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Nuclear Magnetic Resonance Investigation of the Interaction of a ^{13}C-Labeled Quinacrine Derivative with DNA†

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ABSTRACT: A quinacrine derivative with [^{13}C]methyl groups on both the aliphatic (side chain) and aromatic (acridine) nitrogens was prepared from quinacrine using [^{13}C]methyl iodide. As expected, the ^{13}C nuclear magnetic resonance spectrum of this compound had two major signals corresponding to the two labeled methyl groups. On adding this compound to sonicated calf thymus DNA at molar ratios of 1:4 (drug to DNA nucleotides) and less (intercalation binding), both methyl signals were dramatically reduced in intensity at low ionic strength. As the ionic strength was increased, the side chain methyl signal became significantly more intense and approached the free solution line width at ionic strength greater than 1.0. Both the ring and side chain methyl signals were also reduced in intensity at low ionic strength with glucosylated T_4D bacteriophage DNA as with calf thymus DNA. These results can be interpreted in terms of a model that involves intercalation and immobilization of the quinacrine aromatic ring even at high ionic strength. The side chain is tightly bound at low ionic strength (presumably through interaction with the DNA phosphate groups) but has a considerably shorter group correlation time at high ionic strength suggesting that the side chain has considerable freedom of movement even though the molecule remains bound to DNA through the acridine ring. These results indicate that a single intercalated molecule has a bimodal interaction with DNA. The acridine ring and side chain groups have different binding constants for DNA and this difference in binding for a *single* molecule can be accentuated by increased salt concentrations. At molar ratios of 1:1 the intercalation sites are saturated but at low ionic strengths the acridine methyl group signal remains at low intensity relative to the unbound compound. The side chain methyl signal, however, has appreciable intensity in this complex. As the ionic strength is increased, both the side chain and aromatic methyl signals increase in intensity suggesting dissociation of the complex. These data are consistent with a binding model involving stacking and immobilization of the acridine ring in a self-association type complex induced by the negatively charged DNA phosphate-deoxyribose backbone. The side chain in this 1:1 complex must possess a considerable degree of rotational freedom to account for its relative short correlation time. Addition of either sodium or magnesium ions disrupts this complex due to competition with DNA phosphate binding sites.

Acridine derivatives display a wide spectrum of biological properties which result from their ability to form complexes with intracellular DNA (Peacocke, 1973). Complexation of the acridine, quinacrine (Figure 1), with DNA in vivo is convincingly demonstrated by its use as a chromosomal fluorescence stain and by its ability to eliminate bacterial plasmids (Lurquin, 1974). In addition, quinacrine and related derivatives possess antimalarial (Henry, 1973) and antineoplastic activity (Cain et al., 1976) which is thought to result from their ability to complex with DNA. Intercalation is the predominant interaction mode of many acridines and related planar aromatic compounds with DNA (Lerman, 1961; Waring, 1970; Blake & Peacocke, 1968). For compounds without cationic substituents, such as proflavin (Lerman, 1964), acridine orange (Blake & Peacocke, 1966), and coralyne (Wilson et al., 1976), intercalation simply involves insertion of the planar aromatic ring between DNA base pairs with local extension and unwinding of the double helix. Very little of the drug aromatic ring system protrudes into either the major or minor groove of DNA. Other compounds such as 2-hydroxystilbamidine (Festy & Daune, 1973), netropsin (Wartell et al., 1974), and

† From the Department of Chemistry, Georgia State University, Atlanta, Georgia 30303. *Received July 12, 1977; revised manuscript received June 5, 1978.* Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, the Research Corporation, and the Georgia State University Arts and Sciences Research Fund for support of this work.

