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Topography of Nucleic Acid Helices in Solutions

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Topography of Nucleic Acid Helices in Solutions

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SUMMARY

A number of reporter molecules of the structure $R-(CH_2)_nN^+(CH_3)_2(CH_2)_mN^+(CH_3)_3 \cdot 2Br^-$, where R is a chromophore absorbing in the 300-500 mμ region, have been synthesized. The effect of DNA and RNA on the absorption, induced circular dichroism, and proton magnetic resonance spectra is reported. A red shift and a hypochromic effect on the absorption spectra of the bound chromophore is observed. In all cases where R is an "unsymmetrical" 4-nitroaniline chromophore, it is found that DNA and RNA induce an opposite CD in the absorption band of the bound reporter molecules. These results together with PMR studies are interpreted in terms of the structure of the nucleic acid systems in solutions.

INTRODUCTION

We have recently reported on the use of reporter molecules specifically designed to interact strongly with polyanions, e.g., nucleic acids [1-4]. The reporter labeled polycations, I, $R-(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$, where R is a chromophore absorbing in the 300-500 mμ region, have been

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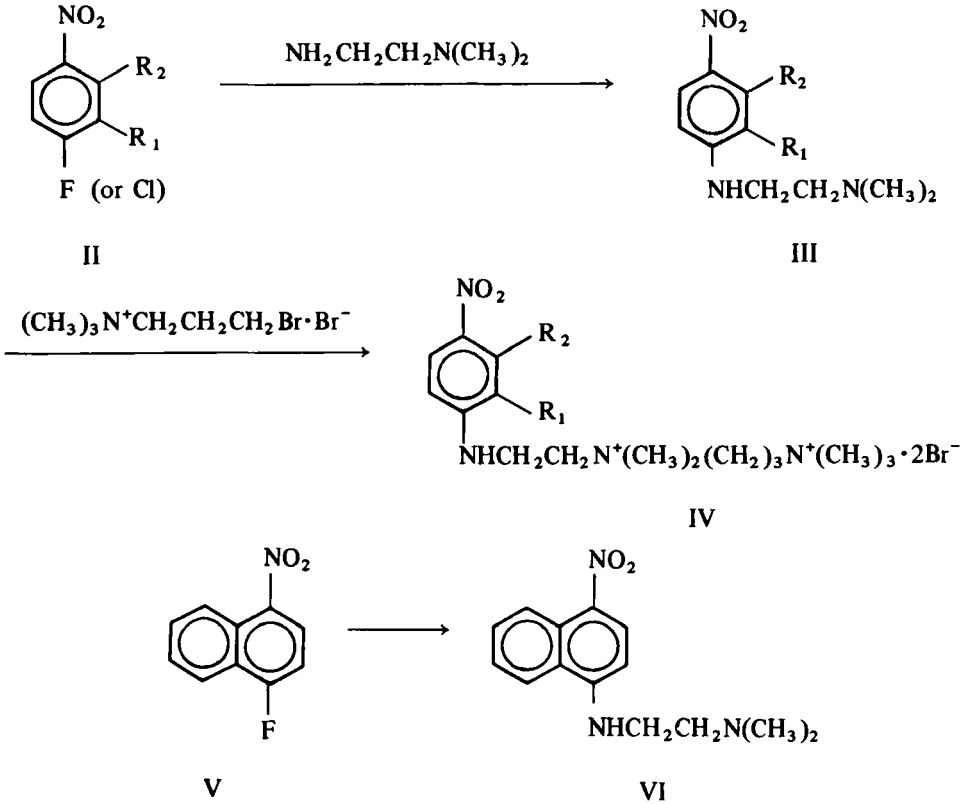
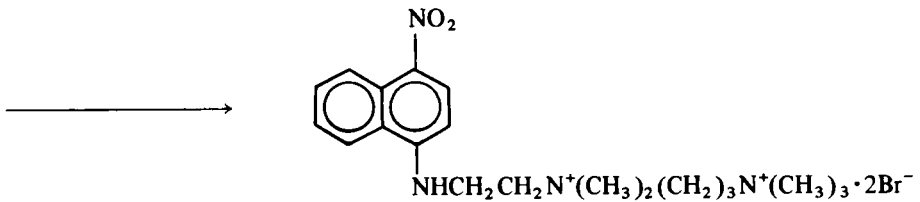




