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Determination of Internuclear Angles of DNA Using Paramagnetic-Assisted Magnetic Alignment

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Paramagnetic ions have been used to assist the magnetic alignment of DNA. The anisotropy of the binding sites is sufficient to give rise to significant alignment of the DNA with the observed proton–carbon dipolar couplings spanning a 70-Hz range. The dipolar couplings have been used to determine the positions of the axial and rhombic alignment axes. The positions of the alignment axes relative to the positions of the binding sites of the paramagnetic europium ions have also been determined.

Key Words: **magnetic alignment; DNA; paramagnetic; NMR; quadruplex DNA.**

Diffraction maps contain information about both the distances between the electron densities of atoms and their relative angles, in three-dimensional inverse space, and this combination of scalar and vectorial data gives rise to the power of crystallography. The determination of the structures of molecules in solution by NMR methods typically relies, to a considerable degree, on the information provided by the nuclear Overhauser effect which is related to the internuclear distances. Angular information would provide a significant enhancement of the utility of solution state NMR methods particularly in biomolecular applications (*1–5*). Partial alignment of molecules in large magnetic fields can give rise to dipolar couplings which are dependent on the orientation of the internuclear vectors relative to the magnetic alignment axis (*6–11*). However, many molecules do not have enough magnetic susceptibility anisotropy to overcome Brownian motion. The large magnetic moments of paramagnetics can provide a route to significant magnetic alignment as long as the environments of the paramagnetics are sufficiently anisotropic (*8*). The magnetic susceptibility anisotropy arises from having a preferred orientation of the magnetic moment of the paramagnetics relative to the molecular framework.

To demonstrate the use of paramagnetic-assisted magnetic alignment of biomolecules we first determined the positions of the two binding sites of paramagnetic europium ions to d(G-GTTGGTGTGGTTGG) which is known to form a quadruplex structure in the presence of potassium (*12, 13*). Europium was chosen since it has a relatively short electron relaxation time and hence shifts more than broadens resonances. Also, the binding of europium to DNA displaces some of the waters of hydration potentially giving rise to an asymmetric site (*14*). Following the methods we previously used for the determination of the positions of the manganese binding sites to this DNA (*15*) it was found that the two europium ions bind to the two narrow grooves as shown in Fig. 1. The intereuropium distance is 23.3 Å.

The heteronuclear ^{13}C–^{1}H couplings of the protons of the DNA–europium complex were determined at natural abundance at 400 and 750 MHz as described previously (*4*) using standard heteronuclear correlation experiments. The observed couplings are the sum of the dipolar and scalar couplings with the dipolar coupling dependent on the square of the field strength (*6–11*). The observed dipolar couplings are listed in Table 1. The presence of the europiums increases the alignment by more than an order of magnitude relative to that observed for the free DNA (*4*). The aromatic and methyl sites were chosen for this study as these contain information about the orientation of the aromatic bases and the chemical shifts and linewidths of most of the aromatic resonances are the same in the presence and in the absence of the europiums. The ribose sites are also of interest, though many are shifted by the presence of the europiums, but were not examined due to limited access to high-field spectrometer time.

The sum of the squares of the deviations of all the dipolar couplings was determined as a function of the position of the alignment axis relative to the molecular framework. The dipolar coupling, D_{CH}, for a C–H internuclear vector is given by

$$D_{CH} = [(-h\gamma_C\gamma_H)\mu_0 S/32R^3\pi^3] \times [2A_a(3\cos^2\theta - 1) + 3A_r(\sin^2\theta\cos 2\phi)]$$

where h Planck's constant, γ_C the gyromagnetic ratio for ^{13}C, γ_H is the proton gyromagnetic ratio, θ and ϕ are the polar angles of the C–H vector relative to the magnetic alignment axis, R is the length of the particular C–H bond, the order parameter is S, μ_0 is the vacuum magnetic permeability, A_a is

