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Interaction of diamidino-2-phenylindole (DAPI) with natural and synthetic nucleic acids

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ABSTRACT

The interaction of DAPI with natural and synthetic polydeoxynucleotides of different base content and sequences was studied with circular dichroism, ultracentrifugation, viscosity and calorimetry. All the polymers show two types of binding. The strength of the interaction and its resistence to ionic strength are related to the content of AT clusters in the chain. On the other hand, sedimentation measurements rule out an intercalation mechanism. A model of DAPI interaction with DNA, similar to that displayed by distamycin and netropsin, is proposed.

INTRODUCTION

4'-6-Diamidino-2-phenylindole.2 HCl (DAPI), first synthesized as an analogue of Berenil (1), is a fluorescent dye having the structure shown in Fig.1. It binds reversibly to DNA chains, showing a marked increase in fluorescence quantum yield (2-5). Studies with synthetic polydeoxynucleotides of different base sequences have shown that DAPI forms fluorescent complexes only with AT, AU and IC clusters of double-stranded deoxynucleic acids (6). No such complexes have been found with single-stranded DNA (7) nor with simple or double stranded RNAs, poly rI being the only exception (6). Because of these properties DAPI is currently used in a number of biochemical and cytochemical investigations (2-4, 7-17) and a staining procedure for the selective visualization of DNA in the presence of RNA has been developed (18).

In a thorough investigation (4,6) it was shown that binding of DAPI to natural and synthetic DNAs can be described by two different modes: the first is characterized by a high affinity constant, specificity toward regions rich in AT (AU, IC) sequences and a large increase in fluorescence quantum yield; the second exhibits a lower binding constant, no dependence on base sequence and only a very slight increase in fluorescence quantum yield. The nature of the former type of binding is still obscure: intercalation (4,6,8,19) and outside binding (9,20) have been postulated as possible mechanisms of inter-

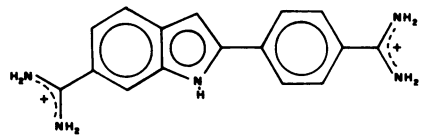

Figure 1. Molecular structure of DAPI

action.

In order to clarify this aspect and to gain some insight into the molecular nature of the reported specificity of DAPI toward AT base pairs we have carried out a series of physico-chemical measurements on the interaction of DAPI with several polydeoxynucleotides (including natural DNAs) and oligodeoxynucleotides of different base composition and/or sequence.

MATERIALS AND METHODS

Calf thymus DNA (Sigma, type I),*Micrococcus lysodeikticus* DNA (Miles),Col E1 plasmid DNA (Boehringer) were used without further purification. PM2 DNA was a kind gift of Dr. Zunino (Istituto Tumori, Milan). Poly(dAdT) was a Miles product, whereas polydA-polydT, poly(dGdC), poly(dAdC)-poly(dGdT) were obtained from P-L Biochemicals. $(dAdT)_3$ and $(dCdG)_3$ were prepared as described earlier (21). DAPI was obtained from Boehringer and used without further purification. All the reagents used were of the highest purity available, and triply distilled water was used throughout. The concentration of the solutions was obtained by using the following extinction coefficients: calf thymus DNA (6,600), *M.lysodeikticus* DNA (6,800), Col E1 DNA (6,600), PM2 DNA (6,600), polydA-polydT (6,000), poly(dAdT) (6,600), poly(dGdC) (8,400), poly(dAdC)-poly (dGdT) (6,500), DAPI (23,000).

All the measurements were performed at 25°C unless otherwise stated. Spectrophotometric measurements were carried out with a Cary 219 spectrophotometer. CD measurements were performed with a Jasco J500A dichrograph. Sedimentation experiments were carried out with a Beckman model E ultracentrifuge using UV absorbance and the schlieren method to determine the boundary position. Measurements were done at 20°C and at 40,000 rpm. Viscometric measurements were performed with an Ubbelohde viscometer in an AVS (Schott-Geräte) automatic apparatus. Enthalpic measurements were carried out with an LKB batch microcalorimeter. The method employed has been described (22).

