^{67}Zn Solid-State NMR Spectroscopy of the Minimal DNA Binding Domain of Human Nucleotide Excision Repair Protein XPA

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Historically it has not been possible to directly observe the *native* zinc metal of Zn^{2+} metalloproteins by liquid- or solid-state NMR methods. Even with today's 21 T magnets, if solution NMR methods afforded observable zinc resonances in a metalloprotein, the resulting line widths (due to quadrupole relaxation) would obscure the determination of site specific isotropic chemical shifts. In the absence of this shielding information, the utility of this experiment is significantly weakened. Even in the case of a single site, the *isotropic* quadrupole coupling constant extracted from the data by itself is not as easily correlated with structure in the absence of knowing the full quadrupole tensor. That level of detail can only be obtained by a solid-state NQR or NMR experiment. The liquid-state data can be further complicated by the presence of distinct, multiple sites. In the absence of independent information the resulting spectra are at best difficult to interpret.

Due to the difficulty in observing such a broad, low-frequency resonance via solution NMR spectroscopy 30 years ago, a surrogate probe strategy was developed.[1−3] The strategy was the replacement of the metal of interest, in this case Ca^{2+} and/or Zn^{2+}, with Cd^{2+}. With almost 30 years of literature behind it cadmium substitution seems to be working and is well understood. The solid-state ^{113}Cd experiments afford a direct comparison of shielding tensors with X-ray structural data, thus providing the means to probe structure and function. However, the purpose of this communication is to demonstrate that this surrogate probe strategy may no longer be necessary.

After the studies of Oldfield[4] and Bastow[5] there has been little work done on solid-state NMR of noncubic zinc compounds, until the recent observations of ^{67}Zn-enriched model complexes utilizing both magic angle spinning (MAS)[6] and static spin−echo methods.[7,8] However, to make these measurements routinely on a natural abundance or biological sample where the metal center is in a dilute environment, e.g., a metalloprotein, a different strategy has to be employed. For example, consider the model compound for the active site of carboxypeptidase A (CPA), zinc diimidazole diacetate, $Zn(Im)_2(OAc)_2$. For an equal mass, going from the model compound to the protein (CPA) a dilution of a factor of 110 of observable zinc has occurred. The loss in sensitivity caused by dilution must be regained in such a way that the structural information, the value of the quadrupole, shielding tensors, and their relative orientations, is retained. We recently outlined a strategy for gaining back this dilution factor.[7] This can be obtained by a combination of low-temperature NMR experiments,[9] cross-polarization methods (CP),[10] and spin−echo techniques.[11−13]

Central to this strategy is the development of an NMR probe utilizing a cryostat (supplied by Oxford Instruments) to observe zinc at low temperatures (5−250 K).[14] Employing this new probe and spin−echo methods outlined previously[13] we have improved the sensitivity of ^{67}Zn NMR spectroscopy sufficiently to observe the ^{67}Zn signal in a metalloprotein in the solid state. Figure 1 represents the first direct observation of a Zn^{2+} site in a protein by methods other than X-ray/EXAFS. The protein investigated was the minimal DNA binding domain of xeroderma pigmentosum A (XPA-MBD), a 14.7 kDa protein and an essential component in the multienzyme nucleotide excision repair (NER) pathway. The Zn^{2+} site is structural in XPA rather than catalytic, which was one of the reasons for its selection. The NMR solution structure of XPA-MBD was recently determined.[15] An isotopically enriched ^{67}Zn XPA-MBD was prepared as described previously[16] except 75 μL of 0.5 M ^{67}Zn-labeled (88%) zinc acetate was used when inducing protein expression in 750 mL of minimal medium. The purified XPA-MBD was exchanged into 10 mL of buffer (15 mM K_2HPO_4, 2 mM DTT, pH 7.3) before lyophilization to dryness. The sample was rehydrated by vapor diffusion, and the final mass was 217 mg which is 18% water by weight.