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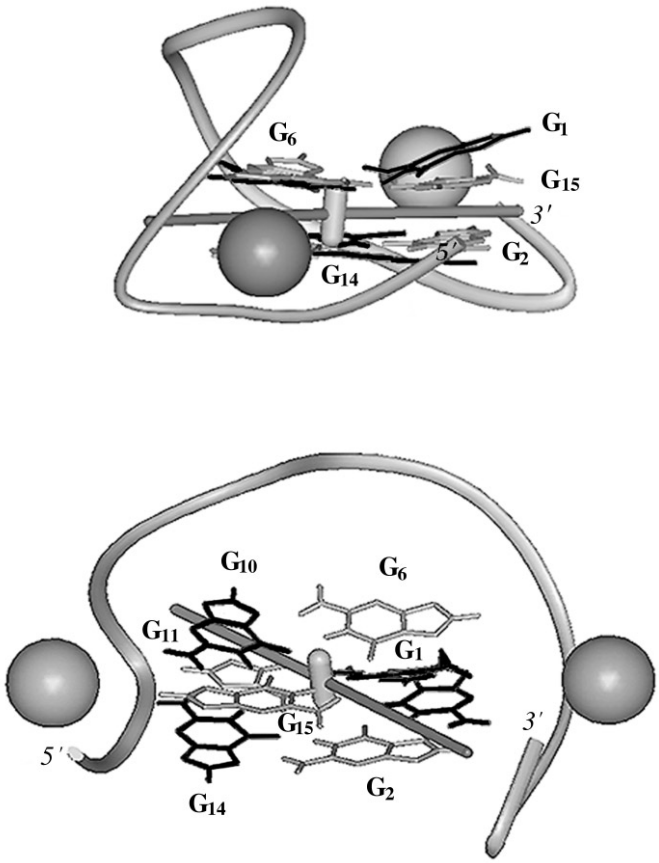

FIG. 1. The backbone of d(GGTTGGTGTGGTTGG) is depicted as a ribbon and the guanine residues involved in the two quartets are shown as lines. The positions of the europium ions are shown as spheres and the magnetic alignment axes are shown with the axial alignment axis as the thinner cylinder and the rhombic axis as the thicker cylinder. The lengths of the cylinders correspond to the ratio of A_a to A_r. Two views of the complex are shown. The coordinates of the aptamer–europium complex shown have been deposited at the Protein Data Bank. In this coordinate system the alignment axis, in spherical coordinates, is ζ of 26° and ψ of 355°. The inverted alignment with ζ at 154° and ψ at 175° is also at a minimum. ζ and ψ define the orientation of the alignment axis relative to the center of mass molecular coordinates and θ and ϕ the angles between the alignment axis and a particular internuclear vector.

TABLE 1
Experimental and Predicted Couplings

	$(D + J)$ 400 MHz	$(D + J)$ 750 MHz	Experimental D	Predicted D
G_1 H8	208	215	10	9.5
G_2 H8	207	224	24	21.7
T_3 Me	104	118	20	20.5
T_4 Me	104	117	18	21.2
G_5 H8	208	219	8	18.3
G_6 H8	192	205	18	15.7
T_7 H6	196	181	−21	−35.0
T_7 Me	103	118	21	8.6
G_8 H8	182	215	46	44.7
T_9 H6	184	175	−13	−27.1
T_9 Me	104	117	18	8.4
G_{10} H8	210	225	21	18.9
G_{11} H8	200	214	20	22.7
T_{12} H6	178	179	1	6.4
T_{12} Me	103	118	21	16.6
T_{13} Me	104	117	18	17.9
G_{14} H8	210	224	20	18.2

Note. $D + J$ is the sum of the experimental dipolar, D, and scalar, J, couplings. D is the dipolar coupling at 750 MHz. All of the values are in hertz.

the axially symmetric portion of the magnetic alignment tensor, and A_r is the rhombic component using the nomenclature previously proposed (*1*). Table 1 contains the predicted and observed dipolar couplings.

The deviation between the observed, D_{obs}, and predicted dipolar, D_{pred}, couplings was calculated as $(D_{obs}\text{-}D_{pred})^2$ divided by the number of dipolar couplings used in the calculation. The plot of this deviation between the predicted and experimental dipolar couplings as a function of the orientation of the alignment axis is shown in Fig. 2.

