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Binding of 5-Fluorotryptamine to Polynucleotides as a Model for Protein–Nucleic Acid Interactions: Fluorine-19 Nuclear Magnetic Resonance, Absorption, and Fluorescence Studies†

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ABSTRACT: Fluorine-19 nuclear magnetic resonance (^{19}F NMR), optical absorption, and fluorescence spectroscopy have been used to study the interaction of 5-fluorotryptamine (5FTA) with polynucleotides as a model for protein–nucleic acid interactions. In the presence of DNA, denatured DNA, poly(A), and poly(A)·poly(U), the ^{19}F resonance of 5FTA shifted 0.3–0.6 ppm upfield while the presence of poly(I)·poly(C) had little effect on the chemical shift. Differences in the ^{19}F chemical shift induced upon changing from H_2O to ^2H_2O indicate differences in the solvent accessibility of 5FTA bound to the various polynucleotides. ^{19}F NMR relaxation experiments were carried out for free 5FTA and in its nucleic acid complexes, and the results were interpreted by using a two correlation time model that included contributions to relaxation from dipolar coupling and chemical shift anisotropy. Values for the internal motion correlation time and the overall motion correlation time are reported. The effect of 5FTA on the melting transition of the double-stranded polynucleotides and on the quenching of 5FTA fluorescence was also studied. The ^{19}F NMR results support the model of partial intercalation of the 5FTA chromophore into the polynucleotides, and the implications for protein–nucleic acid interactions are discussed.

Over the past 2 decades a great deal of experimental work has been focused on the binding of proteins to nucleic acids. These studies have examined the factors involved in the recognition process through the use of model protein systems, such as mono-, di-, and tripeptides (Dimicoli & Hélène, 1974a,b), as well as more complex systems such as the *lac* operon (Caruthers, 1980) and gene 32 protein (Kelly & von Hippel, 1976; Kelly et al., 1976). The forces involved may include electrostatic interactions, hydrogen bonding, and hydrophobic interactions. Of particular interest is the interaction of planar aromatic amino acids, tryptophan, phenylalanine, and tyrosine, with nucleic acids. It is thought that these amino acids may bind DNA in a manner similar to the classical intercalators ethidium bromide and proflavin.

As a probe of the interaction of aromatic amino acids with polynucleotides, we have used optimal absorption, fluorescence, and fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectroscopy to study the interaction of 5-fluorotryptamine (5FTA), Figure 1, with single- and double-stranded DNA and RNA. ^{19}F NMR is a convenient probe of noncovalent in-

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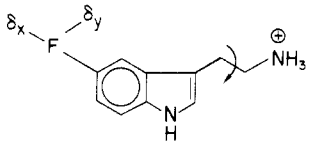

FIGURE 1: The structure of 5-fluorotryptamine (5FTA). Relaxation parameters were calculated by assuming rotation about the C–C bond as indicated in the figure and discussed under Theory, which would permit 5FTA to slide between the bases of the polynucleotide. The principal axes of the ^{19}F chemical shift are also shown with δ_z normal to the plane of the indole ring.

teractions due to the lack of obscuring resonances from the solvent or the macromolecule and the sensitivity of the fluorine chemical shift to environmental factors. Also, since the fluorine NMR relaxation parameters can be related to molecular motions, we may probe the dynamics of this model for a protein–nucleic acid complex. We have previously studied the binding of fluorinated intercalators to nucleic acids (Bolton et al., 1981; P. A. Mirau, R. H. Shafer, T. L. James, and P. H. Bolton, unpublished results); the objective of this study is to compare the mode of binding of 5FTA to the intercalation model.

Theory

The motions of 5FTA bound to polynucleotides may be investigated by ^{19}F NMR. The spin–lattice relaxation time (T_1), line width ($W_{1/2}$), nuclear Overhauser effect (NOE), rotating frame spin–lattice relaxation time in the presence of an off-resonance field ($T_{1\rho}^{off}$), and off-resonance peak intensity ratio (R) are related to the molecular motions through the spectral density functions as previously described (Bolton et al., 1981). The relaxation is usually dominated by dipolar coupling to neighboring protons, but relaxation due to chemical shift anisotropy can be important in some situations (Hull & Sykes, 1975).

