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9-AMINOACRIDINE INHIBITS THE B-Z TRANSITION OF POLY(dA-dT)

Pere Garriga, David Garcia-Quintana and Joan Manyosa*

Unitat de Biofísica, Departament de Bioquímica i de Biologia Molecular, Edifici M, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Catalonia, Spain

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9-Aminoacridine is the parent compound of a family of pharmacologically active model substances that bind to DNA through intercalation between base pairs. In the present study we show that 9-aminoacridine inhibits the B-to-Z isomerization of poly(dA-dT) in conditions that otherwise cause it to occur (5 M NaCl and 123 mM $Ni(ClO_4)_2$). Higher concentrations of $Ni(ClO_4)_2$ (155 mM) are able to induce the Z-form due to the disruption of the drug-polynucleotide interaction by the metal ion. Additionally, the dye reverses the Z-form in certain conditions. Thus, the data from this study indicate that 9-aminoacridine binds preferentially to the B-form of poly(dA-dT).

Acridine dyes are classical model substances for studying the binding of small, pharmacologically active molecules to DNA. These kind of molecules have also been widely used as molecular probes for nucleic acids structure (1). Their proposed mode of action at the molecular level is intercalation between the DNA base pairs (2). 9-Aminoacridine has been important in early X-ray studies of its complex with DNA dinucleotides that provided further validation for the intercalation hypothesis (3). Molecular mechanical studies analyzed the interaction between 9-aminoacridine and d(CGCGCGC) duplex and found no stereochemical preference for the neighbor-exclusion principle (4).

On the other hand, the interest in the study of the effect of metal ions on nucleic acids structural transitions has spectacularly increased in the last years as a result of the expected biological significance of this DNA polymorphism (5). It has been a puzzle that AT containing sequences have offered a great resistance to enter the left-handed conformation. This resistance has been associated to the

* To whom correspondence should be addressed.

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lack of the amino exocyclic group in the case of poly(dA-dT) and to its different hydration pattern when compared to GC sequences (6). In spite of this, it has recently been shown that the B-Z transition of poly(dA-dT) can be induced in 5 M NaCl and in the presence of $NiCl_2$ using UV and CD (7), and Raman spectrsocopies (8). Interestingly, in this case both a high salt content and the presence of Ni^{2+} are needed to promote the right- to left-handed isomerization, while with most other synthetic polynucleotides only one of this requirements is needed. We report here the effect of 9-aminoacridine on this conformational isomerization as studied by absorption and fourth derivative spectrophotometries.

Absorption spectroscopy has been widely used to study drug-nucleic acids interactions (9). This technique has been applied to the study of complexes of acridine orange with different natural and synthetic nucleic acids (10). We have previously shown that fourth derivative spectrophotometry is a useful technique in the study of conformational isomerizations of polynucleotides allowing to obtain more information than classical absorption spectrophotometry (11). Moreover, we have been able to associate some of the fourth derivative peaks in the spectra of polynucleotides to stacking interactions (11). In the present study we take advantage of the resolving power of the fourth derivative technique to study the drug-polynucleotide interaction in spite of the overlapping of the absorption spectra of both molecules in the region analyzed (220-320 nm). It has to be taken into account that the intensity of the fourth derivative peaks is proportional to the inverse of the fourth power of the bandwidth at half-height (12). Thus, one of its main advantages is that it favors the narrow components of the absorption spectra while eliminating the contribution from the broader ones.

MATERIALS AND METHODS

Poly(dA-dT) was purchased from P.L. Biochemicals and used without further purification. It was dissolved in 50 mM Tris/HCl 0.1 M NaCl pH 7.5 (buffer solution). Ni^{2+} was added as $Ni(ClO_4)_2 \cdot 6H_2O$, from Fluka. 9-Aminoacridine was from Sigma. The polynucleotide concentrations were determined by using an absorption coefficient $\epsilon_{260} = 6600\ M^{-1}\ cm^{-1}$.

UV absorption spectra were recorded at 20°C with a 320-model Perkin-Elmer spectrophotometer interfaced to a microcomputer in order to allow posterior treatment. Fourth derivative spectra were obtained using the standard least-squares method by Savitzky and Golay (13); the derivative parameters used were interval 3 and number of points 11.