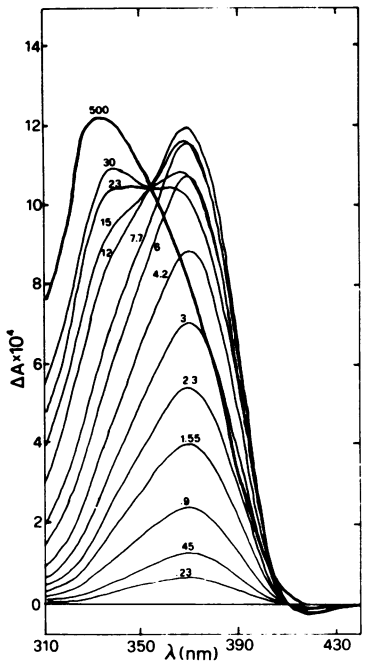

Figure 2. CD spectra of DAPI in buffer A in the presence of incrasing amounts of calf thymus DNA. The figures above each spectrum indicate the DNA/drug ratio. The limiting spectrum of the type I binding is indicated in boldface. The DAPI concentration is constant throughout the series and equal to 6.9×10^{-5} M. 1 cm cell. T = 25°C.

RESULTS

Circular Dichroism

DAPI is an optically inactive molecule which becomes active as a result of an interaction with dissymmetric molecules such as nucleic acids.

A display of the CD results obtained with calf thymus DNA (58% AT) in 0.1 M NaCl-0.1 M Tris at pH 7.4 (buffer A) is shown in Fig.2. The curves were obtained by adding increasing amounts of the polymer to a solution of DAPI. Constancy of DAPI concentration was achieved by adding a DNA solution containing DAPI at the same molarity as that of the initial, DNA free, solution. Early addition produced a CD band centered at 370 nm whose intensity progressively increased. Later additions led to the appearance of first a shoulder and then a new band with a maximum at around 335 nm. Starting at a DNA/DAPI molar ratio of about 12, a well-defined isodichroic point at 355 nm was detected. This indicated that, for DNA/DAPI molar ratios higher than 12, practically all DAPI molecules (>99%) were bound to DNA and were distributed between two binding modes, each characterized by its own CD spectrum. The molar ellipticity value at the isodichroic point ($\Delta\varepsilon = 15\ M^{-1}cm^{-1}$) was used to calculate the amounts of bound and free drug throughout the range of DNA/DAPI ratios less than 12, where a detectable concentration of free DAPI was present. The results were

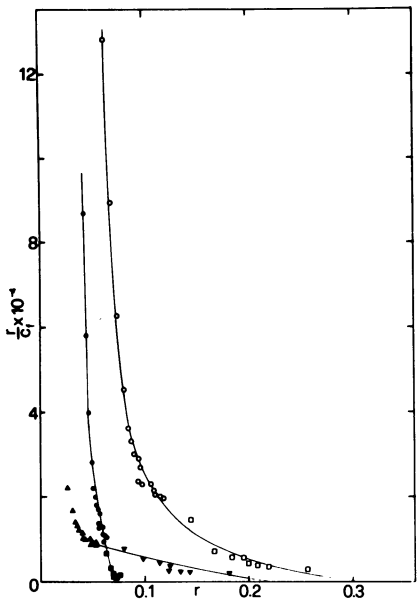

Figure 3 Overall Scatchard plot for DAPI/calf thymus DNA in buffer A (open symbols). The circles and squares refer to two sets of measurements obtained with different DAPI concentration, 6.9×10^{-6} (low r values) and 6.9×10^{-5} (high r values). Full symbols represent the resolved Scatchard plots for the two types of binding according to the method described in the text.

plotted according to the usual Scatchard procedure (23) (Fig.3, open symbols), and clearly indicate the presence of a stronger (type I) and a weaker (type II) binding, with a total number of sites per phosphate residue (n) equal to about 0.3.

