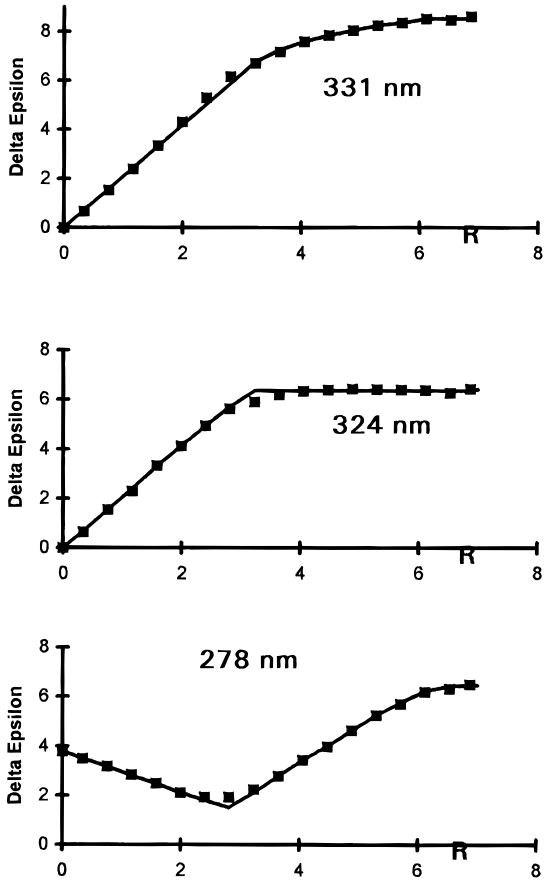

FIGURE 2: CD titration of the double-stranded form of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ with distamycin at several wavelengths. The experimental conditions are those of Figure 1A.

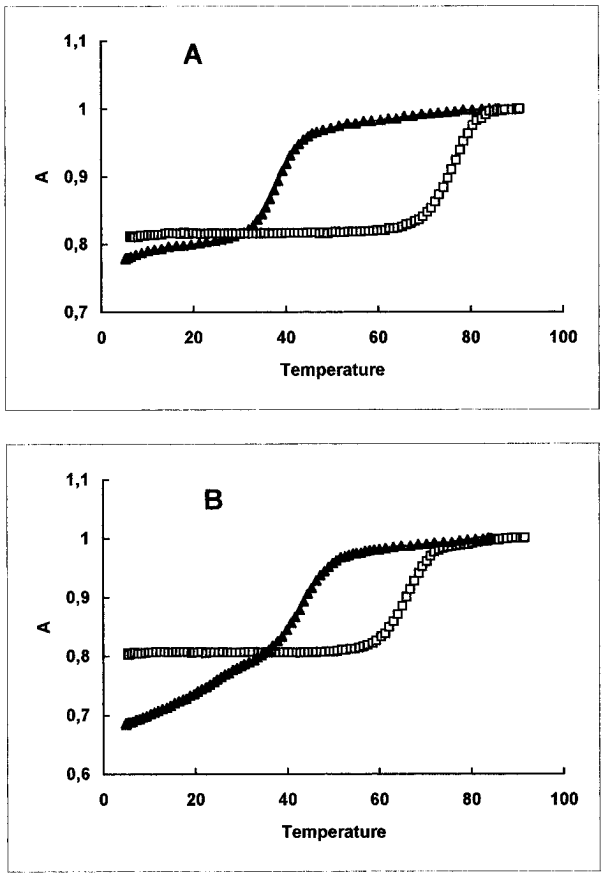

FIGURE 3: UV melting curves at 260 nm of the compounds in the absence (▲) and in the presence (□) ($R = 6.8$) of distamycin. (A) $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the absence of NaCl salt. (B) The mixture $(dA)_{12} + (dT)_{12} + (dT)_{12}$ in the presence of 1 M NaCl. For convenient comparison, the curves were normalized to an absorbance of 1 at high temperature.

