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Berenil recognizes and changes the characteristics of adenine and thymine polynucleotide structures

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Abstract

Using circular dichroism, differential scanning calorimetry and susceptibility to DNAse I cleavage assays, we show that the interaction of berenil, a minor-groove binding drug, with poly(dA–dT) · poly(dA–dT) and poly(dA) · poly(dT) involves important changes in the polynucleotide conformation. The effect of berenil on poly(dA–dT) · poly(dA–dT) comprises a clear alteration in CD spectra even at drug/DNA ratios smaller than the stoichiometric value. Berenil recognizes and binds to the alternating-B conformation of DNA changing it to a new conformation which appears to show some of the peculiarities of poly(dA) · poly(dT), possibly through a modification in the helical parameters at the TpA and ApT steps. Such alteration is accompanied by a small calorimetric enthalpy change. Moreover, the calorimetric enthalpy does not change significantly whatever the input ratio of drug to poly(dA–dT) · poly(dA–dT), indicating that berenil binding does not substantially alters the enthalpy of transition. In addition to increasing the melting temperature of the polynucleotide, berenil reduces the cooperativity of the poly(dA–dT) · poly(dA–dT) transition slightly more than either distamycin or netropsin.

Keywords: Berenil; DNA–drug interactions; Polynucleotide structures

1. Introduction

There are several conformational variations in DNA which can exist under physiological conditions. DNAs containing only adenine plus thymine base pairs have been suspected to differ in structural aspects from the ideal B-DNA model. The helical periodicity of poly(dA) · poly(dT) in solution (10 base pairs/turn) is markedly different from the value of 10.6 observed for other DNAs [1]. Recent crystal structures of oligo(dA) · (dT) tracts within dodecanucleotides [2,3] have revealed a high propeller twist in the base pairs which produces an increase in base stacking interactions, as well as a novel system of bifurcated hydrogen bonds, and a narrowed minor groove which appears to be stabilised by a spine of hydration [4,5]. Poly(dA–dT) · poly(dA–dT) has

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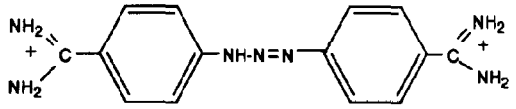
been proposed to adopt a characteristic alternating-B structure, where the helical twist angle is smaller at ApT steps than at TpA steps [5,6]. These structural variations could serve as recognition signals in the biological functions of DNA where A and T containing regions become, for example, important to explain helical bends [7] and it has also been reported that nucleosomes will form on poly(dA–dT) · poly(dA–dT) but not on poly(dA) · poly(dT) [8]. AT-rich DNAs are also present in the anomalous drug binding to some polynucleotides, including a premelting conformational transition of poly(dA) · poly(dT) [9,10]. Therefore the different properties displayed by poly(dA) · poly(dT) and poly(dA–dT) · poly(dA–dT) upon binding of several drugs is of special interest in order to gain insight into unusual DNA structures, and the mechanism involved in drug–DNA interactions.

We sought new information about AT-rich DNAs using the minor groove binding drugs berenil and distamycin (Fig. 1) which are known to recognize A and T containing tracts in DNA [2,11–19]. Distamycin appears to bind preferentially to homopolymeric AT-rich DNA [2,11,12] while berenil would bind better to AATT sites [13–19]. We have used the deviations observed between the conformations of the DNA sequences in the presence and absence of distamycin and berenil to analyze the relationship between the peculiar conformations of poly(dA) · poly(dT) and poly(dA–dT) · poly(dA–dT) and the canonical B-DNA. Berenil produces a pronounced effect on poly(dA–dT) · poly(dA–dT) which is not observed with distamycin and other minor-groove binders.