The experiment was performed at 25 K with a combination of CP[10] and spikelet echo (or QCPMG)[13] pulse sequences.[17] A cryogenic (77 K, N_2 (l)) preamplifier supplied by Miteq (model AFS, 25−30 MHz) with a nominal noise figure of 0.2 dB was used to detect the NMR response, which afforded a 2-fold gain in S/N over a standard preamplifier configuration.[9,14] The proton field strength used was 60 kHz with a matching field of 20 kHz for zinc and a 30 ms contact time. The proton reservoir was fully recovered at 120 s; however, the data were collected every 60 s with use of a proton flip back pulse. The spectrum shown in Figure 1a was collected with 1090 transients (overall time of ∼24 h). The selective π pulse was 4 μs with an interecho separation of 500 μs (a spike separation of 2 kHz in the frequency domain). Figure 1b illustrates the CP/QCPMG spectrum of ^{67}Zn-XPA-MBD

(1) (a) Coleman, J. E.; Vallee, B. L. *J. Biol. Chem*. **1960**, *235*, 390. (b) Coleman, J. E.; Vallee, B. L. *J. Biol. Chem*. **1961**, *236*, 2244. (c) Lindskog, S. *J. Biol. Chem*. **1963**, *238*, 945. (d) Vallee, B. L.; Williams, R. J. P. *Proc. Natl. Acad. Sci*. **1968**, *59*, 498. (e) Latt, S. A.; Auld, D. S.; Vallee, B. L. *Proc. Natl. Acad. Sci*. **1970**, *67*, 1383.

(2) (a) Summers, M. F. *Coord. Chem. Rev*. **1988**, *86*, 43 and references cited within. (b) Coleman, J. E. In *Metallobiochemistry, Part D*; Riordan, J. F., Vallee, B. L., Eds.; Methods in Enzymology No. 227; Academic Press: San Diego, CA, 1993; pp 16−43.

(3) McAteer, K.; Lipton, A. S.; Ellis, P. D. *Cadmium-113 NMR: A Surragate Probe for Zinc and Calcium in Proteins*; Grant, D. M., Harris, R. K., Eds.; Encyclopedia of Nuclear Magnetic Resonance; John Wiley & Sons: New York, 1996; Vol. 2, pp 1085−1091 and reference cited within.

(4) Kunwar, A. C.; Turner, G. L.; Oldfield, E. *J. Magn. Reson.* **1986**, *69*, 124.

(5) Bastow, T. J.; Stuart, S. N. *Phys. Status Solidi B.* **1988**, *145b*, 719.

(6) (a) Wu, G. *Chem. Phys. Lett*. **1998**, *298*, 375. (b) Sham, S.; Wu, G. *Can. J. Chem*. **1999**, *77*, 1782.

(7) Larsen, F. H.; Lipton, A. S.; Jakobsen, H. J.; Nielsen, N. C.; Ellis, P. D. *J. Am. Chem. Soc.* **1999**, *121*, 3783.

(8) Larsen, F. H.; Skibsted, J.; Jakobsen, H. J.; Nielsen, N. C. *J. Am. Chem. Soc.* **2000**, *122*, 7080−7086.

(9) (a) Styles, P.; Soffe, N. F.; Scott, C. A.; Cragg, D. A.; Row: R.; White, D. J.; White, P. C. *J. Magn. Reson*. **1984**, *60*, 397. (b) Styles, P.; Soffe, N. F. *J. Magn. Reson.* **1989**, *84*, 376.

(10) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys*. **1972**, *56*, 1776.

(11) (a) Carr, H. Y.; Purcell, E. M. *Phys. Rev*. **1954**, *94*, 630. (b) Meiboom, S.; Gill, D. *Rev. Sci. Instrum*. **1958**, *29*, 688.

(12) Garroway, A. N. *J. Magn. Reson*. **1977**, *28*, 365.

(13) Larsen, F. H.; Jakobsen, H. J.; Ellis, P. D.; Nielsen, N. C. *J. Phys. Chem*. **1997**, *101*, 8597.