330 nm, and three clear isoelliptic points are observed at 291, 263, and 244 nm. Upon further increasing the ratio between 3 and 7, the wavelength of the maximum shifts to 335 nm, and three isoelliptic points are observed at 324, 294, and 242 nm (Figure 1A). For ratios larger than 7, the induced CD spectrum does not change any more, indicating that saturation has been reached.

The presence of these two steps in the binding process can be visualized by following the CD signal at several wavelengths. Up to a distamycin to oligonucleotide ratio of 3, a linear variation is observed at all wavelengths (Figure 2). For larger ratios, a more progressive variation of the intensity different from the previous one is observed. When the process is followed at a wavelength corresponding to the isoelliptic point of the second step (for example, 324 nm in Figure 2), a sharp break near a drug to oligonucleotide ratio of 3 followed by a plateau is observed, which indicates that the binding process associated with the first step is strong.

The position as well as the intensity of the induced CD spectrum is in agreement with previously published data on the binding of distamycin A to poly(dA)·poly(dT) (Luck *et al*., 1974; Wanhert *et al*., 1975; Zimmer & Wähnert, 1986).

The stability of the complex formed between distamycin and the oligonucleotide was studied using circular dichroism and absorption. Upon increasing temperature, the induced CD signal decreases to vanish at 90 °C. At this temperature, the remaining CD signal is located below 300 nm and corresponds to that which has been previously observed for the denatured state of the oligonucleotide (Durand *et al*., 1992b). Both absorption and CD experiments indicate that a single melting process is observed (Figure 3). Comparison with the melting of the oligonucleotide alone at this ionic strength shows that the binding of distamycin is accompanied by a strong stabilization of the double-stranded structure ($T_m = 77$ °C versus $T_m = 38$ °C).

Binding of Distamycin to the Trimolecular Triplex $(dA)_{12}$·$(dT)_{12}$·$(dT)_{12}$. In the presence of 1 M NaCl, the mixture $(dA)_{12} + 2(dT)_{12}$ can form a triple-stranded structure. At 0 °C the major part of the mixture is in triple-stranded structure although the transition to this state is not complete (Durand *et al*., 1992b).

When distamycin is added to this triplex, an induced CD spectrum appears, indicating that the drug binds to this compound (Figure 1B). When the CD spectra obtained during the binding process are compared to those obtained in the previous experiment with the oligomer $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in a double-stranded structure, a similar pattern is observed at least for wavelengths longer than 300 nm: a first step with a spectrum showing a maximum at about 330 nm until the distamycin to oligonucleotide ratio reaches 3, followed for larger ratios by a step where the maximum of the CD signal is located at 320 nm and isoelliptic points are observed at 324, 294, and 242 nm. When the CD spectra obtained for ratios between 3 and 7 are compared with those of the previous experiments (Figure 4), the similarity is striking and leads us to believe that upon addition of

