Communications to the Editor

Nuclear Magnetic Resonance Studies of a Paramagnetic Metallo DNA Complex

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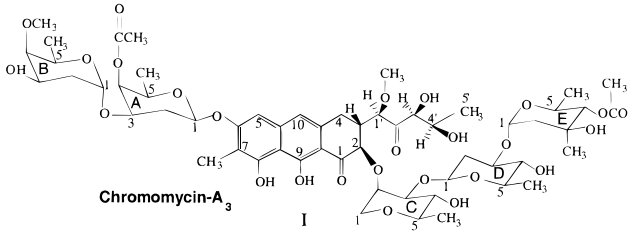
Standard nuclear magnetic resonance (NMR) spectroscopy exploits the nuclear Overhauser effect (NOE) to obtain interproton distance restraints. Structures of RNA and DNA are relatively imprecisely determined, due to the shortage of medium and long-range NOEs compared to those of proteins. Long-range constraints are ideally needed to define the structures more precisely. We report here on the NMR study of a ternary paramagnetic complex consisting of a DNA oligomer, a drug, and a divalent cation. The purpose of the study is to utilize pseudocontact shifts as structural restraints in solution NMR of macromolecules.[1,2] By assigning the paramagnetic spectrum of the DNA complex, we have obtained long-range constraints out to 25 Å from the metal ion.

The complex consists of d(TTGGCCAA)$_2$ bound to two molecules of chromomycin-A$_3$ (I) in the minor groove at the G4C5 step.[3−5] A single Co^{2+} cation is strongly bound to the O1 and O9 atoms of two chromomycins and to two water molecules, forming a pseudo-octahedral coordination site.[6] The



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Co^{2+} cation is high spin with three unpaired electrons. A large magnetic susceptibility anisotropy in the complex gives rise to isotropic dipolar or pseudocontact shifts of the nuclear resonances. Observed shifts depend on both the distance ($\sim 1/r^3$) and angular orientation of the nuclei with respect to the magnetic susceptibility tensor.[7,8] The tensor is defined by five parameters, the axial and rhombic molecular susceptibility anisotropies, χ_{ax} and χ_{rh}, and three Euler angles, α, β, and γ, which define its orientation with respect to the molecular axis system.

The large dispersion of resonances in the ^{1}H and ^{31}P NMR spectra of the DNA−chromomycin−Co^{2+} complex is shown in Figure 1. Paramagnetic line broadening is limited chiefly to Curie relaxation, because of the short electronic relaxation time (10^{-12} s). Almost no line broadening is observed in the ^{31}P NMR spectrum, due to the lower nuclear gyromagnetic ratio

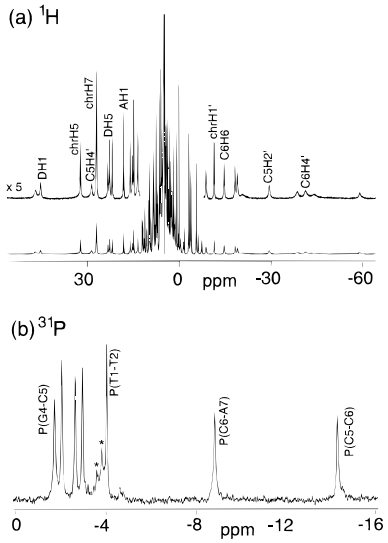

Figure 1. One-dimensional spectra of 2.3 mM d(TTGGCCAA)$_2$ complexed to 1 equiv of Co^{2+} and 2 equiv of chromomycin-A$_3$ in D_2O, pH 5.9, 25 °C on a 500 MHz spectrometer. The ^{1}H spectrum was run with 128 scans, spectral width 70 kHz, and referenced to (trimethylsilyl)propionic acid (TSP); the ^{31}P spectrum was run with 128 scans, 5 kHz spectral width, and referenced to trimethyl phosphate (TMP). An asterisk denotes residual uncomplexed DNA.

