NMR Studies of a Cobalt-Substituted Zinc Finger Peptide†

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Abstract: Nuclear magnetic resonance studies of the complex between the consensus zinc finger peptide CP-1 and the paramagnetic ion Co(II) have allowed the orientation and anisotropy of the magnetic susceptibility tensor to be determined. Two approaches were used. First, 70 resonances were assigned for the CP-1–Co(II) complex using a combination of 2-D NMR techniques and characterization of sequence variants. The tensor was determined by maximizing the agreement between observed and calculated chemical shifts while varying the orientation and anisotropy of the tensor. The second approach compared the observed and calculated integrated spectra of the Co(II) complex while optimizing the tensor parameters. Both methods found the axial and equatorial anisotropies of the tensor to be $\chi_{ax} = \chi_{zz} - 0.5(\chi_{xx} + \chi_{yy}) = 2280 \pm 400$ VVk and $\chi_{eq} = \chi_{xx} - \chi_{yy} = 280 \pm 310$ VVk. The largest value of the magnetic susceptibility tensor lies approximately along the bisector of one of the sulfur–Co(II)–nitrogen angles. The knowledge of this tensor will allow refinement of the three-dimensional structure of the peptide and its complexes with DNA with constraints not available from traditional NMR studies.

Paramagnetic ions hold great promise as probes in magnetic resonance studies.[1-3] Zinc finger peptides represent a natural system to investigate these effects. These small peptides (25–30 amino acids) bind one Zn(II) or another similar ion to fold into a stable structure.[4-6] The Zn(II) complexes of such peptides have been studied extensively by NMR methods.[7,8] We report herein the first detailed NMR studies of a zinc finger peptide substituted with the paramagnetic ion Co(II). Using a combination of 2-D NMR techniques, characterization of sequence variants, and novel refinement methods, we have been able to determine the orientation and anisotropy of the magnetic susceptibility tensor.

Experimental Section

All peptides were synthesized with the use of a Milligen/Biosearch 9050 peptide synthesizer and purified and reduced as described previously.[8] All experiments were performed under an atmosphere of 2–5% hydrogen balanced with nitrogen to avoid cysteine oxidation. NMR samples were prepared with 1.1–1.2 equiv of cobalt(II) and adjusted to the desired pH with deuterated Tris (MSD Isotopes). ^{1}H NMR data were obtained on Varian XL400 and Bruker AM600 NMR spectrometers and processed with software supplied with the spectrometers or with FELIX (Hare Research, Inc., Woodinville, WA). Magnitude COSY[9,10] and TOCSY[11,12]