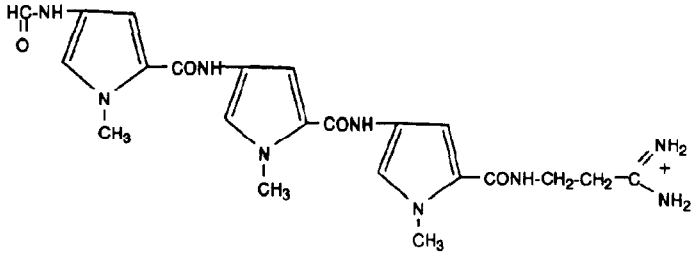
2. Materials and methods

Berenil (Hoechst, Frankfurt) was kindly provided through Hoechst Iberica, Spain. Distamycin was a gift from Dr S. Penco (Farmitalia, Italy). Daunomycin was kindly provided by Farmitalia, Italy. Poly(dA) · poly(dT) and poly(dA–dT) · poly(dA–dT) were purchased from Boehringer Mannheim and Pharmacia Iberica.

Experiments were carried out using about 30 μM basepair polynucleotide in 10 mM cacodylate buffer (pH 7.0) containing 50 mM NaCl at 25°C. DNA concentrations, in base pairs, were determined spectrophotometrically by using the



Berenil



Distamycin

Fig. 1. Chemical formulae of berenil and distamycin.

following molar extinction coefficient ($M^{-1}cm^{-1}$) values: ε_{260}(poly(dA–dT)) = 13 200, ε_{260}(poly(dA) · poly(dT)) = 12 000. Drug concentrations were determined using ε_{303}(distamycin) = 37 000, ε_{370} (berenil) = 34 400, ε_{480}(daunomycin) = 11 500.

2.1. Determination of binding stoichiometry

Absorbance measurements were recorded on a Zeiss DMR-11 spectrophotometer thermostatted at 25°C. Wavelength scans and spectrophotometric measurements were performed in semimicrocuvettes of 5 cm path length.

The stoichiometry of drug-DNA binding was determined by monitoring the change in absorbance of poly(dA) · poly(dT) and poly(dA–dT) · poly(dA–dT) solutions as each drug was titrated into them. This determination was performed using different concentrations of the polymers and each drug to obtain different input ratios of drug to DNA, between 0 and 1.1, keeping constant the polynucleotide concentration. The stoichiometry was determined from the break in the straight lines resulting from the profiles of absorbance at 315 nm (distamycin) and at 380 nm (berenil) versus the molar ratio (r) of the concentrations of the ligand and polynucleotide (in base pairs) [20].

2.2. Circular dichroism measurements

CD spectra were recorded with either a Jasco J-20C or a Jasco J-720 spectropolarimeter using 1 and 2 cm path length cuvettes. Experiments were carried out at 25°C using 20–35 μM DNA in 10 mM cacodylate buffer (pH 7.0) containing 50 mM NaCl and 0.1 mM EDTA. CD data are given as $\Delta\varepsilon$ (M^{-1} cm^{-1}).

2.3. Differential scanning calorimetry

Calorimetric measurements were performed using a Microcal MC-2 Scanning Calorimeter (Microcal Inc.). The polynucleotides were dissolved in 10 mM cacodylate buffer, pH 7.0, containing 5 mM NaCl and 0.1 mM EDTA, to a final concentration of 0.3–0.4 mM (in base pairs). A heating rate of 0.5°C min^{-1} was used. Data adquisition, excess heat capacity versus temperature, and analysis were performed using appropriate software (DA-2, purchased from Microcal). A model assuming $\Delta H_{cal} \neq \Delta H_{vH}$ was used to analyze the melting curves.

Drugs dissociation enthalpies where calculated by substracting the transition enthalpy of the duplex from the transition of the drug-bound