A two correlation time model was used to relate the ^{19}F NMR relaxation data to the molecular motions of 5FTA bound to polynucleotides; a model utilizing a single correlation time was incapable of explaining all of the NMR data. The overall isotropic motion, which is thought to be related to bending motions in polynucleotides with lengths greater than their persistence length (Barkley & Zimm, 1979; Bolton & James, 1979, 1980a), has the correlation time τ_0. In addition to the overall motion, faster internal motions may give rise to ^{19}F relaxation. We have constructed a model for the internal motions of polynucleotide–5FTA complexes from model building studies with dinucleotides. One possible orientation of 5FTA bound to nucleic acids is with the amine side chain aligned along the phosphate backbone, which would allow 5FTA to slide between the base pairs by rotation about the C–C bond shown in Figure 1. This motion has the correlation time τ_i. Other internal motions may be visualized such as rotation about an axis in the plane of the chromophore but are less amenable to mathematical analysis. Both the long-range and local motions induce reorientation of the fluorine–proton internuclear vectors and the chemical shift tensor and thus affect the NMR relaxation parameters of 5FTA.

The dipolar relaxation of the fluorine nucleus is presumed to arise from coupling to the two adjacent ring protons of 5FTA, which are 0.26 nm away from the fluorine nucleus. The angle between the F–H internuclear vector and the axis of internal rotation (C–C bond) is 109.5°. The chemical shift anisotropy contribution to relaxation depends on the principal axis of the chemical shift tensor as well as the asymmetry. Since the chemical shift tensor for 5FTA has not yet been determined, the data for fluorobenzene is used as a reasonable

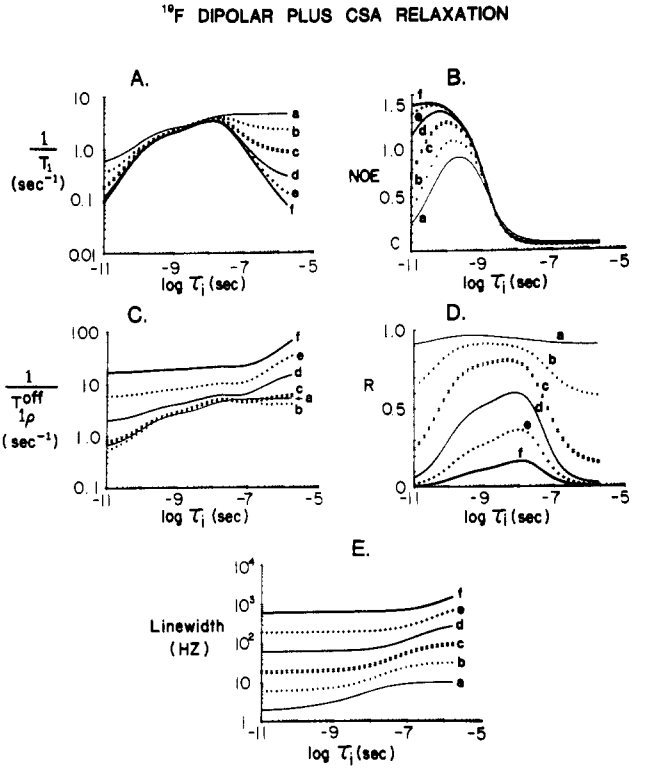

FIGURE 2: Theoretical dependence of the (A) spin–lattice relaxation rate, (B) nuclear Overhauser effect, (C) off-resonance rotating frame spin–lattice relaxation rate, (D) off-resonance intensity ratio, and (E) line width for ^{19}F at 94.1 MHz. The relaxation parameters were calculated by assuming the two correlation time model presented under Theory. The calculations account for a contribution to relaxation from chemical shift anisotropy as well as dipolar interactions, with the exception of the NOE curves which were calculated by assuming only dipolar interactions. The various plots were calculated for values of the overall correlation time of (a) 3×10^{-8}, (b) 10^{-7}, (c) 3×10^{-7}, (d) 10^{-6}, (e) 3×10^{-6}, and (f) 10^{-5} s.