VII

Compound	Derivitive of	R_1	R_2
7	IV	NO_2	H
9	IV	H	H
21	IV	CH_3	H
23	IV	H	CH_3
25	VII	–	–

Figure 1

Table 1. Summary of the Absorption Spectra of Reporter Molecules in 95% Ethanol and in Water[a,b]

	95% Ethanol				H_2O/buffer[b]			
Reporter	$\lambda_{m\mu}^{max}$	ϵ^{max}	$\lambda_{m\mu}^{max}$	ϵ^{max}	$\lambda_{m\mu}^{max}$	ϵ^{max}	$\lambda_{m\mu}^{max}$	ϵ^{max}
7	338	16,600	–	–	350	16,600	–	–
9	366	16,500	–	–	383	16,260	–	–
21	371	14,480	–	–	387	14,440	–	–
23	366	13,600	–	–	381	13,600	–	–
25	414	15,360	333	2800	428	14,400	322	2500

[a]All spectra were taken in 10 mm cells using a Cary 14 Spectrophotometer at 25.0 + 0.2°C.
[b]In 0.01 M sodium phosphate buffer (0.01 M in Na^+), pH 6.50.

demonstrated to differentiate between 1) single stranded polypyrimidines and polypurines [1], 2) single and double stranded ribose containing polynucleotides [1], and 3) double stranded RNA and DNA helices [2-4]. In this paper we report a summary of the absorption and induced circular dichroism of the bound reporter molecules to DNA and RNA helices. The results of 1) relative binding studies of the reporter molecules to RNA and DNA, 2) base composition of the DNA on the induced circular dichroism of the bound reporter, and 3) preliminary studies of the proton magnetic resonance spectra of the bound reporter molecule are also reported.

RESULTS AND DISCUSSION

The synthetic scheme for the preparation of the reporter molecules is straightforward and is outlined in Fig. 1. An activated haloaromatic (II) is treated with N,N-dimethylethylene diamine to afford the corresponding tertiary amines, III. The latter upon treatment with N,N,N-trimethyl-N-3-bromopropyl ammonium bromide yields the corresponding bis-quaternary ammonium salts, IV. These particular compounds have been synthesized since it is well known that polyammonium salts interact strongly with nucleic acids [5-10]. In fact, considerable evidence have been presented which indicate that bis-ammonium salts of the type I bind to adjacent phosphate anions on the same chain [10, 11]. This conclusion has also been corroborated by our recent studies using reporter molecule 7. For example, the single stranded homopolymers, i.e., polyriboadenylic (rA), polyriboinosinic (rI), polyribocytidylic (rC), and polyribouridylic (rU) acids interact strongly with 7 as indicated by the hypochromic effect on the absorption band of the bound reporter molecule [1]. Removal of the positive charges from the side chain of the reporter molecule, e.g., $4\text{-}NO_2C_6H_5NHCH_2CH_2OH$, abolishes the affinity to the polynucleotides as indicated by the fact that the absorption spectra is unchanged in the presence and absence of the homopolymers [3, 4].

The absorption spectra of the free and bound reporter molecules are shown in Tables 1 and 2, respectively. Several interesting points may be made. 1) The transition in the 350 mμ region is probably a $^*\pi \leftarrow \pi$ type since it has an extinction coefficient, ϵ^{max}, of 15,000 characteristic of an allowed transition, and a red shift in the maximum is observed in going from 95% ethanol to 0.01 M sodium phosphate buffer (Table 1). 2) Interaction of the reporter molecule with the nucleic acids leads to a red shift and a hypochromism in the absorption band of the reporter. The extent

Table 2. The Effect of Ribo- and Deoxyribonucleic Acids on the Absorption Spectra of Reporter Molecules[a-c]

	Calf thymus DNA			Yeast RNA		
Reporter	$\lambda_{m\mu}^{max}$	ϵ^{max}	%H[d]	$\lambda_{m\mu}^{max}$	ϵ^{max}	%H
7	355	10,000	66	354	12,300	35
9	393	12,200	33	393	13,080	24
21	399	10,260	41	397	11,430	26
23	392	11,200	22	391	12,400	10
25	442	12,400	16	440	12,700	13

[a]All spectra were taken in 10 mm cells using a Cary 14 Spectrophotometer at 25.0 + 0.2°C.