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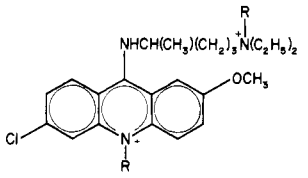

R=-H, QUINACRINE

R=-CH_3, DIMETHYLQUINACRINE

FIGURE 1.

distamycin (Zimmer, 1975), which are also thought to exert their biological effects by complexation with DNA, do not insert between DNA base pairs but bind externally in one of the DNA grooves. Simple diamines and more complex polyamines (spermidine, spermine, etc.) also bind outside of the stacked base pairs in one of the DNA grooves (Liquori et al., 1962; Gabbay, 1968). At relatively low ionic strengths and high molar ratios of compound to DNA nucleotides, many planar aromatic drugs have saturated the intercalation binding sites and can also bind externally along the DNA helix (Bradley & Wolf, 1959). This type of complex primarily involves self-association (stacking of the cationic drug aromatic ring system) which is induced by the negatively charged DNA phosphate groups.

Many important drugs, such as quinacrine (Figure 1), propidium (Waring, 1970), tilorone (Chandra et al., 1972), daunorubicin (Gabbay et al., 1976), and hycanthone (Carchman et al., 1969; Waring, 1970), which are known to intercalate, have both a planar aromatic ring system and a cationic substituent (side chain). In this case the binding model involves insertion of the planar aromatic ring system between successive DNA base pairs with the side chain projecting into one of the grooves of the double helix. In the quinacrine–DNA complex, for example, the side chain is postulated to lie in the minor groove with a strong electrostatic interaction between the aliphatic nitrogen cation and the DNA phosphate groups (Krey & Hahn, 1974). With daunorubicin, on the other hand, X-ray fiber diffraction investigations (Pigram et al., 1972) and model building studies (Henry, 1976) have suggested that the amino sugar cation lies in the major groove and also involves strong electrostatic interaction with the phosphate groups. Structurally, these drugs are hybrids of intercalating and externally binding compounds and must be analyzed from this dual viewpoint.

As can be seen from the above list of drugs, some of the most active compounds against a wide range of disease are in this mixed aromatic–cationic side chain class. Binding studies with these compounds have generally assumed that the ring system and side chain interact with DNA as a unit regardless of the solvent conditions. In order to understand the biological effects of these compounds and to develop models which can precisely explain their complexation with DNA, this assumption must be tested under a variety of conditions. For monitoring specific molecular regions in complexes of this type, NMR is a more powerful method than other spectroscopic techniques. ^{13}C NMR is normally hampered by its low sensitivity relative to ^{1}H NMR but the synthesis of specifically labeled ^{13}C derivatives along with proton noise decoupling and NOE[1] enhancement in Fourier transform NMR can eliminate this sensitivity difference (Levy & Nelson, 1972). ^{13}C NMR has two major advantages over ^{1}H NMR: (i) the chemical shift range of carbon nuclei is approximately tenfold greater than that of protons; and (ii) the HDO signal that is always present with biological samples in proton NMR does not appear in ^{13}C NMR. To overcome the sensitivity problem we have synthesized a ^{13}C-labeled methylated quinacrine derivative (Figure 1) and accumulated a large number of free induction decays before Fourier transformation. The interactions with DNA of the aromatic and aliphatic molecular regions of this compound have been investigated as a function of ionic strength and molar ratio of MQ to DNA nucleotides. This allows an independent determination of side chain and ring interactions with DNA for both intercalated and externally bound MQ at concentrations similar to those used in other investigations (such as viscometric titrations, ultraviolet-visible spectroscopy, etc.).

Experimental Section

Materials

DNA. Calf thymus DNA (Worthington lot no. 35M614) was sonicated as previously described (Davidson et al., 1977a), and lyophilized for storage. The molecular weight average (viscosity), spectral properties, and hyperchromicity of DNA prepared in this manner were consistent with published values (Bloomfield et al., 1974; Davidson et al., 1977a). Calf thymus DNA concentrations were determined using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm and are expressed in terms of nucleotide equivalents per liter.

Bacteriophage T_4D and its host *Escherichia coli* B/1,5 (resistant to phages T_1 and T_5) were kindly provided by Dr. William B. Wood. Phage were grown and isolated by the procedures outlined by Adams (1959). T_4D DNA was isolated and purified by the method of Mandell & Hershey (1960) and phenol was removed by exhaustive dialysis of the DNA against 1.0 M NaCl, 0.05 M Tris, 10^{-3} M EDTA (pH 8.0). DNA was stored in this buffer with a few drops of chloroform. Purified T_4D DNA has a A_{260}/A_{280} ratio between 1.85 and 1.92, and A_{260}/A_{230} ratio of 2.43 and a total hyperchromicity at 260 nm of 29.5%. DNA concentrations were determined using an extinction coefficient of 6460 M^{-1} cm^{-1} at 260 nm (Rubenstein et al., 1961).