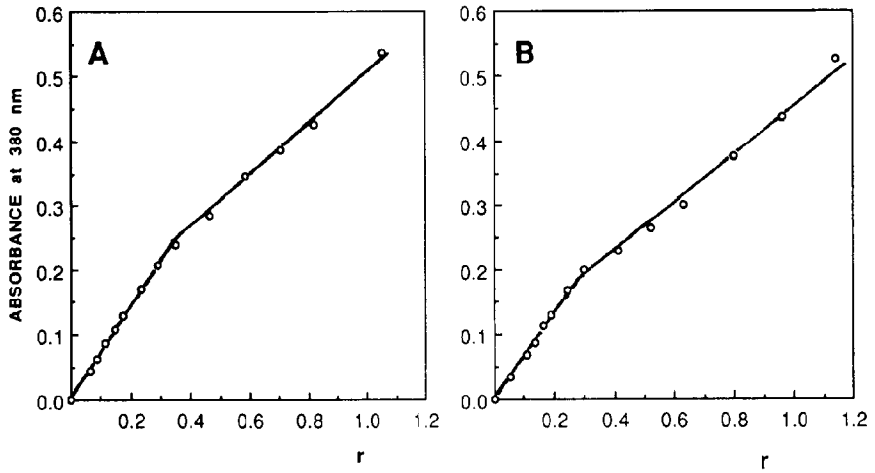


Fig. 2. Plots used to determine the binding stoichiometries for (A) the berenil–poly(dA) · poly(dT) and (B) berenil–poly(dA–dT) · poly(dA–dT) interactions; r represents the concentration input of drug per base pair of polynucleotides. The drug was added to the polynucleotide whose concentration was kept constant. The ratio corresponding to the break point, calculated by a least-squares fit, gives the stoichiometric value [20].

duplex [21,22], corrected for the different input ratios of drug to DNA (r) values.

2.4. Deoxyribonuclease I digestion studies

Deoxyribonuclease I (DNase I) was purchased from Boehringer Mannheim and stored at −20°C at a concentration of 7200 units ml^{-1} in a solution containing 150 mM NaCl and 1 mM $MgCl_2$. Aliquots of this enzyme stock solution were diluted to working concentrations immediately before use.

DNase I digestion studies were performed by the procedure of Kunitz [23]. Samples of the different polynucleotides (33 μM in base pairs) were digested with a small aliquot of the DNase I stock solution in 10 mM cacodylate buffer (pH 7.0) containing 50 mM NaCl and 5 mM $MgCl_2$. The enzyme was adjusted to yield a final concentration between 6 and 50 units ml^{-1}. Initial rates were determined from the slope of the linear portions of a plot of absorbance versus time. V_{max} and K_m values were estimated by linear least-squares fit of the initial rate data cast into the form of a Lineweaver–Burk plot.

3. Results

3.1. Binding stoichiometry

Binding stoichiometries were determined as described in Section 2, by least squares fitting of the experimental data. Representative examples for the determination of the binding saturation with berenil are shown in Fig. 2. Berenil showed two slightly different binding stoichiometric values ($r = 0.33 \pm 0.05$ for poly(dA)·poly(dT) and 0.28 ± 0.04 for poly(dA–dT)·poly(dA–dT)). Under the same experimental conditions, distamycin