(14) Lipton, A. S.; Sears, J. A.; Ellis, P. D. *J. Magn. Reson.* Submitted for publication.

(15) (a) Buchko, G. W.; Ni, S.; Thrall, B. D.; Kennedy, M. A. *Nucleic Acid Res.* **1998**, *26*, 2779−2788. (b) Buchko, G. W.; Daughdrill, G. W.; Rao, S.; Isern, N. G.; de Lorimier, R.; Lingbeck, J.; Taylor, J.-S.; Wold, M. S.; Gochin, M.; Spicer, L. D.; Lowry, D. F.; Kennedy, M. A. *Biochemistry* **1999**, *38*, 15116−15128. (c) Ikegami, T.; Kuraoka, I.; Saijo, M.; Kodo, N.; Kyogoku, Y.; Morikawa, K.; Tanaka, K.; Shirakawa, M. *Nature Struct. Biol.* **1998**, *5*, 701−706.

(16) Hess, N. J.; Buchko, G. W.; Conradson, S. D.; Espinosa, F. J.; Ni, S.; Thrall, B. D.; Kennedy, M. A. *Protein Sci*. **1998**, *7*, 1970−1975.

(17) Experiments were performed on a Varian Infinity *Plus* console with a home-built probe described elsewhere.[14] The overall gain in S/N ratio of the combination of CP with QCPMG is on the order of 30 relative to a simple quadrupole echo experiment at a given temperature.

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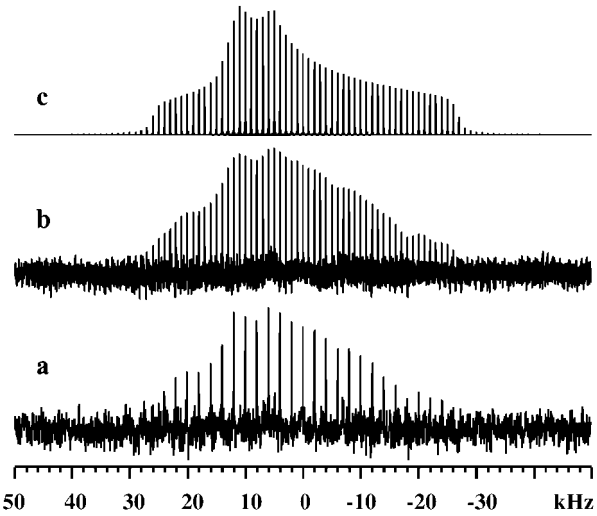

Figure 1. (a) Experimental ^{67}Zn NMR spectrum of XPA-MBD at 9.4 T and 25 K, with 25 Hz conventional line boadening (LB), (b) data from part a apodized with 5 Hz conventional and 1 kHz matched LB and zero filled to double the echo spacing, and (c) simulation utilizing parameters described in the text with 25 Hz conventional and 1.5 kHz matched LB. All spectra are conventionally zero filled to 512k points.

resulting from application of a periodic apodization function matched to the echo spacing and zero filling between the echoes.[14] Also shown in Figure 1 is the simulated line shape, using a quadrupole coupling constant, Cq, of 4.9 MHz, an asymmetry parameter, η_q, of 0.84, and an isotropic chemical shift of 327.6 ppm relative to 0.5 M $Zn(OAc)_2$ (aq) solution. While it is clear that the simulation is not optimal, the possible contribution of chemical shift anisotropy (CSA) cannot be determined accurately at this field strength. It was also noted by Buchko et al. that the lyophilized protein has some disorder around the metal site.[18]

