ENDOR Determination of the Distance between Bleomycin-Bound Iron and ^{19}F of 2′-Fluorocytidine in a DNA Target Sequence

Dmitriy Lukoyanov,[§,†] Richard M. Burger,[#,§] and Charles P. Scholes*[,§]

Department of Chemistry and Center for Biological Macromolecules, State University of New York at Albany Albany, New York 12222
Public Health Research Institute, 455 First Avenue New York, New York 10016

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In the presence of Fe(II), O_2, and reductant, activated bleomycin (Act-BLM) transiently arises as the kinetically competent form leading to DNA cleavage. Act-BLM and its final product, ferric bleomycin (Fe(III)-BLM), are low-spin ferric complexes.[1,2b] Act−BLM has g_{max}, g_{inter}, g_{min} = 2.26, 2.17, 1.94, and Fe(III)-BLM has g_{max}, g_{inter}, g_{min} = 2.45, 2.18, 1.89.[2] Understanding bleomycin action[3] requires knowing the physical and electronic nature of the BLM−oligonucleotide complexes. NMR-derived structures were first determined from nonparamagnetic, nonactive Zn-BLM[4a,b] and CO-Fe(II)-BLM forms.[4c] Next, NMR structures of stable, nonparamagnetic HOO-Co(III)-BLM−oligonucleotide complexes showed proximity of the hydrogen on the hydroperoxy HOO^- metal ligand to the sugar H4′.[5] With iron-containing Act-BLM, a spontaneous oxidative attack abstracts this H4′.[6]

Fe(III)-BLM and Act-BLM have not been crystallized, either by themselves or as oligonucleotide complexes. Furthermore, these Fe(III)-containing forms are not amenable to structural NMR because the ferric ion is a strong relaxer and Act-BLM is unstable in solution. However, electron nuclear double resonance (ENDOR) can reveal structural and electronic information on these paramagnetic Fe(III) forms. ENDOR has resolved nearest-neighbor hyperfine structure from nitrogen ligands, from axial exchangeable H, and, for Act-BLM, from the bound hydroperoxide derived from $^{17}O_2$.[7a] ENDOR has demonstrated bleomycin interaction with a DNA 10-mer duplex, d(GGAAGCTTCC)$_2$, containing a 5′-G-pyr-3′ sequence favored for DNA cleavage.[7b] DNA ^{31}P-to-Fe(III) dipolar couplings indicated an Fe(III)-to-^{31}P phosphate (unassigned[7b]) distance of 7.4 Å.[8]

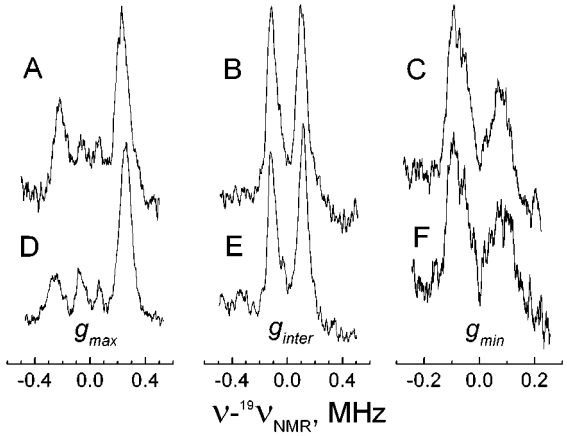

Figure 1. ^{19}F ENDOR spectra centered at the appropriate $^{19}\nu_{NMR}$ were obtained under adiabatic rapid passage conditions with small (∼0.12 G) 100 kHz field modulation, ∼0.25 μW microwave power, and ∼20 W radio frequency power, in a sweep time of 5 s. Each spectrum for Act-BLM was compiled in ∼30 min, and each Fe(III)-BLM spectrum was collected in ∼1 h. Spectra A−C from Act-BLM were (A) at g = 2.26, H = 1.08 T; (B) at g = 2.17, H = 1.12 T; (C) at g = 1.94, H = 1.26 T. Spectra D−F from Fe(III)-BLM were (D) at g = 2.43, H = 1.00 T; (E) at g = 2.18, H = 1.12 T; (F) at g = 1.89, H = 1.29 T.

Our aim is now to measure the distance and orientation from Fe(III) in both Act-BLM and Fe(III)-BLM to the target cytidine sugar in the 5′-G-C-3′ sequence. To pinpoint this cytidine, this sugar was labeled with ENDOR-detectable ^{19}F replacing H2″ at its 2′ carbon; thus, the oligonucleotide d(GGAAGC^{F}TTCC)$_2$ was used, where C^F is 2′F-cytidine.[9] Samples of Fe(III)-BLM and Act-BLM in deuterated solvent and in a slight molar excesses of (GGAAGC^{F}TTCC)$_2$ were prepared as described previously for d(GGAAGCTTCC)$_2$[7b] (see Supporting Information). Samples (∼50 μL) contained 0.6 mM Act-BLM or 1.4 mM Fe(III)-BLM. The respective g-values of Fe(III)-BLM and Act-BLM were unchanged by these oligonucleotides. The Q-band spectrometer[10] and conditions[7b] were as previously described.