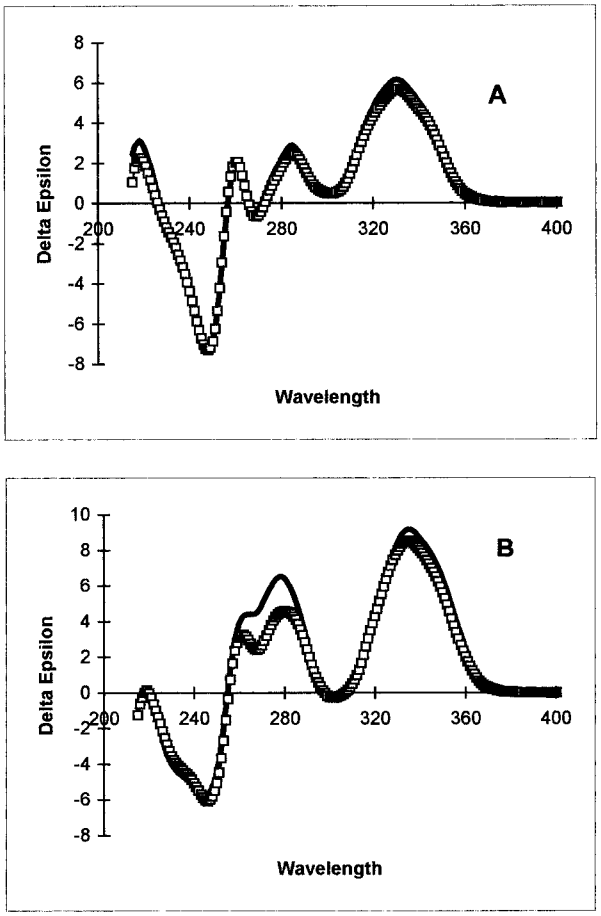

FIGURE 4: Comparison between the CD spectra of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ (represented by the open squares, □) and of the mixture $(dA)_{12} + (dT)_{12} + (dT)_{12}$ (represented by the dark solid line) at a distamycin to oligonucleotide ratio of 2.8 (A) and 6.8 (B). Conditions were those of Figure 1.

distamycin the triple-stranded structure has been converted to a double-stranded one.

To further confirm this point, melting experiments using circular dichroism and absorption were performed. In both cases, only one transition was observed with a melting temperature at about 68 °C (Figure 3B). For the complex in the absence of distamycin, the double-stranded-to-coil transition is observed at 43 °C. This confirms that the main effect of distamycin on this triplex was to convert it to a duplex form and to stabilize this duplex.

Binding of Distamycin to the Monomolecular Triple-Stranded Form of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$. The next step was to study the binding to $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ under conditions where it is triple stranded. This was done in the presence of 1 M NaCl at 0 °C. Under these conditions, the triplex-to-duplex transition of the oligonucleotide alone occurs at 66.5 °C and that of the duplex-to-coil at 70 °C (Durand *et al.*, 1994b). When followed by UV absorption, these two transitions cannot be differentiated and gave rise to a broad melting curve around 68 °C.

Upon addition of distamycin, an induced CD signal of increasing intensity with a first band located at 330 nm occurs, demonstrating the binding of the drug to the oligonucleotide (Figure 5A). A second induced CD band, which can be attributed to the distamycin molecule, occurs near 300 nm. At shorter wavelengths, modifications of the CD spectra due to both the oligonucleotide and the dye are also observed.

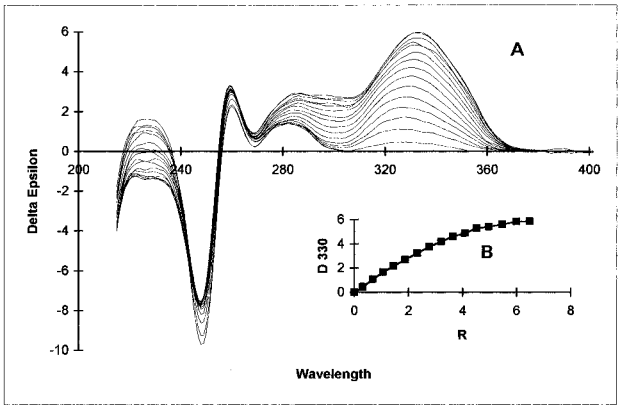

FIGURE 5: CD spectra of the triplex form of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ at 0 °C in the presence of 1 M NaCl with increasing concentrations of distamycin. The ratio of drug/oligonucleotide varied in order of increasing CD intensity signal in the 300−370 nm region from 0 to 6.5. The salt conditions are those of Figure 1. The nucleotide unit concentration was 9.5×10^{-5} M. Inset B: CD titration followed at 330 nm.

In contrast to what has been observed in the two previous cases [$(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the absence of NaCl salt and $(dA)_{12} + 2(dT)_{12}$ in the presence of 1 M NaCl salt], the titration does not seem to be a multistep process. When the titration was followed at several different wavelengths in the region of the induced signal (wavelength longer than 300 nm), similar curves were obtained (Figure 5B).