Quinacrine Methylation. A solution of quinacrine (0.28 g; 0.7 mmol) and methyl iodide (0.4 g; 2.8 mmol) in 0.5 mL of methanol was placed in a tightly capped 3-mL Reacti-Vial (Pierce Chemical Co.) and was heated for 4 h at 50 °C with no stirring. The solution was then stirred and cooled, and a mixture of the dimethylated quinacrine and the hydroiodide of quinacrine methylated only on the side chain precipitated. MQ was purified by three recrystallization from methanol–ethyl acetate in the presence of approximately equimolar amounts of triethylamine (final yield: 15–20%). The purity of MQ was monitored by thin-layer chromatography on silica gel in the upper phase of butanol/acetic acid/water (5:1:4). Anal. Calcd for $C_{25}H_{26}ClI_2N_3O$: C, 43.91; H, 5.31; N, 6.15. Found: C, 43.99; H, 5.22; N, 5.91. Purified MQ decomposed at 224 °C (uncorrected). Both ^{1}H and ^{13}C NMR spectra were consistent with the proposed structure. The ^{13}C-labeled MQ derivative was prepared by the same procedure using ^{13}C-labeled methyl iodide (90% ^{13}C) from Merck.

Methods

Lyophilized sonicated calf thymus DNA and MQ were dissolved as concentrated stock solutions (2.1×10^{-2} and 3×10^{-3} M, respectively) in standard buffer (1.5×10^{-2} M NaH_2PO_4, 10^{-4} M EDTA, adjusted with NaOD to a pH meter reading of 7.0 in D_2O). All NMR spectra were recorded

[1] Abbreviations used: MQ, dimethyl quinacrine; NOE, nuclear Overhauser effect; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time.

at MQ concentrations of either 3×10^{-4} or 1.5×10^{-3} M. Solutions in standard buffer for experiments at different molar ratios of MQ to DNA were prepared from the above stock solutions. Dioxane was added (as indicated in figure legends) to provide a constant intensity and chemical shift standard (67.4 ppm relative to Me_4Si) for a particular set of instrumental parameters. The dioxane concentration was increased in experiments using 1.5×10^3 M MQ to assure the same ratio of signal intensities between dioxane and the MQ methyl signals. For experiments at higher sodium ion concentration, solid NaCl was added to an aliquot of the MQ–DNA solution.

The ^{13}C NMR experiments were performed on either a JEOL FX-60 or FX-60Q (Digital Quadrature Detection) Fourier transform spectrometer operating at a frequency of 15.04 MHz. Data were accumulated on a Texas Instruments 980B computer using 8192 computer words with a 2500-Hz spectral width yielding 0.6-Hz data point resolution. Samples in 10-mm NMR tubes (Wilmad 513-7PP) were pulsed approximately 2.5×10^4 times utilizing a 45° pulse angle and a 2.5-s repetition time. Temperature was maintained in all cases at 35 °C using a JEOL variable temperature module. All experiments utilized broad band proton noise decoupling. To increase signal-to-noise, an exponential filter was applied to the free induction decay data before it was Fourier transformed to yield the resultant frequency domain spectrum (Farrar & Becker, 1971). The enhanced sensitivity is also accompanied by line broadening which is dependent on the magnitude of the exponential function. Care was taken to keep all adjustable instrumental variables constant to facilitate comparison of spectra within each experiment.

