Intercalation Complex of Proflavine with DNA: Structure and Dynamics by Solid-State NMR

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The structure of the complex formed between the intercalating agent proflavine and fibrous native DNA was studied by one- and two-dimensional high-resolution solid-state nuclear magnetic resonance (NMR). Carbon-13–labeled proflavine was used to show that the drug is stacked with the aromatic ring plane perpendicular to the fiber axis and that it is essentially immobile. Natural abundance carbon-13 NMR of the DNA itself shows that proflavine binding does not change the puckering of the deoxyribose ring. However, phosphorus-31 NMR spectra show profound changes in the orientation of the phosphodiester grouping on proflavine binding, with some of the phosphodiesters tilting almost parallel to the helix axis, and a second set almost perpendicular. The first group to the phosphodiesters probably spans the intercalation sites, whereas the tilting of the second set likely compensates for the unwinding of the DNA by the intercalator.

A WIDE RANGE OF MUTAGENS, drugs, and carcinogens can interact with double-stranded nucleic acids by intercalation (*1*) and thereby inhibit both replication and transcription; several such agents are important in clinical pharmacology. However, the molecular nature of the structural change in DNA that allows the interbase spacing to essentially double to accommodate the intercalator has been unclear. Models for the intercalation complex have involved several diverse distortions of the basic DNA helix. Most such have been based on crystallography of small oligomers complexed with intercalators. An early model (*2*) suggested that the DNA double helix surrounding the intercalator might have an asymmetrical arrangement of the backbone, with an alternating C3′-endo–C2′-endo sugar pucker. Theoretical calculations suggested that the mixed C3′-endo (3′-5′)–C2′-endo pucker was intrinsically more stable than the uniform C3′-endo conformation (*3*). However, the crystal structure of a daunomycin-d (CGTACG) complex has sugar puckers that fall within the C2′-endo family (*4*), while the phosphodiester linkages along the DNA backbone have a tg^- conformation with respect to the C5′, instead of the g^-g^- conformation of an idealized B-DNA duplex. Bis-intercalators, such as triostin, can also alter the type of hydrogen bonding found in DNA base pairs; some purines are in the syn conformation and Hoogsteen base pairing coexists with Watson-Crick base pairs in the crystal structures of DNA oligomers complexed to triostin A (*5*, *6*). Solution NMR studies have confirmed this result (*7*, *8*).

Both of these techniques have, however, the major disadvantage that they cannot be applied to native DNA, whereas fiber diffraction, which can, is not at present applicable to most intercalators. Two-dimensional (2-D) solid-state NMR methods (*9*), in which cross polarization and magic angle spinning (CP-MAS) are used, can in principle determine orientations of each of the individual residues in a complex oriented solid (*10*, *11*); these can be applied to highly oriented DNA films produced by wet spinning methods and can be used to determine backbone orientations. Simple 1-D ^{13}C CP-MAS NMR of DNA fiber samples is in itself sufficient to determine the ring pucker (*12*). We applied these methods to the DNA intercalation complex of proflavine (3,6-diaminoacridine), an antibacterial and mutagenic agent that is one of the simplest intercalating agents known.