approximation; the chemical shift anisotropy is 51.2 ppm and the asymmetry is −1.27 ppm (Mehring et al., 1974; Hull & Sykes, 1975). For the internal motion of 5FTA, the Euler angles for the chemical shift anisotropy are $\beta = 19°$ and $\gamma = 30°$.

Theoretical curves illustrating the dependence of the relaxation parameters on the internal motion correlation time for a series of overall motion correlation times are shown in Figure 2. The curves contain contributions from both dipolar interactions and chemical shift anisotropy with the exception of the NOE curves which contain only dipolar interactions. The calculations are for a magnetic field strength of $2.35T$ and a radio-frequency field strength of 0.23 G applied 5.6-kHz off-resonance. The calculations show that the relative importance of the dipolar and chemical shift anisotropy contributions to the ^{19}F relaxation process depends on the particular features of the molecular motion.

Materials and Methods

5-Fluorotryptamine, calf thymus DNA, synthetic polynucleotides, and buffer reagents were obtained from Sigma Chemical Co. and used without further purification. All experiments were performed in 0.01 M NaCl–0.01 M cacodylate buffer at pH 7.0. For NMR experiments the buffer contained 10% or 90% ^2H_2O as indicated. Synthetic polynucleotides were prepared by extensive dialysis against the buffer, and concentrations were determined by using previously reported extinction coefficients (Janik, 1971). DNA was dissolved in a high salt buffer containing ethylenediaminetetraacetic acid (EDTA), briefly sonicated, and dialyzed against the buffer

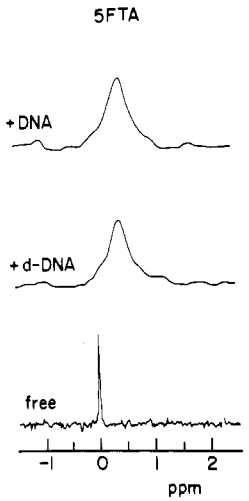

FIGURE 3: ^{19}F NMR spectra (94.1 MHz) of 5FTA free and in the presence of DNA and denatured DNA at 37 °C. The data were gathered in 2K data points by using a sweep width of ±2 kHz, a delay of 0.75 s, between pulses, a 60° nonselective rf pulse, and broad band proton decoupling. The samples contained 2 mM 5FTA, 10 mM d-DNA, and 20 mM DNA and required about 30 min of signal averaging.

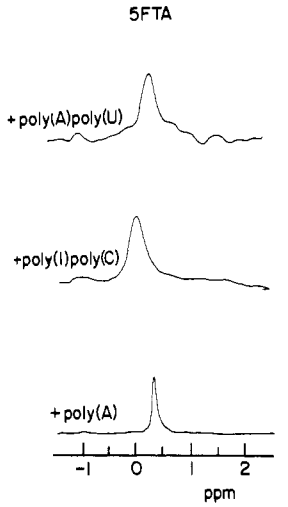

FIGURE 4: ^{19}F NMR spectra of 5FTA in the presence of single- and double-stranded polyribonucleotides at 25 °C. The samples contained 2 mM 5FTA, 10 mM poly(A), 20 mM poly(I)·poly(C), and 20 mM poly(A)·poly(U). See Figure 3 for details.

described above. Denatured DNA was prepared by heating the sonicated DNA to 100 °C for 10 min, followed by rapid cooling to 0 °C.