Spin–lattice relaxation times (T_1) were measured for MQ (1.5×10^{-3} M) and MQ–DNA complexes (1.5×10^{-3} and 6×10^{-3} M, respectively) at various ionic strengths using the inversion–recovery method (Freeman & Hill, 1971; Farrar & Becker, 1971) with the samples deoxygenated and sealed under nitrogen. For each $180°-\tau_1-90°-\tau_2$ pulse sequence, 6 to 9 values of τ_1 were utilized with τ_2 values being greater than five times the longest T_1 value and 10^4 accumulations for each τ_1 were collected. T_1 was determined by a standard least-squares regression analysis using software supplied by JEOL. The NOE enhancement factors were measured on MQ using the NOE supress gated decoupling technique (Freeman et al., 1972) a 90° pulse angle, a pulse repetition time of 20 s, and 1125 accumulations for each spectrum. To increase accuracy, these experiments were performed at tenfold higher concentrations (3×10^{-3} M) than the dilute solution binding studies with DNA. The error of both the T_1 and NOE values is ±20% due to the relatively low signal-to-noise ratio.

Results and Discussion

At the MQ concentrations used in this research only signals corresponding to the enriched side chain [^{13}C]methyl (47.5 ppm) and aromatic [^{13}C]methyl (36.8 ppm) substituents can be detected above baseline noise (Figure 2). The chemical shifts of the peaks were assigned through synthesis of the unlabeled dimethyl analogue and a derivative with only the side chain methylated (Davidson et al., 1977b). The side chain carbon resonance is split into a triplet due to coupling with the aliphatic nitrogen. Increasing salt at constant MQ concentration and increasing MQ concentration (from 3×10^{-4} to 3×10^{-3} M) in standard buffer (not shown) cause negligible changes in the MQ ^{13}C NMR spectrum. It should be emphasized that some sacrifice in signal-to-noise has been made in these and following experiments to allow utilization of DNA and drug concentrations similar to those of other physical measurements. This allows direct comparisons among different biophysical methods without possible concentration induced artifacts (such as drug aggregation).

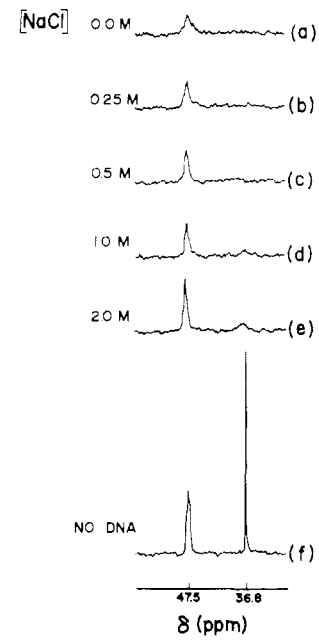

FIGURE 2: Effect of NaCl on the spectrum of intercalated MQ–DNA complex at a molar ratio of 0.25 (1.5×10^{-3} M MQ, 6×10^{-3} M DNA). (a) No added NaCl, (b) 0.25 M NaCl, (c) 0.5 M NaCl, (d) 1.0 M NaCl, (e) 2.0 M NaCl, and (f) reference spectrum without DNA or added NaCl (0.15% dioxane). All spectra were accumulated for 2.5×10^4 pulses and all samples were made in standard buffer with added solid NaCl where appropriate. The side chain methyl signal is a triplet at 47.5 ppm and the aromatic methyl signal is at 36.8 ppm. There is no significant change in these chemical shift positions on adding DNA or salt in this or any of the following figures. A 0.7-Hz broadening function (exponential filter) was applied to each free induction decay before Fourier transformation (Farrar & Becker, 1971).

The T_1 values for the side chain and ring methyl groups of MQ in the absence of DNA are 0.70 and 1.1 s, respectively. For compounds of this size the ^{13}C spin–lattice relaxation times are generally dominated by dipole–dipole interactions for protonated carbons (Levy, 1973). We have confirmed this for MQ by determining NOE values which depend on the fraction of the relaxation that is occurring due to dipole–dipole interactions (Noggle & Schirmer, 1971). Within experimental error both of the NOE values for the protonated carbons are greater than 1.9, compared with the theoretical maximum of 1.99, indicating that their relaxation is due to dipole–dipole interactions (Levy & Nelson, 1972) within experimental error.