Complexes of isotopically labeled proflavine with native B-DNA films can be readily prepared (*13*–*16*); we can verify that the proflavine signal arising from the drug-DNA sample indeed arises from bound proflavine by an inversion recovery–cross polarization pulse sequence. In this sequence, the proton magnetization M is inverted with a 180° pulse. After a variable relaxation delay τ, the proton magnetization is detected by cross polarization to ^{13}C. The results of the application of this sequence of DNA are shown in Fig. 1. The top spectrum, where τ is much greater than the proton relaxation time T_1, shows resonances both from labeled proflavine and natural abundance DNA. The proflavine signal is composed of a center band and a set of rotational side bands whose intensities can be used to determine the principal values of the chemical shielding tensor (*17*). The ^{13}C-9 chemical shift tensor principal values of free proflavine hydrochloride (σ_{11} = 229 ppm, σ_{22} = 171 ppm, and σ_{33} = 15 ppm) and those of proflavine bound to DNA (σ_{11} = 228 ppm, σ_{22} = 169 ppm, and σ_{33} = 31 ppm) are similar. The 4-ppm downfield shift in the drug-bound form may reflect hydrogen-bonding changes; the shift appears to be localized in the σ_{33} tensor element. The near identity of the two downfield tensor elements between free and bound proflavine means that the molecule is not undergoing significant motion in the DNA bound state, and in particular is not free to rotate about the ring normal. Deuterium NMR data corroborate this finding, suggesting that the pronounced in-plane motion detected in some crystallographic studies of DNA oligomer-proflavine complexes (*18*) may not be so significant in native DNA. The bottom spectrum in Fig. 1 (τ = 100 ms) shows both proflavine and DNA signals inverted. The time constants for the recovery of the signals of both the drug and the DNA as a function of τ are identical within experimental error, implying that both drug and DNA are being cross polarized by the same pool of protons. This requires that the proflavine protons and the DNA and water protons in the sample be able to spin-diffuse to equilibrium in a time short compared with T_1, and thus that the sample is microscopically homogeneous.

A 2-D CP-MAS ^{13}C spectrum of an oriented B-DNA film sample containing bound proflavine, obtained at a spinning speed ν_R of 2.6 kHz, is shown in Fig. 2A. Displayed are slices through the 2-D spectrum parallel to the ω_2 axis at intervals of $M\nu_R$ in ω_1. In the ω_1 dimension, only even-numbered slices are plotted because there is no significant signal intensity at odd-integer multiples of the spinning speed in the ω_1 dimension when the sample axis is oriented

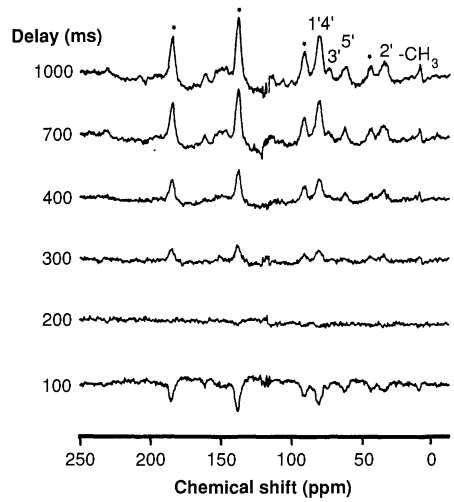

Fig. 1. An inversion-recovery CP-MAS spectrum of [9-^{13}C]proflavine-DNA as a function of the recovery period τ. The mole fraction of proflavine to DNA base pairs is 0.20; the ^{13}C-9 resonance of proflavine and its rotational side bands are marked with *.

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at 90° to the rotor axis (*9*). The presence of positive and negative side bands from the proflavine in the $M \neq 0$ slices shows that the molecules have some preferential orientation with respect to the rotor axis. These side band intensities can be fit to an orientational distribution function (ODF) of the shielding tensor, with the use of a spherical harmonic expansion by methods previously described (*9*, *11*). This function describes the probability of finding the chemical shielding tensor principal axis system at some particular orientation relative to the fiber axis, expressed as two polar angles χ and ø, where χ is the angle between the σ_{33} principal axis and the fiber axis, and ø is the angle between the projection of the fiber axis on the $\sigma_{11} = \sigma_{22}$ plane, and the σ_{11} axis. For aromatic carbons, these principal axes are simply related to the molecular geometry: the σ_{33} axis is parallel to the ring normal, whereas the σ_{11} axis is along the C–H bond (*19*).

The proflavine C-9 ODF is represented as a contour plot in Fig. 2B. The strong maximum at $\chi = 0°$ indicates that the ring normal is parallel to the fiber axis. This is consistent with the proflavine binding in an intercalative mode. The width of the distribution function is similar to that obtained for the base pairs in B-DNA.

