Journal: *Biochemistry*

Characterization of the Product Radical Structure in the Co^{II}-Product Radical Pair State of Coenzyme B_{12}-Dependent Ethanolamine Deaminase by using Three-Pulse ^2H ESEEM Spectroscopy

Kurt Warncke*

Department of Physics, N201 Mathematics and Science Center, 400 Dowman Drive, Emory University, Atlanta, Georgia 30322

SUPPORTING INFORMATION

Comparison of simulation models corresponding to different active site populations

Figure S1 shows the Fourier transform of ESEEM collected for the condition, τ=303 ns and B_0=388.0 mT, and the overlaid Fourier transforms of ESEEM simulated for two different models. The τ=303 ns condition was chosen for illustration, because H_s, $H_{\beta a}$, and $H_{\beta b}$ features are all clearly shown in the Fourier transform. The "Two Site Model" (top, Figure S1) corresponds to two separate populations of ethanolamine deaminase enzyme active sites in the sample, and is the model that best accounts for the observed ESEEM frequencies and amplitudes, as described in the text. The ESEEM for this model was simulated and combined according to eq 3 in the text. The hyperfine couplings in the two populations of enzyme active sites are as follows: (1) H_s, $H_{\beta a}$,, H_w and H_w, and (2) H_s, $H_{\beta b}$, H_w and H_w. The "One Site Model" (bottom, Figure S1) corresponds to a single type of enzyme active site population in the sample. This population is different from either of the two populations in the Two Site Model. The ESEEM for the One Site Model was simulated and combined according to eq 2 in the text. The single

enzyme active site population includes the following hyperfine couplings: H_s, $H_{\beta a}$, $H_{\beta b}$, H_w and two H_w. The inclusion of the four types of hyperfine coupling is required to reproduce all of the spectral features observed in the τ=303 ns and five other τ and B_0 conditions.

The match of the simulation to experiment is superior for the Two Site Model relative to the One Site Model. For each model, the frequency positions of the spectral features appear over the correct ranges, because the hyperfine coupling parameters are the same for each model. However, the spectral amplitudes are reproduced well for the Two Site Model, but are poorly reproduced by the Single Site Model. The difference is caused by the method of combination of the ESEEM. For τ=303 ns and B_0=388.0 mT, the relative individual simulated modulation amplitude (which is characterized by the "modulation depth", the peak-to-trough amplitude of the modulation divided by the constant amplitude of the electron spin-echo envelope) for the $H_{\beta a}$ coupling is large (maximum modulation depth at τ+T>160 ns, 0.7) relative to H_s (0.3), $H_{\beta b}$ (0.1) and H_w (0.01). In the product modulation for the One Site Model, and for the fraction f of the Two Site Model, the $H_{\beta a}$ hyperfine coupling contribution is dominant. The amplitude of the $H_{\beta a}$ features are therefore too large, relative to the H_s, $H_{\beta b}$, and H_w features. In the population of sites in the Two Site Model that correspond to the fraction, 1-f, the H_s modulation is dominant. Therefore, the sum taken in eq 3 leads to a larger relative contribution of H_s to the total modulation [$E_{tot}(\tau, T)$], and thus achieves the observed reproduction of the experimental ESEEM and Fourier transform.

<u>Sensitivity of the simulated ESEEM to small variations in hyperfine coupling parameters</u>

Figures S2, S3 and S4 show the influence of a variation of individual hyperfine coupling simulation parameters that is equal to a decrease and increase in the best-fit value by 10% of the

