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Insights into copper coordination in the EcoRI–DNA complex by ESR spectroscopy

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Abstract

The EcoRI restriction endonuclease requires one divalent metal ion in each of two symmetrical and identical catalytic sites to catalyse double-strand DNA cleavage. Recently, we showed that Cu²⁺ binds outside the catalytic sites to a pair of new sites at H114 in each sub-unit, and inhibits Mg²⁺-catalysed DNA cleavage. In order to provide more detailed structural information on this new metal ion binding site, we performed W-band (~94 GHz) and X-band (~9.5 GHz) electron spin resonance spectroscopic measurements on the EcoRI–DNA–(Cu²⁺)₂ complex. Cu²⁺ binding results in two distinct components with different g_{zz} and A_{zz} values. X-band electron spin echo envelope modulation results indicate that both components arise from a Cu²⁺ coordinated to histidine. This observation is further confirmed by the hyperfine sub-level correlation results. W-band electron nuclear double resonance spectra provide evidence for equatorial coordination of water molecules to the Cu²⁺ ions.

Keywords

W-band ENDOR; ESEEM; metal ion coordination; Cu²⁺ inhibition; restriction endonuclease

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1. Introduction

Restriction endonucleases are produced in archaea and bacteria to protect against invading foreign DNA [1]. These enzymes catalyse double-strand DNA cleavage at specific recognition sites, four to eight base-pairs in length, by hydrolytic reactions that invariably require divalent metal ions as cofactors. The catalytic sites are generally similar to those of many other phosphotransferases that catalyse synthesis or hydrolysis of phosphodiester bonds. Because of their extraordinarily high ability to distinguish between closely related base sequences, the restriction nucleases have been favoured models for studying the relationship between substrate recognition specificity and catalytic specificity [1–4].

The EcoRI restriction endonuclease is a well-studied example. It carries out staggered cleavage of the DNA sequence 5'-GAATTC-3' in both strands, generating single-strand overhangs. Cleavage rates are as much as 10^6 -fold lower at DNA sites that differ in one base-pair from the canonical GAATTC [2–5]. The enzyme is a symmetrical homodimer with two catalytic centres, in each of which Mg^{2+} coordinates to carboxylate side chains of E111, D91, and the scissile phosphate GpAATTC [4,6]. The binding of Mg^{2+} in the two catalytic sites exhibits cooperativity [4,7]. The catalytic rates of different divalent metal ions vary according to the sequence $Mg^{2+} \approx Mn^{2+} > Co^{2+} \gg Zn^{2+} \gg Cd^{2+} > Ni^{2+}$ [8]. Recently, we found that Cu^{2+} does not support DNA cleavage by EcoRI [7], but profoundly inhibits Mg^{2+} -dependent catalysis [7]. Ca^{2+} was known to inhibit by competing with Mg^{2+} [4,8], so it was unexpected that Cu^{2+} does not coordinate to the same site as Mg^{2+} [7]. Based on a novel use of Cu^{2+} -based distance constraints derived from electron spin resonance (ESR) and double electron–electron resonance (DEER) experiments together with site-directed mutagenesis and molecular dynamics (MD) simulations, we showed that Cu^{2+} interacts with H114 in the EcoRI–DNA complex [7], and inhibits catalysis by displacing protein side chains and water molecules that were earlier proposed [4] to have roles in catalysis, and by affecting the DNA distortion that is required for catalysis.

The ESR spectra of the EcoRI–DNA–(Cu^{2+})₂ complex showed two distinct components with different g_{zz} and A_{zz} values [7]. However, in that study, only Cu^{2+} –H114 coordination was confirmed experimentally, whereas the identity of other ligands was inferred solely from MD simulations. Based on the research of Cu^{2+} binding mode in different systems by high field ESR spectroscopy [9–12], here we present the application of W-band electron nuclear double resonance (ENDOR) spectroscopy, X-band electron spin echo envelope modulation (ESEEM), and hyperfine sub-level correlation (HYSCORE) experiments to clarify further details of Cu^{2+} -coordination in the EcoRI–DNA complex. W-band ENDOR spectra are used to identify the existence of equatorial as well as axial water ligand(s) of the Cu^{2+} ions in both states reflected in the two spectral components. The Cu^{2+} –histidine coordination in the two states is also discussed based on X-band ESEEM and HYSCORE spectra at different magnetic fields.

