

## Conformation of Single Stranded Poly(dA) and Its Interaction with 4',6-Diamidino-2-phenylindole

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We studied the interaction of 4',6-diamidino-2-phenylindole (DAPI) with single stranded poly(dA) using optical spectroscopic methods, including absorption, circular dichroism (CD), and fluorescence spectroscopy. The temperature-dependent conformation of poly(dA) was also investigated. The conformation of poly(dA) varied with temperature, which is explained by the stacking-restacking process of the adenine bases, resulting from the sugar conformation. The hypochromicity and red-shift in the absorption spectroscopy, the lack of CD change in the drug absorption region, and the fluorescence behavior, especially a great accessibility of the I<sub>1</sub> quencher to the poly(dA)-bound DAPI, suggest that DAPI binds to the outside of poly(dA). The Job plot for the DAPI-poly(dA) mixture demonstrated that a stoichiometry of one DAPI molecule binds to the one phosphate of poly(dA).

### Introduction

The interaction of 4',6-diamidino-2-phenylindole (DAPI) (Figure 1) and DNA has been intensively studied since it was synthesized.<sup>1</sup> The drug was initially synthesized as an analogue of berenil, to be used as a trypanocidal agent. However, the special spectroscopic properties (DAPI forms a fluorescent complex with DNA) made it useful as a DNA probe in electrophoresis,<sup>2,3</sup> in cytofluorometry,<sup>4-11</sup> and for staining chromosomes.<sup>12-14</sup>

Evidence that DAPI binds to DNA preferentially at the minor groove of AT-rich regions has been confirmed by linear dichroism (LD),<sup>15,16</sup> fluorescence,<sup>11,17</sup> NMR, and viscometry.<sup>18-20</sup> Footprinting experiments have demonstrated that DAPI prevents cleavage of DNA at 3-4 continuous AT base pairs, which suggests that DAPI is bound to the AT sequences of 3-4 contiguous base pairs.<sup>21</sup> An X-ray crystal structure of DAPI bound to the synthetic B-DNA oligonucleotide C-G-C-G-A-A-T-T-C-G-C-G shows that DAPI locates in the minor groove of the four AT base pairs at the center of the duplex by forming a hydrogen bond between the indole N-H and the thymine O<sub>2</sub> atoms of the two central base pairs.<sup>22</sup> DAPI can also bind to a GC sequence. In the GC-rich region of DNA, it binds either in the major groove<sup>23</sup> or through intercalation.<sup>19, 20, 24-26</sup>

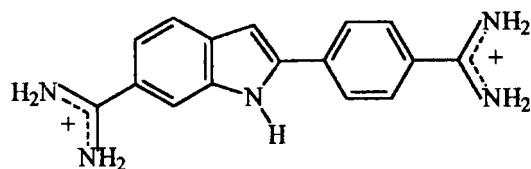
In this work, we studied the interaction of DAPI with poly(dA), a single stranded DNA. Single stranded DNA plays a significant role in biological functions such as the re-

plication and transcription processes. The conformation of a single stranded poly(dA) was identified from CD and NMR results.<sup>27,28</sup> However, relatively few studies have been reported on the interaction of small molecules and single stranded DNA.<sup>29</sup> Such studies may provide a rapid and simple clarification of the role of this interaction in biological functions by uncovering a reagent that gives a unique spectroscopic signal in the presence of single stranded DNA. In this study, we used optical spectroscopic techniques to examine the temperature-dependent conformational changes of poly(dA); we then investigated the interaction between DAPI and single stranded poly(dA).