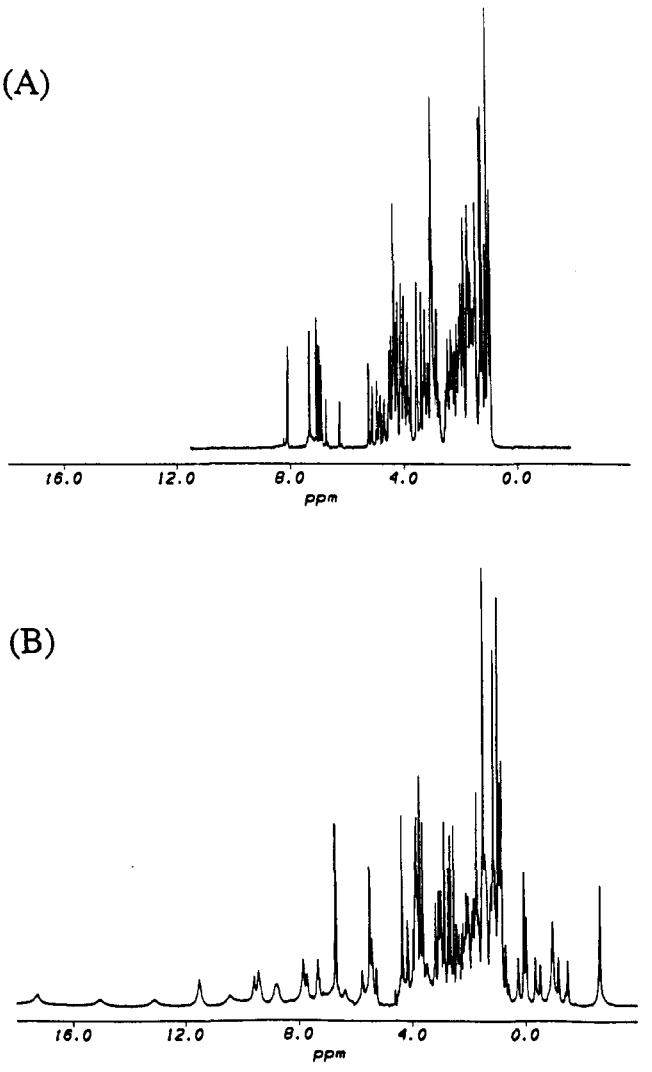

Figure 1. The 600 MHz one-dimensional ^{1}H NMR spectrum of the (A) Zn(II) and (B) Co(II) complexes of CP-1 in D_2O, 2.1 mM, pH 7.20 and 7.05, respectively, at 25 °C. Additional resonances shifted further downfield due to very large dipolar and/or contact shifts have been detected but are not shown.

† Dedicated to Professor Richard H. Holm on the occasion of this 60th birthday.

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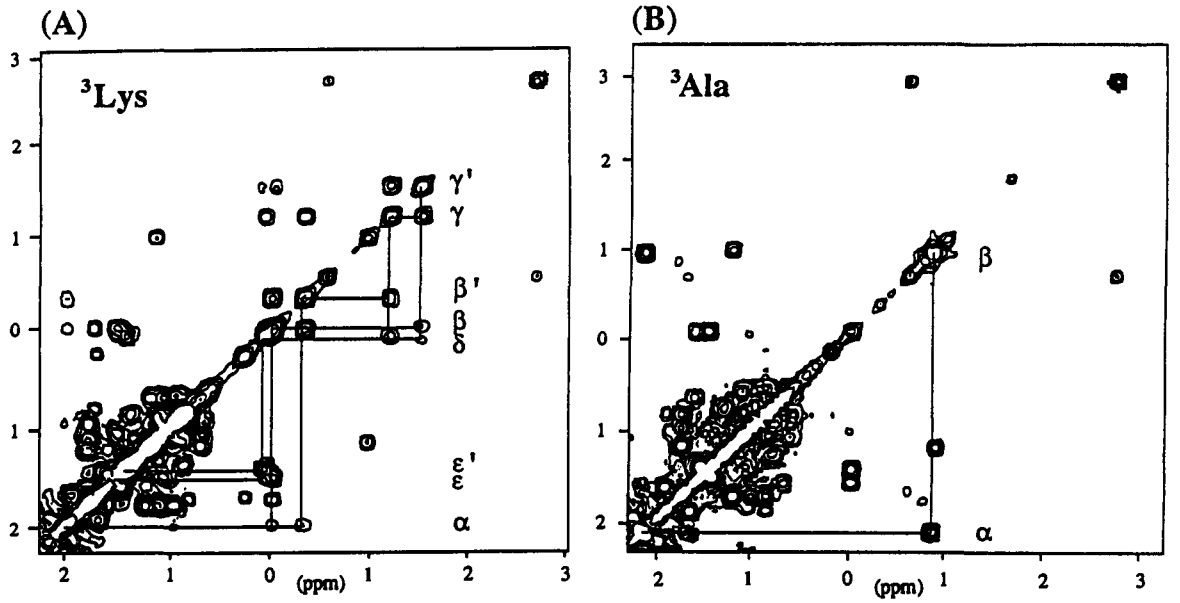

Figure 2. A portion of the 400-MHz magnitude COSY spectrum of the Co(II) complex of (A) CP-1, 2.1 mM, pH 7.05 and (B) CP(K3A), 6.78 mM, pH 6.55 in D_2O, at 25 °C. Assignments for the appropriate resonances are indicated.