2. Materials and methods

2.1. Enzyme expression and purification

The EcoRI protein was expressed from a maltose-binding protein–EcoRI (MBP–EcoRI) fusion construct [7,13]. Details of generation of the fusion gene, expression and cleavage of the fusion protein are provided in the supporting information available with the online version of this paper [7]. The EcoRI protein was isolated, purified, and characterised as described in [7,13].

2.2. Cu^{2+} –EcoRI–DNA complex preparation

A solution of EcoRI (5 μM) plus TCGCGAATTCGCG (25 μM) was exchanged into buffer (30 mM N-ethylmorpholine, NEM; 0.3 M NH_4Cl ; 10% dioxane; 30% deuterated glycerol (D8); 65% D_2O ; pH 8.0). The solution was then concentrated. The final concentrations of EcoRI and DNA were 380 μM and 1.5 mM, respectively. Isotopically enriched $^{63}\text{CuCl}_2$ (Cambridge Isotope Labs, Inc.) was added at a 4:1 molar ratio (Cu^{2+} :protein dimer). Based on our isothermal titration calorimetry (ITC) results [7], the stoichiometry for Cu^{2+} binding is 2 Cu^{2+} per EcoRI dimer. We used a 4:1 (Cu^{2+} :dimer) ratio in these experiments to ensure that the protein is fully loaded with Cu^{2+} , given that the apparent K_D is 14 ± 6 μM for Cu^{2+} coordination to the EcoRI–DNA complex. Excess free Cu^{2+} is present as ESR silent $[\text{Cu}(\text{OH})_2]_n$ in the NEM buffer (pH 8.0) that was used for the experiment [14]. The sample was stored at -80 °C and flash-frozen before each ESR experiment [7,15].

2.3. Spectroscopic measurements

X-band ESR experiments were performed on a Bruker Eleksys 580 spectrometer. W-band ESR experiments were performed on a Bruker Eleksys 680 spectrometer. All the pulse experiments were performed at 20 K.

The X-band three pulse ESEEM signals were collected by recording the stimulated electron spin echo intensity as a function of T using the pulse sequence $\pi/2-\tau-\pi/2-T-\pi/2-\tau$ -echo. The $\pi/2$ pulse length was 16 ns. The τ values at different magnetic fields are: 166 ns at 2810 G, 154 ns at 3050 G, 152 ns at 3116 G, 148 ns at 3189 G, and 140 ns at 3369 G. The values of τ were chosen in order to suppress the ^1H ESEEM signal. The initial T was incremented from 400 ns with a step size of 16 ns, for a total of 1024 points. A four-step phase cycle was used to eliminate unwanted signals [16]. The resulting time domain signals were baseline subtracted by using a polynomial fit.

The HYSCORE signals were collected by recording the electron spin echo intensity as a function of T_1 and T_2 using the pulse sequence $(\pi/2)-\tau-(\pi/2)-T_1-(\pi)-T_2-(\pi/2)-\tau$ -(echo), on the Cu^{2+} -bound EcoRI–DNA complex. The durations of the $(\pi/2)$ and (π) pulses were 16 and 32 ns, respectively. The interpulse delay τ was fixed at 200 ns. The time intervals T_1 and T_2 were incremented from 400 ns with a step size of 16 ns, for a total of 512 points. A four-step phase cycling was employed to eliminate unwanted signals. The spectra were simulated by using EasySpin software package [17].

For the ^1H ENDOR experiments at $B_0 = 32,625$ G, we did Davies ENDOR using the pulse sequence $\pi 1-T-\pi/2-\tau-\pi-\tau$ -echo with a radio frequency (rf) pulse applied during T . The duration of the $\pi 1$, $\pi/2$, and π pulses was adjusted to be 256, 16, and 32 ns, respectively. The rf pulse length was 10 μs . The interval T was 13 μs , τ was 600 ns.