of ^{31}P. The spectral dispersion caused by the Co^{2+} makes it useful for eliminating overlap in the ^{31}P NMR resonances and for defining the phosphate backbone. The paramagnetic component of the shift was determined experimentally as the difference in chemical shift between a Co^{2+} and a Zn^{2+} complex. Assignment of the diamagnetic Zn^{2+} complex was straightforward owing to the existing assignment of the Mg^{2+} complex.[4] The nonexchangeable proton shift differences observed between Zn^{2+} and Mg^{2+} complexes were negligible,[9] confirming that no structural change occurs on metal substitution. The assignment of the DNA−chromomycin−Co^{2+} spectrum will be the subject of another paper; 95% of the paramagnetic spectrum has been assigned.

The NMR structure of d(AAGGCCTT)$_2$ with chromomycin and Mg^{2+} has been reported,[6] and the coordinates of the chromomycin sugar residues and central four bases were used in defining the susceptibility tensor for this complex with d(TTGGCCAA)$_2$. The tensor was determined by a least squares fit between observed shifts of 94 protons and shifts calculated from the coordinates. For a molecular axis system defined with *z* along the C_2 symmetry axis and *y* in the plane formed by the metal and the two O1 oxygen atoms of the chromophore, the susceptibility tensor obtained was $\chi_{ax} = 6329(354)$ ppm Å^3, $\chi_{rh} = -415(1201)$ ppm Å^3, $\alpha = 82.0(2.6)°$, $\beta = 90.4(0.6)°$, and $\gamma = 0.4(6.2)°$ with the numbers in parentheses indicating the standard deviation.[10] The large standard deviation in the susceptibility resulted from an extreme sensitivity to the coordinates. The rmsd (root mean square deviation) between observed and calculated data was 1.63 ppm over a data range from −64 to +44 ppm. This result indicates that the observed pseudocontact shifts are in agreement with the existing NMR structure with probably small adjustments.

(1) Gochin, M.; Roder, H. *Protein Sci.* **1995**, *4*, 296−305.
(2) Banci, L.; Bertini, I.; Bren, K. L.; Cremonini, M. A.; Gray, H. B.; Luchinat, C.; Turano, P. *JBIC, J. Biol. Inorg. Chem.* **1996**, *1*, 117−26.
(3) Gao, X. L.; Patel, D. J. *Biochemistry* **1990**, *29*, 10940−56.
(4) Gao, X.; Patel, D. *Biochemistry* **1989**, *28*, 751−62.
(5) Itzhaki, L.; Weinberger, S.; Livnah, N.; Berman, E. *Biopolymers* **1990**, *29*, 481−9.
(6) Gao, X. L.; Mirau, P.; Patel, D. J. *J. Mol. Biol.* **1992**, *223*, 259−79.
(7) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin/Cummings: Menlo Park, CA, 1986.
(8) Harper, L. V.; Amann, B. T.; Kilfoil Vinson, V.; Berg, J. M. *J. Am. Chem. Soc.* **1993**, *115*, 2577−80.

(9) Shift differences were ≤0.03 ppm for all except G4H1′ (0.07 ppm) and C_5H_6 (0.05 ppm), both protons being at the site of complex formation.
(10) Alternative equivalent tensor: $\chi_{ax} = -3839$ (821) ppm Å^3, $\chi_{rh} = -5845$ (493) ppm Å^3, $\alpha + \gamma = 81.4$ (3.1)°, $\beta = 0.15$ (0.34)°.

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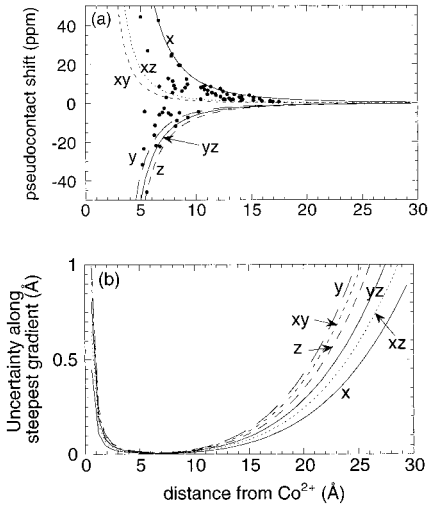