Table I. Observed versus Calculated Shifts

	Zn_{obs}	Co_{obs}	$(\Delta H/H)^{dip}$	Co_{calc}		Zn_{obs}	Co_{obs}	$(\Delta H/H)^{dip}$	Co_{calc}
^{1}Pro α	4.27	3.87	−0.33	3.94	^{14}Lys N	8.76	8.44	−0.30	8.46
^{1}Pro β	2.31	1.85	−0.39	1.92	^{14}Lys α	3.07	2.73	−0.35	2.72
^{1}Pro β′	1.85	1.27	−0.52	1.33	^{14}Lys β	1.54	1.40	−0.16	1.38
^{1}Pro γ	1.98	1.64	−0.29	1.69	^{14}Lys β′	1.13	0.87	−0.17	0.96
^{1}Pro γ′	1.91	1.48	−0.37	1.54	^{15}Ser N	8.71	8.42	−0.34	8.37
^{1}Pro δ	3.34	3.05	−0.27	3.07	^{15}Ser α	3.92	3.74	−0.30	3.62
^{2}Tyr N	8.99	8.21	−0.62	8.37	^{15}Ser β	3.86	3.61	−0.35	3.51
^{2}Tyr α	4.66	3.97	−0.64	4.02	^{15}Ser β′	3.81	3.66	−0.26	3.55
^{2}Tyr β	3.10	2.44	−0.77	2.33	^{16}Asp N	6.86	6.38	−0.66	6.20
^{2}Tyr β′	2.72	1.90	−0.96	1.76	^{16}Asp α	4.39	3.80	−1.03	3.36
^{2}Tyr δ	7.17	6.67	−0.43	6.63	^{16}Asp β	2.98	2.09	−1.47	1.51
^{2}Tyr ε	6.86	6.75	−0.16	6.78	^{16}Asp β′	2.77	2.34	−0.88	1.89
^{3}Lys N	8.81	7.67	−1.06	7.75	^{17}Leu N	7.08	6.25	−0.86	6.22
^{3}Lys α	5.08	2.04	−3.17	1.91	^{17}Leu α	3.24	1.04	−1.61	1.63
^{3}Lys β	1.59	−0.34	−2.36	−0.77	^{17}Leu β	2.08	1.54	−0.54	1.54
^{3}Lys β′	1.44	−0.01	−1.60	−0.16	^{17}Leu β′	1.26	−0.03	−1.40	−0.14
^{3}Lys γ	1.17	−1.19	−0.96	0.21	^{17}Leu γ	1.63	1.77	0.47	2.10
^{3}Lys γ′	1.17	−1.52	−1.03	0.14	^{17}Leu δ	0.99	0.83	1.02	2.01
^{10}Ser N	8.03	7.63	0.50	8.53	^{18}Val N	8.19	7.70	−0.38	7.81
^{10}Ser α	5.25	3.07	−1.02	4.23	^{18}Val α	3.66	3.91	0.39	4.05
^{10}Ser β	3.50	2.65	−0.28	3.22	^{18}Val β	1.98	1.83	−0.12	1.86
^{11}Phe N	8.80	7.41	−1.19	7.61	^{18}Val γ	0.98	1.12	0.15	1.13
^{11}Phe α	4.75	4.18	−1.01	3.74	^{18}Val γ′	0.89	0.96	0.12	1.01
^{11}Phe β	3.50	2.71	−1.31	2.19	^{19}Lys N	7.45	6.68	−0.66	6.79
^{11}Phe β′	2.67	1.48	−1.41	1.26	^{19}Lys α	3.91	3.18	−0.59	3.32
^{12}Ser N	9.21	8.78	−0.70	8.51	^{19}Lys β	1.84	1.08	−0.97	0.87
^{12}Ser α	4.45	4.14	−0.43	4.02	^{19}Lys β′	1.73	0.97	−0.84	0.89
^{12}Ser β	4.05	3.89	−0.29	3.76	^{19}Lys γ	1.39	0.70	−0.45	0.94
^{12}Ser β′	4.05	3.82	−0.32	3.73	^{19}Lys γ′	1.39	0.70	−0.52	0.87
^{13}Gln N	8.14	7.68	−0.61	7.53	^{23}Thr α	4.13	1.72	−2.23	1.90
^{13}Gln α	4.91	4.62	−0.36	4.55	^{23}Thr β	4.03	−0.53	−4.23	−0.20
^{13}Gln β	2.23	2.00	−0.32	1.91	^{23}Thr γ	1.17	−2.63	−3.57	−2.40
^{13}Gln β′	2.00	1.73	−0.43	1.57	^{26}Gly N	8.37	7.70	−0.43	7.94
^{13}Gln γ	2.46	2.25	−0.29	2.17	^{26}Gly α	4.02	3.62	−0.47	3.55
^{13}Gln γ′	2.41	2.19	−0.32	2.09	^{26}Gly α′	3.96	3.81	−0.07	3.89