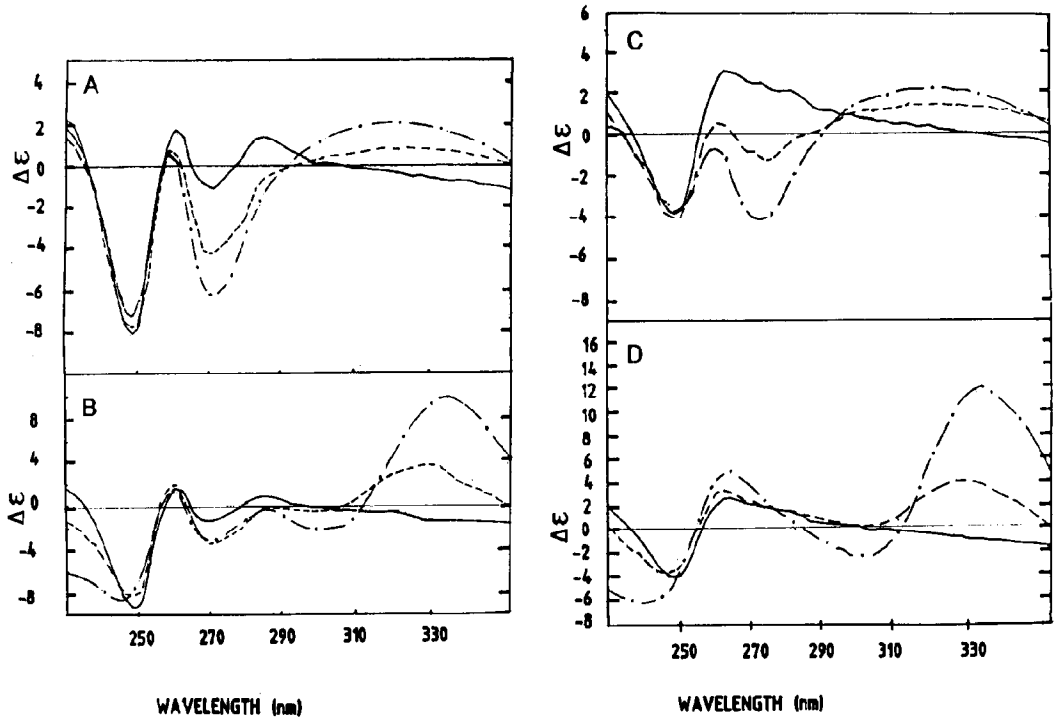


Fig. 3. Circular dichroism spectra (25°C) of poly(dA)·poly(dT) (3A and B) and poly(dA–dT)·poly(dA–dT) (3C and D), in the absence and presence of berenil (A and C), at $r = 0$ (———), $r = 0.05$ (— — —) and $r = 0.15$ (·–·–·); or distamycin (3B and D) at $r = 0$ (———), $r = 0.05$ (— — —) and $r = 0.10$ (·–·–·).

presents (plots not shown) an r value of 0.14 ± 0.03 with either poly(dA) · poly(dT) or poly(dA–dT) · poly(dA–dT), in good agreement with previously reported data [24].

3.2. Circular dichroism

Circular dichroism spectra of poly(dA) · poly(dT) and poly(dA–dT) · poly(dA–dT) in the absence and presence of different amounts of berenil or distamycin are displayed in Fig. 3. In poly(dA) · poly(dT) berenil induces a deep effect on the CD negative band around 272, which is comparable to the effect of other minor groove binders [25], though it seems a little more pronounced. The CD spectra of poly(dA–dT) · poly(dA–dT) in the absence of the ligands are of a B-DNA-type spectrum (Figs. 3C and 3D)with a negative maximum at 245 nm, a broad positive maximum around 265 nm and a shoulder at 285 nm. Increasing amounts of berenil added to the polynucleotide (Fig. 3C) caused an inversion of the initial positive CD signal of the polynucleotide around 260–280 nm, with a negative maximum centred at 272 nm, while the berenil molecule alone has an slight intrinsic positive CD spectrum in the 260–320 nm region. The binding of berenil is substantiated by the appearance of a small, broad, extrinsic Cotton effect around 320 nm (Fig. 3C), which resembles those reported for other minor-groove binding ligands [24–26]. The binding is also characterized by an isodichroic point around 248 nm. However, the induction of a negative band around 272 is very pronounced even at small drug/DNA ratios, whereas the addition of other minor groove ligands (Fig. 3D, and refs. [25] and [26]) renders spectra in which both the positive and the negative peaks change to the same extent. These CD characteristics were observed even when working below the stoichiometry calculated from UV absorption (see above). For distamycin, Figs. 3B and 3D reveal the presence of a defined shift of the CD maximum, accompanied at high r by a positive CD maximum around 325 nm and a negative one at 300 nm and isodichroic points. In the distamycin-poly(dA–dT) · poly(dA–dT) spectra the negative CD signal around 270 nm is not observed (cf. Figs. 3C and 3D).

It is noteworthy that upon berenil binding the spectra obtained with poly(dA–dT) · poly(dA–dT) somewhat resemble the spectra displayed by poly(dA) · poly(dT) (cf. Figs. 3A and 3C). Since we have analyzed the CD spectra under non-saturing amounts of drug (below the stoichiometric value), we can confidently consider that the