### Experiment

**Materials.** DAPI was obtained from Sigma and used without further purification. Poly(dA), purchased from Pharmacia, was dissolved in 1 mM Na<sub>2</sub>HPO<sub>4</sub> buffer containing 100 mM Na<sub>2</sub>HPO<sub>4</sub> and 1 mM EDTA at pH 7.0, then dialyzed against 1 mM Na<sub>2</sub>HPO<sub>4</sub> with 10 mM NaClO<sub>4</sub> buffer at pH 7.0 and 4 °C. All measurements were performed in this buffer. The chemical concentrations were determined spectrophotometrically using the molar absorption coefficients: ( $\epsilon_{270\text{ nm}}=8,600\text{ M}^{-1}\text{ cm}^{-1}$  for poly(dA) and  $\epsilon_{442\text{ nm}}=27,000\text{ M}^{-1}\text{ cm}^{-1}$  for DAPI in water.<sup>30</sup> The mixing ratio, R, was defined as the total number of added DAPI molecules per nucleotide base. All measurements were performed at room temperature except for the temperature-dependent conformational changes of poly(dA).

**Absorption and CD spectroscopy.** The absorption spectra were recorded either on a Cary 2300 or a Hewlett Packard 8452A diode array spectrophotometer. The CD spectra were measured on a Jasco J-720 or J-500C spectropolarimeter and the signal was averaged over an appropriate number of scans. The CD spectra of the ligand-poly nucleotide adducts provide information on two levels. The conformation of the polynucleotide itself can be ex-



**Figure 1.** Molecular structure of 4',6-diamidino-2-phenylindole (DAPI)

aminated through the CD of the intrinsic polynucleotide absorption near 260 nm. Also, although the drugs are all achiral molecules, they acquire an induced CD signal when they form complexes with a polynucleotide. The CD is induced by the interaction between the bound ligand and the chirally arranged base transitions and is dependent upon the position and orientation with respect to the polynucleotide bases.

**Fluorescence measurement.** The fluorescence spectra were measured on a Perkin-Elmer LSB50B spectrofluorometer. The emission spectrum of the free DAPI was measured using a 342 nm excitation and the DAPI-poly(dA) complex 360 nm. Slit widths were 6/6 nm for both excitation and emission. If the fluorescence intensity of the quenched fluorophore is negligible compared to when it is unquenched, the fluorescence intensity in the presence ( $F$ ) and absence ( $F_0$ ) of quencher is expressed by the Stern-Volmer equation:<sup>21</sup>

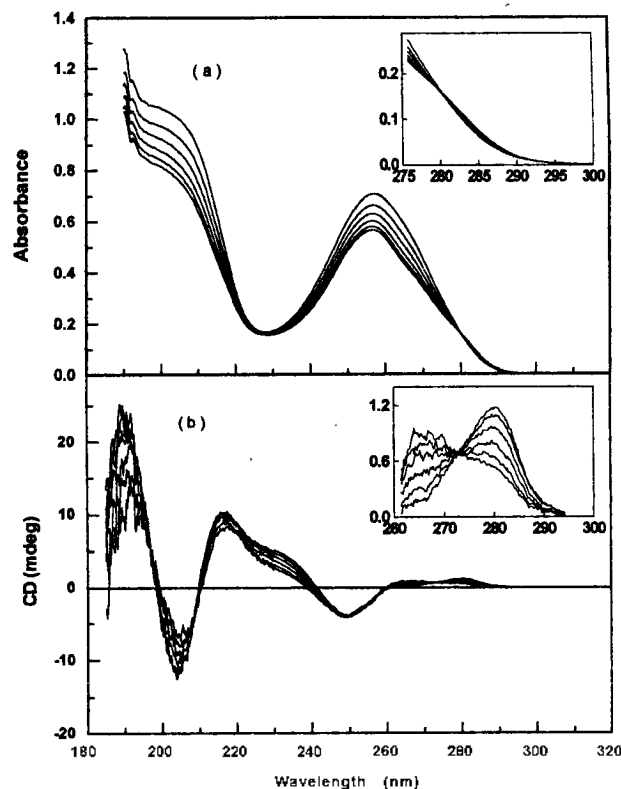
$$F_0/F = 1 + K_s [Q]$$

where  $K_s$  is the Stern-Volmer quenching constant and  $[Q]$  is the quencher concentration. This reflects how accessible the quencher is to the fluorophore and thus, in our system, this method can be used to investigate how DAPI is exposed to or hidden from solvent. We measured the quenching of DAPI fluorescence by the iodine molecule ( $I_2$ ) by adding aliquots of concentrated  $I_2$  solution to a sample containing DAPI or poly(dA)-DAPI complex (up to 70  $\mu$ L to 2900  $\mu$ L of the sample solutions). The measured intensity was corrected for the volume changes. Fresh  $I_2$  solution was prepared prior to each quenching experiment.