^{19}F NMR experiments were performed on a Varian XL-100 spectrometer equipped with a Nicolet Fourier-transform accessory at 94.1 MHz. The temperature was maintained by blowing cooled nitrogen over the sample. Polynucleotide samples for ^{19}F NMR and thermal denaturation experiments were prepared to contain a 5-fold excess of binding sites. Therefore, the double-stranded polynucleotides contained a 10-fold excess of moles of phosphate, and the single-stranded polynucleotides contained a 5-fold excess. Actual concentrations are given in the appropriate figure legends. Control experiments were performed at higher ratios of phosphate to 5FTA to eliminate the possibility that free 5FTA contributed to the measured NMR properties of the polynucleotide complexes. A 20-fold excess of moles of phosphate led to no appreciable change in the chemical shift or line width of the complexes. In addition, the large excess had no effect on the spin–lattice relaxation time or the solvent-induced shift of the 5FTA–poly(A) complex. The relaxation measurements were performed as described elsewhere (James, 1975; James et al., 1978). For the off-resonance experiments a 0.23 G field applied 5.6-kHz off-resonance was utilized. Chemical shifts are reported with respect to the chemical shift of free 5FTA at pH 7.0 in 10% ^2H_2O; upfield shifts are reported with a positive sign.

Fluorescence measurements were performed on a Perkin-Elmer MPF-2A fluorescence spectrophotometer at room temperature (22–24 °C). The fluorescence measurements were made by exciting at 305 nm (to minimize inner filter effects from the nucleotides) and monitoring at 360 nm. The reported data are uncorrected for absorption by the nucleotides. Absorption measurements were made on a Beckman Acta CIII equipped with water-jacketed cells for temperature regulation. The temperature was measured by insertion of a thermistor probe into the reference cell.

Results

^{19}F Chemical Shifts. The ^{19}F resonance of 5FTA is sensitive to the presence and nature of added polynucleotides. Figure 3 shows the effect of denatured DNA (d-DNA) and native DNA on the 94.1-MHz ^{19}F NMR spectra of 5FTA. Both polynucleotides induce upfield shifts of the ^{19}F resonance, and the shift is accompanied by an increase in line width from ~1 Hz for free 5FTA to 60 Hz for the DNA complex. As shown in Figure 2, the line width is especially sensitive to the slower overall motions; thus, the observed line widths indicate that 5FTA is intimately associated with the nucleic acid. Figure 4 shows the effect of single- and double-stranded RNAs on the ^{19}F spectra of 5FTA. Poly(A) and poly(A)·poly(U) induce similar upfield shifts on the ^{19}F resonance (0.3 ppm) while poly(I)·poly(C) has no significant effect. Similar to the DNA samples, the RNAs induce line broadening of the ^{19}F resonance of 5FTA. The induced chemical shifts for all the nucleic acid complexes are compiled in Table I. As mentioned above, a large excess of nucleic acid ($P/D > 20$) led to no further changes in the ^{19}F chemical shift or line width. These data indicate that 5FTA binds to all the nucleic acids examined but that the binding to poly(I)·poly(C) is qualitatively different from the others. Although the induced shifts are in the direction and of the magnitude predicted from ring-current shifts which might result from stacking of 5FTA with the bases of the polynucleotides, the sensitivity of fluorine chemical shifts to environmental factors such as solvation precludes this as an unambiguous explanation (Gerig, 1978).

Table I: Induced Chemical Shifts and Solvent-Induced Shifts (SIS) for 5FTA and Its Nucleic Acid Complexes

sample[a]	$\Delta\delta$ [b]	SIS[c]
5FTA	0.0	0.22
5FTA + poly(A) (P/5FTA = 5, 25 °C)	0.34	0.18
5FTA + poly(A)·poly(U) (P/5FTA = 10, 25 °C)	0.33	0.13
5FTA + poly(I)·poly(C) (P/5FTA = 10, 25 °C)	0.06	0.22
5FTA + d-DNA (P/5FTA = 5, 37 °C)	0.31	0.01
5FTA + DNA (P/5FTA = 10, 37 °C)	0.61	0.01

[a] Samples were prepared in 0.01 M NaCl–0.01 M cacodylate at pH 7.0. P/5FTA refers to the ratio of moles of polynucleotide phosphates to 5FTA. [b] Chemical shifts are reported with respect to free 5FTA in buffer containing 10% ^2H_2O. Upfield shifts are reported positive. [c] The solvent-induced shift (SIS) is the chemical shift in 10% H_2O–90% ^2H_2O minus that in 90% H_2O–10% ^2H_2O.