The plot illustrates that there is a well-defined minimum which allows the position of the alignment axis to be well defined. The rhombic contribution to the fit was 7%. The position of the alignment axis relative to the molecular framework is shown in Fig. 1. The alignment axis was determined assuming that all internuclear vectors have an S value of unity.

The effect of molecular motion on the determination of the

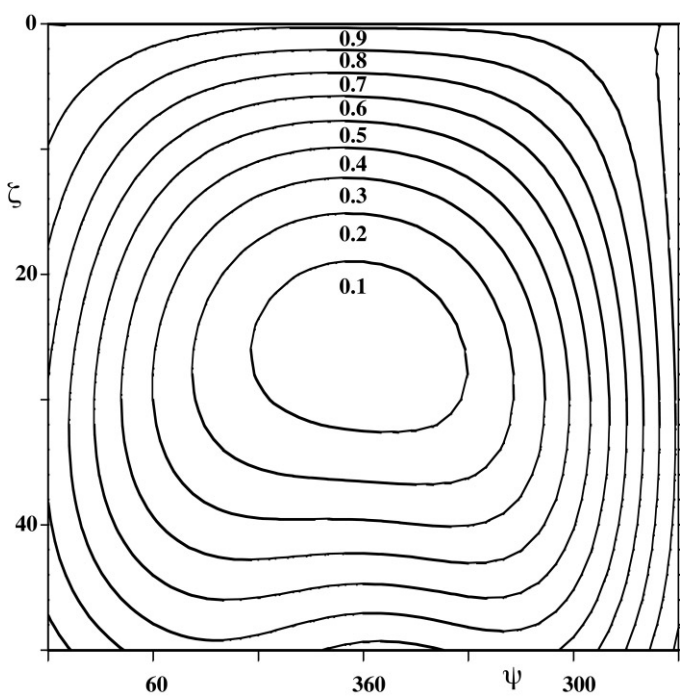

FIG. 2. The contour map shows the sum of the square of the deviations between the experimental and predicted dipolar couplings of the 15mer–europium complex. The magnitudes of the contours are indicated as a function of the spherical coordinates ζ and ψ. The values of the sum of the deviations were determined by a grid search.

