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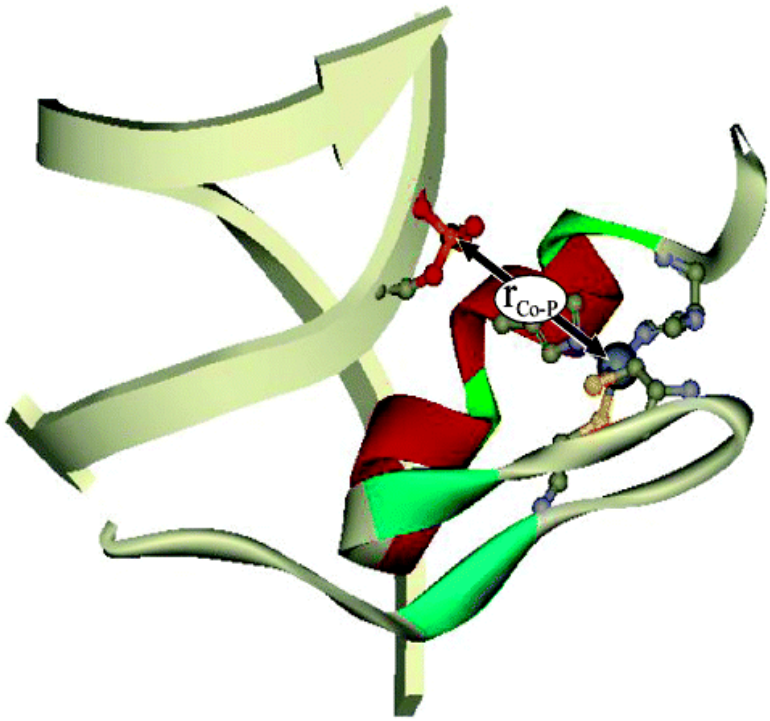

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Cobalt-Substituted Zinc Finger 3 of Transcription Factor IIIA: Interactions with Cognate DNA Detected by ^{31}P ENDOR Spectroscopy

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Zinc metalloenzymes are extremely abundant in nature, but the spectroscopic silence of the Zn(II) ion (d^{10}, $S = 0$; $I = 0$ isotopes, 95.9%) hinders their study. One way of circumventing this problem is substitution of Zn(II) by the spectroscopically rich Co(II),[1] which typically produces a high-spin Co(II) center that can be studied by optical and EPR spectroscopies. Although low-spin Co(II) systems provide highly resolved EPR spectra and are amenable to study by electron nuclear double resonance (ENDOR),[2] high-spin (hs) Co(II) complexes tend to have broad, unresolved EPR spectra,[3] and there is no published ENDOR study of the hs-Co(II) coordination environment in a biological system.[4] We demonstrate that ENDOR can be equally well applied to hs-Co(II), with a study of DNA binding to the Co-substituted Cys_2/His_2 single Zn-finger domain, Finger 3 (F3), from the prototypical zinc finger protein, transcription factor IIIA (TFIIIA) from *Xenopus laevis*.

TFIIIA is a nine-finger DNA binding protein that associates with the internal control region (ICR) of the 5S ribosomal RNA gene and acts to upregulate its transcription into ribosomal RNA.[5−8] Finger 3 is thought to contribute centrally to the affinity of TFIIIA for the ICR.[9] The NMR structure of the adduct between fingers 1−3 and their cognate DNA within the ICR defined the Cys_2/His_2 coordination geometry of the metal ion and also how the protein interacts with bound DNA (Figure 1). The structure disclosed a number of specific amino acid/DNA major-groove contacts,[8,10−13] with F3 making the largest number.[11] In this paper, we use 35 GHz pulsed ^{1}H, ^{14}N, and ^{31}P ENDOR of Co(II)-F3 to characterize the metal-ion coordination sphere and to measure the distance from the metal ion to the nearest phosphodiester of bound DNA. Of particular importance to the use of Co(II) substitution for Zn(II), the ENDOR method shows that Co(II)-F3 undergoes sequence-specific binding to its cognate DNA.

