TOPOGRAPHY OF NUCLEIC ACID HELICES IN SOLUTIONS. XIV.

A PMR STUDY OF THE INTERACTIONS OF REPORTER MOLECULES WITH POLYNUCLEOTIDES.*

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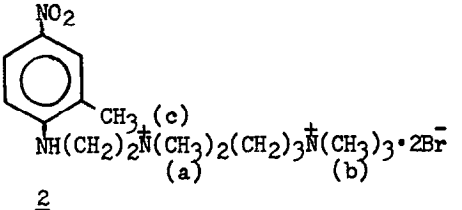
Summary

This paper reports the preliminary results of the proton magnetic resonance (pmr) spectra of the free and nucleic acid-bound reporter molecules 2 and 3.



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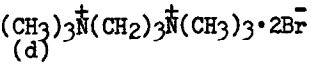

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It is found that the DNA and RNA-reporter 2 complexes result in an upfield shift and considerable line broadening for the c- and a-methyl signals as compared with the free reporter molecule. The chemical shift of the b-methyl signal remains unchanged for the free and the bound reporter 2 complex. Similar results are obtained for the d-methyl signals of reporter 3 in the free and bound state. The results are discussed and shown to be consistent with previous work utilizing absorption and circular dichroism techniques (see below).

We have reported on the use of reporter molecules specifically designed to interact strongly with polyanions, e.g., nucleic acids (Gabbay and Mitschelle, 1969; Gabbay, 1968; Gabbay, 1969; Gabbay and Malin, 1969). The reporter labelled polycations, I, R-$(CH_2)_n\overset{+}{N}(CH_3)_2(CH_2)_3\overset{+}{N}(CH_3)_3 \cdot 2Br^-$, where R is a chromophore absorbing in the 300-500 nm region, have been demonstrated to differentiate between (a) single stranded polypyrimidines and polypurines nucleotides, (b) single and double stranded RNA and DNA helices, and (c) single and double

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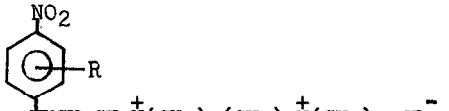
stranded ribose containing polynucleotides. The above studies were based on the results of the absorption spectra and induced circular dichroism of the bound reporter molecules.

The earlier work with diquaternary ammonium salts of the general structure, $R_1R_2R_3\overset{+}{N}(CH_2)_n\overset{+}{N}R_1R_2R_3 \cdot 2\bar{B}r$ has indicated that these salts bind electrostatically to adjacent phosphate anions on the same strand of polynucleotides (Gabbay, 1966; Gabbay, 1967; Gabbay and Shimshak, 1968). Moreover, recent work with reporter molecules, I, has shown that additional hydrophobic type interactions are observed with DNA and to a lesser extent with RNA nucleic acid systems. For example, Gabbay (1969), and Gabbay and Malin (1969) have observed that ribose containing double stranded nucleic acids, e.g., yeast RNA, torula RNA, rI-rC, and rA-rU induce a positive CD in the absorption band of the 4-nitroaniline transition of I irrespective of the nature of the substituents on the ring of the latter. Calf thymus DNA and salmon sperm DNA exhibit a positive



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induced CD in the "symmetrical" unsubstituted 4-nitroaniline reporter molecule, and a negative CD for the bound unsymmetrical "substituted" 4-nitroaniline reporter molecules, i.e., 2,4-dinitroaniline, 2-CH_3-4-nitroaniline, 3-CH_3-4-nitroaniline, 2-cyano-4-nitroaniline, 2-CF_3-4-nitroaniline and 2-carboxyamido-4-nitroaniline. Moreover, the interaction of RNA and DNA with reporter 1 leads to a positive and a negative CD for the 450 nm 4-nitroaniline transition,