**Job's method.** Job's method was applied to determine the binding stoichiometry.<sup>22-24</sup> The concentrations of the two reactants varied, although the sum of their concentrations was kept constant. We detected an absorption against the mole fraction of the DAPI. Such plots may reveal discontinuities at certain mole fractions of DAPI which reflect the stoichiometry of the DAPI-poly(dA) complex. A typical Job's method was used for the binding of DAPI to poly(dA). The sum of the DAPI and poly(dA) concentrations was 30  $\mu$ M and the absorbance was measured at 342 nm.

## Results

**Absorption and CD spectra of poly(dA) at various temperatures.** Figure 2 shows the absorption and CD spectra of poly(dA) at various temperatures. In the absorption spectra of poly(dA) (Figure 2a), the intensity at 258 nm increased and decreased at 285 nm when the temperature was increased. An isosbestic point at 280 nm was clearly observed, suggesting that the transition between the two state occurs where the stacking of the adenine bases of poly(dA) differs. The spectral transitions in the CD spectra are depicted in Figure 2b. The overall shape of the CD spectrum of poly(dA) agrees with those in previous studies. Small but steady changes were found in the long wavelength region (260-290 nm) following temperature changes: a positive band around 280 nm was observed at a low temperature (2  $^{\circ}$ C). As the temperature was increased, a new positive band began to develop around 265 nm. At the highest temperature (75  $^{\circ}$ C), this 265 nm band was dom-