RESULTS AND DISCUSSION

Figure 1 shows the absorption and fourth derivative spectra of poly(dA-dT) in the B- and Z-forms. The B-form spectrum obtained in buffer solution is characterized by main fourth derivative peaks at 262, 272 and 282 nm, the latter being associated with stacking interactions of the adenine bases (11). Regarding the nature of this electronic transition, although components of absorption spectra of polynucleotides are usually assigned to $\pi \rightarrow \pi^*$ electronic transitions we have discussed the possibility of an $n \rightarrow \pi^*$ character for this transition in view of previous physicochemical studies (11). Recently, an $n \rightarrow \pi^*$ electronic transition in the 280-300 nm region has been proposed for the adenine cromophore (14) which reinforces our tentative assignment.

When the polymer is brought to 5 M NaCl no changes in the derivative spectrum are observed. The addition of the required amount of nickel salt results in the appearance of a new fourth derivative peak at 295 nm also observed in the Z-form of poly(dG-dC)[+]. The profile of this conformational change is shown in Figure 2 where a plot of the fourth derivative parameter R ($R = h_0/h_1$, see Figure 1) versus nickel salt concentration leads to an abrupt transition between 102 and 123 mM with a midpoint at approximately 115 mM. An anomalous behavior of the curve above 123 mM may be indicative of supramolecular forms of Z-DNA due to the effect of the metal ion (15). The higher nickel salt concentration needed to obtain the Z-form in comparison with a previous work where the reagent used was $NiCl_2$ (95 mM, reference 7) shows the different role of the counterion upon the structural transition. The importance of specific interactions involving ClO_4^- has been suggested in the case of the B-Z transition of poly(dG-dC) (16).

9-Aminoacridine has a strong absorption in the 220-270 nm region and main peaks at 250 and 261 nm in the fourth derivative spectrum. Nevertheless, no significant fourth derivative peaks can be seen in the 280-320 nm region, so that it remains useful to monitor the conformational change by using the 295 nm

[+] Unpublished results.