The values of T_1 and T_2 for protonated carbons in the region of extreme narrowing are given by eq 1 (dipolar relaxation):

$$\frac{1}{T_1} = \frac{1}{T_2} = N\hbar^2\gamma_C^2\gamma_H^2 r_{CH}^6 \tau_C \qquad (1)$$

where N is the number of directly attached protons, γ_C and γ_H are the magnetogyric ratios for ^{13}C and ^1H, r_{CH} is the C–H bond length, and τ_C is the effective correlation time for rotational reorientation (Doddrell et al., 1972). If the molecule has internal motion in addition to the overall molecular rotation, τ_C is the average of the two and is given by eq 2:

$$\frac{1}{\tau_C} \simeq \frac{1}{\tau_R} + \frac{1}{\tau_G} \qquad (2)$$

where τ_R is for overall molecular reorientation and τ_G is for internal motion of a group in the molecule (Doddrell & Allerhand, 1971). As the overall molecular motion becomes slower, the measurable effects of τ_G increase.

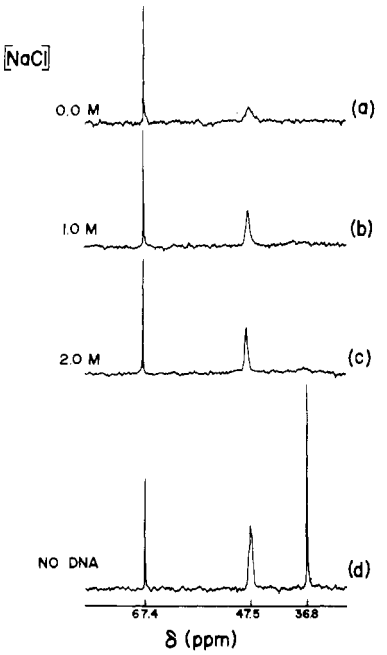

FIGURE 3: Effect of NaCl on the spectrum of intercalated MQ-DNA complex at a molar ratio of 0.1 (1.5×10^{-3} M MQ, 1.5×10^{-2} M DNA). (a) No added NaCl, (b) 1.0 M NaCl, (c) 2.0 M NaCl, and (d) reference spectrum (0.15% dioxane). Other conditions are identical with those described in Figure 1.

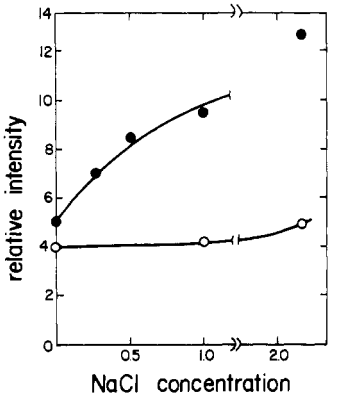

FIGURE 4: Relative intensities of the MQ side chain signal (47.5 ppm) as a function of added NaCl. (○) Data collected from Figure 3 at a molar ratio of 0.1, and (●) data collected from Figure 2 at a molar ratio of 0.25.

Spectra for the interaction of MQ with DNA are shown in Figure 2. At low salt concentrations the aromatic methyl signal has completely disappeared (Figures 2a–c) and the side chain methyl signal has broadened so that it is barely detectable above baseline noise. There are two general possible reasons for the changes in these signals as DNA is added: (i) a decrease in molecular and group correlation times; (ii) slight differences in chemical shifts of the methyl groups bound in different environments on the DNA double helix. Some combination of these two is also possible. The decrease in intensity due to (i) can occur due to an increase in peak width resulting from dipolar broadening, and also from a decrease in NOE as the tumbling rate is drastically reduced when MQ binds to DNA (Doddrell et al., 1972). The effect of increasing salt concentration on the MQ–DNA complex is also illustrated in Figure 2. Under these conditions (a molar ratio of 0.25; MQ to DNA nucleotides), only the intercalation complex is present in significant amounts. In standard buffer without any added NaCl (Figure 2a) the aromatic methyl signal is affected as discussed above. As the salt concentration is increased from 0.25 to 1.0 M (Figure 2b–d), the side chain signal becomes more narrow and its intensity increases but no aromatic signal can be detected above the noise level. At 2.0 M salt (Figure 2e) the side chain signal has again increased and a broad aromatic signal is barely visible above the noise level. No change in the chemical shift of either peak can be detected within experimental error at any salt concentration, suggesting that the peak broadening is due to a tumbling effect leading to dipolar broadening. In the absence of DNA, changing salt concentration has a negligible effect on the MQ ^{13}C NMR spectrum. Using the intercalation model, these results suggest that at low salt the aromatic ring is bound between base pairs without mobility on the NMR time scale and the side chain lies in one of the DNA grooves with its mobility greatly restricted by electrostatic interaction with the DNA phosphate groups. As the sodium ion concentration is increased, competition occurs with the cationic drug side chain for electrostatic interaction with the negatively charged DNA phosphate but little effect is produced for the acridine ring. An increasing fraction of the side chain is released from the strong complex with the DNA phosphate groups and becomes more analogous to the MQ side chain free in solution, even though the MQ aromatic ring remains intercalated at these salt concentrations. The aromatic methyl signal does not reappear until a salt concentration is reached which allows total dissociation of a significant fraction of the complex.