As a model for this coordination sphere, Zn^{2+} bound to 4 sulfurs, we have examined tetrakis(thiourea)zinc nitrate. The crystal structure is known[19] and Sham and Wu reported the ^{67}Zn MAS NMR spectrum at 9.4 T.[6b] The quadrupole tensor parameters reported were Cq = 3.15 MHz with $\eta_q = 1$. This complex was prepared via methods previously described (at natural abundance) and a static, quadrupole echo experiment was acquired at 18.8 T (50.060 MHz for ^{67}Zn and 800 MHz for ^{1}H), the results of which are depicted in Figure 2. As shown in Figure 2b (simulation with Cq = 3.08 and $\eta_q = 0.9$), the line shape at this higher field strength cannot be described by quadrupole coupling alone. The simulation in Figure 2c has the same quadrupole parameters used in Figure 2b with a CSA (following the convention of Spiess)[20] of −94.5 ppm and $\eta_{cs} = 0.56$ included. The Euler angles relating the two tensor frames, α, β, and γ, are 0, 21, and 50, respectively, and the isotropic shift is 325 ppm from 0.5 M $Zn(OAc)_2$ (aq) solution.

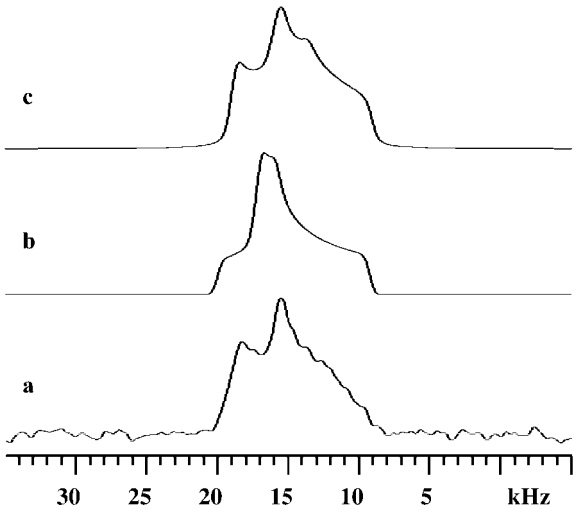

Figure 2. Experimental (a) and simulated (b and c) spectra of $Zn[SC(NH_2)_2]_4(NO_3)_2$ at 18.8 T.

Comparing the line shape parameters from the protein and the model it is evident that both have similar environments. The isotropic chemical shifts of each are indicative of sulfur coordination (more deshielded than an all oxygen environment). From the crystal structure of the thiourea salt, there are two bond lengths, 2.36 and 2.32 Å; and the EXAFS data of XPA-MBD show an average Zn−S bond length of 2.34 Å.[16] While this has led to similar isotropic shifts, it is clear that the quadrupole tensor is more sensitive to the coordination geometry. Work is already underway in our laboratory to correlate the zinc quadrupole tensor and structural information by using single-crystal experiments.[21]

The data described here represent the first direct observation of a Zn^{2+} site in a metalloprotein by NMR methods. However, the techniques utilized to obtain these data are not optimized. The temperature chosen, 25K, was selected for T_1 considerations. By utilizing paramagnetic dopants to shorten T_1, lower temperatures may become more practical. This affords signal gain that can be translated into a larger dilution of the metal. One can also envision going to high field to gain sensitivity and thereby increase the molecular weight of the observable protein further. These data represent a novel means to characterize the structure and bonding associated with the Zn^{2+} in a metalloprotein. The use of solid-state ^{67}Zn as a means to characterize Zn^{2+} has the potential of providing a new level of understanding of the role of this metal in biology. Finally, it is also clear that this same strategy can also be applied to Mg^{2+}- and Ca^{2+}-dependent proteins as well.

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(18) Buchko, G. W.; Hess, N. J.; Kennedy, M. A. *Carcinogenesis* **2000**, *21*, 1051−1057.

(19) Vega, R.; López-Castro, A.; Márquez, R. *Acta Crystallogr.* **1978**, *B34*, 2297−2299.

(20) Spiess, H. W. Rotation of Molecules and Nuclear Spin Relaxation. In *NMR Basic Principles and Progress*; Diehl, P., Fluck, E., Kosfeld, R., Eds.; Springer: New York, 1978; Vol. 15, pp 55−214.

(21) Lipton, A. S.; Smith, M.; Adams, R. D.; Ellis, P. D. Unpublished results.