For the ^1H ENDOR experiments at the other two lower magnetic fields (30,130 G, 29,500 G), we did Mims ENDOR using the pulse sequence $\pi/2-\tau-\pi/2-T-\pi/2-\tau$ -echo with an rf pulse applied during T . The length of the $\pi/2$ pulse was 28 ns, and that of the rf pulse was 25 μs . The interval T was 50 μs and τ was 128 ns.

For the ^2H ENDOR experiments at $B_0 = 32,625$ G, we did Mims ENDOR using the pulse sequence $\pi/2-\tau-\pi/2-T-\pi/2-\tau$ -echo with an rf pulse applied during T . The length of the $\pi/2$ pulse was 16 ns, and that of the rf pulse was 10 μs . The interval T was 13 μs and τ was 200 ns.

The frequency scales of the ^2H Mims ENDOR spectra were multiplied by a factor 6.5144 ($\gamma^{\text{H}}/\gamma^{\text{D}}$) in order to compare directly with the ^1H ENDOR data.

2.4. ENDOR simulations

The ENDOR spectra were simulated by the GENDOR 50 program from Hoffman's group, which can be downloaded from http://chemgroups.northwestern.edu/hoffman/endor_files/simulationprograms.htm.

2.5. ESEEM simulations

For computation in the case of $\text{Cu}^{2+}-^{14}\text{N}$ ESEEM signal, the Hamiltonian used to describe the system is

$$\hat{H} = \beta \vec{B} \cdot \vec{g} \cdot \hat{S} - g_n \beta_n B_0 \hat{I}_z + \hat{S} \cdot \vec{A} \cdot \hat{I} + \hat{I} \cdot \vec{Q} \cdot \hat{I}, \quad (1)$$

where β is Bohr magneton, $\hat{S}$ and $\hat{I}$ are the electron spin and nuclear spin operators, and $\vec{g}$, $\vec{A}$, and $\vec{Q}$ are the g -tensor of electron, hyperfine tensor, and nuclear quadrupole tensor, respectively. The relative orientation of the g -tensor and external magnetic field is described by two angles θ and ϕ . The relative orientation of the principal axis system (PAS) of the Cu^{2+} g -tensor and the Q -tensor, as well as the orientation of the PAS of the g -tensor and A -tensor, is described by six Euler angles, $(\alpha_1, \beta_1, \gamma_1)$, $(\alpha_2, \beta_2, \gamma_2)$ (Figure 1).

The three nuclear-quadrupole interaction (NQI) transitions of ^{14}N in the ESEEM spectrum are given by [18]

$$\nu_- = \frac{e^2 q Q (3 - \eta)}{4h}; \nu_0 = \frac{2\eta e^2 q Q}{4h}; \nu_+ = \frac{e^2 q Q (3 + \eta)}{4h}, \quad (2)$$

where e is the electron charge, q is the electric gradient at the nuclear site, Q is the nuclear quadrupole moment, η is the asymmetry parameter, and h is Planck's constant. The three principal components of the quadrupole Q -tensor relate to the asymmetry parameter η and the quadrupole coupling constant $e^2 Q q / h$: $\eta = (Q_x - Q_y) / Q_z$, $e^2 Q q / h = 2I(2I - 1)Q_z$, where I

is the nuclear quantum number. Therefore, the three Q -tensor principal values can be estimated from the X-band ESEEM spectra directly.

The g -tensor is obtained from the continuous wave ESR (CW-ESR) spectrum. The principal values of the A -tensor were estimated from literature values [19–21]. For axial symmetric A - and g -tensors, only β_2 and γ_2 affect the simulation results. In the simulation, the variables to be optimised are the hyperfine tensor A , the nuclear quadrupole tensor Q , the Euler angles describing the orientation of the PAS of g -tensor and A -tensor, as well as the orientation of the PAS of g -tensor and Q -tensor.

The robustness of the optimised variables was further tested by fitting ESEEM spectra at five different external magnetic fields.