experiments were performed on peptide samples with concentrations in the range of 1 to 7 mM in D_2O (Aldrich, 100.0%), pH 6.5–7.5, 298 K. Magnitude COSY and NOESY[13-15] experiments were preformed on peptide samples in 10% D_2O/90% H_2O, pH 5.6–5.8, respectively. The mixing times for the NOESY and TOCSY experiments were 100 and 21 ms, respectively. The water signal was suppressed by presaturation.

Results and Discussion

Studies were performed with the concensus zinc finger peptide CP-1 which has the sequence ProTyrLysCysProGluCysGlyLysSerPheSerGlnLysSerAspLeuValLysHisGlnArgThrHisThrGly.[8] The one-dimensional ^{1}H NMR spectra of the Zn(II) and Co(II) complexes of this peptide are shown in Figure 1. Note the large shifts of resonances to positions from −2.64 to +17.3 ppm in the Co(II) complex spectrum. These shifts are due to dipolar interactions between protons and the

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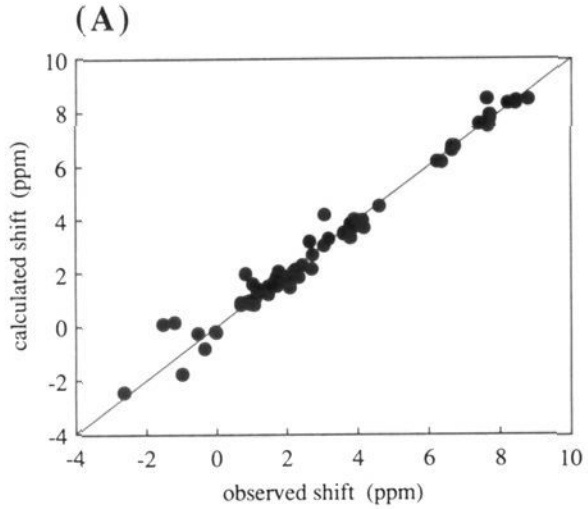

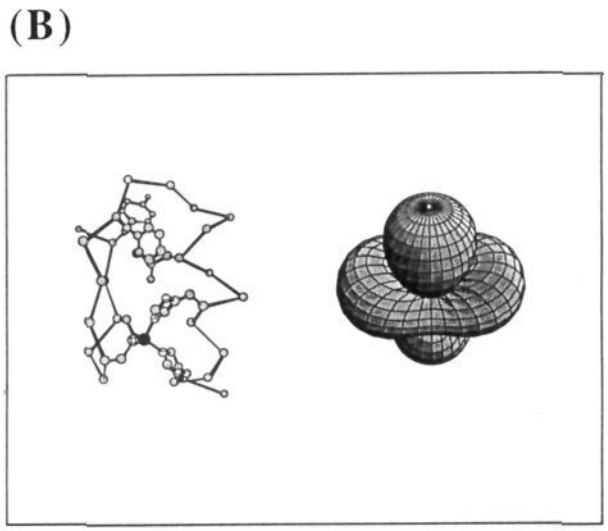

Figure 3. *(A) The observed versus calculated NMR shifts for the Co(II)* complex of CP-1. (B) An isoshift surface denoting the distance and orientation necessary for a 1 ppm downfield or upfield shift. The surface is in the same orientation as the accompanying structure of the zinc finger peptide complex.

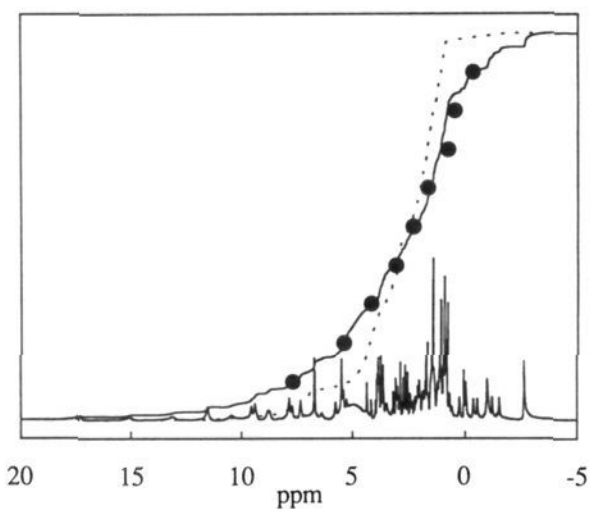

Figure 4. The observed (solid line) versus calculated (closed circles) integrated NMR spectra for the Co(II) complex of CP-1.