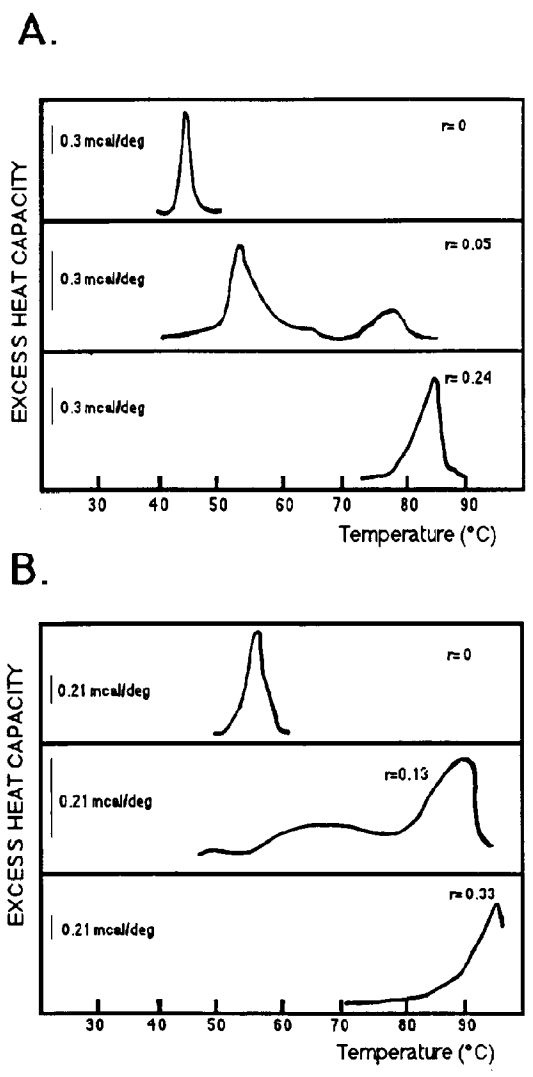


Fig. 4. Differential scanning calorimetry curves of (A) poly(dA) · poly(dT) and (B) poly(dA–dT) · poly(dA–dT) in the presence of berenil at the indicated drug-to-DNA ratios.

change in the 272 nm CD band, Fig. 3C, is reflecting distinct conformational properties of the polynucleotide. It is possibly due to changes in the symmetry of the helix in the vicinity of the berenil binding sites, including variations in the helical parameters. Because, at the r values used (about one berenil molecule per six base pairs) the berenil molecules are widely separated, the negative band cannot be caused by the interaction between externally stacked drug molecules.

3.3. Differential scanning calorimetry

Ultraviolet light spectroscopy has often been used to study the melting of DNA and synthetic oligonucleotides, but it is not the best technique by which to study DNA-interactions, since many DNA-binding drugs, such as berenil, absorb ultraviolet light, thus interfering with any melting analysis. To circumvent this problem, and in order to obtain information on the effect of berenil on poly(dA–dT)·poly(dA–dT) and poly(dA)·poly(dT), we have studied the effect of berenil, and the structurally related distamycin, on the stepwise melting of these polymers by differential scanning calorimetry, see Fig. 4. This method allowed us to calculate the calorimetric enthalpy accompanying the melting of the double helix. Tables 1 and 2 summarize the estimates for the calorimetric enthalpy data of the poly(dA–dT)·poly(dA–dT) and poly(dA)·poly(dT) helix–coil transitions. Figure 4 shows that whereas the overall transition of the unsaturated duplex, with any of the studied drugs, proceeds by two different subtransitions, only one monophasic transition is detected for the drug-saturated complexes. The addition of berenil to either poly(dA–dT)·poly(dA–dT) or poly(dA)·poly(dT) causes the appearance of a high temperature subtransition and the abolition of the low temperature transition. We have centred our attention on poly(dA–dT)·poly(dA–dT) since it shows clear changes in its CD spectra in the presence of berenil but not in the presence of distamycin. Berenil binding at an r value of 0.24 (saturation) increases the thermal stability of the duplex by 41°C. In addition to affecting the melting temperature, berenil and distamycin alter the nature of the transition as shown by the increase in the transition width. Berenil also reduces the cooperativity of the poly(dA–dT)·poly(dA–dT) as indicated by the N_0 values displayed in Table 1. They represent the average length of the polynucleotide segment that undergoes the transition from double helix to single strand in the presence/absence of the drugs. The berenil-induced increase in thermal stability of poly(dA–dT)·poly(dA–dT) is accompanied by a small increase in the overall transi-

Table 1

Differential scanning calorimetry data for the drug-free ($r = 0$) and drug-bound poly(dA–dT)·Poly(dA–dT). ΔH_D = drug dissociation enthalpy at T_m. N_0 = size of the cooperative unit ($\Delta H_{vH}/\Delta H_{cal}$), where ΔH_{vH} is the van't Hoff calorimetric enthalpy, and ΔH_{cal} the calorimetric enthalpy