3. Results and discussion

3.1. W-band field-swept electron spin echo (FS-ESE) spectrum confirms the existence of the two Cu^{2+} components

Figure 2(a) shows the W-band (~ 94 GHz) FS-ESE spectrum (black solid line) and the simulation result (black dashed line). Two Cu^{2+} components with different g_{zz} and A_{zz} values were observed: component 1 (cp1) with $g_{zz} = 2.289$, $g_{xx} = g_{yy} = 2.055$, $A_{zz} = 163$ G; component 2 (cp2) with $g_{zz} = 2.227$, $g_{xx} = 2.072$, $g_{yy} = 2.035$, $A_{zz} = 143$ G. In order to clearly distinguish the two components, we also show the first derivative of the FS-ESE spectrum in Figure 2(b). The W-band data showed a much higher resolution than the X-band data [7]. The A_{zz} values of cp1 and cp2 are consistent with the results observed at the X band (~ 9.5 GHz) [7] and within the range of type II Cu^{2+} complex [22], indicating that both components have four equatorially coordinated ligands. The unequal g_{xx} and g_{yy} values of cp2 may be attributed to the distortion of the planarity of the equatorial coordinated ligands [7]. Since the axially coordinated ligands have a small impact on the g -tensor [23], the difference of the g -tensor principal values in the two components suggests either different types of equatorially coordinated ligands or different bond lengths and angles among these ligands and the Cu^{2+} centre [23,24].

3.2. W-band ENDOR spectra prove the existence of equatorial coordinated water molecule

Based on extensive MD simulations and a DEER-based triangulation approach, we had previously postulated that the N_ϵ from H114 imidazole, carboxylate oxygen from E170 and E245, and oxygen from a water molecule (W_ϵ) form the equatorial coordination plane in cp1, as shown in Figure 3(a) [7]. Meanwhile, we did not observe ^{31}P ENDOR signal at W-band (data not shown). This result combined with distance measurements indicate that Cu^{2+} does not interact with DNA in the EcoRI–DNA system. Furthermore, our ITC titrations showed that the stoichiometry for Cu^{2+} binding was two Cu^{2+} ions to each dimeric EcoRI–DNA complex. On the other hand, we had proposed that the N_ϵ from H114 imidazole and oxygens from two water molecules ($\text{W}_{\epsilon 1}$ and $\text{W}_{\epsilon 2}$) form an equatorial coordination plane in cp2, as shown in Figure 3(b) [7]. The two carboxylate oxygens from E170 coordinate to Cu^{2+} at the same time. These two oxygens are not in the plane formed by the other three ligands. This observation may explain the abnormal g -tensor of cp2 in the EcoRI–DNA

complex. Both proposed coordination environments have two axially coordinated water molecules (W_{a1} and W_{a2}).

In this work, we employ W-band ENDOR spectroscopy to prove the existence of the equatorially and/or axially coordinated water molecules. First, we measured ^1H ENDOR spectra of the Cu^{2+} –EcoRI–DNA complex in H_2O at different magnetic fields. Different types of ^1H atoms with different hyperfine values are observed. These hyperfine interactions give us information on the distance between ^1H atom and the Cu^{2+} centre, which can help us identify the possible origin of these ^1H atoms in the protein–DNA complex. Second, we detected the ^1H ENDOR spectra of the Cu^{2+} –EcoRI–DNA complex exchanged with D_2O . The decrease in the intensity of ENDOR signals of solvent-exchangeable protons helped in the assignment of peaks observed in ^1H ENDOR. Third, the existence of solvent-exchangeable protons was confirmed by the scaled-up ^2H ENDOR spectrum of the sample in D_2O .

Figure 3(c) shows the W-band ^1H Davies and Mims ENDOR obtained at three different magnetic fields: 32,625 G, 30,130 G, and 29,500 G (shown by arrows in the inset) for the Cu^{2+} –EcoRI–DNA complex in H_2O .

At 32,610 and 30,130 G, both components contribute to the ENDOR signals. At 29,500 G, only cp1 contributes to the ENDOR signal. The two spectra at the higher magnetic fields consist of a relatively broad doublet at $\sim\pm 5.0$ MHz and splittings below $\sim\pm 2.5$ MHz centred at the proton Larmor frequency, ν_L (^1H). Because of the low signal-to-noise ratio at the lowest magnetic field (29,500 G), the relatively broad doublet at $\sim\pm 5.0$ MHz is hard to identify.