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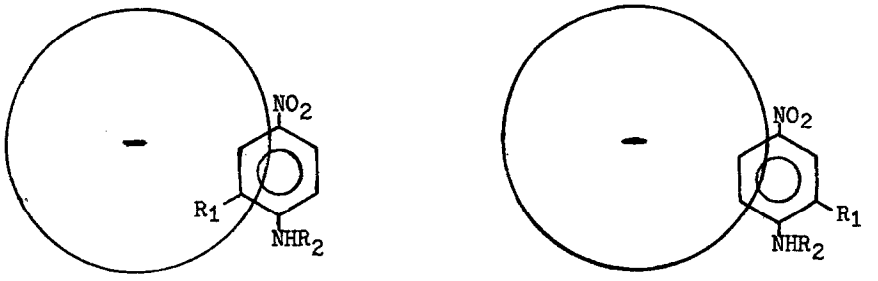
respectively. However, only the DNA complex shows optical activity associated with the naphthylamine transition of 1 at 340 nm. These results are best

interpreted in terms of an in geometry of the substituent (in this case a fused 2,3-phenyl ring) for the DNA complex. In line with this interpretation,



(a) in geometry (b) out geometry

Top View of Nucleic Acid Helix-Reporter Complex.

competitive binding studies between DNA and rI-rC or torula RNA revealed that the reporter molecules are bound more strongly to DNA by a factor of 5-10.

In order to shed further light on the nature of the reporter-nucleic acid complex we report the preliminary pmr results of the interactions of reporter molecules 2 and 3 with DNA and RNA systems. Figure 1 shows the partial pmr spectra of the free and the nucleic acid-bound reporter molecules at various temperatures in D_2O solution. The results in terms of chemical shifts from 2,2-dimethyl-2-silapentane-sulfonic acid (DSS) and line width at half height $\Delta\nu_{\frac{1}{2}}$, are summarized in Table 1.

Several interesting points may be made. (1) The pmr spectra of the simple diquaternary ammonium salt, 3, $(CH_3)_3\overset{+}{N}(CH_2)_3\overset{+}{N}(CH_3)_3 \cdot 2\bar{Br}$, is identical for the free as well as for the DNA-bound molecule at 31.0°C. (2) The pmr spectra of the DNA-bound reporter molecule 2 at 31.0°C shows considerable variation in the chemical shifts and line width of the a, b, and c-methyl protons. As can be seen from Table 1 and Figure 1 the resonance signal of the a-CH_3 protons shifts upfield by 0.13 ppm and coalesces with the b-CH_3 signal which remains unchanged at 3.17 ppm. A more dramatic effect is observed for the resonance signal of the ring -CH_3 (C-CH_3) where it appears to be broadened by at least 20 Hz and shifted upfield by 0.5 ppm at 31.0°C. These results may be explained in terms of (a) restricted motions (tumbling or rotations) in