Two important observations which differ from previous experiments must be emphasized:

(i) The shape of the CD spectrum differs from that which has been obtained for the binding to the double-stranded structure of the 36-mer oligonucleotide. This is particularly striking around 300 nm.

(ii) The binding curve does not show any sharp break whatever the wavelength used to follow it. When compared to the previously obtained binding curves [$(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the absence of NaCl salt and $(dA)_{12} + 2(dT)_{12}$ in the presence of 1 M NaCl salt], a more progressive variation of the intensity is observed. However, a limit is clearly reached for the highest distamycin to oligonucleotide ratios we have used.

The stability of the complex was studied using circular dichroism (Figure 6) and absorption (Figure 7). Upon increasing the temperature, the CD signal decreases until vanishing at the highest temperature reached in these experiments. During this decrease, the shape of the CD spectra varies in a complex way. Two steps can be determined, however, particularly if we focus on the longer wavelengths corresponding only to the induced signal of distamycin. For example, at wavelengths near 300 nm, the CD signal decreases up to 35 °C, and then shows only little variation. On the contrary, the signal around 330 nm shows only limited changes up to 50 °C, and then it shows a drastic decrease. The absorption melting curve shows two transitions (Figure 7).

Together with the absorption melting curve, these changes can be interpreted without ambiguity as reflecting first a triple-to-double-stranded transition followed by a double-stranded-to-coil one. Therefore, this indicates a destabilization of the triple-stranded structure and a stabilization of the duplex. This result also indicates that contrary to what has been observed with the mixture $(dA)_{12} + 2(dT)_{12}$ in 1 M

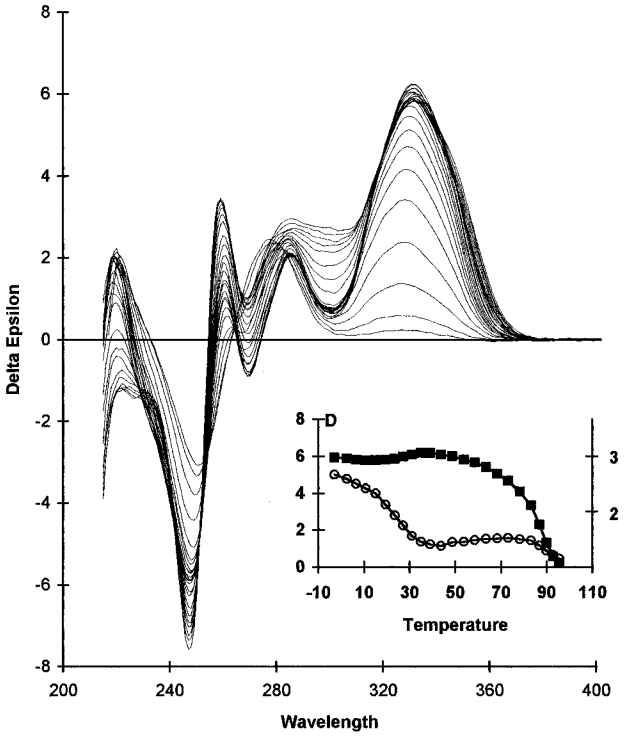

FIGURE 6: Influence of the temperature on the CD spectrum of the distamycin−$(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ complex at a drug to oligonucleotide ratio of 6.5. Concentration and salt conditions are those of Figure 5. Inset: CD intensity followed at 330 nm (■, left scale) and 300 nm (○, right scale).