Drug	r (mol drug/mol base pairs)	ΔH_{cal} (kJ/mol base pairs)	T_m (°C)		N_0	ΔH_D (kJ/mol drug)
			1st	2nd		
–	0.00	33.47	44.0	–	58	–
Berenil	0.05	35.98	53.4	76.4	–	–
	0.10	36.02	58.8	81.5	–	–
	0.24	35.15	–	85.0	27	7.10
	(Average)	35.71				7.10 [b]
Distamycin	0.11	40.59	–	85.0	32	64.43 [c]
Netropsin [a]	0.20	41.00	–	90.0	39	44.77

[a] Data adapted from ref. [21].

[b] Calculated from the average ΔH_{cal} value.

[c] Average value (two experiments), normalized to $\Delta H_{cal} = 33.47$ kJ/mol base pairs.

tion enthalpy, which is at variance with the results obtained for distamycin and netropsin (Table 1), and it is clearly independent of the berenil concentration. If we assume that the heat capacity effects are small, as for netropsin [21], the dissociation enthalpies at the melting temperature, displayed in Table 1, should be similar in magnitude but opposite in sign to the drug binding enthalpies obtained by isothermal bath calorimetry at room temperature. In this case, the value obtained for the berenil dissociation enthalpy (around 8 kJ/mol) is clearly different from the values obtained for netropsin [21]. Assuming that the heat capacity effects are small, the distamycin's enthalpy of dissociation, Table 1, can be extrapolated at 25°C to calculate the drug binding enthalpy (i.e. a ΔH_{bound} equal in magnitude but opposite in sign) rendering a value which is not at variance with the published data [27] (we estimate that an experimental error of about 10% should be considered for our calorimetric data).

3.4. Deoxyribonuclease I digestion studies

Since CD spectra show evidence of a strong effect of berenil on poly(dA–dT) · poly(dA–dT) and to a lesser extent on poly(dA) · poly(dT), we sought further characterization of this effect using DNase I. This enzyme is a good conformation probe since its cleaving efficiency is susceptible to changes in the minor groove size upon ligand binding [12–14,28,29]. DNase I digestions of poly(dA–dT) · poly(dA–dT) and poly(dA) · poly(dT) in the presence of different concentrations of berenil and distamycin are displayed in Fig. 5. Poly(dA–dT) · poly(dA–dT) shows a $V_{max} = 0.30\ \Delta A_{260}$/sec and $K_m = 60\ \mu M$, while Poly(dA) · poly(dT) shows a V_{max} value of 0.66 ΔA_{260}/sec and $K_m = 1000\ \mu M$. For the sake of comparison, we also performed DNase I digestions in the presence of daunomycin, since this intercalating drug is believed to convert poly(dA) · poly(dT) into a different conformation, more susceptible to DNase I cleavage [10]. Figure 5 shows that at relatively low drug-to-DNA ratios, the presence of daunomycin increases the susceptibility of poly(dA) · poly(dT) to enzyme digestion, while either berenil or distamycin protects it. At any given drug/DNA ratio distamycin protects both polynucleotides against cleavage better than berenil. The r value needed to provoke the inhibition effect is likely to reflect the different binding constants, which seem to be higher for distamycin [11,24] than for berenil [17]. Although the differences in protection against DNase I cleavage are not only a function of the binding stoichiometry but also of the binding kinetics, it is worth noting that the differences in minor groove width are crucial to understand the preference of these drugs for the AT-rich DNA [30], and they are also the major parameter determining the global sequence dependence of the cleavage by DNase I [28,29]. In fact, a narrowing of the minor groove can be produced by either overwinding of

Table 2

Differential scanning calorimetry data for the drug-free ($r = 0$) and drug-bound poly(dA)·Poly(dT). ΔH_D = drug dissociation enthalpy at the T_m. N_0 = size of the cooperative unit ($\Delta H_{vH}/\Delta H_{cal}$), where ΔH_{vH} is the van't Hoff enthalpy, and ΔH_{cal} the calorimetric enthalpy