In earlier work we showed that we can reliably distinguish between 3′-endo and 2′-endo ring puckers in DNA on the basis of the ^{13}C chemical shifts at the 3′ and 5′ positions. The 3′-endo conformers have 3′ and 5′ chemical shifts significantly (5 to 10 ppm) upfield of comparable 2′-endo conformers. The deoxyribose region of the ^{13}C NMR spectra of B-DNA (Fig. 3A) and of proflavine-DNA (Fig. 3B) are similar, with only slight differences in chemical shift in the sugar carbon resonances, and no evidence of signals at the positions expected for 3′-endo sugars. (The apparent weak signals along the baseline of both spectra are rotational side bands from the DNA-base carbon resonances.) This finding conflicts with early alternating pucker models, but is consistent with more recent x-ray structures of larger oligomers complexed with daunomycin (*4*) and triostin (*5*, *6*), which also failed to show 3′-endo puckering.

The ^{31}P 2-D–NMR spectra of B-DNA (Fig. 4A) and proflavine-bound DNA (Fig. 4, B and C) were obtained by the same method as used in Fig. 2. At low levels of proflavine (Fig. 4B) the spectrum is qualitatively similar to that of B-DNA for both the center slice and the side bands in the ω_1 dimension. However, for higher levels (Fig. 4C) there are marked changes in the side band intensities in the center and side band slices, indicating a major change in the phosphodiester group orientation in the proflavine-DNA complex.

The ODF of the phosphorus tensor of B-DNA is depicted in Fig. 4D. It shows a broad maximum at $\chi = 55°$, ø = 5°. For comparison, we superimposed the ø and χ angles obtained by a computer fit of a straight helix to the B-DNA dodecamer (*20*). Our measured ODF corresponds reasonably well in both overall orientation and width to the range of angles observed in the oligomer, albeit with a somewhat smaller ø angle. The high-proflavine ODF (Fig. 4F) is totally different, with pronounced maxima at $\chi = 0°$ and $\chi = 90°$, ø = 0°. In Fig. 4 it is compared with phosphate orientations calculated for the triostin-DNA octamer complex. The lower proflavine concentration (Fig. 4B) is intermediate between Fig. 4A and Fig. 4C, with a maximum centered near that of native B-DNA, but pronounced tails approximately directed toward the maxima for highly proflavine-doped DNA. Each of these ODFs was obtained by weighted averaging of data from several 2-D spectra obtained at different spinning speeds; data calculated from individual spectra and from different samples are highly reproducible; in addition, the standard deviation of the orientation distribution in no case exceeds 10%.