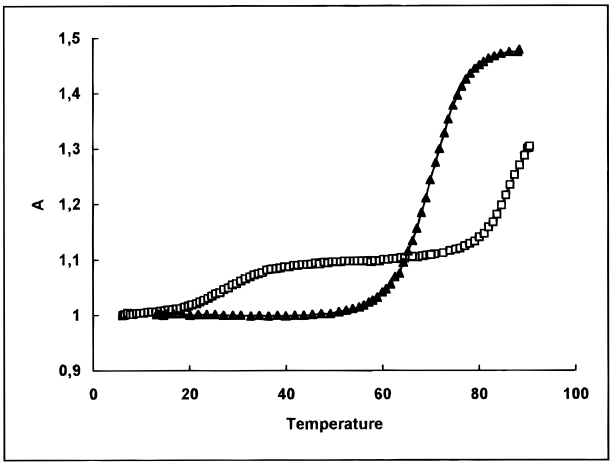

FIGURE 7: UV melting curves at 260 nm of $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the absence (▲) and in the presence (□) of distamycin at a drug to oligonucleotide ratio of 6.5. Concentration and salt conditions are those of Figure 5. For convenient comparison, the curves were normalized to an absorbance of 1 at high temperature.

NaCl salt, after addition of distamycin the oligonucleotide is still in the triple-stranded conformation.

DISCUSSION

Our CD results show that the distamycin molecule can bind to the duplex form of the oligonucleotide to form two types of complexes. The linearity of the first part of the titration curves, whatever the wavelength (Figure 2), indicates that the first binding process is strong. From the sharp break, it can be deduced that it involves three drugs on the $(dA)_{12}$·$(dT)_{12}$ sequence. Crystallographic (Coll *et al.*, 1987) as well as NMR studies (Klevit *et al.*, 1986; Pelton & Wemmer, 1988, 1989, 1990) have shown that the binding site of distamycin on the double-stranded structure is about 5 base pairs. In our experiments, the binding of three distamycins to an oligonucleotide of 12 bases pairs indicates that 4 base pairs are enough to maintain the binding. We may envisage two explanations to our smaller value. There might be some overlapping of the distamycin molecule, or alternatively the two molecules at each of the extremities of the sequence may protrude from the duplex. The sequence A_n·T_n is not the strongest binding site of distamycin A, but it is among the strongest. We cannot determine the binding constant from the titration curve. However, taking into account the concentration used, we can estimate that it is larger than 10^9 M^{-1}, a value in agreement with previously reported data on poly(dA)·poly(dT) (Breslauer *et al.*, 1987).

With the double-stranded form of the oligonucleotide, a second binding process is observed which involves another type of circular dichroism change. We think that it reflects the second mode of binding of distamycin which has been described by Pelton and Wemmer (1989, 1990) in which two drugs bind simultaneously, overlapping in the minor groove with antiparallel orientations. An interesting point is that during this second binding process an isoelliptic point is observed at a wavelength where only the drug contributes to the CD spectrum ($\lambda > 300$ nm) (Figure 1A). The presence of this point can be explained by considering that there is an equilibrium between the two types of complexes (complex I, formed by one monomeric distamycin, and complex II, formed by two distamycins) with the total amount remaining constant. Addition of distamycin does not increase the total amount of complex but converts complex I into complex II. Such an equilibrium can give rise to an isoelliptic point at a wavelength where the CD signal of complex I is identical to that of complex II.

Addition of distamycin to the trimolecular triplex dissociates it to give a double-stranded structure. Above a drug to oligonucleotide ratio of 3, the CD spectra obtained with the mixture $(dA)_{12} + 2(dT)_{12}$ are identical to those obtained with the oligonucleotide $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the double-stranded conformation (Figure 4). The triplex-to-duplex transition is therefore induced by the first binding process.

Upon addition of distamycin, the oligonucleotide $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the presence of 1 M NaCl remains in the triple-stranded structure as demonstrated by its melting behavior. The appearance of an induced CD spectrum demonstrates that the distamycin molecule interacts with the triplex to form a complex. In the 300−350 nm wavelength range, the shape of the induced CD spectrum resembles that which is observed upon binding to the double-stranded structure, but is not identical (Figures 1A and 5). The most likely explanation of this result is that the geometries of the bound molecule and its surroundings are not absolutely identical in the duplex and in the triplex. The presence of a third strand in the major groove of the duplex has induced modifications such that new constraints are applied to distamycin for binding in the minor groove of the triplex.