best fit value. The τ=303 ns, B_0=388.0 mT condition was chosen for illustration of H_s, $H_{\beta a}$, and $H_{\beta b}$ features, because they are all clearly shown in the Fourier transform. The τ=300 ns, B_0=313.0 mT condition was chosen for illustration of the H_w feature, because it is clearly shown in the Fourier transform. The τ=303 ns, B_0=388.0 mT best-fit simulation is presented in Figure S3 (bottom), for comparison. Figure S2 shows that the ±10% variation of r for H_s, $H_{\beta a}$ and $H_{\beta b}$ produces significant deviations from the best-fit simulation of the H_s, $H_{\beta a}$ and $H_{\beta b}$ features. Figure S3 shows that the ±10% variation of A_{iso} for $H_{\beta a}$ and $H_{\beta b}$ produces significant deviations from the best-fit simulation of the $H_{\beta a}$ and $H_{\beta b}$ features. Figure S4 (top spectra) shows that the ±10% variation of r for H_w also produces significant deviations from the best-fit simulation of the H_w (see below for further description of the constraints on H_w simulations). These results show that the line shapes of the ESEEM frequency spectra are sensitive to small changes of ≤10% in the hyperfine coupling simulation parameters. Comparable results are obtained for the other τ and B_0 conditions. Therefore, the simulation parameters are well-constrained by the frequency positions and amplitudes of the corresponding features in the experimental spectra.

Requirements for the weak hyperfine coupling

The H_w nucleus is the most weakly coupled of the four nuclei necessary to simulate the ESEEM and ESEEM Fourier transforms. The weak individual modulation depth of the H_w coupling relative to the individual modulation depths of H_s, $H_{\beta a}$ and $H_{\beta b}$ reduces the modulation and spectral amplitude of H_w in the combined ESEEM. Further, the τ values, which were chosen to suppress the strong, interfering ^{1}H matrix contribution to the ESEEM (see description under Experimental Procedures/ESEEM Spectroscopy), coincide with essentially full suppression of the ^2H_w features near to the ν_{2H} frequency for τ=525 and 975 ns at B_0=313.0 mT and τ=424 and

787 ns at B_0=388.0 mT. In other words, the τ-suppression weighting factor for the modulation, which can be approximated by $\sin^2[\pi\nu_{\alpha/\beta}/\tau]$ (references #36 and #37), where $\nu_\alpha \approx \nu_\beta \approx \nu_{2H}$, is close to 0 under these conditions. For τ=300 ns at B_0=313.0 mT and τ=303 ns at B_0=388.0 mT, the values of $\sin^2[\pi\nu_{\alpha/\beta}/\tau]$ are 0.84 and 0.50, respectively, and H_w modulation amplitude is thus present. The τ=300 