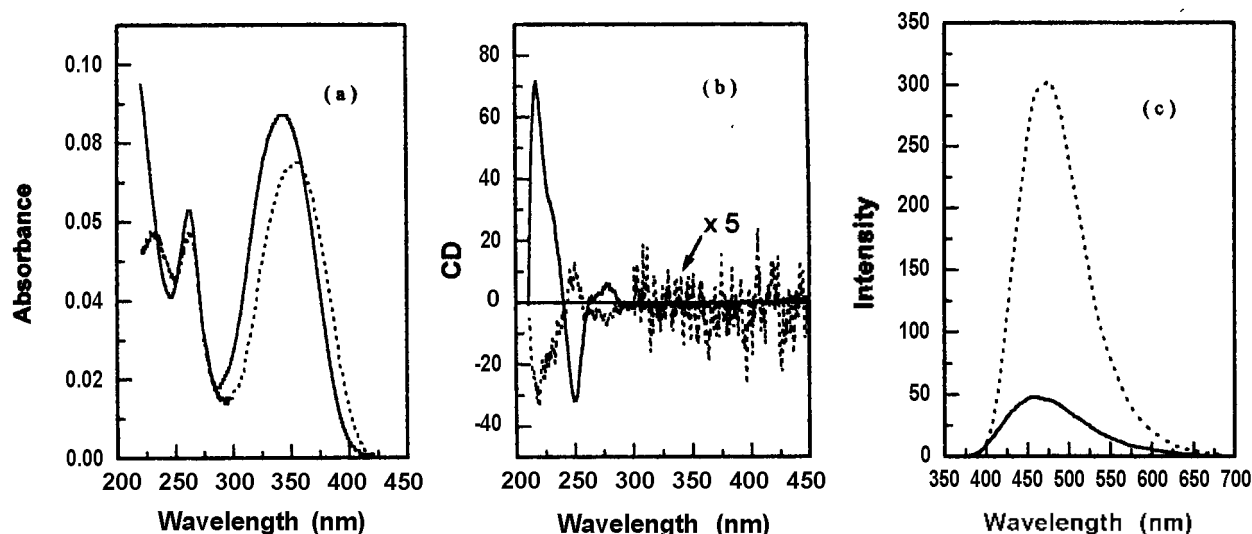


**Figure 2.** (a) Absorption. (b) CD spectra of poly(dA) at various temperatures (2, 15, 30, 45, 60, and 75  $^{\circ}$ C from bottom to top at 258 nm for absorption and 265 nm for CD spectra). Enlargements of the absorption spectra in the 260-300 nm range and CD spectra at 270-300 nm are inserted. Isosbestic points at 280 nm in absorption spectra and at 273 nm for the CD spectra are clearly observed in the inserted figures.

inant and the band around 280 nm became smaller. The negative peak at 249 nm retained considerable intensity; the intensity at 230 nm decreased with temperature. The four isosbestic point at 197, 209, 258, and 273 nm in the CD spectra strongly suggest that this transition occurs between the two different conformations of poly(dA).

### Spectral properties of DAPI bound to poly(dA).

Figure 3a shows a comparison between the absorption spectrum of free DAPI and of DAPI bound to poly(dA) at a mixing ratio ( $R$ ) of 0.05 (which corresponds to one DAPI molecule per 20 adenine bases). The absorption spectrum of the corresponding polynucleotide was subtracted to facilitate the comparison. The DAPI bound to poly(dA) shows a 14% hypochromism in the long wavelength band and a 15 nm red-shift. These spectral changes indicate the interaction between DAPI and poly(dA). The CD spectra of DAPI complexed with poly(dA) are shown in Figure 3b. In contrast to the DAPI-double stranded polynucleotide complexes,<sup>16,23,27-29</sup> we observed no induced CD band in the DAPI absorption region; however, in the DNA absorption region, we observed a decrease in the CD intensity of the positive band around 220 nm and 280 nm and an increase in the negative band around 250 nm (Figure 3b). These changes in the CD spectrum in the DNA absorption region following DAPI binding are similar to the change in the CD spectrum in the presence of high concentrations of  $Na^+$  ions (data not



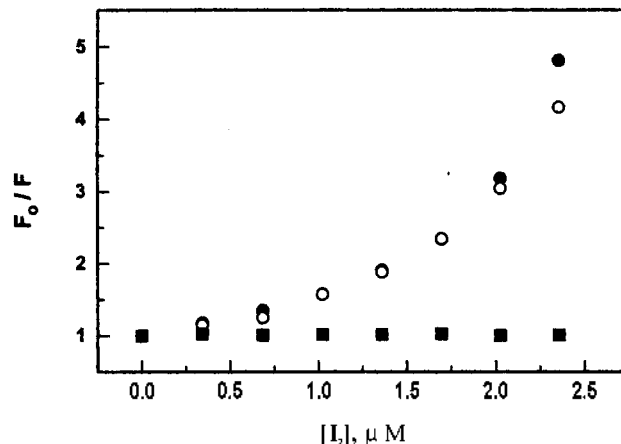
**Figure 3.** (a) Absorption, (b) CD, and (c) fluorescence emission spectra of DAPI in the presence (dotted curve) and absence (solid curve) of poly(dA). The corresponding polynucleotide spectrum was subtracted to facilitate comparison. The DAPI concentration was 5 mM and the pol(dA) concentration was 100 mM. Excitation wavelength was 360 nm for the emission spectrum. Slit width was 6.6 nm.

shown). Thus, these CD changes may reflect a change in the poly(dA) conformation upon DAPI binding.

