TOPOGRAPHY OF NUCLEIC ACID HELICES IN SOLUTIONS. XXIV

INTERCALATION SPECIFICITIES OF DNA AND THEIR POSSIBLE ROLE IN THE RECOGNITION PROCESS.

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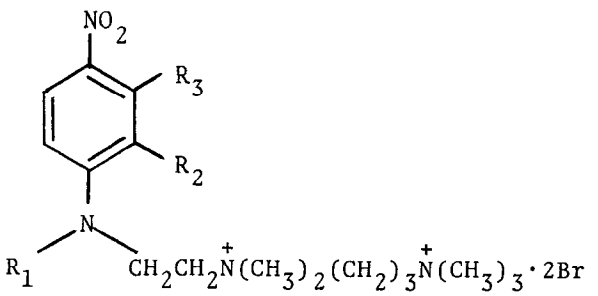
Summary

Through the use of reporter molecules, I, it is shown that more than one type of intercalating site must exist in DNA. Although this finding is completely consistent with the DNA structure it has not yet been demonstrated. The significance of the presence of different intercalating sites in DNA may be of extreme importance in the recognition of nucleic acid-protein systems.

It is well known that planar molecules such as acridine orange, ethidium bromide, proflavine, and actinomycin may intercalate between base pairs in DNA(1-4). This phenomenon leads to an increase in the length of the helix and is usually accompanied by an increase in the viscosity of the solution. Previous work from this laboratory has shown that reporter molecules, I, bind to DNA via electrostatic and hydrophobic



I

1, $R_1=R_3=H$; $R_2=NO_2$
2, $R_1=R_3=H$; $R_2=CN$
3, $R_1=R_3=H$; $R_2=CH_3$
4, $R_1=R_3=H$; $R_2=CF_3$

type forces (5-10). In particular, considerable evidence has been presented that the 4-nitroaniline ring of I may intercalate between

base-pairs in DNA. This paper reports further studies of the interaction of reporter molecules with DNA and the evidence for the presence of more than one type of intercalating site in nucleic acid.

As a consequence of the right handed Watson-Crick-Wilkins double helix of DNA with the A-T and G-C base pairs, there are 10 distinctly different intercalating sites. These are illustrated schematically in figure 1 and shown from top view in figure 2 for the intercalating

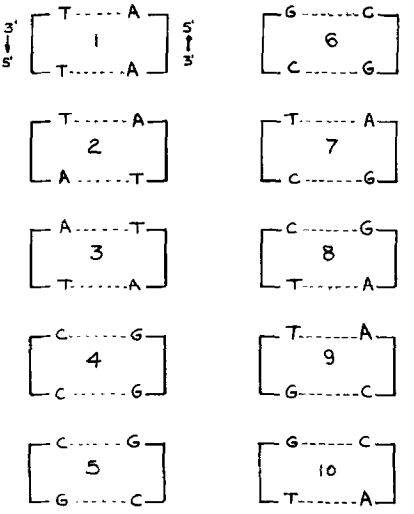


Figure 1. Schematic drawings of the ten possible intercalating sites in DNA.

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sites designated as 1, 2, and 3, i.e., 3',5'—TpT—3',5'-dApdA, 3',5'-TpdA—3',5-TpdA, and 3',5'-dApT—3',5'-dApT, respectively. It is clear from inspection of figure 2 that the intercalation sites 1, 2, and 3 are not equivalent and present different environments for the intercalating molecule. Thus, it is theoretically possible that the reporter molecules I would have different affinities for each of the ten possible sites shown in figure 1.

In order to test the above hypothesis we have examined the interactions of various reporter molecules, i.e., 1-4,with salmon