[b]In 0.01 M sodium phosphate buffer (0.01 M in Na^+), pH 6.50.

[c]P/R ratio (polynucleotide phosphorus/reporter) used in these studies is approximately 74-80. Under these conditions the reporter is fully bound.

[d]% Hypochromicity. $(\%H) = [\epsilon_{H_2O}^{max}/\epsilon_{p}^{max} - 1]\ 100$, where $\epsilon_{H_2O}^{max}$ and ϵ_{p}^{max} are the extinction coefficients in the absence and presence of the polynucleotides.

of hypochromism appears to depend on the nucleic acid system, e.g., the per cent hypochromicity in the absorption band is greater for the DNA as compared with the RNA complex. This finding is significant and consistent with the idea that the ring chromophore is bound more closely to DNA than to RNA. The hypochromic effect may be explained in terms of coupling of the transition moments of the chromophore with the transition moments of the bases in the nucleic acid helix [3, 12].

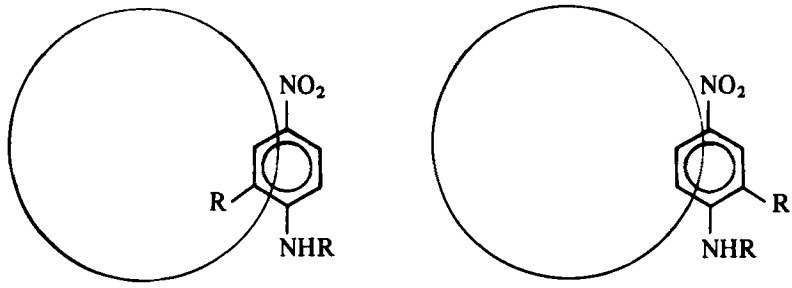
An induced circular dichroism (CD) in the absorption band of the bound reporter molecule is also observed. The results are summarized in Table 3. The following observations may be made. 1) Ribose containing double stranded nucleic acids, e.g., yeast RNA (Table 3), rI-rC[1], and rA-rU[1] induce a positive CD in the 4-nitroaniline transition of I irrespective of the nature of substituents on the ring of the latter. Calf thymus DNA (Table 3) as well as other DNA systems [1-4] exhibit a positive induced CD in the

Table 3. Effect of Various Nucleic Acids on the Induced CD of the Bound Reporter Molecules[a,b]

Reporter	Chromophore	Calf thymus DNA				Yeast RNA	
		$\lambda^{t}_{m\mu}$	$[\theta]^{t} \times 10^{-3}$	$\lambda^{p}_{m\mu}$	$[\theta]^{p} \times 10^{-3}$	$\lambda^{p}_{m\mu}$	$[\theta]^{p} \times 10^{-3}$
7	2,4-Dinitroaniline	360	-9.40	–	–	357	7.80
9	4-Nitroaniline	–	–	388	2.51	395	4.06
21	2-CH_3-4-Nitroaniline	412	-4.08	–	–	393	5.04
23	3-CH_3-4-Nitroaniline	402	-3.0	–	–	391	6.35
25	4-Nitronaphthylamine	450	-7.20	323	6.00	440	6.48

[a]CD curves were measured in a Cary 60 Recording Spectropolarimeter equipped with a Model 6001 CD accessory at 26.0 ± 0.4°C in 10 mm cells. The solution contained 1.67×10^{-4} M of the reporter molecule in sodium phosphate buffer (0.01 M in sodium) pH 6.40-6.50.

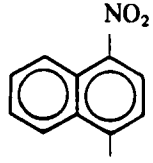
[b]The P/R ratio used in these studies ranged from 22 to 24.



(a) in geometry (b) out geometry

Fig. 2. Top view of nucleic acid helix-reporter complex.

"symmetrical" unsubstituted 4-nitroaniline reporter molecule, i.e., 9, and a negative CD for the bound unsymmetrical "substituted" 4-nitroaniline reporter molecules (Table 3). 3) Interaction of RNA and DNA with Reporter 25 leads to a positive and a negative CD for the 450 mμ 4-nitroaniline transition, respectively. However, only the DNA complex shows optical activity associated with naphthylamine transition of 25 at 340 mμ. These results are interpreted in terms of an in geometry of the substituent (in this case a fused 2,3-phenyl ring) for the DNA complex (Fig. 2).