Attribution of the 335 nm band to the stronger and of the 370 nm band to the weaker binding comes out straightforwardly. Since a constant CD spectrum for DAPI was obtained when very large amounts of DNA were added (Fig.2, boldface spectrum), we have assumed this spectrum to be that of the more strongly bound drug (the presence of a few percent of "weakly" bound molecules in this case is practically irrelevant). Direct experimental measurement of the pure "weaker mode" CD spectrum was not possible, since this type of binding was always associated with a significant proportion of the stronger one. However, upon adding DAPI to a DNA solution, there was a wavelength near 335 nm where, after an initial increase, the signal remained constant notwithstanding the further increase of the 370 nm band (data not shown). Evidently, at these high DAPI/DNA ratios only the weakly bound molecules increased, the strong sites being almost saturated. This finding suggested that at 335 nm the molar ellipticity of the weakly bound species is nearly zero, and as a consequence, the 335 nm CD signal is attributable to the stronger binding mode only. Thus the deconvolution of the Scatchard plot into two component binding modes from the CD titration was easily achieved and the data are plotted in Fig.3 (full sym-

bols). From inspection of these plots the numbers of binding sites are obtained: rather clearly for the first mode (n_1=0.07 drug/phosphate) and, less accurately, for the second one (n_2=0.2 or slightly more). Of the two resolved plots, only that attributable to the strong binding was fairly linear, giving a k_1 value of about 5×10^6 M^{-1} and a corresponding free energy change, ΔG_1, of -9±0.5 kcal/mol. In contrast, the weak binding showed a concave resolved plot with k_2 about 10^5 M^{-1} (ΔG_2=-7.0±0.5 kcal/mol) in the nearly linear portion at high r values.

The CD results obtained with *M.lysodeikticus* DNA (28% AT), polydA-polydT, poly(dAdT) in buffer A, processed by the same method, gave the parameters reported in Table I. Qualitatively similar CD behaviour was obtained also by adding poly(dGdT)-poly(dAdC) and poly(dGdC) to a DAPI solution in buffer A. However a quantitative difference was clearly evident in the region of high polymer/drug ratios (10-100), where the 335 nm band showed an intensity markedly lower than those elicited in the case of natural DNAs and fully AT-containing polymers. The low intensity of the 335 nm band could not be ascribed to incomplete DAPI binding, because further addition of both polymers to a solution at r=0.01 did not change substantially the intensity and shape of the CD band. A direct consequence of the low intensity was the practical impossibility of deriving reliable Scatchard plots from CD measurements. However, visual inspection of the CD spectra of both polymers showed that the intensity of the 335 nm band was always higher than that at 370 nm, indicating that the number of type I binding sites was not much lower than 0.1. Of course, no reliable k_1 values could be obtained, but 10^5 M^{-1} could be confidently taken as the lower limit, being >90% of DAPI bound when its concentration is 10^{-5} and that of the polymers $\sim 10^{-3}$.

Table I reports also the molar ellipticity of the 335 nm CD band (type I binding) obtained with the various natural and synthetic polymers at very high polynucleotide/drug ratios. The $\Delta\varepsilon_{335}$ value is clearly a function of AT content, and even more of base sequence. Clusters of AT base pairs induce on a DAPI molecule (bound with mode I) a 335 nm band whose intensity is higher than that produced by isolated AT base pairs, but even in the clusters, homonucleotidic sequences are more effective than alternating ones.

A similar dependence on base content and sequence appeared also for k_1 values, at least for those polymers for which reliable k_1 figures could be derived. This trend has already been observed by Chandra and Mildner (19), notwithstanding the too simple (and unrealistic) assumptions made by these authors in dealing with their experimental data. These results strongly suggest

Table I. Binding Parameters of DAPI for Different Polydeoxynucleotides in Buffer A.

Polymer	$k_1(M^{-1})$	n_1	ΔG_1(kcal/mol)	$\Delta\varepsilon_{335}(M^{-1}cm^{-1})$
polydA-polydT	$\sim 10^7$	0.06	-9.5±0.5	26
poly(dAdT)	$\sim 10^7$	0.08	-9.5±0.5	19
calf thymus DNA	5×10^6	0.07	-9.0±0.5	17
M.lysodeikticus DNA	7×10^5	0.06	-8.0±0.5	11
poly(dGdT)-poly(dAdC)	$> 10^5$	~0.1	<-6.8	6
poly(dGdC)	$> 10^5$	~0.1	<-6.8	1.5

that DAPI binding is not specific for clusters of AT base pairs, as indicated by fluorescence measurements (6), but rather that type I interaction is stronger for polymers with a higher content of AT clusters. On the other hand, the number of strong binding sites seems to depend very little on base content and sequence: n_1 values between 0.06 and 0.08 were found for all polymers for which a Scatchard plot could be derived. Such values correspond to one DAPI molecule bound with mode I to 6-8 base pairs. As regards mode II, the number of sites seems to be higher (or at least equal) for all polymers.