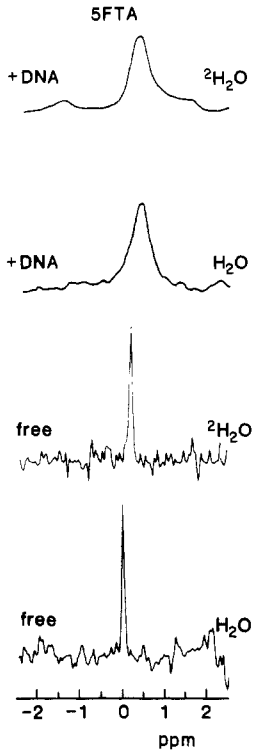

FIGURE 5: Solvent-induced shifts for 5FTA free and in the presence of DNA at 37 °C. The samples contained 2 mM 5FTA and 20 mM DNA and were recorded in either 90% H_2O–10% ^2H_2O or 10% H_2O–90% ^2H_2O.

Solvent-Induced Shifts. As noted above, fluorine resonances are sensitive to environmental factors. It has been shown, for example, that the chemical shift is sensitive to the isotopic composition of the solvent, with the chemical shift in H_2O usually being downfield with respect to that in ^2H_2O (Hull & Sykes, 1976). The sensitivity of the resonance to the solvent composition may be regarded as a measure of the solvent accessibility of the fluorine atom. Figure 5 shows the effect of solvent composition on the ^{19}F NMR spectra of 5FTA free in solution and in the DNA complex. The solvent-induced shifts (SIS) for these and all other complexes listed in Table I are determined as the chemical shift in 10% H_2O–90% ^2H_2O minus that in 90% H_2O–10% ^2H_2O. The maximum SIS, 0.22 ppm, is observed for 5FTA free in solution. In the DNA and d-DNA complexes the SIS was effectively quenched, indicating that the fluorine atom is isolated from solvent in these complexes. The binding of 5FTA to poly(A) or poly(A)·poly(U) less effectively shields the fluorine atom from the environment while 5FTA in the poly(I)·poly(C) complex appears accessible to the solvent.

^{19}F NMR Relaxation. Under Theory, we point out that the relaxation of the ^{19}F resonance is related to the molecular motions of 5FTA. Table II lists the relaxation parameter values for free 5FTA and its nucleic acid complexes. In all cases the presence of the polynucleotide has a significant effect on the relaxation parameters. The results have been interpreted with a two correlation time model in which 5FTA undergoes a sliding motion parallel to the bases with the characteristic internal motion correlation time τ_i and an overall isotropic motion τ_0 which is thought to be characteristic of bending motions in polynucleotides. The values of the correlation times in the polynucleotide complexes are compiled in Table III. For DNA, d-DNA, poly(A)·poly(U), and poly(A) at 10 °C, all of the relaxation parameters are well described by an internal motion correlation time of about 1 ns. This indicates that in these complexes the internal motion, here modeled as a rotation about the bond shown in Figure 1, is severely restricted relative to free 5FTA. For 5FTA free in solution this motion is expected to have a correlation time of about 0.01 ns. In the poly(A) complex at 25 °C this motion is less severely restricted while the presence of poly(I)·poly(C) has only a small effect on this motion.