paramagnetic ion. The differences between the chemical shifts for corresponding resonances in the Co(II) and Zn(II) complexes

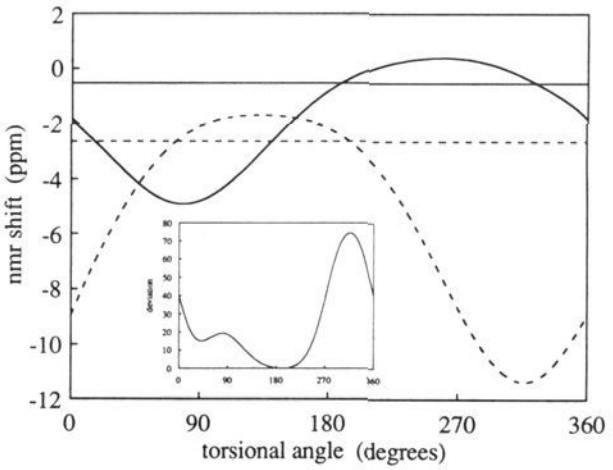

Figure 5. Calculated NMR shifts versus torsional angle for ^{23}Thr in the Co(II) complex of CP-1. The dotted line is for the γ-methyl group and the solid line the β-hydrogen. The angle is defined as C_γ–C_β–C_α–CO. The horizontal lines denote the respective observed NMR shifts. The inset shows the combined deviation as a function of torsional angle.

can be characterized by an effective magnetic susceptibility tensor. Attempts to locate signals due to the contact-shifted metal-binding residues expected outside this range have been unsuccessful. Further studies at lower field strengths may be useful in this regard.

In order to determine the tensor properties, two approaches were taken. The first method required that a number of proton resonances be assigned in the Co(II) complex. The spectrum of the Zn(II) complex had been assigned previously.[8] Partial assignment of the Co(II) was achieved by magnitude COSY experiments on the Co(II) complexes of CP-1 and on a series of peptides for which selected residues were replaced by alanine. The variants studied included two single variants, CP-1(K3A) and CP-1(P5S), and two double variants, CP-1(E6A, T23A) and CP-1(K9A, T25A). Chemical shifts for ^{3}Lys and ^{23}Thr were assigned by comparing the COSY peak patterns of the Co(II)-bound variants with those from the CP-1–Co(II) complex. This is illustrated for the CP-1(K3A) in Figure 2. Assignments were not found for ^{6}Glu, ^{9}Lys, and ^{25}Thr and the resonances for these residues were assumed to be too broad to appear in the COSY spectra under the conditions used. Although peaks were found associated with ^{5}Pro, they have not yet been unambiguously assigned. A minimalist zinc finger peptide MZF,[16] whose sequence is LysTyrAlaCys(Ala)$_2$Cys(Ala)$_3$Phe(Ala)$_2$Lys(Ala)$_2$Leu(Ala)$_2$His(Ala)$_3$HisAlaLys, proved to be particularly useful in that resonances for the hydrophobic core residues, underlined, were able to be assigned. The shifts for these resonances are very nearly identical for all of the peptides studied, supporting the conservation of both the three-dimensional structure and the electronic properties of the Co(II) ion among the variant peptides. The resonances for two residues, ^{13}Glu and ^{18}Val, were assigned by observing the similarity of their chemical shifts and COSY peak patterns to those found in the CP-1–Zn(II) complex. Using all of the above assignments as well as the *NH_i–NH_{i+1}, αN, βN, and NH_i–NH_{i+3} NOESY patterns observed* in H_2O samples of CP-1 complexed with Zn(II) and with Co(II), a total of 70 resonances were assigned. These are listed in Table I.