Some information about the nature and the strength of type I and II binding modes could be derived by studying the influence of the ionic strength on the stability of the DNA-drug complexes. Addition of NaCl always caused a decrease in the intensity of both CD signals (370 and 335 nm). Fig.4 shows that, while most of the salt effect on the weak binding was, for all polymers, produced at low NaCl concentrations (0-0.3 M), only for polymers not containing AT clusters was the CD signal completely canceled below 2 M. The influence of NaCl concentration on the strong binding is shown in the inset of Fig.4. The molar ellipticity at 335 nm (measured at very high polymer/drug ratios) showed for calf thymus DNA and poly(dAdT) a lower dependence on salt concentration than poly(dGdT)-poly(dAdC) and poly(dGdC).

In summary, these results suggest that:

a) both types of binding contain an electrostatic component which appears higher for type II
b) only polymers with AT clusters bind DAPI (with both modes) even at quite high ionic strength.

These conclusions are supported also by binding experiments carried out with two different hexadeoxynucleotides, $(dAdT)_3$ and $(dCdG)_3$. When progressive

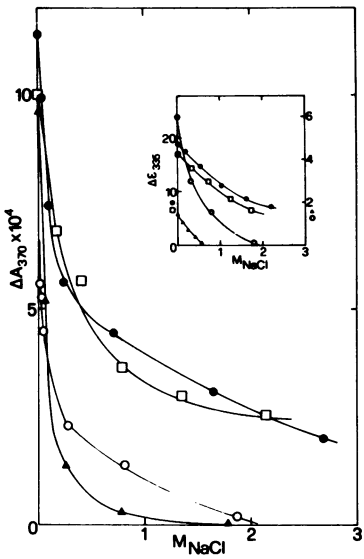

Figure 4. Effect of the ionic strength on the 370 nm CD signal (type II binding) for different polymers. (●) poly(dAdT), [DAPI] = 7.7×10^{-5}, r_a=0.43; (□) calf thymus DNA, [DAPI] = 7.7×10^{-5}, r_a=0.43; (○) poly(dGdT)-poly(dAdC), [DAPI]= 5.4×10^{-5}, r_a=0.6; (▲) poly(dGdC), [DAPI]= 1.5×10^{-4}, r_a=0.83. 0.5 cm cell for all systems. The inset shows the salt effect on type I binding for the same polymers. In this case $\Delta\varepsilon$ corresponds to DAPI that can be considered almost completely bound.

amounts of $(dAdT)_3$ in buffer A at 0°C (to favour the formation of $(dAdT)_3$ duplexes) were added to a solution of DAPI in the same solvent, a CD pattern different from that displayed by the system DAPI/DNA (Fig.2) was obtained. Already from early additions of $(dAdT)_3$ a band with a maximum at 338 nm was developed whereas no band at 370 nm was present (Fig.5). The band increased (with no modification in shape) as the $(dAdT)_3$ concentration was raised, then it leveled off (Fig.5, inset a). Using the highest intensity of the band as a measure of the completely bound DAPI, a Scatchard plot was obtained (Fig.5, inset b). The total number of binding sites (all of type I) was 0.16 per phosphate group (two DAPI molecules bound to each $(dAdT)_3$ duplex). The absence of the weak binding in this case can be interpreted as due to the low electrostatic potential generated by the short chain of $(dAdT)_3$ duplex which prevents the formation of a prevalently electrostatic bond. The low electrostatic potential of the short chain reduces also k_1 by about forty times ($k_1 = 2.5 \times 10^5\ M^{-1}$) relative to that of the corresponding polymer, again indicating the partial electrostatic character of the strong binding. This interpretation was fully confirmed when $(dCdG)_3$ was added to a DAPI solution under the same experimental conditions. No CD bands were developed in the range 300-400 nm, even when an oligonucleotide/DAPI ratio of 100 was reached. This excludes the presence of both binding modes, in agreement with the salt effect shown by poly(dGdC) with respect to both types of binding.