The hyperfine-coupled spin $^1/_2$ nucleus of ^{19}F has first-order ENDOR frequencies, $^{19}\nu_{ENDOR} = |^{19}\nu_{NMR} \pm A/2|$, where $^{19}\nu_{NMR}$ is the ^{19}F NMR frequency (48.06 MHz at 1.200 T) and A is the hyperfine coupling. Figure 1 shows ^{19}F ENDOR spectra centered at $^{19}\nu_{NMR}$ for the d(GGAAGC^{F}TTCC)$_2$ complexes with Act-BLM (spectra A−C) or Fe(III)-BLM (spectra D−F) near their respective g_{max}, g_{inter}, and g_{min}. ^{19}F provided distinctly anisotropic ENDOR splittings. For Act-BLM and Fe(III)-BLM, respectively, the hyperfine splittings, measured as splittings between maxima of the ENDOR spectra, were as follows: at g_{max}, 0.45 ± 0.01 and

* To whom correspondence should be addressed.
§ SUNY at Albany.
# Public Health Research Institute.
† On leave from the MRS Laboratory, Kazan State University, 420008, Kazan, Russian Federation.

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(8) NOESY cross peaks used in constraining interproton distances define shorter distances than those that ENDOR can define between paramagnetic metal centers and ^{19}F or ^{31}P.
(9) d(GGAAGC^{F}TTCC), containing C^F as 2′F-cytidine and all other sugars in their deoxy form, was purchased from Trilink Biotechnologies (San Diego, CA) with double HPLC purification. The melting temperature of d(GGAAGC^{F}TTCC)$_2$ and d(GGAAGCTTCC)$_2$ was 32 °C, determined spectrophotometrically. Prior to preparation of BLM−oligonuclotide complexes, 20 mM d(GGAAGC^{F}TTCC) was dissolved in 20 mM pH 7.8 deuterated buffer, heated to 75 °C, and slowly chilled over 1 h to provide 10 mM d(GGAAGC^{F}TTCC)$_2$.
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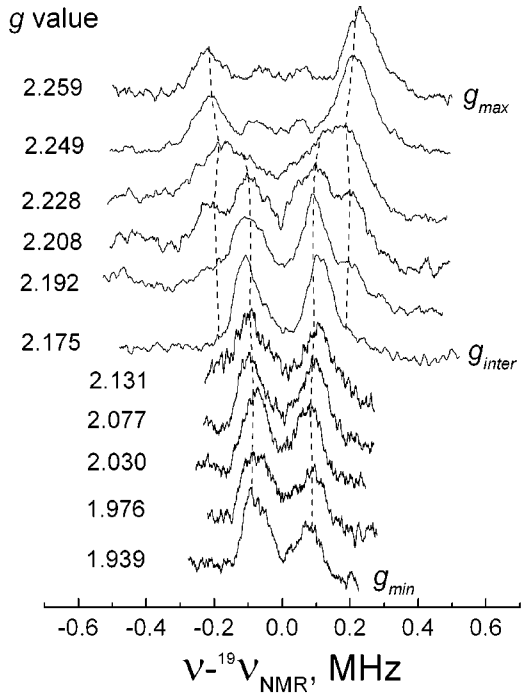

Figure 2. The g value dependence of ^{19}F ENDOR features from Act-BLM with d(GGAAGC^{F}TTCC)$_2$. The experimental conditions were as for Figure 1. The dashed lines show the values predicted for outlying splittings modeled from an anisotropic hyperfine tensor having A_z, A_y, $A_x = 0.48$, -0.23, -0.20 MHz and the Fe(III)$-^{19}$F vector (**R**) pointing at 10° to the g_{max} axis in the $g_{max}-g_{min}$ plane. The signal-to-noise was approximately 2-fold better in the $g_{max}-g_{inter}$ region, where the underlying EPR signal is larger. In the $g_{inter}-g_{min}$ region there were no ^{19}F splittings greater than 0.5 MHz.