**Fluorescence measurement.** Both the shape and intensity of the fluorescence emission spectrum of DAPI changed in the presence of poly(dA), indicating that DAPI interacts with poly(dA) (Figure 3c). The fluorescence intensity of the emission spectrum of DAPI increased by a factor of six in the presence of poly(dA). It also red-shifted by 18 nm compared to that of free DAPI. The spectral half-width of free DAPI was 120 nm, whereas that of DAPI bound to poly(dA) was 85 nm. The narrowing in the emission spectrum was similar to that observed in DAPI complexed with various double helical polynucleotides.<sup>35</sup> However, the red-shift in the emission spectrum was observed only in the DAPI-poly(dA) mixture; the DAPI-double helical polynucleotide complexes usually exhibit blue-shift.<sup>36</sup>

In Figure 4, the ratio of the fluorescence intensity without quencher to that with it is shown as a function of the  $I_2$  concentration. Free DAPI was effectively quenched: 2  $\mu$ M DAPI was almost fully quenched in the presence of 3  $\mu$ M  $I_2$ . The DAPI bound to poly(dA) demonstrated an accessibility similar to free DAPI, indicating its accessibility to the quencher even when bound to poly(dA). In contrast, DAPI bound to poly[d(A-T)]<sub>2</sub>, where DAPI is known to bind in the narrow minor groove of the duplex and become well-shielded from the solvent,<sup>37,38</sup> exhibited no quenching at all. Both free DAPI and the DAPI-poly(dA) complex exhibited upward-bending quenching curves, which suggests that both the static and dynamic quenching mechanisms were active.<sup>31</sup> A detailed analysis of the quenching mechanism was omitted from this study because we are concerned only with the relative accessibility of the large quencher to DAPI.

**Job plot.** Changes in the absorbance at 342 nm with respect to the mole fraction of DAPI in the DAPI-poly(dA) mixture (the Job plot) are shown in Figure 5. The break in this plot appears at the 0.5 mole fraction of DAPI. Therefore, a discontinuity at the 0.5 mole fraction of DAPI produces a stoichiometric ratio of DAPI/phosphate (or base)=1 :



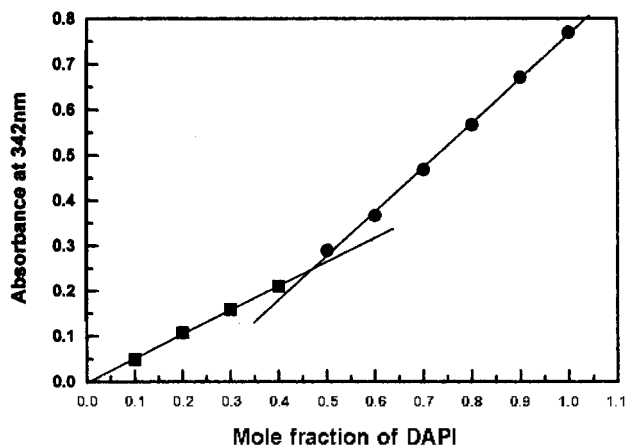
**Figure 4.** Fluorescence quenching of free DAPI (●), DAPI-poly(dA) complex (○), and DAPI-poly[d(A-T)]<sub>2</sub> complex (□) by  $I_2$ . The DAPI concentration was 2 mM, polynucleotide 20 mM. The fluorescence intensity at 470 nm was measured with an excitation at 342 nm in the absence of polynucleotide and at 360 nm with polynucleotide. Slit width was 6.6 nm.

1, which indicates that the DAPI molecule binds to one base or phosphate of poly(dA). The straight line above and below the 0.5 mole fraction of DAPI implies that the complex is homogeneous, consisting of two kinds of DAPI-free and bound.

## Discussion

**Temperature-dependent conformation of poly(dA).** Conformational studies of polynucleotides using optical techniques (ultra-violet absorption, ORD, CD) have been conducted.<sup>37-39,31-36</sup> The vertical helical stack of the base commonly induces appreciable changes in the optical properties. The temperature dependence of these, optical properties can be used to extract the stack-destack equilibrium from nucleotides.