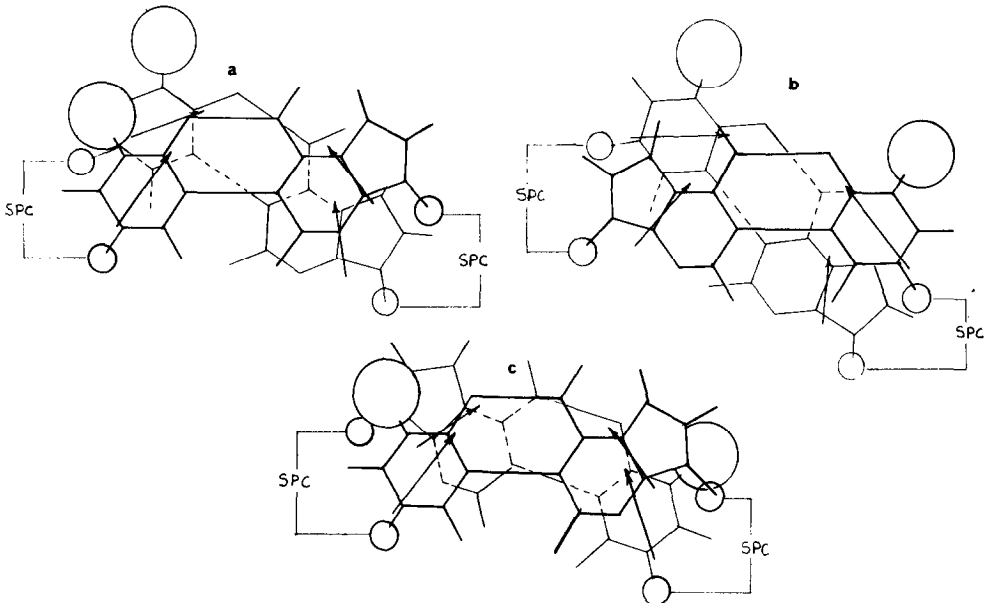

Figure 2. The top view illustration of (a) TpT-dApdA; (b) TpdA-TpdA; and (c) dApT-dApT intercalating sites. The van DerWaal radii of the thymine methyl group is illustrated in each case together with the directions of the transition moments of the 260 nm transition of the bases and the sugar phosphate chain (SPC).

Table 1. Binding Constants and Maximum Number of Strong Binding Sites Per Base-Pair ($1/2\alpha$) in DNA.[1]

	K	$1/2\alpha$
1	$5.62 \pm 0.28 \times 10^5$	$4.59 \pm .13$
2	7.37×10^5	5.44
3	5.04×10^5	5.12
4	2.88×10^5	6.90

[1]The data are obtained by spectral titration studies according to the modified Scatchard type method described by Hyman and Davidson (11). The DNA concentration was held constant at 5.9×10^{-4} and increments of reporter molecules were added. The hypochromic effect of the 4-nitroaniline transition due to the formation of the DNA-reporter complex was recorded at 390 nm. The measurements were carried out at pH 6.2 in 0.01 M 2-(N-Morpholino)ethanesulfonate buffer (MES) and 0.005 M Na^+ at 37.0°C.

sperm DNA utilizing binding, absorption, and circular dichroism studies. The results of the binding studies which were carried out according to the methods described by Hyman and Davidson (11) are shown in Table 1. An interesting observation can be made, i.e., the 2-nitro, 2-cyano and 2-methyl substituted 4-nitroaniline reporter molecules intercalate to a greater extent than the corresponding 2-trifluoromethyl- derivative since the former reporter molecules give a maximum number of binding sites of one per five base-pairs and the latter yield only one reporter per seven base-pairs. The data are indicative that the reporter molecule 4 is more selective for specific intercalating sites of DNA (presumably due to steric and/or electronic factors) than the reporters 1-3.

Induced circular dichroism of the 4-nitroaniline transition of the DNA-reporter complex is also in agreement with the above interpretation. The results of these studies carried out at high DNA to reporter concentration (P/R=70) and at low DNA to reporter concentrations (P/R=5.7 are shown in figure 3. It is instructive to examine first the results shown in figure 3a utilizing the reporter molecule 1, i.e., 2-nitro substituted 4-nitroaniline system. At high P/R ratio [2 x 10^{-3} M in P/1 and 3 x 10^{-5} M of R] a limiting value of the molar ellipiticity, $[\theta]_M$, equal to -8700 is obtained, i.e., further excess of DNA gives the same value of [θ]. Under these conditions the reporter molecule 1 is fully intercalated and presumably could discriminate between the 10 possible sites since the base-pair to reporter ratio is 35/1. At low P/R ratio [3.2 x 10^{-4} M P/1 and 5.55 x 10^{-5} M R] a lower value of [θ] is obtained. If it is assumed that all intercalating sites ellicit a negative CD band of the same magnitude in the 4-nitroaniline transition of 1 then it is possible to calculate the concentration of the intercalat reporter molecule at the low P/R ratio, i.e., $[\theta]/[\theta]_M \times [3 \times 10^{-5}]$ which is equal to 1.75 x 10^{-5} M. If this is the case, then under these