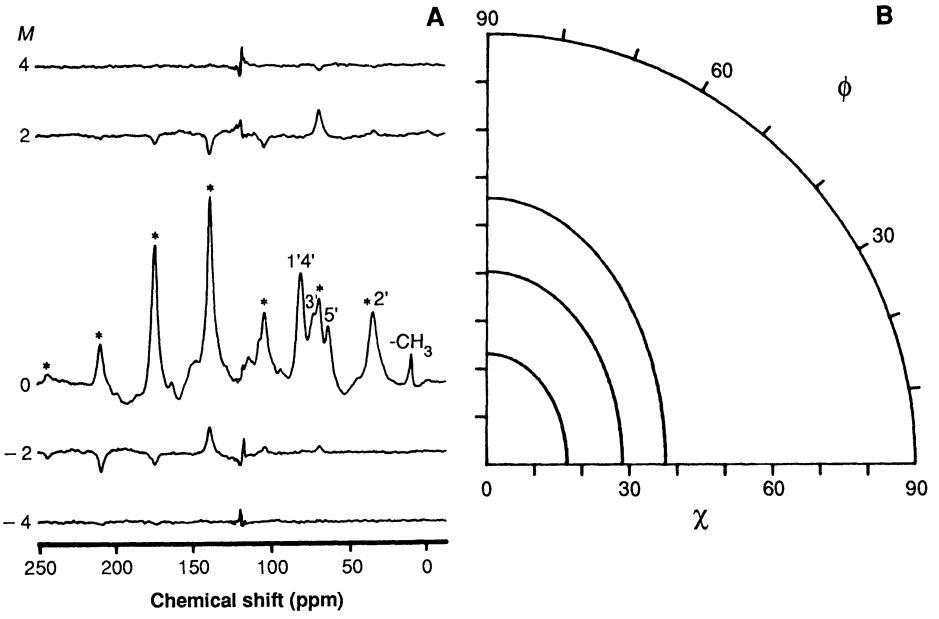

Fig. 2. (**A**) Slices parallel to the ω_2 axis from a 2-D ^{13}C CP-MAS NMR spectrum of the oriented proflavine-DNA complex [mole ratio of proflavine (P) to B-DNA (B) = 0.20], obtained at a spinning speed of 2.666 kHz. The proflavine ^{13}C-9 resonance and its rotational side bands are marked with *; the DNA sugar carbons and thymine methyl carbon are also labeled. (**B**) Contour diagram of the orientation distribution function of the ^{13}C shielding tensor of the C-9 of proflavine intercalated to DNA, obtained by a least-squares fit to experimental 2-D MAS data such as those shown in (A). The contours are spaced at intervals of 1 unit. For comparison, a purely isotropic ODF would have an intensity of 1 unit everywhere. χ and ø are the polar angles relating the fiber axis to the frame of reference of the chemical shielding tensor, χ being the angle between the most shielded principal axis (perpendicular to the ring plane) and fiber axis, and ø the angle by which the fiber axis is rotated out of the tensor xz plane (which contains the C–H bond axis).

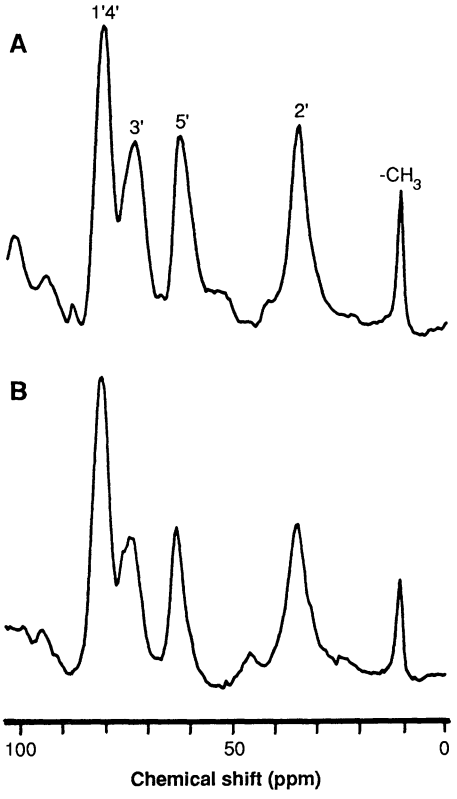

Fig. 3. (**A**) Aliphatic region of the ^{13}C CP-MAS spectrum of B-DNA. (**B**) The same region of the spectrum of proflavine-DNA. The near identity of the two spectra rules out any significant fraction of 3′-endo sugars in proflavine DNA.