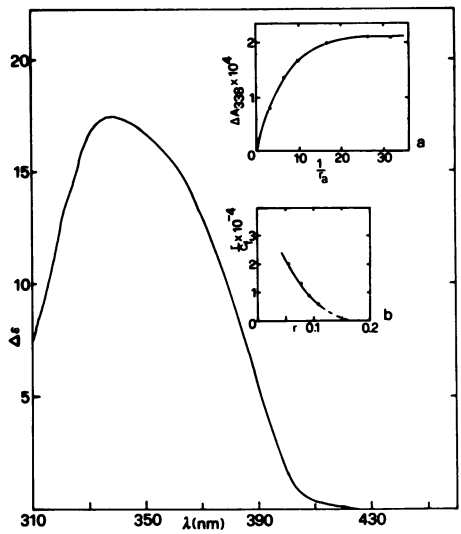

Figure 5. Limiting CD spectrum of DAPI in the presence of $(dAdT)_3$ in buffer A. Inset *a* shows the change of CD signal at 338 nm with the addition of $(dAdT)_3$. [DAPI]= 2.78×10^{-5} M, 0.5 cm cell, T=0°C. r_a is the stoichiometric drug/nucleotide ratio. Inset *b* shows the Scatchard plot for the same system.

The Scatchard plot shown in Fig.5 raises the question of why a $(dAdT)_3$ duplex can accomodate two DAPI molecules bound by the first mode whereas poly (dAdT) can bind to an equivalent stretch of bases approximately one DAPI molecule with mode I and one (or more) with mode II (Table I). On the other hand, Kapuściński and Szer (6) showed a Scatchard plot for poly(dAdT) (using fluorescence enhancement measurements) similar to our overall Scatchard plot and giving a binding site every three AT base pairs.

This behaviour can be rationalized only by considering that:

a) fluorescence enhancement is specific for AT clusters (2-4,6,10), but does not discriminate between the two binding modes, provided DAPI is bound to such clusters

b) competition between type I and type II for adjacent sites is occurring. In other words, it appears that when a cluster of three (or four) AT base pairs has bound a DAPI molecule by type I mode, the adjacent site in the polymer prefers to bind a new DAPI molecule with a type II mode. In the case of $(dAdT)_3$ both molecules are bound with mode I but the concavity of the Scatchard plot (Fig.5) indicates that the second DAPI molecule is bound with lower strength. In this case, however, the event can be facilitated by the

absence of steric hindrance or repulsions or by the presence of end effects because of the chain shortness.

Another indication that fluorescence monitors only binding to AT clusters comes from the comparison of the Scatchard plots obtained by fluorescence measurements with those obtained by CD measurements for the calf thymus DNA-DAPI system. In the former case, a very low number of binding sites (0.03) was found (4) whereas in the latter, n_1 and n_2 values of 0.07 and 0.20, respectively, were found.

The induction of CD bands on DAPI is certainly not limited to the 300-400 nm region. In fact, DAPI shows absorption bands with maxima around 340, 260 and 220 nm (5,19). The interpretation of the changes in CD spectra below 300 nm is complicated by mixing of DAPI-induced CD bands with possible CD effects due to the changes in polymer conformation. Subtraction of the DAPI-free polymer CD spectrum from the overall CD bands in each particular system and at different DAPI/polymer ratios showed no simple and consistent behaviour.

In summary, the CD results suggest these considerations:

i) DAPI binds to DNAs with two different types of interactions characterized by 335 nm (type I) and 370 nm (type II) bands;

ii) both types are present independently of base content and sequence;

iii) type I prevails at low r values and type II at high ones;

iv) the saturation limit for type I binding is about one DAPI molecule for every 6-8 base pairs;

v) the molar ellipticity of the 335 nm band is strongly dependent on base content and sequence, being particularly high when DAPI is bound to AT clusters and very low when bound to GC clusters. The 370 nm band intensity also depends on the base sequence of the polymer but to lesser extent;

vi) k_1 values appear to increase with the AT content of the DNA, but even with poly(dGdC) k_1 is not negligible;

vii) an increase in salt concentration decreases the strength of both binding modes for all complexes, type II being more sensitive than type I; when the nucleic acid contains AT clusters, both binding modes partially persist even at very high ionic strength.