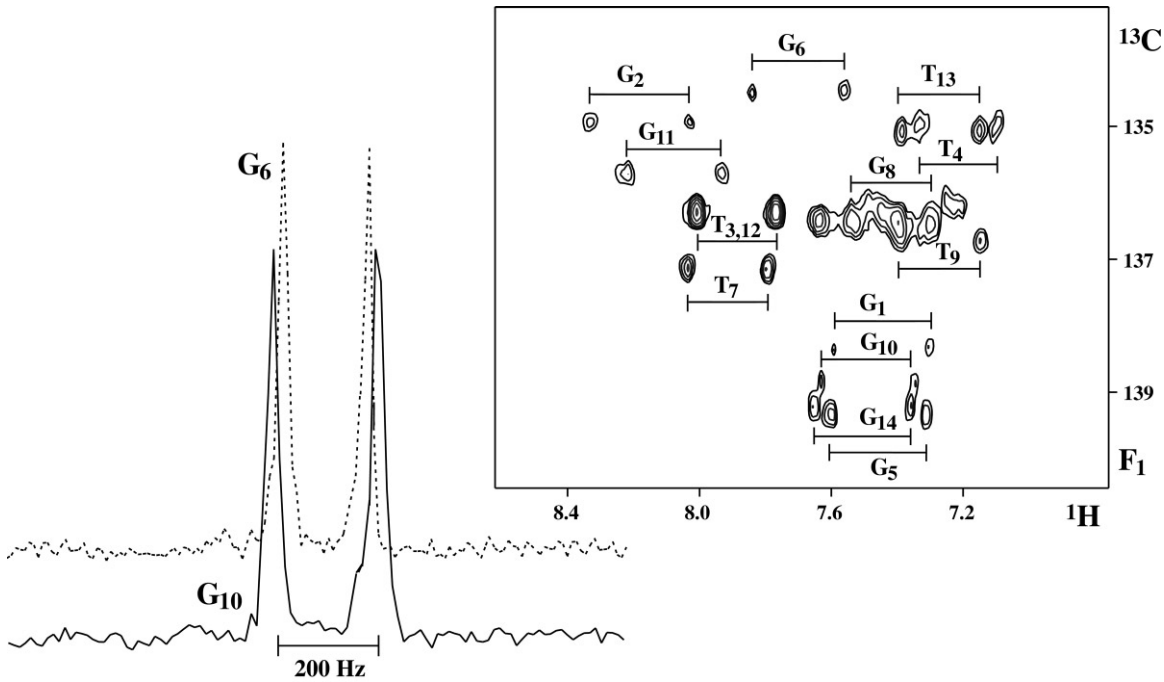

FIG. 3. A region of the 750-MHz HSQC data on the europium–15mer complex is shown. The traces at the left show two representative slices from the data. The 15mer, d(GGTTGGTGTGGTTGG), obtained from Gilead Sciences (Foster City, CA) was purified by HPLC and then lyophilized. The sample was dissolved in 200 ml of 140 mM NaCl, 20 mM perdeuterated Tris, and 5 mM KCl at pH 7.0 and then ethanol precipitated three times. NMR and HPLC results on the samples showed no detectable impurities. The NMR samples consisted of 100 A_{260} of DNA, in 500 ml of 140 mM NaCl, 20 mM perdeuterated Tris, and 5 mM KCl buffer at pH 7.0 in a grade 6 NMR tube (Scientific Glassware; Vineland, NJ). One A_{260} is equivalent to an absorption of 1 at 260 nm with the sample in a 1-cm-pathlength cell. The sample was then dried in the NMR tube using N_2 gas and then redissolved with 500 ml of ^2H_2O. The pH was then checked and adjusted, if necessary, to 7.0. The europium was added as $EuCl_3$ in ^2H_2O to a molar ratio of two Eu to one DNA. Spectra were acquired at 400 MHz on a Varian Unityplus and at 750 MHz on a Bruker DMX at the University of Wisconsin, Madison. All the Varian NMR data were processed using VNMR software and all the Bruker data were processed using Felix 97.0 software. The 400-MHz ^{13}C gradient-enhanced HSQC was run at 27°C. The F_2 spectral width was 5000 Hz and that of F_1 was 3500 Hz. The ^{13}C frequency was set at 120 ppm and delay optimized for a coupling of 200 Hz. 2048 complex points in t_2 were collected during an acquisition time of 0.205 s. 82 increments of t_1 were collected and the data in t_1 linearly predicted to 210 points. Gaussian weighting functions were used and the data were Fourier transformed into 4K × 1K points. The 750-MHz gradient-enhanced HSQC was run at 27°C. The F_2 spectral width was 12,500 Hz and that of the F_1 dimension, 31,250 Hz. The ^{13}C frequency was set at 120 ppm and delay optimized for a coupling of 200 Hz. 2048 complex points in t_2 were collected using an acquisition time of 0.328 s. 512 increments of t_1 were collected. Gaussian weighting was used and the data Fourier transformed into 4K × 1K points.

position of the alignment axis was also examined. Isotropic diffusion on the surface of a cone of ±5° was used to model this motion. It was found that neither the quality of fit nor the optimum position of the alignment axis is highly sensitive to the inclusion of this modest amount of motion.

The differences between the predicted and experimental values are largest for the C6–H of the dT7 and dT9 residues which are in a loop. The 13-Hz difference between the predicted and experimental dipolar couplings corresponds to a change in orientation of the vectors by less than 15°. The positions of these residues were defined by relatively few NOE constraints in the refinement. Inclusion of constraints based on these dipolar couplings will allow the positions of these loop residues to be more precisely refined. To distinguish between motion and orientation more than one dipolar coupling per base is needed. For example, the combination of the dipolar couplings of the C6H and N1H of a thymine would offer two data points to determine the orientation and motion of the base. The loop residues may also be undergoing more local motion than the rest of the molecule which would decrease both *S* and the observed dipolar coupling.