Table II: ^{19}F NMR Relaxation Parameter Values for 5FTA Free and in Its Nucleic Acid Complexes

sample[a]	$W_{1/2}$ (Hz)	T_1 (s)	NOE	$T_1\rho^{off}$ (s)	R[b]
5FTA (25 °C)	1	3.16	1.5		
5FTA + poly(A) (10 °C)	10	0.49	0.78	0.41	0.84
5FTA + poly(A) (25 °C)	8	0.91	1.10	0.86	0.95
5FTA + poly(A)·poly(U) (10 °C)	45	0.66	0.65	0.29	0.44
5FTA + poly(A)·poly(U) (25 °C)	40	0.81	0.82	0.46	0.56
5FTA + poly(I)·poly(C) (10 °C)	60	1.39	1.25	0.49	0.36
5FTA + poly(I)·poly(C) (25 °C)	60	1.40	1.25	0.63	0.45
5FTA + d-DNA (37 °C)	30	0.59	0.89	0.46	0.48
5FTA + DNA (37 °C)	60	0.49	0.58	0.25	0.51

[a] Samples were prepared in 0.01 M NaCl–0.01 M cacodylate–10% ^2H_2O, at pH 7.0, to contain a 5- or 10-fold excess of polynucleotide phosphates as described under Materials and Methods. [b] For measurement of the off-resonance parameters a 0.23 G field applied 5.6-kHz off-resonance was utilized.

Table III: Internal and Overall Motion Correlation Times for 5FTA in Its Nucleic Acid Complexes

sample[a]	T (°C)	τ_i (ns)	τ_0 (ns)
5FTA + poly(A)	10	1	100
5FTA + poly(A)	25	0.3	70
5FTA + poly(A)·poly(U)	10	1	1000
5FTA + poly(A)·poly(U)	25	0.8	800
5FTA + poly(I)·poly(C)	10	0.1	1000
5FTA + poly(I)·poly(C)	25	0.1	800
5FTA + d-DNA	37	1	300
5FTA + DNA	37	1	1000

[a] Samples were prepared to contain a 5-fold excess of nucleic acid as described under Materials and Methods.

Similarly, the correlation time for the overall motion may be inferred from the relaxation parameters. DNA, poly(A)·poly(U), and poly(I)·poly(C) give similar values of about 1000 ns for this motion. The poly(A) and d-DNA complexes have faster overall correlation times of 100 and 300 ns, respectively. The overall motions are similar to the values obtained from ^{13}C and ^{31}P NMR studies on free nucleic acids for DNA, poly(I)·poly(C), and poly(A) (Bolton & James, 1979, 1980a,b) but shorter than the overall correlation times observed for fluorinated intercalators bound to DNA and poly(A) (Bolton et al., 1981; P. A. Mirau, R. H. Shafer, T. L. James, and P. H. Bolton, unpublished results).

Thermal Denaturation and Fluorescence. The effect of 5FTA on the helix to coil transition of the double-stranded polynucleotides has been studied by monitoring the hyperchromism of the polynucleotides as a function of temperature at 260 nm. In all cases the samples contained a 10-fold excess of moles of phosphate to 5FTA. Figure 6 shows the effect of 5FTA on the melting transitions of poly(I)·poly(C) and DNA. In both cases the presence of 5FTA has no measurable effect on the transition. Similar results are observed for the melting of poly(A)·poly(U). Since a change in melting temperature is related to the relative affinity of a ligand to the helix and

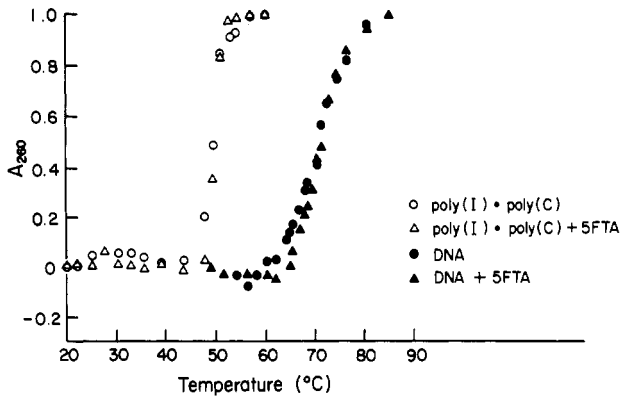

FIGURE 6: Thermal denaturation of poly(I)·poly(C) (○, △) and DNA (●, ▲) in the absence and presence of 5FTA. The data are plotted as the normalized fractional increase in absorption vs. temperature. The concentration of polynucleotides and 5FTA was 7.5×10^{-5} M and 7.5×10^{-6} M, respectively. The absorbance of the polynucleotide was measured at 260 nm.

coil form, the above lack of effect indicates that 5FTA has an equal affinity for the single- or double-stranded nucleic acids. Others have reported little or no effect on DNA thermal denaturation due to the presence of similar amino acid analogues (Gabbay et al., 1972).