Ultracentrifugation

To get some insight into the molecular mechanism of binding at low DAPI/DNA ratios, sedimentation measurements on circular DNAs were performed. It is well known that intercalation of drugs into supercoiled circularly closed DNA causes progressive removal and reversal of supercoiling, which is reflected in a decrease of sedimentation coefficient down to the value of the nicked form,

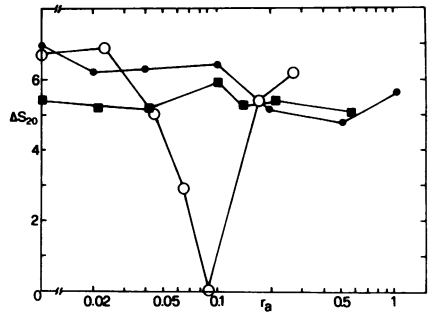

Figure 6. Effect of DAPI on the difference between sedimentation coefficients of closed and nicked form of DNA. PM2 DNA in buffer A (■) and of Col E1 DNA in 14 mM Tris (●). The corresponding results obtained with the system proflavine-PM2 DNA in buffer A are also shown (○).

and then in a subsequent increase (24). The r value corresponding to the minimum in S_{20} depends on the unwinding angle of the DNA helix associated with intercalation, and on the degree of supercoiling of the circular DNA. Fig.6 shows the effect of DAPI on the difference between the sedimentation coefficients of the closed and nicked form of PM2 (59% AT) (25) and Col E1 DNAs (47% AT) in buffer A. No dip was observed. The binding of DAPI therefore did not measurably alter the supercoiled state of the closed circular DNAs and intercalation of DAPI could be confidently ruled out. Kapuściński and Skoczylas (4) were skeptical on the possibility of finding the dip in S_{20} by predicting that the absence of lateral substituents on DAPI structure would produce an unwinding angle similar to that of proflavine which was estimated to be 2/3 of that of ethidium bromide. Fig.6 shows the effect of proflavine on PM2 DNA. Even though the dip is found at r=0.09, a consistent change in ΔS_{20} is evident at lower r values, contrary to what is found for DAPI. The data reported in Fig.6 also exclude intercalation for higher r values in contrast with the proposal of Chandra and Mildner (19).

We therefore conclude that DAPI does not intercalate into DNA, in agreement with the suggestion made by different authors (9,20).

Viscosity

The results of viscometric measurements on the calf thymus DNA-DAPI system at moderate (buffer A) and low (14 mM Tris pH 7.4) ionic strength are shown in Fig.7. The viscosity of DNA increased by about 12%, up to a DAPI/DNA ratio of about 0.06, and then leveled off. The increase corresponded well to the range of r where the type I binding is prevalent. No effect of type II bind-

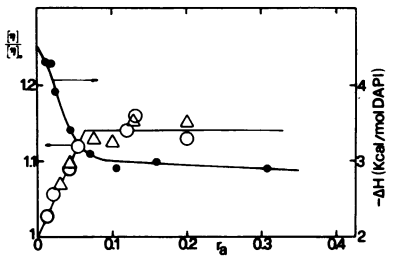

Figure 7. Relative increase in viscosity for sonicated calf thymus DNA as a function of stoichiometric DAPI/polymer ratio. (Δ) in 14 mM Tris; (O) in buffer A.
Enthalpy change in the interaction DAPI-calf Thymus DNA in buffer A (●) as a function of stoichiometric ratio drug/polynucleotide. T = 25°C.

ing upon viscosity was apparent.

An increase in viscosity is generally interpreted as due to an increase in contour length of the chain and/or to an increase in the persistence length (stiffening). Both effects are produced when a drug interacts with DNA through intercalation but similar effects have been shown also in the case of non intercalating molecules. It has been reported, in fact, that netropsin and distamycin A, well known non-intercalators, increase the viscosity of DNA (26-29) by increasing both contour length and persistence length. We propose that the same mechanism is working in the DAPI-DNA system. Viscosity measurements on DNAs of different molecular weight and base composition are under way in our laboratory to obtain the change in length per bound DAPI molecule. At the moment it seems evident that type I binding of DAPI produces some conformational change in DNA chains, in agreement with the CD results in the polymer spectral region.