Drug	r (mol drug/mol base pairs)	ΔH_{cal} (kJ/mol base pairs)	T_m (°C)		N_0		ΔH_D (kJ/mol drug)
			1st	2nd			
–	0.00	38.28	58.0	–	31		–
Berenil	0.13	46.86	65.0	90.0	–		64.01
	0.26	56.07	–	93.0	–		67.36
	0.33	– [a]	–	95.0	–		–
						(Average)	65.69
Distamycin	0.05	51.88 [b]	60.0	90.0	–		–

[a] Data not calculated, since the curve cannot be plotted completely due to instrumental limitations at temperatures about 100°C.
[b] Error is this measure is over 10%.

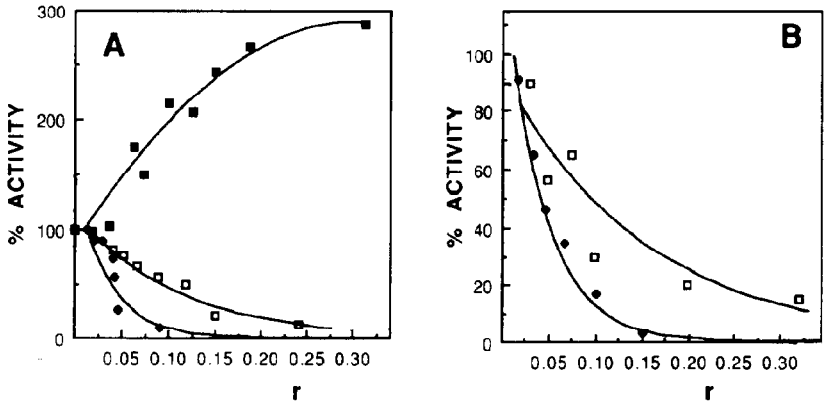


Fig. 5. Effect of berenil (□), distamycin (♦) and daunomycin (■) on the initial rate of DNase I digestion of (A) poly(dA)·poly(dT) and (B) poly(dA–dT)·poly(dA–dT). The plot represents the drug/DNA ratio (r) versus the activity of the enzyme (in %), defined as the percentage of the initial rate remaining after inhibition by the bound drug. Data (the mean value of 2–3 independent experiments) were fitted to an exponential curve, except for daunomycin enhancement of DNAse I cleavage.

the helix (reckoned to affect the circular dichroism spectra) or changes in the base-pair propeller twist angles [19,31].

The binding of either berenil or distamycin did not induce a conformational change comparable to the premelting of poly(dA)·poly(dT) induced by intercalating drugs [9,10], see Fig. 5, in clear agreement with the small changes detected in the CD experiments (Fig. 3). Nevertheless, the situation with poly(dA–dT)·poly(dA–dT) is somewhat more complicated, and informative, because even at r values higher than the stoichiometric ratio determined in Fig. 2, the cleavage by the enzyme never reaches the same extent of inhibition than in the distamycin experiments (Fig. 5B). Therefore, it is plausible that berenil binding results in a structural alteration as detected by the CD spectra presented above, which renders the polynucleotide less susceptible to DNAse I cleavage, though some of the differences in susceptibility might also be a function of the different binding kinetics of the ligands.

4. Discussion

Among the minor-groove binding ligands berenil can be considered a good aid in the analysis of sequence-dependent variations in DNA because of its small size and symmetry [13,16,32]. In this paper we present evidence of a peculiar behaviour of this drug when compared to other minor-groove binding drugs. The CD spectra of poly(dA–dT)·poly(dA–dT) in the presence of berenil shows that this drug recognizes, binds to and changes the conformation of the polynucleotide to a more dramatic extent than distamycin or netropsin [26]. However, it is noteworthy that the behavior of netropsin in CD experiments [26] is closer to that observed here for berenil. It is worth mentioning that the berenil-induced negative CD band around 272 nm is also observed, in the presence of bound drug, using more sequence-averaged DNAs, such as calf-thymus DNA (Portugal, unpublished data) and a B-form oligonucleotide [18]. DNAse I cleavage experiments reflect that the changes induced by berenil binding to poly(dA)·poly(dT) are not equivalent to those produced by intercalating drugs [9,10], but show a protection of the DNA samples from the enzyme digestion. In general, it seems that berenil acts in a peculiar way upon binding to poly(dA–dT)·poly(dA–dT), while it behaves like other minor groove ligands in respect to poly(dA)·poly(dT).