ns, B_0=313.0 mT condition supplies the best constraints on the H_w simulation parameters, because the amplitude is largest for this condition. The H_w hyperfine coupling parameters were therefore determined primarily by simulations for τ=300 ns at B_0=313.0 mT and τ=303 ns at B_0=388.0 mT. The H_w parameters were then incorporated into simulations performed for other τ and B_0 conditions, and the results were consistent with the experimental ESEEM and Fourier transforms.

Figure S4 shows simulations obtained with a single coupled H_w nucleus (Single H_w, middle) and in the complete absence of a coupled H_w nucleus ($-H_w$, bottom). The simulated $-H_w$ Fourier transform reveals that the ^2H_s feature has a shoulder near to the ν_{2H} frequency. In contrast, the experimental Fourier transform does not appear to have a significant shoulder at this position. In the simulations, the shoulder cannot be eliminated without changing the match to the frequency position of the maximum amplitude and of the line width of the experimental ^2H_s feature. (Additional assumptions, such as rhombicity in the hyperfine tensor for H_s, would be required to improve the overall reproduction. This was not pursued in the present study. Deviations of the tensors from axial symmetry would be best addressed experimentally with the higher spectral resolution attainable by using electron-nuclear double resonance spectroscopy of the corresponding ^1H_w nucleus.) The shoulder increases the apparent amplitude of the simulated H_w feature in the combination simulation, which precludes an exact overlaid match with the line shape in the experimental Fourier transform. However, the amplitude and width of the best-fit

simulated ^2H_w feature itself does appear to adequately match the amplitude and width of the experimental ^2H_w feature, as shown in the best-fit simulation in Figure 5, Panel A, Top. Two coupled H_w nuclei, with the parameters in Table 1, are required to provide a satisfactory match to the experimental ^2H_w feature. Figure S4 (middle) shows that a single coupled H_w nucleus, with parameters as given in Table 1, contributes additional amplitude above the H_w shoulder, but the amplitude does not correspond well to both the amplitude and width of the experimental ^2H_w feature. Increasing the distance parameter, *r*, for H_w causes lower intensity and a narrower line width, which produce poorer agreement experiment. Decreasing *r* leads to an increase in amplitude, which, in itself, would obviate the requirement for two H_w nuclei, but the concomitant increase in line width is inconsistent with the experimental ESEEM.