Calorimetry

Calorimetric measurements have often provided useful informations for determining the mechanism of drug binding to DNA. Intercalation, with few exceptions, is associated with a large decrease in enthalpy (30) so that a large negative ΔH associated with drug-DNA interaction can be used as a diagnostic test for intercalation, at least when the only alternative is an external, purely electrostatic binding. Very recently the enthalpy change associated with the interaction of netropsin with poly(dAdT) was measured and a value of -9.2 kcal/mol of drug was found (31). This large and negative ΔH value obtained with a molecule which does not intercalate indicates that the "calori-

metric diagnosis" must be used with caution, especially when the alternative mechanism is not simply electrostatic, external binding. Analogous conclusions can be drawn in the case of the DAPI-DNA system. Fig.7 shows the calorimetric results obtained for the interaction of DAPI with calf thymus DNA in buffer A as a function of the stoichiometric ratio, r_a. A moderately large negative enthalpy change of about -4.5 kcal/mol of DAPI was obtained at low r values (where almost complete binding can be assumed) followed by a drop to a nearly constant value of about -3 kcal/mol. The larger negative value can be confidently attributed to type I binding, and from the ΔG_1 value previously obtained an increase in entropy (ΔS_1) of about 15 eu can be calculated. Tha data shown in Fig.7 are significantly different in magnitude and trend from those reported by Chandra and Mildner (19). We have no explanation for the discrepancy, the only experimental difference lying in the ionic strength (0.01 M Tris vs. 0.1 M NaCl, 0.1 M Tris).

DISCUSSION

The molecular mechanism of the interaction between DAPI and DNA is still obscure, despite the wide use of the drug in biochemistry and cytology. The marked increase in fluorescence quantum yield associated with the binding of DAPI only to polymers containing clusters of AT (AU,IC) base pairs suggested a specific, strong interaction of the drug to such clusters, together with an unspecific, non-fluorescent, weak binding (2-4,6,7). Intercalation between adjacent AT pairs was proposed as the molecular mechanism of the strong specific binding (4). The experimental results reported in this work, however, provide good evidence for a description of the interaction between DAPI and DNA somewhat different from that proposed. In fact, CD studies clearly evidence that DAPI interacts in two distinct modes with all polydeoxynucleotides independently of their base content and sequence, the only difference consisting in the strength of the interaction: the higher the content of AT clusters, the higher the intrinsic constants exhibited, at least for type I binding. The other characteristic variance among various DNAs consists in the different response to ionic strength of both types of binding: again, polymers containing AT clusters bind DAPI even at quite high salt concentrations, whereas, on the contrary, suppression of binding is shown by polymers not containing AT clusters. This property could explain the successful separation in salt gradient of DNAs with different AT content (2).

As for the mechanism of binding, it seems safe to conclude that intercalation of DAPI is very unlikely considering the results of sedimentation meas-

urements on circularly closed DNA. On the other hand, it is hard to see any reason to justify an intercalation of DAPI only between adjacent AT (AU,IC) base pairs.What these base pairs have in common is the absence of the amino group in position 2 of the purine ring, which prevents the third hydrogen bond within the base pair. The difference with respect to a GC base pair, however, does not explain why two adjacent GC pairs could not be a site for intercalation, such a mechanism being mainly governed by π-π and hydrophobic interactions. That difference, however, while not sufficient to justify a specific intercalation, could be the basis for a preferential mechanism of DAPI binding. In fact, the molecular structure of DAPI is characterized by three hydrogen donor groups, the two amidino moieties and the indole NH, which, together with the double positive charge of the molecule, provide a strong driving force for interaction with DNA. In this regard, despite its different structure, DAPI resembles netropsin and distamycin A, two well known antibiotics which strongly interact with DNA, showing experimental results surprisingly similar to those presented by DAPI. In fact, netropsin and distamycin A bind preferentially to AT clusters with bond not exclusively electrostatic (26,32,33), do not relax supercoiled DNA (32,33), increase DNA viscosity (26,27), show a negative enthalpy of binding (31), and increase markedly the DNA melting temperature (26,32). The original model of interaction between DNA and the two antibiotics foresaw the formation in the small groove of hydrogen bonds between amide groups of the drugs and AT base pairs together with electrostatic interactions of the positively charged ends with phosphate groups (32,33). This model has been successfully verified (34,35).