The experimental spectra of poly(dA) at various tem-



**Figure 5.** Job plot for DAPI bound to poly(dA). Absorbance was measured at 342 nm. The sum of the DAPI and poly(dA) concentrations was 30 mM.

peratures are shown in Figure 2. Isosbestic points were found in both the absorption and CD spectra, which suggests temperature-induced conformation changes in poly(dA). These conformational changes may occur through the restacking of the adenine bases in the homo polynucleotide as the temperature increases. One key to understanding the mechanism of these temperature-induced changes is the finding that the two different stacking modes have intrinsically different CD spectra.<sup>27,28</sup> The deoxyribose ring of a nucleotide can have two different conformations, C2'-endo (referred to as S conformation) and C3'-endo (referred to as N conformation). The deoxyribose ring of a nucleotide residue, which is sandwiched between two stacked units, as well as the stacked 5'-terminal residue, appears to adopt an S conformation, whereas the deoxyribose ring in 3'-terminal residue of a stacked sequence retains conformational freedom (S ↔ N). If we extend this to the restacking process in a stacked sequence (--- S(1) · S(2) · S(3) · S(4) ---) with a temperature increase, then upon disruption of the S-S stacking interaction, S(2) and S(3) regain S/N conformational freedom in the deoxyribose ring, because this fragment is no longer sandwiched in a stacked sequence. Consequently, redistribution of the S-S and S-N stacking interactions occurs in the stacked sequence. Thus, random restacking of the S-S stacked units occurs when the temperature is increased. This model explains the CD signal increase at 265 nm. An S-N stack interaction between adenines is evidenced in the CD spectrum by a large positive band at 270-265 nm.

**Conformation of DAPI bound to poly(dA).** The binding of DAPI to poly(dA), single stranded DNA, is manifested by hypochromism, red-shift, and an enhancement in the fluorescence quantum yield. These spectroscopic properties suggest an interaction between the  $\pi$ -systems of neighboring DAPI and those of the adenine bases. However, the DAPI-poly(dA) mixture did not exhibit linear dichroism activity, even though the poly(dA) was well-aligned in the flow orientation system (data not shown). This observation indicates that DAPI may be oriented in a random manner on the surface of a single stranded poly(dA) stem. If DAPI is intercalated between the stacked adenine bases or bound along the phosphate groups, it produces a strong linear di-

chroism signal in the DAPI absorption region.<sup>16,22,27-29</sup> The CD spectrum of the DAPI-poly(dA) complex (Figure 3b) also indicates that DAPI may behave like a Na<sup>+</sup> ion and not directly interact with the adenine bases for two reasons. The CD spectrum of poly(dA) in the presence of DAPI was similar to that in the presence of high concentrations of Na<sup>+</sup>. Also, induced CD in the DAPI absorption region, possibly induced by the interaction between the achiral DAPI molecule and the chirally arranged transition moments of the polynucleotide bases, was not observed. Therefore, the spectral changes we observed in the DAPI molecule in the DAPI-poly(dA) complex may represent an electrostatic interaction between the phosphate group of poly(dA) and the amidino groups of DAPI. From the change in the absorption spectrum in the DAPI absorption region, the equilibrium constant for the DAPI-poly(dA) complex formation was measured at  $1.0 \pm 0.5 \times 10^7 \text{ M}^{-1}$  using Benesi-Hildebrand method. This value of equilibrium constant is much lower than those observed for duplex polynucleotides (e.g., the equilibrium constant for the DAPI-poly[d(A-T)]<sub>2</sub> complex formation is  $5.8 \times 10^7 \text{ M}^{-1}$  and that for poly[d(G-C)]<sub>2</sub> is about  $4.0 \times 10^6 \text{ M}^{-1}$  in similar condition).<sup>29,30</sup> The region for higher equilibrium constant for the duplex polynucleotide may be assigned to the hydrophobic environment of the DAPI binding site as well as to the forming of hydrogen bond between the DAPI molecule and the nucleobases.