The broad doublet with the splitting at $\sim\pm 5.0$ MHz is in the expected range for the interaction of Cu^{2+} with the protons of equatorially bound water molecule(s) [25–27]. Note that equatorial waters in both sites are proposed based on MD simulations (W_e) (see Figure 3(a) and 3(b)). In order to unambiguously identify the origins of these ^1H ENDOR signals, ^1H Davies ENDOR of the Cu^{2+} –EcoRI–DNA complex in D_2O was performed. Given the signal-to-noise ratio, we only performed ^1H and ^2H ENDOR experiments at 32,610 G.

Figure 4 shows that the broad doublet with the splitting at $\sim\pm 5.0$ MHz disappears when the complex is exchanged in D_2O (dotted line). This observation is unambiguously confirmed by the scaled-up ^2H Mims ENDOR spectrum, which includes these missing peaks (Figure 4, dashed line). The frequency scales of the ^2H Mims ENDOR spectra were multiplied by a factor 6.5144 ($\gamma^{\text{H}}/\gamma^{\text{D}}$) in order to compare directly with the ^1H ENDOR data. The presence of a peak at $\sim\pm 5.0$ MHz in the ^2H Mims ENDOR spectrum verifies that this broad doublet arises from the solvent-exchangeable protons. Simulation of this solvent exchangeable ^1H ENDOR signal at two different external magnetic fields, shown as dashed line in Figure 3(c), yields an $A_{\text{iso}} = -1.2$ MHz, and $T_{\text{dip}} = 7.8$ MHz. The Euler angles, which describe the orientation between the A -tensor and g -tensor, are $\alpha = 5^\circ$, $\beta = 72^\circ$, $\gamma = 0^\circ$. These values are similar to the couplings of protons from water molecule(s) equatorially coordinated to the copper centre [25–27]. All these observations explicitly validate the existence of the

equatorial bound water (W_e) in the Cu^{2+} coordination, since there are no solvent-exchangeable protons from the protein close to the Cu^{2+} centre to provide the splitting at $\sim \pm 5.0$ MHz.

The ^1H ENDOR spectrum in D_2O shows that the relative intensity of the signal with a splitting at $\sim \pm 1.7$ MHz decreased, as shown in Figure 4. This result clearly indicates that the solvent-exchangeable as well as the non-exchangeable protons contribute to this peak. Simulation of this peak yields $A_{\text{iso}} = -1.0$ MHz, $T_{\text{dip}} = 2.8$ MHz, and $\alpha = 0^\circ$, $\beta = 5^\circ$, $\gamma = 0^\circ$. The simulations are shown as dotted line in Figure 3(c). These parameters are close to the proton from the axial coordinated water molecule [25–27], which could indicate the existence of axial coordinated water (W_a) molecule(s).

3.3. Field-dependent X-band ESEEM spectra indicate that both Cu^{2+} components have Cu^{2+} –histidine coordination

We have shown that Cu^{2+} coordinates to H114 in the EcoRI-DNA complex [7]. According to the X-band CWESR result [7], the two components contribute to the ESR signal at different magnetic fields with different ratios. In order to provide the direct experimental evidence of the Cu^{2+} –H114 coordination in both components, we employed the X-band three pulse ESEEM experiments at five magnetic fields. Figure 5 shows the ESEEM time domain data and spectrum (solid line) at 2810 G, where only cp1 contributes to the ESEEM signal. In the spectrum, two sharp peaks at ~ 0.67 MHz and ~ 1.5 MHz, a broad shoulder peak at ~ 1.0 MHz, as well as a broad peak at ~ 3.5 MHz were observed. All these peaks are the characteristic transitions of the Cu^{2+} electron spin interacting with the remote ^{14}N nucleus. The peaks below 2 MHz are the typical NQI of the ^{14}N of histidine imidazole [28–31], which indicates Cu^{2+} –histidine coordination in cp1. These NQI transitions can be observed when the nitrogen nuclear Zeeman and hyperfine coupling interactions cancel each other in one electron spin manifold [28,32–37]. To obtain the detailed hyperfine and quadrupole interaction information, we simulated the ESEEM spectrum as shown in Figure 5 (dashed lines). The ESEEM data are best fitted with an asymmetric parameter $\eta = 0.65 \pm 0.01$, the quadrupole coupling constant $e^2Qq/h = 1.51 \pm 0.01$ MHz, $A_N = [1.1, 1.1, 2.5] \pm 0.1$ MHz, $(\alpha_1, \beta_1, \gamma_1) = (90^\circ, 30^\circ, 30^\circ) \pm 5^\circ$, $(\alpha_2, \beta_2, \gamma_2) = (0^\circ, 30^\circ, 35^\circ) \pm 5^\circ$, respectively.