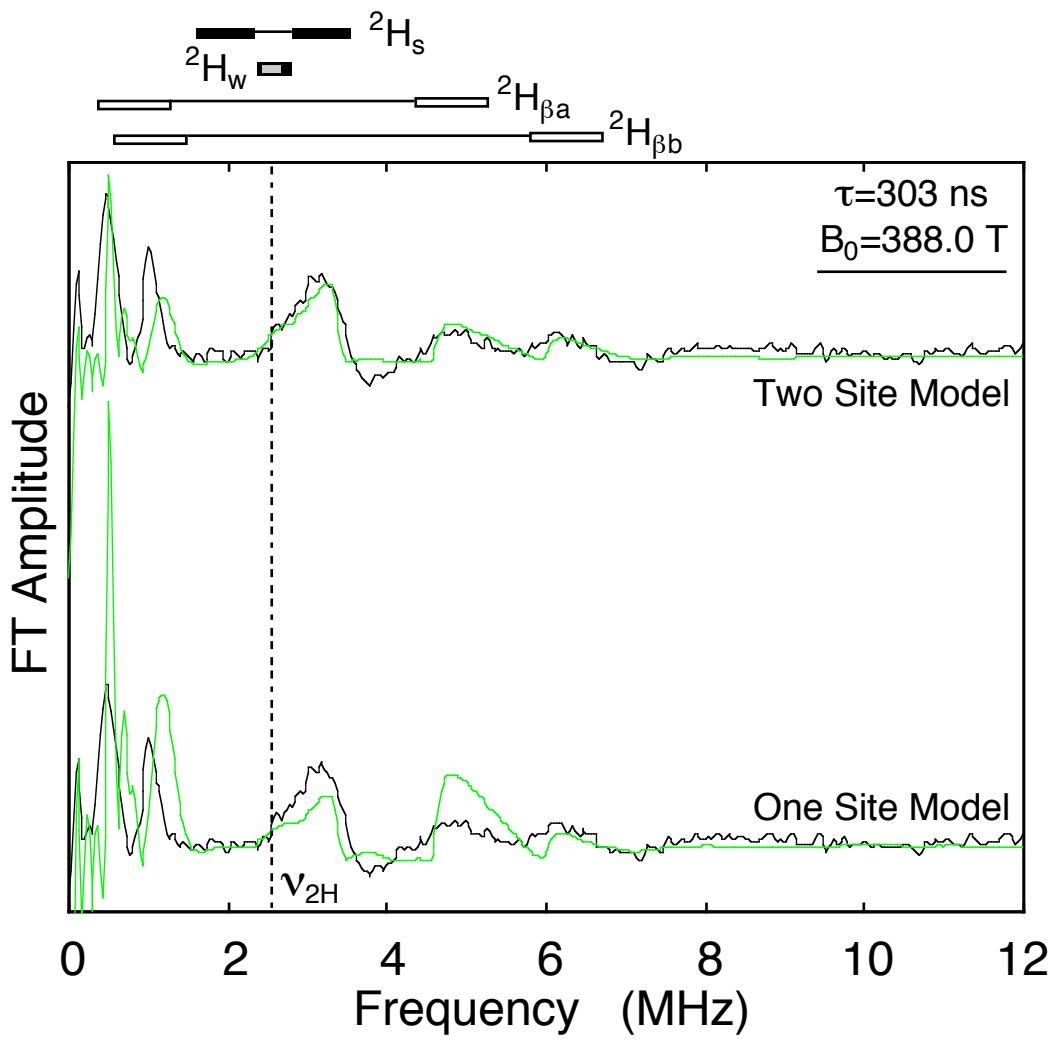

FIGURE S1. Cosine Fourier transforms of the three-pulse $^2H/^1H$ quotient ESEEM from the product radical in the Co^{II}-product radical pair intermediate in ethanolamine deaminase (dark solid line), and overlaid cosine Fourier tranforms of simulated ESEEM (light solid line). The top ESEEM spectral simulation (Two Site Model) corresponds to the model of two separate active site populations (distinguished by the presence of either $H_{\beta a}$ or $H_{\beta b}$), as described in the text, and is based on eq 3. The Two Site Model simulation is identical to the simulation shown in Figure 5, Panel B, τ=303 ns. The bottom ESEEM spectral simulation (One Site Model) corresponds to a single active site population that includes both $H_{\beta a}$ and $H_{\beta b}$. The One Site Simulation is based on eq 2, with i=1-5 corresponding to H_s, $H_{\beta a}$, $H_{\beta b}$, H_w and H_w. Dashed lines mark the positions of the free deuteron resonance frequency (ν_{2H}), and hyperfine coupling assignments are shown. Experimental conditions are described in the legend to Figure 4. The same hyperfine simulation parameters, corresponding to the best-fit values for τ=303 ns and B_0=388.0 mT (see legends to Figures S2-S4), are used for both Two and One Site Model simulations.