For the binding of distamycin to $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$ in the triple-stranded structure, we have no evidence of the formation of a second type of complex, in contrast to the double-stranded form of the same oligonucleotide in the absence of NaCl. We can exclude that this lack of a second complex is related to the presence of 1 M NaCl since in this ionic condition the duplex $(dA)_{12}$·$(dT)_{12}$ also gave two types of complexes. This absence of a second complex is therefore

related to the nature of the host structure. The complex with distamycin bound in a dimeric form, as it has been described by Pelton and Wemmer (1990), requires the expansion of the minor groove to accommodate two distamycin molecules at the same place. It is quite likely that the third strand in the major groove does not allow this expansion and blocks the formation of this type of complex.

Distamycin destabilizes the triplex as reflected by the decrease of the triplex to duplex temperature of transition. This explains why in the case of the mixture $(dA)_{12} + 2(dT)_{12}$ we observe upon binding distamycin a transition from the triplex to the duplex form: the temperature corresponding to the triplex-to-duplex transition has been lowered to below 0 °C. With the modified oligonucleotide due to the stabilization produced by the intramolecular character of the triplex, the destabilization is not large enough to convert the triplex to duplex. This effect on the stability of the various forms of nucleic acid is mainly related to the relative affinity of distamycin to the various structures. The binding is stronger for the double-stranded structure than for the triple-stranded one. This is in agreement with the shape of the titration curves we have obtained (Figures 2 and 5).

It is interesting to compare the results obtained in this study with previous results we have obtained with other minor groove binding molecules, namely, netropsin, Hoechst 33258, and berenil (Durand *et al*., 1992a, 1994a,b). Several differences in behavior exist between these compounds which makes the pattern of interaction more complex than what we expected after studying the interaction of netropsin (Durand *et al*., 1992a).

Netropsin appears to be the only molecule of this group to have exactly the same binding site in triplex and in duplex forms of DNA. The induced CD spectrum of the three other drugs bound in triple-stranded structure shows small differences with that of the corresponding double-stranded structure.

In all cases, the binding of the drug to the triplex at high ionic strength leads to a destabilization of this structure. This destabilization is of the same order of magnitude for netropsin, Hoechst 33258, and distamycin (ΔT_m of about 35 °C), but is much smaller for berenil. It must be mentioned that the magnitude of these effects parallels the stabilization effect on the double-stranded structure, the stabilization by berenil being smaller than that induced by the three other compounds. At low ionic strength, in the absence of NaCl, only berenil promotes the formation of the triple-stranded structure when added to $(dA)_{12}$-x-$(dT)_{12}$-x-$(dT)_{12}$. Such behavior has been attributed to the dicationic nature of berenil as it is known that diamine stabilizes the double-stranded structure. However, it must be pointed out that this cannot be the only explanation since netropsin which is also dicationic does not behave in this way. Geometrical parameters such as, for example, the distance between the charges may also play a role. Similar stabilizing effects of berenil have been reported on a triplex made of RNA and DNA, poly(rA)·2poly(dT) (Pilch & Breslauer, 1994).

The crystallographic structures of the complexes formed by these four drugs bound to double-stranded oligonucleotide have been published (Coll *et al*., 1987; Brown *et al*., 1990, 1992; Pjura *et al*., 1987; Teng *et al*., 1988). However, comparison between the features of the various complexes is difficult since the complexes were obtained with different host oligonucleotides. Three were crystallized with $d(CGCGAATTCGCG)_2$ (netropsin, berenil, and Hoechst 33258); two others were crystallized with $d(CGCAAATTTGCG)_2$ (distamycin and berenil). If one looks at the width of the minor groove, one can see that distamycin induces widening at two positions of the duplex whereas berenil induces little variation of this parameter. The propeller twist at the level of the drug is modified by distamycin whereas it is not by berenil. Such subtle differences in the geometry of the complexes could be at the origin of the difference of behavior of the drug. However, when looking to the complex of the same drug in two different host oligonucleotides, differences are also observed. Clearly, more comparisons will be necessary to deduce rules concerning this point.

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