The enhancement and reduction in fluorescence intensity and the narrowing of the emission spectra of DAPI upon binding to polynucleotides are different outcomes of two forms of protonation in the DAPI molecule.<sup>4</sup> The strong increase in the fluorescence intensity and considerable narrowing of the emission spectra when DAPI binds to poly(dA) (Figure 3c) can be explained as a reduction of the intramolecular proton transfer process by shielding the amidino group of the DAPI molecule. Together with the linear dichroism results, the fluorescence enhancement suggests that one of the DAPI amidino groups may interact with a phosphate group of poly(dA). The fluorescence quenching technique was utilized to study the solvent's accessibility to a fluorophore. A fluorescent probe which is protected from the solvent will not be quenched by an external quencher. Therefore, the fluorescence quenching results indicates that the quenchers are accessible to fluorophore. When the dye DAPI was free, it was effectively quenched by I<sub>2</sub> (Figure 4). In contrast DAPI bound in the minor groove of poly[d(A-T)]<sub>2</sub> exhibited no quenching at all. Surprisingly, the quenching of DAPI bound to poly(dA) was as effective as that of free DAPI, indicating that DAPI is situated on the surface of the polynucleotide.

Poly(dA) is highly-charged polyelectrolyte whose anionic phosphate groups strongly affect their interactions. DAPI has two cations in one molecule. When the drugs are charged, they repel each other electrostatically. If, however, the cations stack along the anionic DNA sugar-phosphate chain, the charge repulsion is decreased; this type of binding leads to a nonspecific outside stacking of planar cations along the helix. DAPI is bound to the phosphate group of poly(dA) by electrostatic attraction. There are two modes of outside binding-DAPI may be bound parallel to the phosphate group, with two positively charged amidino groups held by

the negatively charged phosphate group, or one cation of DAPI may bind to one phosphate of poly(dA). In Figure 5, the Job plot shows a discontinuity at the 0.5 mole fraction of DAPI, suggesting the latter mode. Two other binding modes for DAPI to polynucleotide are known. In the A-T rich sequence, DAPI binds in the minor groove, covering 4-5 continuous base pairs.<sup>14-22</sup> In this binding mode, the first breaking point at the mole fraction of 0.1-0.125 in Job plot should be observed because DAPI would be saturated when its mole fraction reaches 0.2-0.25 ([DAPI]/[nucleotide base pairs]). However, when DAPI is associated in the G-C rich polynucleotide, it exhibits partial intercalation from the major groove.<sup>19-26</sup> A breaking point at 0.25 mole fraction would be expected in this case because of the nearest neighbor exclusion model for a drug-DNA intercalation.

It is note worthy that the spectroscopic properties of the poly(dA)-bound DAPI, which are presented in the Figures 3 and 5, were invariant in the temperature range used in this work (data not shown). This observation indicates that the sugar puckering (and, therefore, base stacking) does not affect the binding mode of DAPI that is associated with the phosphate group of poly(dA).