From footprinting experiments it seems that

berenil produces comparatively smaller changes in DNA structure in regions which are located distal from the binding site [13,16,33]. Nevertheless, the preference for alternating AT sequences detected by several techniques [13,16–19] should be reflected in changes in the twist angles, and other helical parameters coordinately [15,19], thus favouring intermolecular interactions. Ligand-induced perturbations are primarily manifested as an increase in roll and tilt angles, but also in the mean helical twist, which changes by about 2° in a dodecamer [18]. We tentatively consider, therefore, that such helical changes should be larger in poly(dA–dT) · poly(dA–dT), thus explaining the differences in the CD-spectra after berenil binding. In this context it is worth noting that netropsin binding causes an increase in helical twist [34,35], and that variations in twist angles may be the origin of changes in DNA conformation at the A and T containing regions [26,35,36]. Berenil might produce a rather drastic effect that is likely to lead to a DNA-form in which the number of base pairs is close to 10 per turn, as described for netropsin [26] but not for distamycin, and a subsequent change in the minor groove width, which might also be produced by changes in the base pair propeller [19,31], explaining the differences in DNase I cleavage displayed in Fig. 5, yet other factors, as differences in the ligand dissociation rates, would also participate in the phenomenon.

For any minor-groove binder the measured binding enthalpies will comprise both exothermic contributions from specific H-bonding and van der Waals interactions, and an endothermic contribution if a decrease in hydration is produced in the minor groove after binding [21,37]. The changes in helical parameters revealed by our CD and DNase I studies described above and in crystallographic studies [19], are believed to be accompanied by small enthalpy changes [38], in clear agreement with the comparatively low calorimetric enthalpy observed. Interestingly enough, inspection of Table 1 reveals that the enthalpy accompanying the double-to-single strand transition is not significantly affected by the different berenil-to-poly(dA–dT) · poly(dA–dT) ratios, thus the stabilizing effect of berenil does not substantially alter the enthalpy of the transition.

At first glance, we could consider that a smaller endothermic factor would be present with berenil than with distamycin, since the preferred berenil binding sequences contain ApT steps [13–19] but also TpA ones in the alternating polymer, in which, in contrast with poly(dA) · poly(dT), the 'spine of hydration' [39] would be partially disrupted in the free polymer [4]. However, at the melting temperature (DSC experimental conditions) the endothermic contribution to the enthalpy of binding should be small for any minor-groove binder because the spine would be mostly absent (removed). Molecular modelling studies of a berenil–DNA interaction [15] reveal that the redistribution of solvent at the drug binding sites will increase the entropic contribution when compared to other minor-groove binders. Accordingly, the small value obtained for the berenil enthalpy of dissociation at the T_m (Table 1), which assuming $\Delta C_p = 0$ may be extrapolated at 25°C to calculate a drug binding enthalpy of the same order of magnitude [21], lead us to suggest that an entropy-driven process might be a suitable thermodynamic model for berenil binding to poly(dA–dT) · poly(dA–dT). It is not possible to estimate directly from our experiments the exact conformation of the berenil–poly(dA–dT) · poly(dA–dT) complex. The significant change in the calorimetric enthalpy of poly(dA) · poly(dT) in the presence of berenil (Table 2) contrasts with the results with poly(dA–dT) · poly(dA–dT) (Table 1), indicating that the berenil–poly(dA–dT) · poly(dA–dT) complex somewhat differs from the poly(dA) · poly(dT) one, despite the similarities detected by CD and DNase I cleavage.

In this article, we have provided indirect evidence to consider berenil as useful probe for the study of peculiar DNA structures, since it can change the conformation of alternating AT tracts, even at concentrations smaller than its stoichiometric binding to DNA. Further calorimetric studies on DNA–berenil interactions at room temperature will be required to quantify the exact influence of entropy in the total free energy of binding and in order to obtain a complete thermodynamic binding profile.

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