0.51 ± 0.02 MHz; at g_{inter}, 0.21 ± 0.01 MHz and 0.23 ± 0.01 MHz; at g_{min}, 0.16 ± 0.03 and 0.16 ± 0.03 MHz. There was also a smaller ^{19}F splitting of about 0.13 ± 0.02 MHz observed only near g_{max}. Figure 2 shows the systematic shift of ^{19}F ENDOR frequencies of Act-BLM features as the g-value varied from g_{max} to g_{min}. The experimentally most distinct ENDOR features were the outlying features, with frequencies readily computable by angle-selected ENDOR theory[11] (dashed lines, Figure 2). The input parameters for angle-selected computations that predict outlying features were the principal elements of the hyperfine tensor and the angle(s) describing the relative orientation of the g tensor and hyperfine tensor.[12] A comparison of experimental and simulated angle-selected spectra for Act-BLM is shown in the Supporting Information, Figure 1S. A good fit to the overall compendia of angle-selected ^{19}F ENDOR features from the Act-BLM (GGAAGC^{F}TTCC)$_2$ complex was provided by a ^{19}F hyperfine tensor with components A_z, A_y, $A_x = 0.48 \pm 0.01$, -0.23 ± 0.01, -0.20 ± 0.03 MHz, where the principal hyperfine (z) axis defined by the Fe(III)$-^{19}$F vector (**R**) made an angle of 10° in the $g_{max}-g_{min}$ plane with respect to the g_{max} axis. This hyperfine tensor was well explained by dipolar coupling, and the predicted distance from ^{19}F of 2′F-cytidine to Fe(III) of Act-BLM was 7.0 ± 0.2 Å. Angle-selected ENDOR (Supporting Information, Figure 2S) also predicted for Fe(III)-BLM a similar 6.8 ± 0.2 Å distance and collinearity of **R** with g_{max}. In principle, covalency may alter electron spin density on the iron and alter the estimate of **R**; however, if the theoretical estimate of 95% spin on the iron[13] is appropriate, the covalent correction to **R** would be small (~ -0.1 Å). The ^{31}P couplings observed for complexes of d(GGAAGC^{F}TTCC)$_2$ were the same as those previously measured for d(GGAAGCTTCC)$_2$.[7b] Although it was not possible to observe angle-selected ENDOR of the smaller, 0.13 MHz splitting except near g_{max}, such a ^{19}F coupling was expected (see Supporting Information) from the more distant 2′F-cytidine on the complementary oligonucleotide strand.

Our Fe-2′F distances may be compared to analogous distances from plausibly relevant NMR-derived Co-BLM−oligonucleotide structures. For a HOO-Co(III)-BLM complex with a 12 base-pair, covalently linked, gapped duplex, the Co−H2″ distance was 8.0 Å;[5g] with 6-mer[5e] or 10-mer[5a] duplexes it was 6.1 Å. Our directly determined Fe(III)−2′F distance was 6.8−7.0 Å. These NMR structures support our assigning the 7.4 Å Fe(III)−^{31}P distance[7b] to the dC$_6$ 3′-phosphate in d(GGAAGCTTC)$_2$. This dC$_6$-3′ phosphate is adjacent to the 2′F of the present ENDOR study. The 2′F−Fe−3′P angle of 27 ± 5° calculated by combining the ^{31}P hyperfine tensor[7b] with our present ^{19}F tensor (from either Act-BLM or Fe(III)-BLM complexes) lies within the 17−29° range for the analogous H2″−Co−3′P angles of the NMR structures.[5a,e,g] Our ^{19}F dipolar tensor indicates that the Fe−2′F direction nearly coincides with g_{max}. Combining this directional information with the assumption that the Fe and 2′F coordinates approximate those of Co and H2″ coordinates in the Co-BLM−oligonucleotide NMR structure[5g] implies that the g_{max} axis tilts at about 35° to the Fe−proximal−O vector, rather than being collinear with it. (See Supporting Information for the schematic showing g-tensor, Fe, ^{31}P, ^{19}F, and HOO$^-$ geometry.) In summary, the dipolar hyperfine coupling determined between the cytidine-2′^{19}F and the Fe of Act-BLM or of Fe(III)-BLM provides a definitive constraint on the distance between the BLM Fe and the 2′ substituent of the cytidine sugar where H4′ abstraction occurs.

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Supporting Information Available: Preparation of Fe(III)-BLM and Act-BLM complexes; information on the weakly hyperfine coupled 2′F; experimental and simulated angle-selected ENDOR spectra from Act-BLM; angle-selected ENDOR spectra vs g values for Fe(III)-BLM; and schematic showing orientation of the g-tensor, Fe^{3+}, ^{31}P, and ^{19}F of the cytidine sugar, and NMR-determined HOO$^-$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) The point dipolar interaction between a nuclear spin **I** and electron spin **S** separated by a distance **R** is $A_D = \{1/hR^3\}(3(\mu_S\cdot\mathbf{R})(\mu_I\cdot\mathbf{R})/R^2 - \mu_S\cdot\mu_I)$. The nuclear moment is $\mu_I = g_n\beta_n(I_x + I_y + I_z)$, and the electron magnetic moment in the g-tensor principal axis system is $\mu_S = \beta_e(g_xS_x + g_yS_y + g_zS_z)$. g_n is the nuclear g-value (=5.254 for ^{19}F). β_e and β_n are the Bohr magneton and the nuclear magneton, respectively.

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