$NH(CH_2)_2N^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$

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It is likely that the reporter molecules bind to the minor groove. Examination of molecular models of the RNA and DNA reporter complex reveals that the presence of the 2′-hydroxyl group in the minor groove of the former prevents the formation of the in geometry of the substituent at the 2 and 3 positions of the chromophore by steric hindrance, assuming

Table 4. Relative Binding Affinities of the Reporter Molecule 7 to Salmon Sperm DNA and rI-rC in 0.01 M Sodium Phosphate Buffer (0.01 M in Na^+), pH 6.50 at 25.0°C [a]

%DNA	% rI-rC	$K_{DNA}/K_{rI\text{-}rC}$
46.0	54.0	8.4
32.5	67.5	5.1
19.0	81.0	4.0

[a]Relative binding affinities were calculated from the effect of various mixtures of DNA and RNA on the induced CD of Reporter 7, i.e., $[\theta]$ at 355 mμ. CD curves were measured after a 10 min incubation in a Cary 60 Recording Spectropolarimeter equipped with a Model 6001 CD accessory. A thermostatable CD cell compartment was used and the temperature was maintained at 25.0 ± 0.2°C using a Tamson T-9 constant temperature circulator.

that the binding is to the minor groove. In DNA the steric interference to the in geometry is absent. The fact that RNA and DNA may differ in their secondary structure, i.e., A and B form, do not alter the argument.

Competitive binding studies between DNA and RNA for the reporter molecule were carried out using CD techniques for reporters which exhibit opposite induced CD on binding to these systems. The results of this study shown in Table 4 indicate that DNA binds the reporter molecule I more strongly than the ribose containing double-stranded polynucleotides, e.g., rI-rC, by a factor of 5-10 folds.

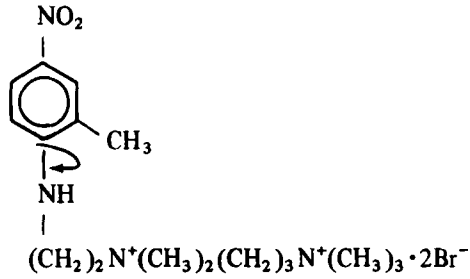
The competitive binding results that indicate a stronger binding to DNA as compared with RNA systems is consistent with the in and out geometries discussed above. Moreover, preliminary evidence utilizing an altogether different spectroscopic approach, i.e., PMR technique, has shown that RNA and DNA complex of 21 causes a 20 cps upfield shift with broadening and total disappearance of ring-CH_3 signal, respectively. The results suggest a tighter binding of 21 to DNA with relatively low rates of rotation of the aromatic ring as compared to the RNA complex [13].

Finally, increasing the G-C content of DNA lowers the affinity of the reporter molecule to DNA and decreases the amount of the in geometry. For example, the effect of base composition on the induced $\overline{CD}$ of the

Table 5. The Effect of Base Composition on the Induced CD of the DNA-Reporter 7 Complex [a]

DNA System	% A-T	$[\theta]^t \times 10^{-3}$	P/R
Poly dAT [b]	100	-9.20	6
Salmon Sperm	58.8	-9.60	24
Calf Thymus	56.0	-9.40	22
M. Lysodeikticus	26.0	-3.86	24
Poly dG-poly dC	00.0	-0.72	6

[a]CD curves were measured in a Cary 60 Recording Spectropolarimeter equipped with a Model 6001 CD accessory at 26.0 ± 0.4°C in 10 mm cells. The solution contained 1.67×10^{-4} M of the reporter molecule in sodium phosphate buffer (0.01 M in sodium) pH 6.40-6.50.
[b]Alternating copolymer.



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DNA-Reporter I complex is shown in Table 5. It is clear that selective interaction of the reporter molecule with specific base-pairs is being observed. As the A-T content decreases the negatively induced CD which is characteristic of the in geometry decreases. This result is in line with the idea that the reporter molecule in the DNA complex lies in the minor groove since the additional H-bond in the G-C base-pair (as compared with A-T) occupies space in the minor groove [3].

The validity of the conclusions reached so far in this work is a matter which requires further investigation. This is in progress.

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