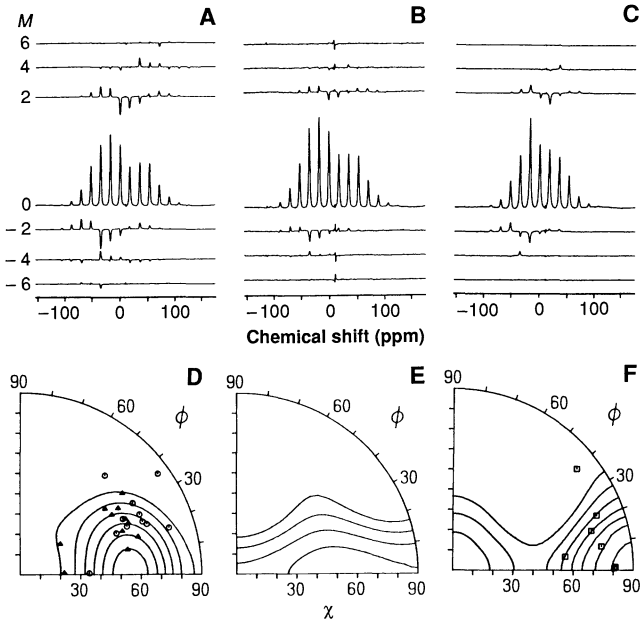

Fig. 4. Experimental ^{31}P 2-D CP-MAS spectra of oriented DNA and proflavine-treated DNA showing slices through the spectrum at intervals of $M\nu_R$ parallel to the ω_2 axis. (**A**) Pure B-DNA; (**B**) DNA with a low mole fraction of proflavine per base pair (P/B = 0.04); (**C**) DNA with a high mole fraction of proflavine per base pair (P/B = 0.20). (**D** through **F**) Contour plots of the orientational distribution functions for the ^{31}P shielding tensor of the phosphodiester grouping of DNA, obtained from 2-D MAS data; plots D, E, and F correspond to the spectra labeled A, B, and C, respectively. Contours are spaced at intervals of 0.5 unit, with the minimum contour plotted at 1 unit.

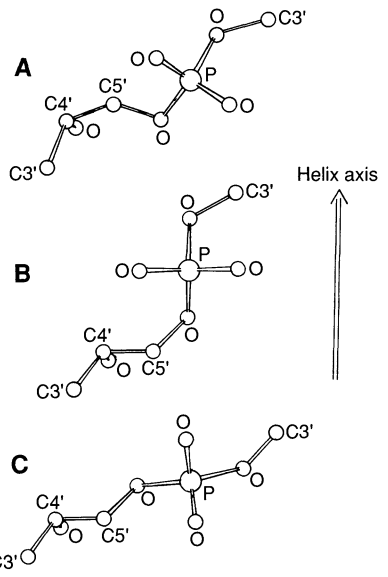

Fig. 5. Orientations of the phosphodiester moiety and adjacent DNA backbone (**A**) in B-DNA (**B**) spanning the intercalated site in proflavine-DNA (**C**) in sites not spanning the intercalator in proflavine-DNA. The phosphodiester orientations are obtained from the contour plots in Fig. 4; the rest of the backbone, in particular the C4′-C5′ torsion angle, is consistent with carbon NMR data.

The phosphodiester σ_{11} (downfield) principal axis lies along a vector connecting the two ester oxygens, whereas σ_{33} connects the two free oxygens (*21*). The data suggest that for the proflavine-DNA complex, there are two predominant orientations (Fig. 5). In one, the phosphodiester linkage lies almost parallel to the helix axis; in the second, almost perpendicular. The parallel orientation is similar to the distribution of orientations for the triostin oligomer complex of Wang *et al.* (*6*), and we believe that it arises from phosphodiesters that link the base pairs surrounding the proflavine intercalator. This orientation makes the longitudinal spacing between the two oxygens nearly maximal. The other major maximum, with a nearly perpendicular orientation of the axis through the phosphodiester oxygens, arises from the phosphodiester groups not directly involved in intercalation. Because intercalation causes local unwinding of the DNA helix, in a high molecular size sample, where rotation of the DNA helices is restricted, unwinding at one region of the DNA must be compensated by tighter winding at others. Such winding could be facilitated by the phosphodiester group lying perpendicular to the helix axis, maximizing the tangential translation per base pair.

What of the rest of the DNA backbone? Pivoting the phosphodiester group alone cannot increase the interbase spacing enough to accommodate an intercalator. However, ^{13}C 2-D CP-MAS data reveal no significant change in the ODFs of the deoxyribose carbons in proflavine-treated samples. These DOFs are principally sensitive to the angle between the C–O bond vectors and the fiber axis. The one major alteration that might go undetected is a change in the orientation of the C5′–O bond from its disposition in B-DNA, where it lies somewhat antiparallel and at an angle to the helix axis, to an orientation parallel to and at the same angle to that axis. This change in orientation might accompany a change in conformation from g^-g^- to tg^- about the C5′–O bond. This conformational change is also shown in Fig. 5. Although one might expect this change to also affect the C5′ chemical shift, it is not clear how large the change would be.