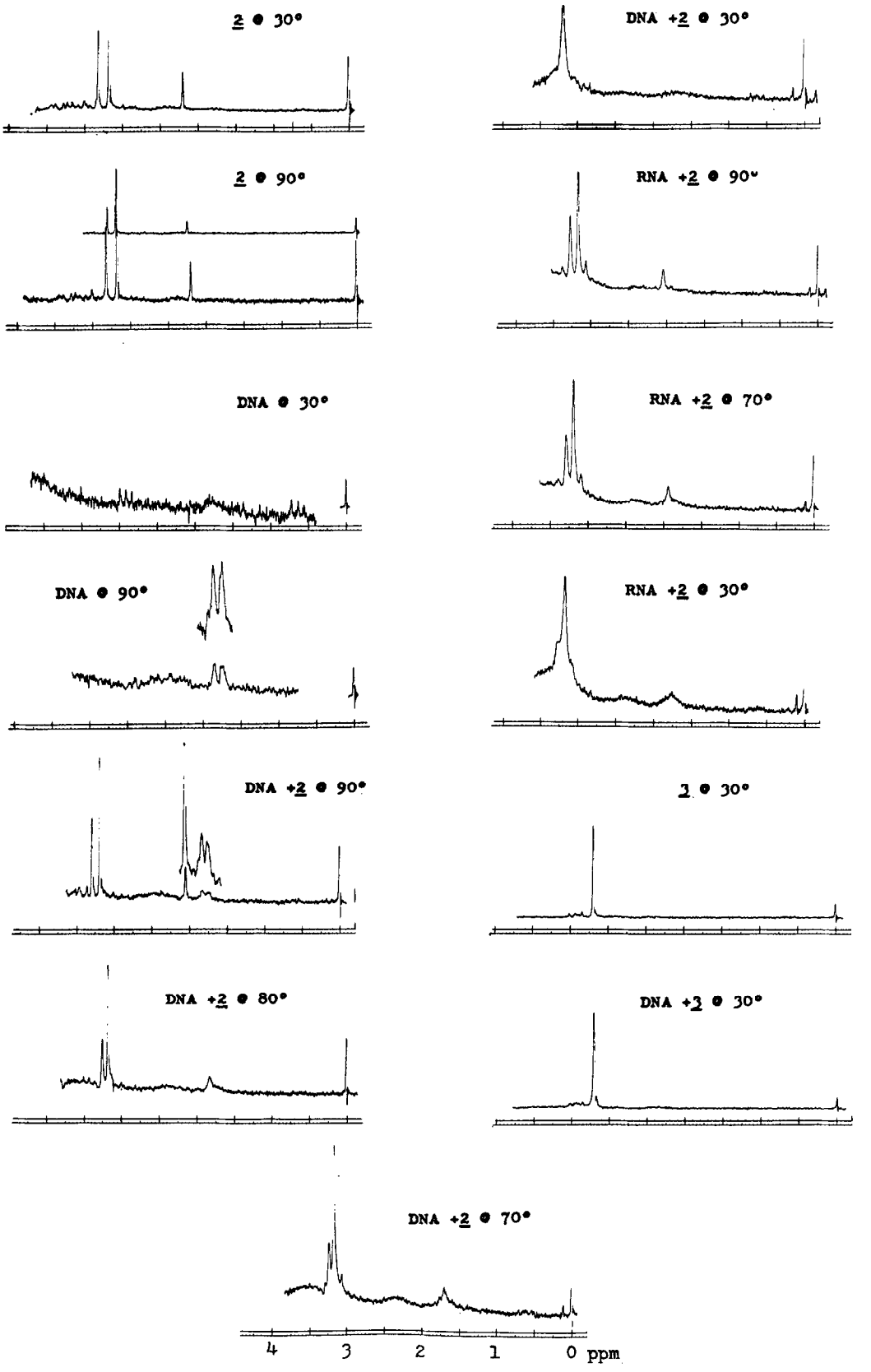


Figure 1. The HA-100D partial pmr spectra of free and nucleic acid bound reporter molecules 2 and 3 at various temperatures.

Table 1. Chemical Shifts (ppm) from DSS (±0.01) and Line Width at Half-Height in Hz ($\Delta\nu_{\frac{1}{2}}$) of Free and Nucleic Acid-Bound Reporter Molecules 2 and 3 at Various Temperatures.[a,b]

System	Temperature	δ ppm from DSS and Line Width ($\Delta\nu_{\frac{1}{2}}$) a-CH_3	b-CH_3	c-CH_3	d-CH_3
2	31°	3.30 (1.6)	3.16 (1.6)	2.19 (1.9)	-
2	90°	3.29 (1.7)	3.18 (1.6)	2.23 (2.5)	-
DNA•2	90°	3.27 (2.0)	3.17 (1.7)	2.05 (2.2)	-
DNA•2	80°	3.24 (2.8)	3.16 (1.8)	1.83 (5.0)	-
DNA•2	70°	3.22 (3.5)	3.16 (2.8)	1.70 (9.0)	-
DNA•2	31°	3.17[c]		1.70 (>20)	-
RNA•2	90°	3.26 (3.2)	3.16 (2.5)	2.04 (4)	-
RNA•2	70°	3.27 (4.7)	3.17 (3.3)	1.93 (6)	-
RNA•2	31°	3.25	3.17	1.75 (16)	-
3	31°	-	-	-	3.18 (1.9)
DNA•3	31°	-	-	-	3.18 (1.9)