The primary sequence of F3[14] corresponds to amino acids 71−100 of TFIIIA.[13,15] It was directly synthesized, purified, and substituted with Co(II) as previously described.[16,17] The cognate DNA oligonucleotide used here corresponds to base pairs 80−92 of the 5S rRNA ICR, d(5′-TGGATGGGAGACC-3′,3′-ACCTACCCTCT-GG-5′), also known as the C-block.[18]

The UV−vis spectrum of Co(II)-F3 has band maxima at 570 and 645 nm; its CW EPR spectrum has g values, $g = [5.2, 3.9, 2.2]$, both consistent with a nominally tetrahedral coordination environment for the Co(II) center.[19,20] Neither optical nor EPR spectra of Co(II)-F3 are affected by the presence of C-block or other DNAs (see below).

A pioneering X-band pulsed EPR study of hs-Co(II) complexes[21] found deep echo envelope modulation and short electron-spin phase memory that abolished the electron-spin-echo at g/g_2, which cast doubt on the possibility of pulsed ENDOR studies of such ions.[22] However, we find that these effects are almost completely eliminated at 35 GHz, where the full EPR envelope of Co(II)-F3 can be detected both by CW and by pulsed techniques (Figure S1). This allows us to carry out the first ^{1}H, ^{14}N, and ^{31}P pulsed ENDOR measurements on such a system.

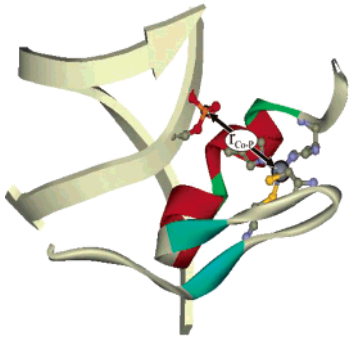

Figure 1. Schematic representation of the interaction of F3 with DNA and the distance to the closest phosphodiester of the DNA backbone to the metal center using coordinates from ref 13.

The ^{1}H Davies pulsed ENDOR spectrum of Co(II)-F3 taken at g_2 (Figure S2) shows a doublet with hyperfine coupling, $A(^1H)_{obs} = 14.6$ MHz, at g_2. The observed coupling, A_{obs}, in a spectrum taken at a particular g value is modified from the intrinsic coupling referenced to the true spin $S = {}^3/_2$, denoted A_{int}; in the simplest case, $A_{obs} = gA_{int}/g_e$. Analysis of a 2D field-frequency pattern comprised of spectra collected across the EPR envelope of Co(II)-F3 shows that the resolved ^{1}H doublet corresponds to an isotropic proton hyperfine coupling, $a_{int} = 7.4$ MHz, which is assigned to the α-protons of coordinated cysteine. The intrinsic coupling for a ligand to hs-Co(II) ($S = {}^3/_2$) is reduced, relative to the equivalent coupling to a $S = 2$ ion (e.g., Cu(II)), by a factor of $(2S)^{-1} = {}^1/_3$. This correction leads to an equivalent coupling, $a(^1H) = 22.5$ MHz, which is comparable to $a = 20-40$ MHz for Cu−S bonds of blue-copper centers.[23]

Lower-frequency ENDOR spectra at g_2 show a pair of broad ^{14}N ENDOR peaks centered at $A_{obs}/2 = 7.0$ MHz and separated by $2\times\nu(^{14}N)$, yielding $A_{int} = A_{obs} \times g_e/g_2 = 7.2$ MHz (Figure S2). This corresponds to an equivalent ^{14}N coupling, $A(^{14}N) = A_{int} \times 3 = 21.6$ MHz, which again is comparable to couplings for ^{14}N of histidine bound to Cu(II) ($S = {}^1/_2$).[23] The high covalency to cysteine and histidine implied by the ^{1}H and ^{14}N ENDOR results supports the expectation that Zn(II) and Co(II) bind to F3 in a very similar manner. No changes in either ^{1}H or ^{14}N ENDOR were detected upon binding Co(II)-F3 to C-block DNA. As the metal center is important in determining and stabilizing the 3D structure of the finger, this result offers new, strong evidence that the interaction of the peptide with its DNA binding site does not involve significant conformational reorganization in the vicinity of the metal ion, and perhaps throughout the domain.[12]

ENDOR spectra of Co(II)-F3 with added cognate DNA show a structured pattern centered at $\nu(^{31}P)$ that arises from the ^{31}P of DNA