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### References

- Dann, O.; Bergen, G.; Demant, E.; Volz, G. *Liebigs Ann. Chem.* **1971**, *749*, 68.
- Kapuscinski, J.; Yanagi, K. *Nucleic Acids Res.* **1979**, *6*, 3519.
- Schwarz, D. C.; Koval, M. *Nature* **1989**, *338*, 520.
- Brown, R. N.; Hitchcock, P. F. *Dev. Brain Res.* **1989**, *50*, 123.
- Chi, H.; Ishibashi, Y.; Shima, A.; Mihara, I.; Otsuka, F. *J. Invest. Dermatol.* **1990**, *95*, 154.
- Hajduk, S. L. *Science* **1976**, *191*, 858.
- Lee, G. M.; Thornthwaite, J. T.; Rasch, F. M. *Anal. Biochem.* **1984**, *137*, 221.
- Russel, W. C.; Newman, C.; Williamson, D. H. *Nature* **1975**, *253*, 461.
- Takata, K.; Hirano, H. *Acta Histochem. Cytochem.* **1990**, *23*, 679.
- Tijssen, J. P. F.; Beckes, H. W.; van Stevenick, J. *Biochim. Biophys. Acta* **1982**, *721*, 394.
- Williamson, D. H.; Fennell, D. J. *Methods Enzymol.* **1979**, *56*, 728.
- Bella, J. L.; Gosalvez, J. *J. Biotech. Histochem.* **1991**, *1*, 44.
- Bernheim, A.; Miglierina, R. *Hum. Genet.* **1989**, *83*, 189.
- Lin, M. S.; Comings, D. E.; Alfi, O. S. *Chromosoma* **1977**, *60*, 15.
- Kubista, M.; Akerman, B.; Norden, B. *Biochemistry* **1987**, *26*, 4545.
- Norden, B.; Eriksson, S.; Kim, S. K.; Kubista, M.; Lyng, R.; Akerman, B. *In the Jerusalem Symposia on the Quantum Chemistry and Biochemistry*; Pullman, B., Jortner, J., Eds.; Kluwer Academic Publishers: Dordrecht, 1990; Vol. 23, p 23.
- Hård, T.; Fan, P.; Kearns, D. R. *Photochem. Photobiol.* **1990**, *51*, 77.
- Manzini, G.; Xodo, L.; Barcelona, M. L.; Quadrifoglio, F. *Nucleic Acids Res.* **1985**, *13*, 8955.
- Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jone, R. L.; Fox, K.; Wydra, R. L.; Streckowski, L. *Biochemistry* **1990**, *29*, 8452.
- Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Streckowski, L.; Boykin, D. W. *J. Am. Chem. Soc.* **1989**, *111*, 5008.
- Portugal, J.; Waring, M. J. *Biochim. Biophys. Acta* **1988**, *949*, 158.
- Larsen, T. A.; Goodsell, D. S.; Cascio, D.; Grzeskowiak, K.; Dickerson, R. E. *J. Biomol. Struct. Dyn.* **1989**, *7*, 477.
- Kim, S. K.; Eriksson, S.; Kubista, M.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 3441.
- Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Wydra, R. L.; Johns, R. L.; Boykin, D.; Streckowski, L. *Anti-Cancer Drug Design* **1990**, *5*, 31.
- Tanious, F. A.; Sychala, J.; Kumar, A.; Greene, K.; Boykin, D. W.; Wilson, W. D. *J. Biomol. Str. Dyn.* **1994**, *11*, 1063.
- Trotta, E.; D'Ambrosio, E.; Ravagnan, G.; Paci, M. *Nucleic Acids Res.* **1995**, *23*, 1333.
- Olsthoorn, C. S. M.; Bostelaar, L. J.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1980**, *112*, 95.
- Olsthoorn, C. S. M.; Bostelaar, L. J.; Derooij, J. F. M.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1981**, *115*, 309.
- Pasternack, R. F.; Brigandi, R. A.; Abrams, M. J.; Williams, A. D.; Gibbs, E. J. *Inorg. Chem.* **1990**, *29*, 4483.
- Kapuscinski, J.; Skozylas, B. *Nucleic Acids Res.* **1978**, *5*, 3775.
- Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983; p 257.
- Job, P. *Ann. Chim. (Paris)* **1928**, *9*, 113.
- Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; Freeman, W. H.; New York, 1980; Vol. III, p 1135.
- Huang, C. Y. *Methods Enzymol.* **1982**, *87*, 509.
- Powell, J. T.; Richards, E. G.; Gratzner, W. B. *Biopolymers* **1972**, *11*, 235.
- Lowe, M. J.; Schellman, J. A. *J. Mol. Biol.* **1972**, *65*, 91.
- Eriksson, S.; Kim, S. K.; Kubista, M.; Norden, B. *Biochemistry* **1993**, *32*, 2987.
- Sehlstedt, U.; Kim, S. K.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 12258.
- Kim, S. K.; Eriksson, S.; Norden, B. *Biopolymers* **1993**, *33*, 1677.
- Loontjens, F. G.; McLaughlin, L. W.; Diekmann, S.; Clegg, R. M. *Biochemistry* **1991**, *30*, 182.
- Szabo, A. G.; Krajcarski, D. T.; Cavatorta, P.; Masotti, L.; Barcelona, M. L. *Photochem. Photobiol.* **1986**, *44*, 143.