In Figure 3 the effects of salt at a lower ratio of MQ to DNA (0.10 MQ to DNA nucleotide) are shown. As with the results in Figure 2, the aromatic methyl signal has totally disappeared while the aliphatic methyl signal is greatly broadened. In this case, however, as the salt concentration is increased to 2.0 M (Figures 3a–c) very little change occurs in the spectrum, indicating a much less pronounced effect of sodium ions on MQ binding, and especially on the side chain binding, at lower ratios of MQ to DNA binding sites. This agrees quite well with binding results that have been observed with quinacrine (Bontemps & Fredericq, 1974; Wilson et al., 1978), indicating pronounced negative cooperativity in the interaction of quinacrine with DNA. This suggests that the quinacrine side chain only becomes dissociable by sodium ions if the binding constant for the side chain is reduced by negative cooperative interactions with other neighboring quinacrine molecules. The results from Figures 2 and 3 illustrate that intercalation of molecules with cationic side chains should be considered as two separate binding events: (i) intercalation of the aromatic ring system, and (ii) electrostatic interaction of the side chain with DNA phosphate groups (hydrogen bonding and van der Waals interactions may also be important and will depend on the side chain structure for any intercalating molecule).

The side chain methyl signal intensity results, as a function of salt concentration, for the MQ side chain are collected from Figures 2 and 3 and presented graphically in Figure 4. This convincingly illustrates the dramatic effect of the molar binding ratio of MQ to DNA on the ability of salt to increase the mobility of the MQ side chain and is a direct proof of the negative cooperativity postulated from an analysis of quinacrine binding models (Wilson et al., 1978; Bontemps & Fredericq, 1974). It should be emphasized that the bimodal interaction referred to here is for a *single* intercalated molecule and does not refer to a two-site theory which has been used primarily to indicate intercalation and some outside stacking mechanism of interaction for different bound ligands. The outside stacking mechanism will be discussed in later figures.

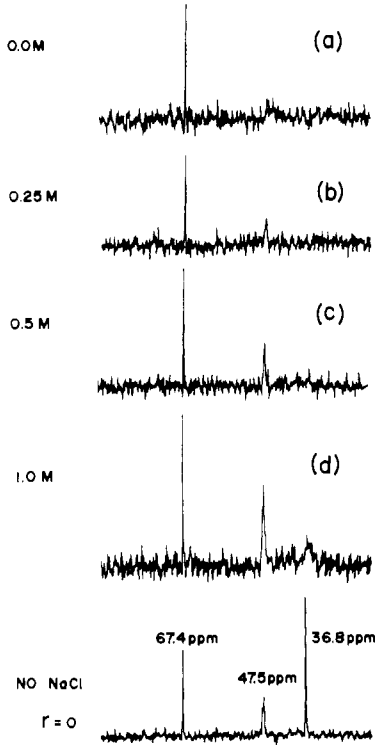

FIGURE 5: Effect of NaCl on the intercalated MQ-DNA spectrum at a lower concentration and molar ratio of 0.25 (3×10^{-4} M MQ, and 1.2×10^{-3} M DNA). (a) No added NaCl, (b) 0.25 M NaCl, (c) 0.5 M NaCl, (d) 1.0 M NaCl, and (e) reference spectrum with no DNA or NaCl (0.03% dioxane). A 0.9-Hz broadening function was applied to each free induction decay signal before Fourier transformation.