Due to the overlap of the two Cu^{2+} components in the ESR spectrum, we cannot obtain the ESEEM signal purely from cp2 at the X-band. In order to get the ESEEM signal of cp2, we collected the ESEEM spectra at four other magnetic fields, 3369 G, 3189 G, 3116 G, and 3050 G, where both components contribute to the ESEEM signals. The ESEEM signal at these fields can be expressed as $V = AV_1 + BV_2$, where V_1 and V_2 are the ESEEM signals of cp1 and cp2, respectively. A and B are the relative ratios of the ESEEM signal from the two components at a certain external magnetic field. The values of A and B under different external magnetic fields can be estimated based on the CW-ESR spectrum at the X-band.

Figure 6(a)–(h) shows the experimental ESEEM time domain data and spectra (solid line) at 3369 G, 3189 G, 3116 G, and 3050 G. The peaks in the spectra below ~ 1.5 MHz and the broad peaks in between ~ 3.5 and ~ 4.0 MHz are the indication of the Cu^{2+} –histidine coordination. We simulated the ESEEM spectra at these four magnetic fields with the consideration of both components contributed to the ^{14}N ESEEM signal. The simulation

results are shown as the dashed lines in Figure 6. These ESEEM spectra are best fitted with the following parameters for cp2: $\eta = 0.73 \pm 0.01$, the quadrupole coupling constant $e^2Qq/h = 1.51 \pm 0.01$ MHz, $A_N = [1.2, 1.2, 2.4] \pm 0.1$ MHz, $(\alpha_1, \beta_1, \gamma_1) = (85^\circ, 62^\circ, 85^\circ) \pm 5^\circ$, and $(\alpha_2, \beta_2, \gamma_2) = (35^\circ, 45^\circ, 0^\circ) \pm 5^\circ$. To verify that both components contribute to the ^{14}N ESEEM signal, we also simulated the spectra with signal from only one component. Poorer quality fits were obtained if only one component was assumed at these four magnetic fields, as shown in Figures A1 and A2 of Appendix 1. This observation prove that cp2 has Cu^{2+} – histidine coordination as well, which is consistent with our previous observation [7].

Compared to the ESEEM technique, the 2D HYSCORE can improve the resolution and provide more detailed spectroscopic information [21,35,38–45]. To provide more detailed structural information and validate the results from the ESEEM spectra, HYSCORE experiments were performed at three different external magnetic fields. Figure 7 shows the HYSCORE spectra of the Cu^{2+} –EcoRI–DNA complex and the simulation results based on the ^{14}N hyperfine and nuclear quadrupole information from ESEEM experiments. All the HYSCORE spectra only contain one broad cross peak around (1.6 MHz, 4.0 MHz). Given the similar hyperfine and nuclear quadrupole values of ^{14}N atom in the two components, it is hard to separate the cross peaks of the two components in the X-band HYSCORE. Simulation results shown in Figure 7 are consistent with this observation. This cross peak, which is due to the correlation between the NQI and double quantum transition of the non-coordinated nitrogen of histidine [21,35,38,39,45], further confirms the Cu^{2+} –histidine coordination.