It has been previously noted that the fluorescence of 5-hydroxy- and 5-methoxytryptamine is quenched in the presence of nucleic acids (Hélène et al., 1971). The fluorescence quenching of 5FTA (uncorrected for inner filter effects) is qualitatively the same as that reported for the above mentioned ligands. In the presence of DNA, for example, a 20-fold excess of nucleotides led to ~8% quenching and a 200-fold excess led to a 50% quenching. The similarity in results observed for 5FTA and the nonfluorinated analogues suggests that fluorine is a nonperturbing probe of the ligand–nucleic acid complex.

Discussion

The ^{19}F chemical shift and relaxation parameters of 5FTA are sensitive to the presence of polynucleotides. With the exception of poly(I)·poly(C), all polynucleotides induce 0.3–0.6-ppm upfield shifts. Binding by intercalation might be expected to induce upfield shifts on the fluorine atom from the ring currents of the bases. However, solvent effects are known to play a role in ^{19}F chemical shifts, and the change from an aqueous environment to a hydrophobic one (i.e., intercalation) is expected to shift the resonance to lower field (Hull & Sykes, 1976). The ^{19}F resonance of fluorinated analogues of the intercalators chloroquine and quinacrine, fluoroquine and fluoroquinacrine, has been observed to shift 1.6 ppm downfield in the presence of DNA. These data indicate a difference in the environment of the fluorine atom in the intercalator complexes and the 5FTA–polynucleotide complexes (Bolton et al., 1981; P. A. Mirau, R. H. Shafer, T. L. James, and P. H. Bolton, unpublished results).

The solvent-induced shifts suggest a difference in solvent accessibility for 5FTA bound to polynucleotides, ranging from fully protected when bound to DNA and d-DNA to partially protected when bound to poly(A) and poly(A)·poly(U) to nonprotected when bound to poly(I)·poly(C). The intrinsic sensitivity of fluorine to such solvent effects is one of the more unique aspects of ^{19}F NMR. For example, the lack of SIS in the 5FTA–DNA complex suggests that simple binding to the outside of the helix is not involved. Similarly, the SIS of fluoroquine was quenched in the presence of DNA and tRNA and less efficiently quenched by poly(A). It is interesting to note this difference in the binding of 5FTA to DNA vs. RNA.

The ^{19}F relaxation parameters are of use in probing the dynamic nature of the complex. The parameters R and the line width are sensitive to the slow motions in the polynucleotides and are clear indicators that a complex is being formed under the conditions of our experiments. The relaxation parameters $T_{1\rho}^{off}$, NOE, and T_1 are sensitive to the internal motion of the indole ring which we have modeled as a rotation about the C–C bond shown in Figure 1. These parameters indicate that DNA, d-DNA, poly(A)·poly(U), and poly(A) at 10 °C have a similar effect on the internal motion, where this motion is restricted to about 1 ns. The internal motion correlation time for 5FTA is similar to that observed for the fluoroquine– and fluoroquinacrine–polynucleotide complexes. This implies that the internal motion of 5FTA is not rigidly coupled to the motions of the bases which is expected to occur on a time scale of about 30 ns (Genest & Wahl, 1978; Barkley & Zimm, 1979). Poly(I)·poly(C) and poly(A) at 25 °C impose a less severe constraint on this motion.