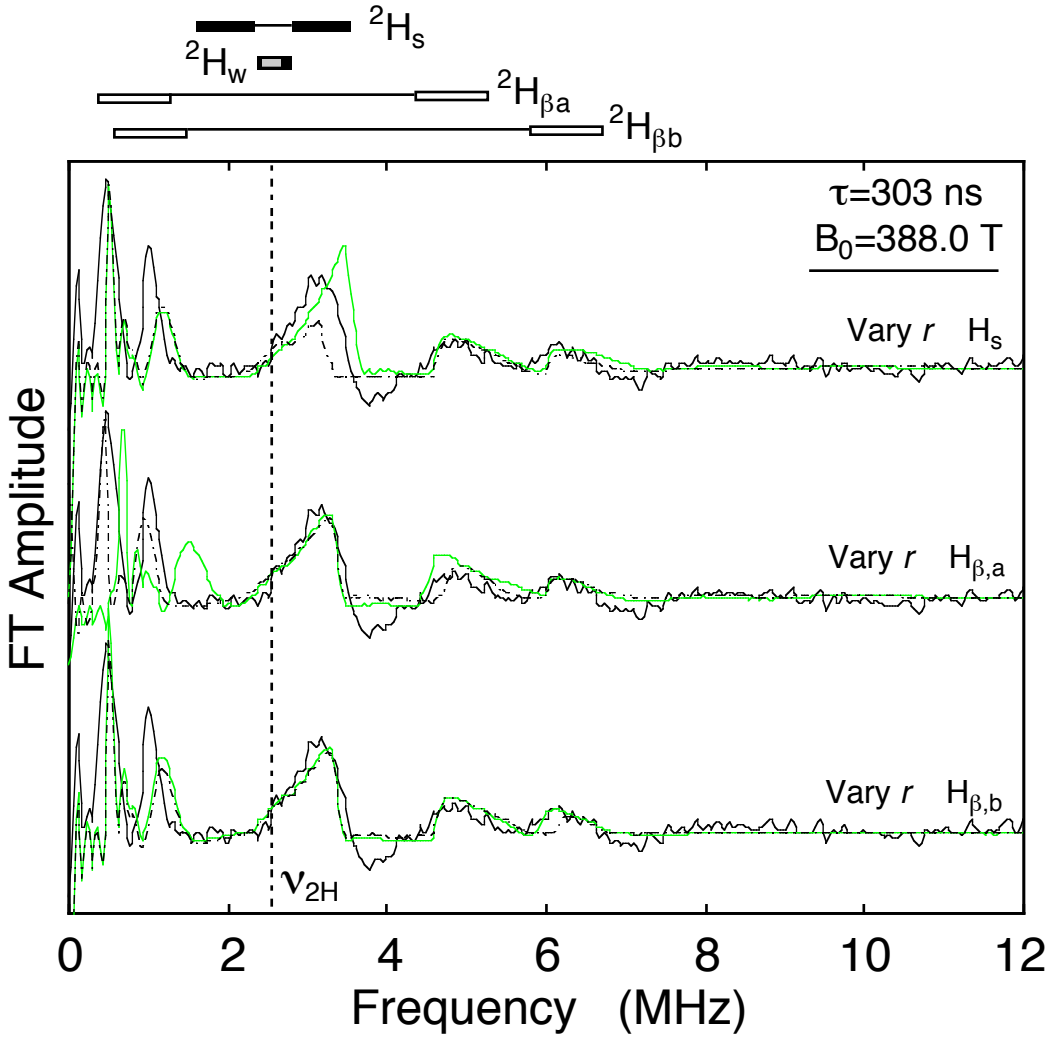

FIGURE S2. Cosine Fourier transforms of the three-pulse $^2H/^1H$ quotient ESEEM from the product radical in the Co^{II}-product radical pair intermediate in ethanolamine deaminase (dark solid line), and overlaid cosine Fourier tranforms of simulated ESEEM. The simulations correspond to a –10% change (light solid line) or a +10% change (dark dash-dot line) in the indicated hyperfine coupling parameter, relative to the best-fit value for τ=303 ns All other hyperfine coupling parameters are held fixed at their best-fit values for τ=303 ns. The best fit value for τ=303 ns and B_0=388.0 mT was averaged with values from the other τ and B_0 conditions to give the average best-fit parameter values presented in Table 1. (Top simulations) The distance parameter, *r*, for H_s was 2.0 Å (-10%) or 2.4 Å (+10%), relative to the best-fit value of 2.2 Å. (Middle simulations) The distance parameter, *r*, for $H_{\beta a}$ was 2.2 Å (-10%) or 2.6 Å (+10%), relative to the best-fit value of 2.4 Å. (Bottom simulations) The distance parameter, *r*, for $H_{\beta b}$ was 2.2 Å (-10%) or 2.6 Å (+10%), relative to the best-fit value of 2.4 Å. Dashed lines mark the positions of the free deuteron resonance frequency (ν_{2H}), and hyperfine coupling assignments are shown. Experimental conditions are described in the legend to Figure 4.