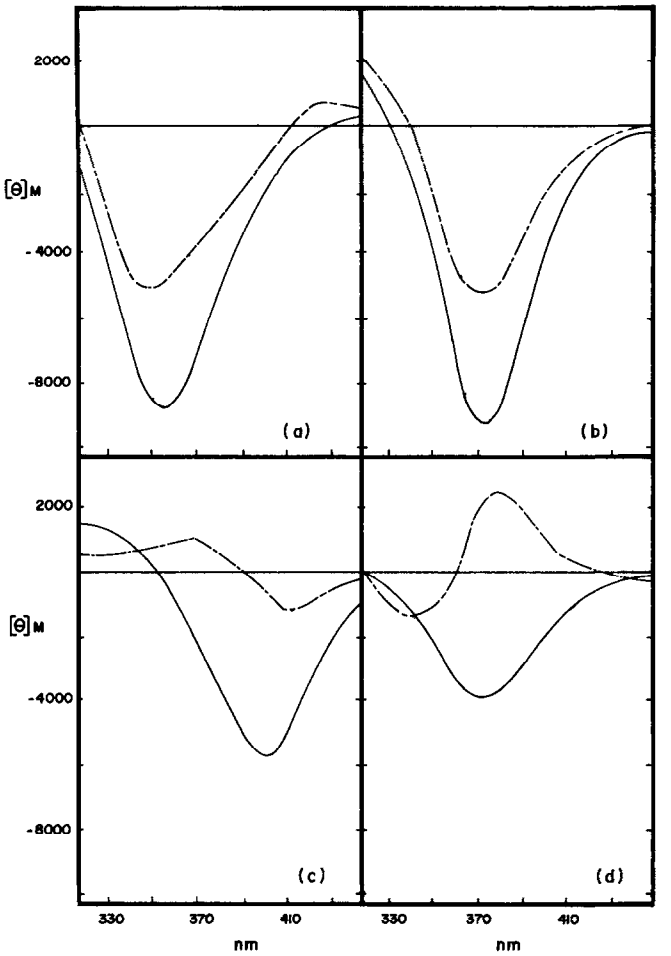

Figure 3. The induced circular dichroism spectra of the DNA-bound reporter molecules 1-4 at high (—) and low (—·—) P/R ratio, i.e. at 70/1.0 and 5.7/1.0: (a) DNA-reporter 1; (b) DNA-reporter 2; (c) DNA-reporter 3; (d) DNA-reporter 4.

conditions there are one reporter molecule per 9 base pairs in DNA. However, under the conditions of the low P/R experiment all possible intercalating sites should be filled (which can be shown by calculation from the measured binding constant given in Table 1). Therefore, there must be at least 1 reporter molecule per 5 base-pairs under the above conditions. Hence, the assumption that all intercalating sites ellicit a negative CD band of the same magnitude at 360 nm is an erroneous one. In line with this is the finding by Gabbay and Gaffney

(12) that poly dAT and poly dG-polydC induce a molar ellipticity, $[\theta]^{360}$ of -9200, and -720, respectively. Similar arguments could also be applied to the induced CD spectra of the DNA-reporter 2 complex shown in figure 3b.

Figure 3c and d dramatically demonstrate the different induced CD of the 4-nitroaniline transition of the DNA-reporter 3 and 4 complexes at high and low P/R ratio. The results seem to strongly suggest that each of the possible intercalating sites may have differing affinities for the reporter molecules and could ellicit significantly different CD signals.

The significance of the presence of differing intercalating sites in DNA may prove to be of great importance in the recognition process involved in protein-nucleic acid interactions. Simple electrostatic and hydrogen bonding type interactions with DNA cannot by themselves be very selective since there are many such sites on the helix. However, in combination with selective binding, i.e., via intercalation of the aromatic residues of the protein, DNA sequence may be recognized. Thus, the "bookmark" hypothesis proposed by Brown (13) is plausible especially if the "bookmarks," i.e., the aromatic residues of the protein, could also recognize the "pages of the book," i.e., the intercalating sites. Work along this area is in progress.

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