Using the first method, the tensor was determined by maximizing the agreement between the observed and calculated NMR shifts for the CP-1–Co(II) complex while varying the anisotropy and orientation of the tensor. The calculated NMR shifts were

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determined by adding the paramagnetic dipolar shift contribution to the previously determined NMR shifts from the Zn(II) complex. The dipolar shift, $(\Delta H/H)^{dip}$, was calculated from the equation

$$(\Delta H/H)^{dip} = -\frac{1}{3N}\left\{\chi_{zz} - \frac{1}{2}(\chi_{xx} + \chi_{yy})\right\}\left\{\frac{3\cos^2\theta - 1}{r^3}\right\} - \frac{1}{2N}\{\chi_{xx} - \chi_{yy}\}\left\{\frac{\sin^2\theta\cos 2\phi}{r^3}\right\}$$

where r is the metal-to-proton distance, θ and ϕ are the spherical polar angles with respect to the principal axes of the susceptibility tensor, and χ_{xx}, χ_{yy}, and χ_{zz} are the principal magnetic susceptibilities. The paramagnetic contact shift contribution, a through bond effect, is expected to be negligible for all residues except those directly bonded to the metal. Since our assignments did not include any of the metal binding ligands, contact shift effects were not considered further. The coordinates used were from the refined NMR structure of the CP-1–Zn(II) complex.[17] The axial and equatorial anisotropies of the tensor were found to be $\chi_{ax} = \chi_{zz} - 0.5(\chi_{xx} + \chi_{yy}) = 2280 \pm 400$ VVk and $\chi_{eq} = \chi_{xx} - \chi_{yy} = 280 \pm 310$ VVk and the principal axes were found to be within 21° of the bisectors of the ligand–metal–ligand axes. This orientation is consistent with results on simple tetrahedral Co(II) complexes.[18] Even though the tensor is nearly axial, the largest principal susceptibility does not lie along the bisector of the unique sulfur–Co(II)–sulfur angle but rather along the bisector of one of the sulfur–Co(II)–nitrogen angles.

In the second method, the agreement between the observed and calculated integrated spectra of the Co(II) complexes was maximized. The observed spectrum was integrated and chemical shift values corresponding to 10%, 20%, etc. of the total integrated area were determined. These values were compared with those calculated assuming particular values for the susceptibility tensor anisotropy. The principal axes of the tensor were assumed to bisect the ligand–metal–ligand angles. The best agreement was observed with anisotropies of $\chi_{ax} = 2400$ VVk and $\chi_{eq} = 500$ VVk. These results are very similar to those from the first method yet required only a one-dimensional NMR spectrum of the Co(II) complex. However, this method did not incorporate enough constraints to allow the orientation of the tensor to be refined. Observed and calculated shifts from both methods are summarized in Figure 3.

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The tensor can be further refined by simultaneously refining the structure. The significant error bars on the anisotropy of the tensor are largely due to the imprecision of the structure. As in any NMR-derived structure, the conformations of the solvent exposed side chains are often not well determined. For example, the side chain of ^{23}Thr was not well constrained by the NMR data of the CP-1–Zn(II) complex alone. The addition of the constraints due to the dipolar shifts uniquely defined the conformation of this side chain. Only one conformation, where the torsional angle is around 166°, can reproduce the observed Co(II) NMR shifts for both the β-hydrogen and the γ-methyl group, as seen in Figure 5.

Conclusions

These results demonstrate the ability to determine the rhombic magnetic susceptibility tensor for a tetrahedral Co(II) complex of a medium sized peptide using only NMR and structural data. One method involved detailed assignment of a number of resonances for the Co(II) followed by tensor refinement. A second method was developed that did not require any assignments for the Co(II) complex but used only the integrated spectrum. Although the second method was not robust enough to allow simultaneous optimization of all tensor parameters, it did yield a preliminary tensor that provided initial predictions for the shifts for the Co(II) complex. Only a few assignments were necessary to resolve the ambiguities from this method. This tensor we have determined will be useful for refining the structures of zinc finger peptide–metal complexes and for examining the structures of zinc finger peptide–DNA complexes. Calculations using the tensor and the structure of the Zif268-DNA complex[1] suggested that shifts up to 2 ppm for certain sugar protons and up to 0.1 ppm for the imino protons of the DNA can be expected via these dipolar interactions even at distances of over 20 Å.

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