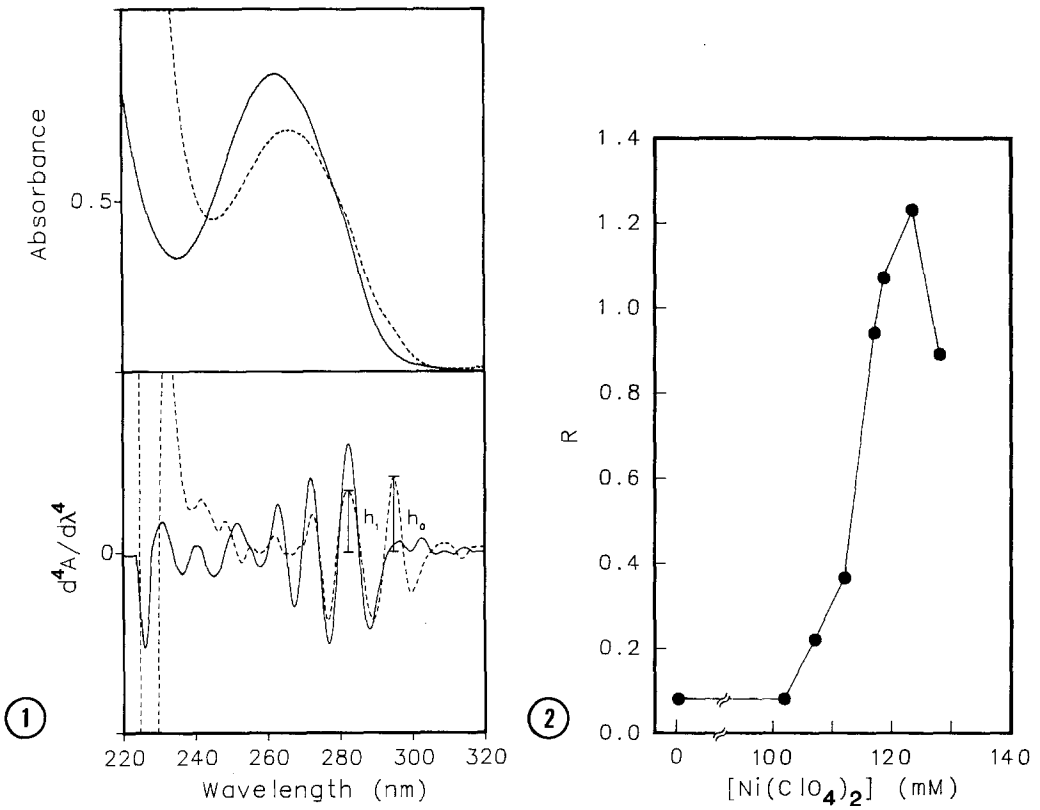

Figure 1. Absorption and fourth derivative spectra of poly(dA-dT) (125 μM) in buffer solution (B-form,—) and in the same buffer plus 5 M NaCl and 123 mM $Ni(ClO_4)_2$ (Z-form,--). h_0 and h_1 are the heights of the 295 and 282 nm fourth derivative peaks, respectively.

Figure 2. Plot of R (see Figure 1 and text) versus $[Ni(ClO_4)_2]$.

fourth derivative peak highly characteristic of the Z-form. Figure 3 shows the expanded spectra of this latter region in order to better observe the appearance of the 295 nm peak. 9-Aminoacridine has no important peaks in this region as stated above (Figure 3a). When aminoacridine is interacting with poly(dA-dT) (1:1, molar ratio of 9-aminoacridine to nucleotide) in 5 M NaCl, the addition of 123 mM nickel salt does not allow the transition to take place (Figure 3b). Further addition of nickel salt to a concentration of 155 mM leads to the left-handed form as monitored by the appearance of the 295 nm peak characteristic of the Z-conformation (Figure 3c). Increase in the drug concentration (4:1, molar ratio of 9-aminoacridine to nucleotide) converts the Z-form back to the B-form as judged by the disappearance of the 295 nm peak (Figure 3d).

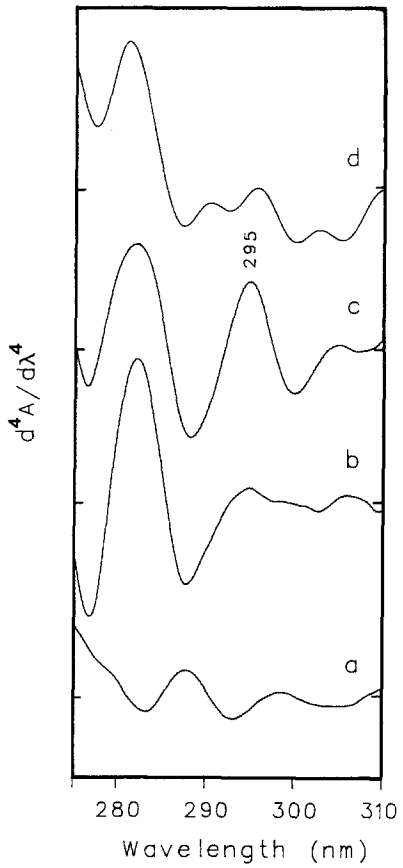

Figure 3. Fourth derivative spectra of (a) 9-aminoacridine; (b) poly(dA-dT) (125 μM) plus 9-aminoacridine (1:1, 9-aminoacridine to nucleotide molar ratio) in 5 M NaCl and 123 mM $Ni(ClO_4)_2$; (c) the same as (b) but with 155 mM $Ni(ClO_4)_2$; (d) the same as (c) but with 9-aminoacridine in excess (4:1, 9-aminoacridine to nucleotide molar ratio).

It is generally accepted that intercalators prefer GC sequences rather than AT sequences in DNA (17). Several studies have reported effects of intercalating drugs upon the B-Z transition but mainly with GC containing polynucleotides (18,19). In particular, proflavin, a molecule closely related to 9-aminoacridine, has been reported to inhibit the thermally induced B-Z transition of poly(dG-m^5dC) (20). Interestingly, in this study we have shown that the interaction between a classical intercalator and a non GC containing polynucleotide, i.e. poly(dA-dT), produces the inhibition of the B-Z isomerization of this polynucleotide.

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