Fluoroquine and fluoroquinacrine also had a much more dramatic effect on the overall bending motions, where 3000-ns overall motion correlation times were observed for the DNA complexes. These are longer than the correlation times for free DNA (1000 ns) as inferred from ^{31}P and ^{13}C NMR measurements (Bolton & James, 1979, 1980a,b). This increase presumably comes from an increase in the persistence length which might be expected to accompany binding by intercalation. For 5FTA, no such increase in the overall correlation time is noted. Also, these intercalators induce a 10–12 °C increase in melting temperature whereas 5FTA has little effect.

The fluorescence of the intercalators is quenched 60–75% with a 20-fold excess of phosphate while much higher ratios are required for quenching of 5FTA. The binding to nucleic acids by intercalation often increases the fluorescent lifetime of the intercalators while even at 50% fluorescent quenching of 5-hydroxytryptamine and 5-methoxytryptamine no change in fluorescent lifetime was observed (Hélène et al., 1971). Intercalators also have the general property of unwinding supercoiled DNA whereas tryptamine, tyramine, phenylalanine, and histamine had no effect on this property (Jacobsen & Wang, 1973).

Taken together, the above data suggest that 5FTA does not bind nucleic acids by the "classical" intercalation mechanism. However, the partial intercalation model (Gabbay et al., 1972), in which the indole ring is partly inserted between the bases, is consistent with our spectroscopic observations. Formation of the partial intercalation complex might be expected to induce changes in the ^{19}F chemical shift due to both ring-current shifts and a change in solvation between the free and the bound states. This model would predict a geometry-dependent quenching of the SIS and a reduction of the mobility of the 5FTA. In particular, the essentially complete protection from solvent in the 5FTA–DNA complex is consistent with insertion of the aromatic end of the ligand between the base pairs. Since the indole ring is not large enough to fill the intercalation "cavity", it is not suprising that 5FTA exhibits some motion relative to the base pairs or shows no great preference for single- vs. double-stranded nucleic acids. Partial intercalation is expected to be less of a perturbation of polynucleotide conformation than is intercalation; this is consistent with our observation that the binding of 5FTA has little effect on the overall motion correlation time. A complex of this geometry would lead to less perturbation of the fluorescence and the fluorescent lifetimes.

These results illustrate several interesting features which may be important in protein–nucleic acid interactions. Perhaps most interesting is the observation of a binding specificity at the amino acid level. With little induced chemical shift, no quenching of the SIS, and little restriction of the internal motion correlation time, it appears that 5FTA binds to the outside of the poly(I)·poly(C) double helix. The differences in the induced chemical shifts and solvent isotope shifts for the various complexes indicate that the details of complex formation are sensitive to the nature and conformation of the polynucleotide. Additional experiments are required to determine if the specificity is due to the interaction of 5FTA with some base or some conformational feature of the nucleic acid. The differences in the complexes with RNA and DNA suggest that tryptophan may play a role in the recognition of nucleic acid binding sites. Since the binding has little effect on the long-range motions, it is not expected that tryptophan binding by itself will induce long-range distortions of the helix.

It is interesting to compare the results from this model study with those obtained from the binding of fluorotyrosine-labeled gene 5 protein to nucleotides (Coleman et al., 1976; Coleman & Armitage, 1977). In the presence of nucleotides the three surface fluorotyrosine resonances were shifted upfield 0.1–0.3 ppm and the NOE was observed to decrease. The similarity between those results and ours presented here suggests that 5FTA may be a reasonable model for studying the role of tryptophan in protein–nucleic acid interactions.

Summary

This report, along with our two previous studies of fluorinated ligands, demonstrates how ^{19}F NMR may be used to discriminate between the various possible binding modes. In particular, the differences in chemical shift and overall correlation time between the 5FTA–DNA complexes and the intercalator complexes suggest that 5FTA does not bind to DNA by the classical intercalation mode. Outside binding, presumably mediated mainly by electrostatic interactions, would not be expected to quench the SIS. Binding by partial intercalation of the fluorine-containing aromatic ring reconciles these differences in a mode of interaction consistent with all experimental data. Work is now in progress in our laboratory to examine the nucleic acid binding properties of more complex fluorine-containing peptides.

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