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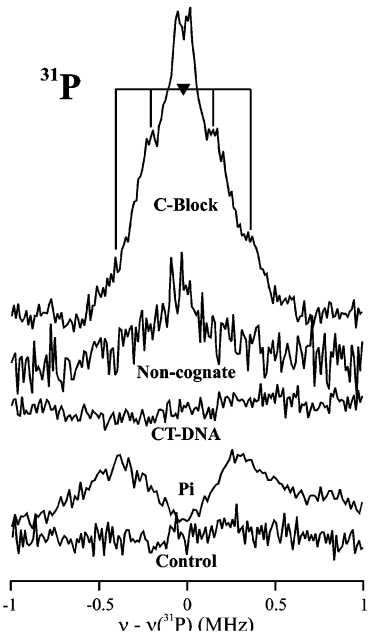

Figure 2. 35 GHz Mims pulsed 31P ENDOR at g_2. Conditions: $T = 2$ K, $\nu_{MW} = 34.9$ GHz, $t = 600$ ns, MW pulse lengths = 80 ns, RF pulse length = 60 μs; scaled to the third ^{1}H ENDOR harmonic.

phosphodiester linkages (Figure 2). The binding of F3 to C-block DNA is specific because no ^{31}P signal is detected in the presence of noncognate calf thymus (CT)-DNA (Figure 2). A noncognate DNA oligonucleotide, d(GGAAGCTTCC)$_2$, may interact weakly, but does not bind specifically; it gives only a weak "distant" ^{31}P signal (Figure 2).[24] The ^{31}P signal (at g_2) from the specifically bound C-block DNA (Figure 2) exhibits a distant ENDOR response near $\nu(^{31}P)$, a pair of peaks corresponding to a coupling, $A_{exp}(^{31}P) = 0.35$ MHz, and a pair of shoulders whose splitting corresponds to $A_{exp}(^{31}P) = 0.7$ MHz. We provisionally assign the peaks and shoulders, respectively, to the perpendicular and parallel features of an axial hyperfine interaction with the nearest phosphodiester of the bound DNA. The intensity nearer to $\nu(^{31}P)$ is assigned to other phosphates of the DNA backbone.

The observed Mims ^{31}P ENDOR pattern can arise in two ways. For a dipolar coupling without an isotropic contribution, the "perpendicular" splitting in the $g_2 = 3.90$ spectrum of this $S = {}^3/_2$ center would correspond to an intrinsic dipolar coupling of $T = (g_e/g_2)A_{exp} = g_e\beta_e g_D\beta_N/r_{Co-P}{}^3$, where the symbols have their usual meanings, and r_{Co-P} is the Co$-^{31}$P distance: $A_{exp} = 0.35$ MHz yields $r_{Co-P} \approx 5.6$ Å. This does not agree with the crystal structure of ZF1-3-DNA,[13] where the shortest Zn(Co)$-$P distance is 8.3 Å. However, a similar pattern would arise for $T = 0.06$ MHz, with an isotropic contribution of $a = 0.24$ MHz. These parameters give $r_{Co-P} = 8.1(3)$ Å, which is in excellent agreement. [25,26]

Interestingly, a ^{31}P ENDOR doublet is observed for Co(II)-F3 in phosphate buffer (Figure 2); the coupling $A(^{31}P) = 0.7$ MHz ($A_{int} = 0.36$ MHz) at g_2 is comparable to that to the nearby phosphate of bound C-block DNA, indicating that inorganic phosphate (Pi) binds at a comparable distance from Co as does the nearest phosphate of DNA, presumably at the same site. In data not shown, the ^{31}P ENDOR spectrum of C-block DNA is independent of added Pi, which suggests that Pi is replaced as the finger binds to the C-block. This may suggest a pathway of reaction for structurally related toxic agents such as $CrO_4{}^{2-}$, which reacts with Zn-F3-C-block in the presence of glutathione.[27]

In summary, ^{1}H, ^{14}N, and ^{31}P ENDOR analysis of a hs-Co(II)-substituted Zn-finger peptide demonstrates that this technique can be a powerful tool to assess ligand structure, peptide conformation, and specific DNA interactions.

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Supporting Information Available: EPR and ENDOR Figures S1 and S2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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