The dipolar couplings experimentally observed are those of the protons attached to ^{13}C. The effect of motion on the dipolar coupling of a methyl group proton can be significant. The rotation of the methyl group will cause averaging of the heteronuclear ^{13}C–^1H dipolar coupling and this will be essentially equivalent to uniform averaging over a cone of 110°. The rotation of a methyl group will remove much, but not all, of the orientation information present in the heteronuclear dipolar couplings of methyl groups. The heteronuclear ^{13}C–^1H dipolar couplings of a methyl group proton will be the same for CH^2H_2 as for a CH_3 group neglecting proton–proton interactions.

The averaging of the dipolar couplings of all of the methyl protons of a molecule can be used to gain information about A_a and the extent of alignment. The experimental dipolar couplings for the methyl protons fall into the range of 19.0 ± 1.5

Hz. These experimental dipolar couplings of the methyls, listed in Table 1, have been averaged and used to calculate an overall average of 19.3 Hz which is close to the value of 15.5 Hz predicted from the fit of the alignment axis and assuming all orientations are equally sampled. This comparison also shows that the methyl proton dipolar coupling results are consistent with those of the aromatic protons.

The use of paramagnetics for magnetic alignment opens up the possibility of determining dipolar couplings at a single field strength. While technically challenging, paramagnetic decoupling experiments are possible and could even be incorporated into multidimensional experiments.

The use of paramagnetic-assisted alignment is limited by the broadening and shifting of resonances caused by the presence of the paramagnetics. However, as we find here almost all of the resonances can be observed and this is likely to apply in other applications as well. The results in Fig. 3 show that most of the aromatic ^{13}C–^{1}H resonances can be observed with linewidths limited by the experimental conditions. It is likely that the use of paramagnetics with relatively long electron relaxation times, such as gadolinium, will lead to a much more unfavorable ratio of observable to unobservable resonances. The method can be applied to many biomolecules with obvious application to those proteins which naturally bind metal ions. The method should also be applicable to the metal binding sites of RNAs, including tRNA, and to a variety of other DNAs. It should also be possible to devise chelates of multiple paramagnetics in highly asymmetric environments which can be rigidly attached to biomolecules.

Some time ago paramagnetic shift reagents were proposed as a means to obtain structural information on molecules in solution. These studies were based on using the through space dipolar fields of the paramagnetics to shift resonances depending on the position and distance of the observed site relative to the position of the paramagnetic. Significant shifts at distances of 5–7 Å can be observed in the best examples. The use of paramagnetics to assist magnetic alignment is based on a very different use of the paramagnetics. In assisted alignment the position, or positions, of the paramagnetics need not be known at any stage of the structure determination. Only the positions of the alignment axes need to be known. In the alignment experiments all of the internuclear vectors are aligned relative to the same axes and hence the dipolar couplings do not depend on the distance from the binding site or sites of the paramagnetics. Thus, dipolar couplings of internuclear vectors which are spatially very distant from the paramagnetic binding sites can be determined without contributions from the contact and relaxation effects which limited the application of shift reagents to biomolecules.

An alternative approach to enhancing alignment is to use a liquid crystalline medium (*16*) or bicelle (*17*) which provides a preferred orientation for the molecules and the liquid crystalline approach has recently been extended to biomolecules (*1, 2*). This approach appears quite promising but may not be suitable for all larger molecules as the liquid crystalline medium may interact directly with the biomolecules or exclude too much volume.

Additional applications to the D_{CH}, D_{NH}, and D_{HH} of DNAs are planned and dipolar couplings of a similar magnitude have been obtained for europium complexes of the quadruplex DNA formed of dimers of d(GGGGTTTTGGGG). Of particular interest is to combine the information in the dipolar couplings with NOE and scalar coupling data to refine solution state structures.

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