[a]Sonicated low molecular weight salmon sperm DNA (M.W. ≤ 500,000) used for the pmr experiments was prepared according to the procedure of Strauss, et al. (1967) from native SS DNA (Worthington Co.). Torula ribosomal RNA (Calbiochem.) was used without further purification (M.W. ≈ 20,000). Nucleic acid solutions were prepared in D_2O in 10^{-4}M sodium phosphate buffer, pD 7.0 ± 0.2 at 75 mg/ml and diluted 4:5 with D_2O or 0.1 M reporter molecule in D_2O. Final concentrations of 2 or 3 and nucleic acid phosphate are 0.02 M and 0.16 M, respectively. Under these conditions the reporter molecules are fully bound (Gabbay, 1968; 1969).
[b]All spectra were determined on a varian HA-100D.
[c]The chemical shift for protons a and b is similar leading to a coalesced signal.

the bound reporter molecule and/or (b) averaging of various chemical shifts due to the numerous local environments that are possible in the complex, i.e., occupied sites adjacent to purines, pyrimidines, minor or major grooves, etc. It is instructive to note that changes in chemical shifts as well as the extent of the line broadening decreases in the following order: c-CH_3 > a-CH_3 > b-CH_3 ≥ 0. Since the CH_3- signal for reporter 3, $(CH_3)_3\overset{+}{N}(CH_2)_3\overset{+}{N}(CH_3)_3 \cdot 2\overset{-}{Br}$, remains unchanged for the free and the bound form, a behavior which is also exhibited by the b-CH_3 of reporter 2, it suggests that the line broadening exhibited by the c-CH_3 and a-CH_3 signals may be due to restricted motions at that end of the molecule in the DNA-complex. In other words, the results may be best explained in terms of a rigid DNA-reporter 2 complex whereby rotations about the 2-methyl-4-nitroaniline

ring plane is slowed presumably due to steric hindrance. This effect is expected to lead to the observed order of line broadening. In line with this interpretation is the observation that the c-CH_3 signal is upfield shifted by 0.5 ppm in the DNA-complex which may be attributed to the anistropic shielding effect of the purine or pyrimidine residues. Molecular framework models indicate that the c-CH_3 group of 2 may in fact lie in the shielding cone of the bases of the nucleic acid. Due to the 36° turn/base-pair in a Watson-Crick type helix, molecules which pertrude into the helix as shown for the in geometry above may partially lie above or below the exposed plane of the base pairs. (Intercalation of the 2-methyl-4-nitroaniline ring of 2 between base-pairs is considered unlikely on steric grounds if the mode of binding to DNA is assumed to be electrostatic in nature and involve the interaction of the diquaternary ammonium salts to adjacent phosphate anions on the same polynucleotide chain. Moreover, experimental evidence in favor of this conclusion has been presented elsewhere by Gabbay (1969)). (3) The pmr spectra of the RNA-bound reporter 2 complex also shows considerable variation in the chemical shifts and line width of the a, b, and c-methyl protons; however, this effect is not as dramatic as that observed for the DNA system. For example, the a-CH_3 proton resonance line is upfield shifted and broadened but does not totally coalesce with the b-CH_3 signal. Similarly, the c-CH_3 resonance line is more clearly distinguishable from the base line noise than in the case for the DNA-complex. These results which indicate more rapid tumbling and/or more efficient averaged chemical shift environments for the a- and b-CH_3 groups of reporter 2 in the RNA vs. the DNA complexes is in line with absorption and circular dichroism studies cited above. (4) Increasing temperatures cause a deshielding and sharpening of the resonance lines for the a, b, and c-CH_3 protons for both the RNA and DNA complexes. For example, at 90°C where partial melting of the DNA and RNA double helical regions has occurred sharp resonance lines characteristic of the free reporter molecule are observed for the a-, b- and c-methyl protons. More rapid tumbling and/or efficient averaging of chemical shift environment is indicated. At this temp-

erature melting of the DNA helix also occurs as evidenced by the appearance of the thymine-methyl peaks (McDonald et al. (1964), McDonald et al. (1967). It is also clear that denatured DNA and RNA at 90°C still bind the reporter molecule as indicated by the 0.02 and 0.18 ppm difference in the chemical shifts of the a- and c-CH_3 protons, respectively.

Further work in this area is in progress.

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