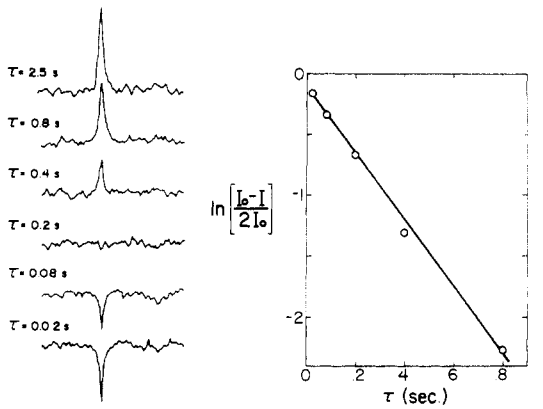

FIGURE 6: Spin-lattice relaxation measurement (T_1) for the side chain methyl signal (47.5 ppm) of the MQ-DNA complex at a molar ratio of 0.25 and a NaCl concentration of 1.0 M. Six τ_1 values of 2.5, 0.8, 0.4, 0.2, 0.08, and 0.02 s were used with a τ_2 value of 3 s. The right-hand portion of the figure illustrates a graph of the equation: $\ln (I_0 - I/2I_0) = -1/T_1\tau$ where I_0 is the side chain signal intensity at τ^∞ (2.5 s), I is the signal intensity at τ_i, and T_1 is the spin-lattice relaxation time. The slope of this equation when plotted as $\ln (I_0 - I/2I_0)$ vs. τ equals $-1/T_1$. The spectra for each τ value were accumulated for 10^4 transients and a 2.3-Hz broadening function was applied to each free induction decay signal before Fourier transformation.

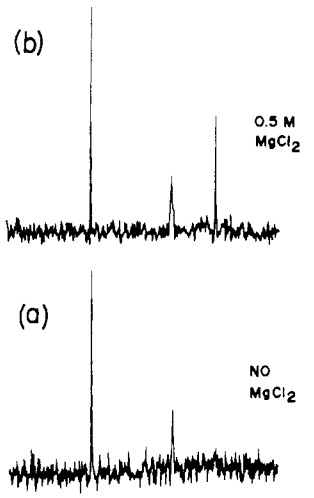

FIGURE 7: Effect of $MgCl_2$ on the 1:1 MQ-DNA complex (3×10^{-4} M MQ, 3×10^{-4} M DNA). (a) No added $MgCl_2$; (b) 0.5 M $MgCl_2$ (0.1% dioxane). A 0.9-Hz broadening function was employed.

An experiment analogous to that depicted in Figure 2 but at one-fifth the concentrations of MQ and DNA is shown in Figure 5. Qualitatively, the results are the same although, as expected, the signal-to-noise is less. This concentration, however, is similar to that used in most biophysical studies of DNA–ligand interactions and indicates that the NMR results are directly comparable to these other investigations. An experiment at the same concentrations of MQ as in Figure 5 but at a molar ratio of 0.2 was also conducted (not shown) and indicated that the MQ side chains also increased in intensity at this ratio as a function of salt. The increase was somewhat less than obtained at the ratio of 0.25 but was significantly greater than that obtained at the 0.1 molar ratio (Figure 3).

To obtain a better idea of the mobility of the side chain of a bound MQ molecule, the T_1 (spin–lattice relaxation) value was measured in an inversion–recovery experiment at a ratio of MQ to DNA of 0.25 and a salt concentration of 1.0 M NaCl. The T_1 value obtained was 0.35 s and the results are illustrated in Figure 6. The T_1 value of the unbound MQ molecule is 0.70 s indicating that the side chain of the free molecule has enhanced tumbling ability relative to the side chain of the bound molecule at high salt concentrations. The restrictions on the tumbling rate of the side chain methyl group can arise from several factors including reduced molecular motion due to intercalation of the acridine ring, proximity to the anionic DNA phosphate groups, and the physical restriction imposed by the local environment in the DNA minor groove.