4. Conclusion

The Cu^{2+} -binding sites in the EcoRI–DNA–(Cu^{2+})₂ complex have been examined by the pulsed ESR experiments at W- and X- bands. The W-band ENDOR spectra indicate the existence of an equatorially coordinated water molecule and the possible existence of an axial water ligand. The X-band ESEEM spectra at different external magnetic fields show that two distinct spectral components both represent Cu^{2+} coordination to histidine, consistent with the Cu^{2+} –H114 coordination inferred in our previous study [7]. This conclusion is further confirmed by the HYSCORE spectra. These results demonstrate the utility of W-band ENDOR, field-dependent ESEEM, and HYSCORE to characterise details of Cu^{2+} coordination in the EcoRI–DNA complex, and foreshadow future high field ENDOR, field-dependent ESEEM, and HYSCORE experiments with isotopically labelled protein to further refine the role of protein in Cu^{2+} -coordination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix 1

Figure A1.

The experimental (solid line) and simulated (dotted line) three pulse ESEEM time domain data and spectra of the Cu^{2+} -EcoRI-DNA complex at external magnetic field 3369 G (a and b), 3189 G (c and d), 3116 G (e and f), and 3050 G (g and h). In the simulation, only signal from cp1 is included.

Figure A2.

The experimental (solid line) and simulated (dotted line) three pulse ESEEM time domain data and spectra of the Cu^{2+} -EcoRI-DNA complex at external magnetic field 3369 G (a and b), 3189 G (c and d), 3116 G (e and f), and 3050 G (g and h). In the simulation, only signal from cp2 is included.

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Figure 1.

The definition of the relative orientation between external magnetic field and PAS of g -tensor, PAS of A -tensor and g -tensor, and PAS of Q -tensor and g -tensor. The relative orientation between external magnetic field and PAS of g -tensor is described by θ and ϕ . The relative orientations between the PAS of Q -tensor and g -tensor and between the PAS of A -tensor and g -tensor are described by two sets of Euler angles $(\alpha_1, \beta_1, \gamma_1)$ and $(\alpha_2, \beta_2, \gamma_2)$, respectively.

Figure 2.

(a) The experimental (black solid line) and simulated (black dashed line) W-band (~94 GHz) FS-ESE spectrum of the Cu^{2+} -EcoRI-DNA complex at 20 K. (b) The first derivative of the W-band FS-ESE spectrum in (a). Experimental result is shown by the black solid line and simulated result is shown by the black dashed line. The best-fitting simulations were obtained with the following parameters: (cp 1) $g_{xx} = g_{yy} = 2.055$, $g_{zz} = 2.289$, $A_{xx} = A_{yy} = 20$ G, $A_{zz} = 163$ G and (cp2) $g_{xx} = 2.072$, $g_{yy} = 2.035$, $g_{zz} = 2.227$, $A_{xx} = A_{yy} = 20$ G, $A_{zz} = 143$ G. The relative ratio of the two components is close to 1:1.

Figure 3.

(a) and (b) Two Cu^{2+} coordination environments postulated from the MD simulations, described in detail by Yang *et al.* [7]; (c) W-band ^1H Davies (32,610 G) and Mims ENDOR (30,130 G and 29,500 G) spectra in H_2O and simulated spectra (dashed and dotted lines) for equatorial and axially coordinated water. The inset is the W-band FS-ESE spectrum of the Cu^{2+} -EcoRI-DNA complex. The magnetic fields at which the ENDOR data were collected are indicated by arrows in the inset.

Figure 4.

^1H Davies ENDOR spectra of the Cu^{2+} –EcoRI–DNA sample in H_2O (solid line) and D_2O (dotted line) at 32,610 G. The dashed line shows the scaled-up ^2H Mims ENDOR spectrum. The inset is the unscaled ^2H Mims ENDOR spectrum.

Figure 5.

The experimental (solid line) and simulated (dashed line) three pulse ESEEM time domain data and spectrum of the Cu^{2+} -EcoRI-DNA complex at 2810G.

Figure 6.

The experimental (solid line) and simulated (dashed line) three pulse ESEEM time domain data and spectra of the Cu^{2+} -EcoRI-DNA complex at external magnetic field 3369 G (a and b), 3189 G (c and d), 3116 G (e and f), and 3050 G (g and h).

Figure 7.

The experimental (a, c and e) and simulated (b, d, and f) HYSCORE spectra of the Cu^{2+} – EcoRI–DNA complex at external magnetic field 3369 G (a and b), 3189 G (c and d), 3116 G (e and f). The intensity of the simulated spectra is graded from black (0.05) to white (0.5).