These data illustrate a new experimental technique that may be of immense value in determining the structure and dynamics both of drugs bound to DNA and of the DNA itself. These studies exemplify two of the strengths of solid-state NMR: that it can be used to probe material of arbitrary molecular size and that it gives orientational information that is complementary to distance information from diffraction and solution NMR.

REFERENCES AND NOTES

1. L. S. Lerman, *J. Mol. Biol.* **3**, 18 (1961).
2. A. H.-J. Wang, J. Nathans, G. van der Marel, J. H. van Boom, A. Rich, *Nature* **276**, 471 (1978).
3. A. Dearing, P. Weiner, P. A. Kollman, *Nucleic Acids Res.* **9**, 1483 (1981).
4. A. H.-J. Wang, G. Ughetto, G. J. Quigley, A. Rich, *Biochemistry* **26**, 1152 (1987).
5. A. H.-J. Wang *et al.*, *Science* **225**, 1115 (1984).
6. A. H.-J. Wang, G. Ughetto, G. J. Quigley, A. Rich, *J. Biomol. Struct. Dynam.* **4**, 319 (1986).
7. X. Gao and D. J. Patel, *Biochemistry* **27**, 1744 (1988).
8. D. E. Gilbert, G. A. van der Marel, J. H. van Boom, J. Feigon, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3006 (1989).
9. P. Tang, R. A. Santos, G. S. Harbison, *Adv. Magn. Reson.* **13**, 225 (1989).
10. G. S. Harbison and H. W. Spiess, *Chem. Phys. Lett.* **124**, 128 (1986).
11. G. S. Harbison, V.-D. Vogt, H. W. Spiess, *J. Chem. Phys.* **86**, 597 (1987).
12. R. A. Santos, P. Tang, G. S. Harbison, *Biochemistry* **28**, 9372 (1989).
13. [9-^{13}C]proflavine was synthesized from [^{13}C]formic acid by a modification of published methods (*14*). Calf thymus DNA in the sodium form was purchased from Sigma. To replace the sodium with a lithium counterion, the DNA was dissolved in 0.4 M LiCl solution and dialyzed three times against 2.5 M LiCl. The material was precipitated with ethanol, dried, and stored at 66% humidity, giving B-form DNA (*15*). Oriented LiDNA films produced by wet-spinning (*11*, *16*) were immersed for 1 week in a bath solution composed of 80% aqueous alcohol, 0.25 M LiCl, and a defined proflavine concentration. The sample was dried over silica gel and equilibrated in an atmosphere of 66% relative humidity for several weeks before NMR measurements. The samples were mounted in MAS rotors with the order axis oriented perpendicular to the rotor axis. The methods used to obtain and interpret 1-D and 2-D spectra of oriented DNA have been described previously (*9*, *12*). Typical sample quantities were 80 mg; the repetition times are 4 s; usually 1024 and 32 transients were averaged for each free induction decay in ^{13}C and ^{31}P 2-D MAS experiments, respectively.
14. A. Albert, *J. Chem. Soc.* **1941**, 121 (1941).
15. A. Rupprecht and B. Forslind, *Biochem. Biophys. Acta* **204**, 304 (1970).
16. A. Rupprecht, *Biochem. Biophys. Res. Commun.* **12**, 163 (1963).
17. J. Herzfeld and A. E. Berger, *J. Chem. Phys.* **73**, 6021 (1980).
18. A. K. Aggarwal and S. Neidle, *Nucleic Acids Res.* **13**, 5671 (1985).
19. See, for example, W. Veeman, *Prog. NMR Spectrosc.* **16**, 193 (1984).
20. R. E. Dickerson and H. R. Drew, *J. Mol. Biol.* **149**, 761 (1981).
21. J. Herzfeld, R. G. Griffin, R. A. Haberkorn, *Biochemistry* **17**, 2711 (1978).

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