With this in mind we have tried to check the fitness of the model also to DAPI-DNA system. As indicated by the behaviour exhibited in the presence of increasing amounts of salts, both DAPI binding modes are not merely electrostatic but mode I is less influenced by the ionic strength. It is conceivable that binding mode I could be characterized by two hydrogen bonds. These can be allowed by AT pairs in the narrow groove, N3 on the purine ring and O2 on the pyrimidine ring being accessible. A 14-15 Å long DAPI molecule cannot make both hydrogen bonds within the same AT pair without disruption of the polymer structure but can accomodate into the narrow groove following its slope and establish hydrogen bonds with adjacent AT pairs. Whether this is possible without a slight conformational change of the polymer is difficult to predict, also because it is not established whether the DAPI molecule is planar or a certain degree of rotation is allowed to the bond joining the phenyl and the indole rings. Viscosity increase and CD curves in the polymer spectral region, howev-

er, suggest some conformational rearrangement of the chain induced by DAPI binding. An additional contribution to the strength of the interaction derives from the electrostatic bonds that one (or both) charged amidino group(s) can form with neighbouring phosphate group(s) or even with the highly negative surface of the narrow groove, as calculated by Pullman and Pullman (36). This model of interaction implies also that one DAPI molecule would span about 3 base pairs, in agreement with the experimental results on type I binding, and does not appear contradictory with the preclusion of the next neighbouring site to a DAPI molecule bound with the same mechanism, owing to the repulsions of charged amidino groups of two adjacent molecules. The model would also explain the decrease in enthalpy and the rather large increase in entropy. The latter effect should be connected to the release of structured water molecules both from DAPI and from the narrow groove where the drug should sit. In this connection it has recently been observed that in the AT rich regions of the DNA B structure a "spine" of structured water molecules is present (37). Were this proposed mechanism correct a bound drug molecule would experience an hydrophobic environment with a consequent increase in fluorescence quantum yield in agreement with the occurrence of the same result when DAPI is dissolved in organic solvents (5). The hydrophobic environment could also explain the decrease in enthalpy on binding as hydrogen bonds would be formed in a medium at lower dielectric constant than bulk water with consequent more favourable energy. Analogous conclusions have been reported for peptide hydrogen bonds (38,39) and for binding of netropsin to poly(dAdT) (31).

While the model of mode I DAPI binding to AT clusters, although speculative, is supported by not conflicting experimental facts, it is much more difficult to outline an equivalent model for the same type of binding to base sequences different from AT clusters. A crucial point is the meaning of the 335 nm CD band. If such band is connected with the formation of two hydrogen bonds, in the absence of at least two AT base pairs, this result can be obtained only in the major groove with the N7 of the purine rings. A lower fitness and a less hydrophobic environment would explain the lower stability of such interaction and the higher electrostatic component. Alternatively, if the DAPI 335 nm CD band is due to a chirality induced in the molecule by the geometrical requirements involved in the minor groove fitting, in this case the lower strength of interaction and the greater sensitivity to salts could be ascribed mainly to the absence of good hydrogen bonds with GC base pairs.

Type II binding seems characterized by a prevalent electrostatic character. The continuous increase in the 370 nm band up to stoichiometric ratios of 0.4-

0.5, and even 0.8 for poly(dGdC) (data not shown) and the n_2 value slightly grater than 0.2 for calf thymus DNA suggest a crowding of DAPI molecules on the surface of DNA chains at saturation. This probably implies not a geometrical defined interaction but a mixing of different possibilities, in which electrostatic bonds are prevalent but not unique. More work must anyway be carried out to better elucidate the main aspects of all the possible bonds that DAPI molecule can form with DNA.

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