As the ratio of quinacrine to DNA nucleotides is increased from 0.25 to 1.0, the intercalation sites are saturated but at low salt quinacrine continues to bind to DNA (Krey & Hahn, 1974) in an external stacked type of complex (two-site binding) as discussed above. In Figure 7a, the MQ-DNA complex spectrum is shown at 1:1 molar ratio (MQ to DNA nucleotides). Other conditions are the same as in Figure 5. The MQ ring methyl signal is totally broadened in the 1:1 complex at low salt. Although a peak can be seen for the side chain methyl, it is reduced in intensity compared with the molecule free in solution (Figure 2f) but is increased compared with the intercalated compound (Figure 2a).

Adding 0.5 M $MgCl_2$ causes significant dissociation of this stacked 1:1 complex as evidenced by the appearance of a sharp aromatic methyl signal (Figure 7b). Sodium chloride has similar effects (not shown) but the salt concentrations required to attain the same signal intensities are higher. The first effect noticed is a narrowing of the side chain signal indicating increased mobility of the group at relatively low ionic strength. This is followed by the appearance of the aromatic methyl signal indicating dissociation of the complex at higher ionic strength.

The interaction of MQ with T_4D bacteriophage DNA, which has the major groove sterically blocked with α- and β-D-glucose residues convalently linked to 5-hydroxymethylcy-

tosine, was also investigated. For intercalated MQ, the spectrum was essentially identical to Figure 5. At a 1:1 molar ratio, the spectrum was quite similar to Figure 7. Differences in both cases were within experimental error. This suggests that the quinacrine side chain lies in the minor groove and interacts strongly with phosphate groups in DNA at low salt concentration. The results at higher drug to DNA ratio suggest that MQ can also stack in the minor groove of DNA, although some MQ could still be stacked in the major groove in the vicinity of A:T rich regions where there are no glucose residues.

Quinacrine does not obey the simple (noncooperative) neighbor exclusion binding model for intercalating compounds (Bontemps & Fredericq, 1974; Wilson et al., 1978). The molecule displays pronounced negative cooperativity in its interaction with DNA suggesting inhibition of quinacrine binding as site saturation is increased. Ethidium bromide, which has no cationic side chain, does obey the noncooperative neighbor exclusion model (Bauer & Vinograd, 1970). The results of our NMR investigations suggest that the reason for this difference between ethidium and quinacrine is the strong interaction of the quinacrine side chain with DNA phosphate groups through the minor groove. This would cause repulsion of binding of other cationic quinacrine molecules, even at alternate base pair positions as suggested in the neighbor exclusion model. Our results demonstrate, however, that as the ionic strength is increased the side chain–DNA phosphate interaction decreases and at high salt concentration quinacrine should approach binding by the neighbor exclusion intercalation model.

A model for the interaction of MQ and quinacrine with DNA which is consistent with our NMR results and other investigations on the quinacrine–DNA complex involves intercalation of the acridine ring system and strong interaction of the aliphatic side chain in the minor groove with the DNA phosphates. This latter interaction is more easily disrupted by salt than the former and is largely eliminated at 2.0 M NaCl at MQ to DNA ratios of 0.25. At lower molar ratios more salt is required to displace the side chain, again suggesting negative interactions among bound drug molecules. As more MQ is added to DNA, it can stack externally along the DNA sugar–phosphate helix. In this complex the aromatic ring is still tightly bound, but the side chain is much closer to free MQ even at lower salt. This external complex is more sensitive to salt than the intercalated complex. In the intercalated complex both the aromatic ring and the side chain bind to DNA as a unit at physiological ionic strength at molar ratios up to the intercalation saturation limit.

The experiments in this paper have been conducted at concentrations similar to those used in other biophysical studies. We feel that the addition of ^{13}C labeled methyl groups (through $^{13}CH_3I$ and nonquaternary nitrogens) offers an excellent method for adding sensitive probes for monitoring biopolymer structure and interactions with small molecules while maintaining the advantages of ^{13}C NMR discussed above.

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