Figure 2. (a) Plot of the pseudocontact shift calculated from the tensor given in the text along various molecular axes in the complex (*xy* bisects the *x* and *y* axes in the *xy* plane, etc.). Experimental data is superimposed for the drug and four central residues GGCC. (b) Plot of the positional change (*dl*) which would occur along the steepest gradient in the shift according to the equation for steepest gradient[16] $|d\delta_{pc}| = \{|d\delta_{pc}/dr| + |d\delta_{pc}/r d\theta| + |d\delta_{pc}/(r \sin\theta\, d\phi)|\}$ dl, where $d\delta_{pc} = (1/(2\pi T_2))$ (for Co^{2+} complex) + 0.02) ppm (set at a minimum of 0.05 ppm).

The largest principal susceptibility anisotropy does not lie along the unique symmetry axis but perpendicular to it and close to one of the $Co^{II}-O$ bonds. This is a similar result to that found for the Co^{2+}-substituted zinc finger.[8] An analysis of the susceptibility tensor in terms of the shift magnitudes and estimated precision along the molecular axes is shown in Figure 2. Figure 2a depicts the observed shifts as a function of distance from Co^{2+} along with calculated shifts out to 30 Å. The figure demonstrates the variability of shift size with orientation. Predicted pseudocontact shifts can be as large as 1.5 ppm 20 Å away from the Co^{2+}. The precision with which positions may be determined is a function of the gradient of the shift tensor and the accuracy with which the experimental shifts can be determined. These two features complement each other, since close nuclei will experience more line broadening, but are in a region of higher positional sensitivity. To account for the interdependence of (*x*, *y*, *z*) due to the molecular bonding network, we estimate the precision from the steepest gradient of the shift tensor at a given position, allowing for the variation in line widths (Figure 2b). A true determination of precision can only follow from a complete molecular dynamics simulation on the system. Nevertheless, it appears that subangstrom precision is possible as far as 25 Å from the metal.

An example of the structure definition possible in nucleic acids can be seen by comparing ^{31}P NMR pseudocontact shifts for standard B-DNA and those determined in this experiment. ^{31}P NMR pseudocontact shifts calculated for standard B-DNA fit poorly to the observed shifts (RMSD = 3.7 ppm) because of the distortion of DNA caused by complexation. The observed ^{31}P NMR shifts agree qualitatively with those calculated from the published structure (RMSD = 0.9 ppm), but refinement can be improved since no information on the backbone torsion angles was available from the NOEs or from $^{31}P-^{1}H$ NMR heternuclear correlation experiments.[6]

In summary, the NMR study of the paramagnetic $Co^{2+}-$DNA complex has yielded global structural restraints. These restraints can be measured on ^{1}H, ^{13}C, ^{15}N, or ^{31}P, independent of gyromagnetic ratio. It is of importance for chemists to identify or construct tight metal-binding sites in nucleic acids which can then be used to obtain global structural information. The presence of large magnetic anisotropy also makes these complexes amenable to the introduction of additional structural constraints such as residual dipolar coupling[11,12] and cross-correlation effects.[13−15]

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(11) Tolman, J.; Flanagan, J.; Kennedy, M.; Prestegard, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9279−83.
(12) Tjandra, N.; Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* **1996**, *118*, 6264−72.
(13) Bohlen, J.-M.; Wimperis, S.; Bodenhausen, G. *J. Magn. Reson.* **1988**, *77*, 589−95.
(14) Bertini, I.; Luchinat, C.; Tarchi, D. *Chem. Phys. Lett.* **1993**, *203*, 445−9.
(15) Qin, J.; Delaglio, F.; LaMar, G. N.; Bax, A. *J. Magn. Reson., Ser. B* **1993**, *102*, 332−6.
(16) Feng, Y.; Roder, H.; Englander, S. W. *Biochemistry* **1990**, *29*, 3494−504.