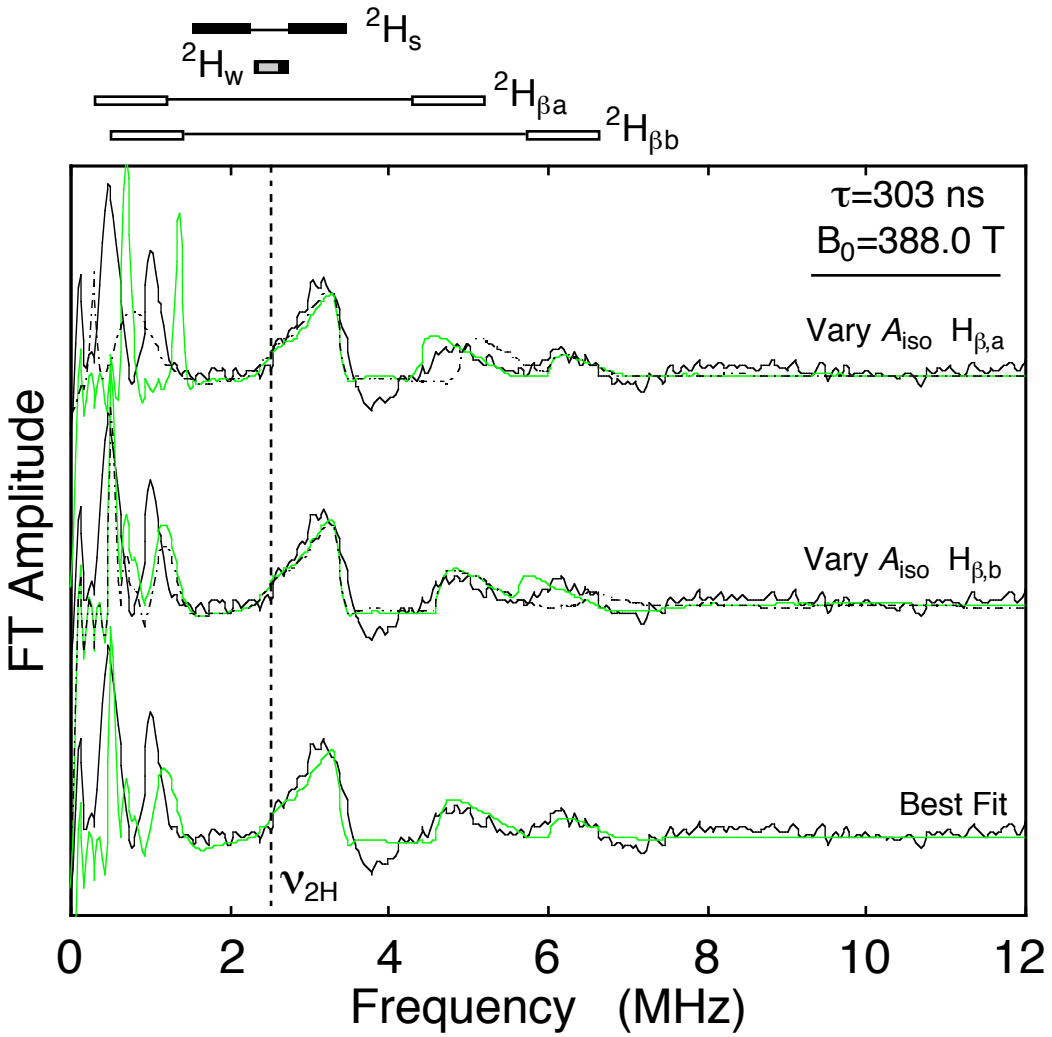

FIGURE S3. Cosine Fourier transforms of the three-pulse $^2H/^1H$ quotient ESEEM from the product radical in the Co^{II}-product radical pair intermediate in ethanolamine deaminase (dark solid line), and overlaid cosine Fourier tranforms of simulated ESEEM. The simulations in the top and middle spectra correspond to a –10% change (light solid line) or a +10% change (dark dash-dot line) in the indicated hyperfine coupling parameter, relative to the best-fit value for τ=303 ns All other hyperfine coupling parameters are held fixed at their best-fit values for τ=303 ns. The best fit value for the τ=303 ns and B_0=388.0 mT condition was averaged with values from the other τ and B_0 conditions to give the average best-fit parameter values presented in Table 1. (Top simulations) The parameter, A_{iso}, for H_s was 4.5 MHz (-10%) or 5.5 MHz (+10%), relative to the best-fit value of 5.0 MHz. (Middle simulations) The parameter, A_{iso}, for H_s was 7.0 MHz (-10%) or 8.6 MHz (+10%), relative to the best-fit value of 7.8 MHz. (Bottom simulation) Reproduction of the best-fit simulation (light solid line) presented in Figure 5, Panel B, Top. Dashed lines mark the positions of the free deuteron resonance frequency (ν_{2H}), and hyperfine coupling assignments are shown. Experimental conditions are described in the legend to Figure 4.

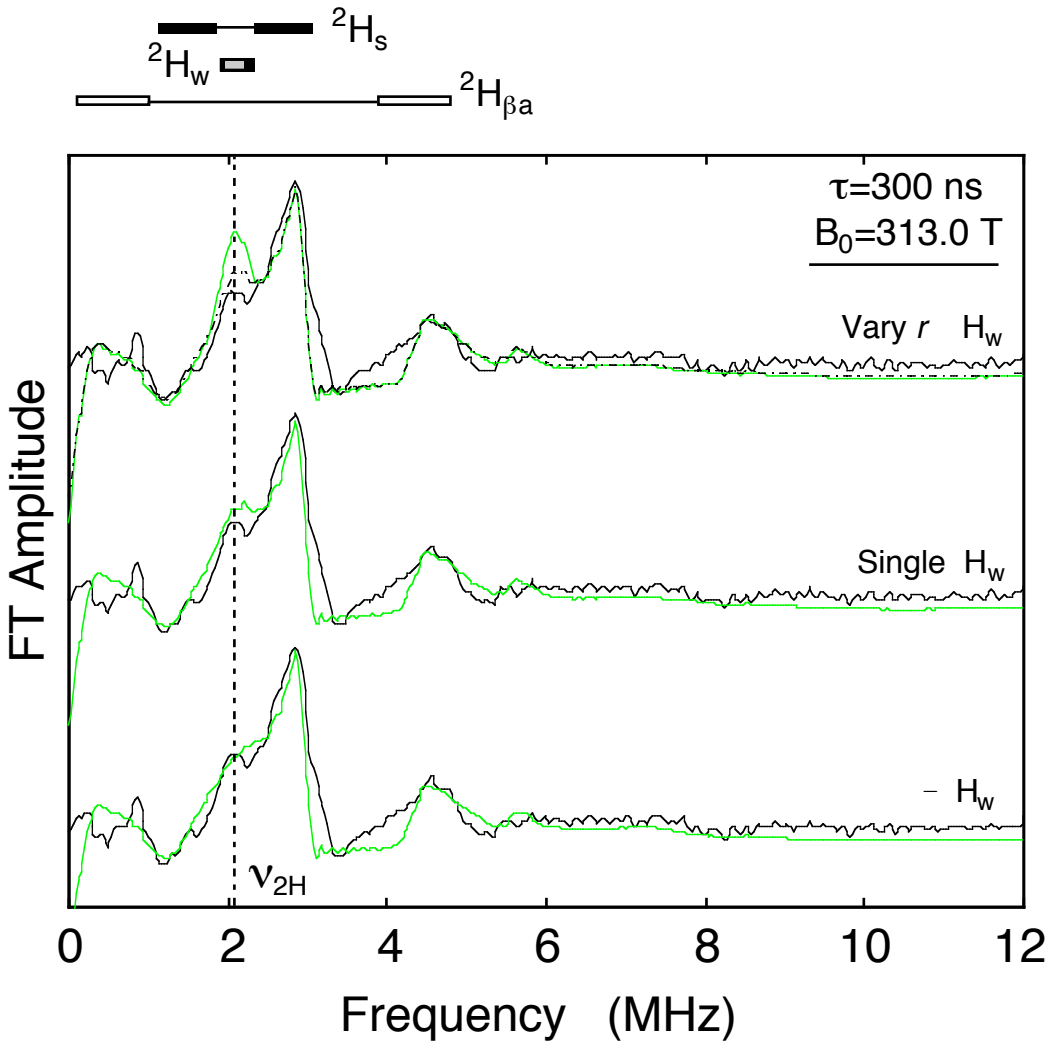

FIGURE S4. Cosine Fourier transforms of the three-pulse $^2H/^1H$ quotient ESEEM from the product radical in the Co^{II}-product radical pair intermediate in ethanolamine deaminase (dark solid line), and overlaid cosine Fourier tranforms of simulated ESEEM. The experimental and simulated ESEEM correspond to τ=300 ns and B_0=313.0 mT (Top simulations) The simulations correspond to a –10% change (light solid line) or a +10% change (dark dash-dot line) in the distance parameter, r, for H_w, relative to the best-fit value for τ=300 ns. All other hyperfine coupling parameters were held fixed at their best-fit values for τ=300 ns. The distance parameter, r, for ^2H_w was 3.8 Å (-10%) or 4.6 Å (+10%), relative to the best-fit value of 4.2 Å. The simulations correspond to two coupled ^2H_w nuclei, as described in the text. (Middle simulation) Simulation obtained for a single coupled ^2H_w nucleus (light solid line). The simulation is otherwise identical to the best-fit simulation in Figure 5. (Bottom simulation) Simulation obtained by omitting the ^2H_w hyperfine coupling. The hyperfine coupling parameters correspond to the best fit hyperfine coupling parameters for τ=300 ns.