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Letter to the Editor: Assignment of the backbone resonances of oxidized Fe-superoxide dismutase, a 42 kDa paramagnet-containing enzyme

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Abbreviations: HSQC, heteronuclear single quantum coherence; kDa, kilodalton; MES, morpholino ethanesulfonic acid; PFG, pulsed field gradient; rf, radio frequency; SOD, superoxide dismutase.

Biological context

Superoxide dismutases (SODs) disproportionate $2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$, thus helping to forestall aging and protect aerobic organisms against the byproducts of respiration (reviewed in Miller and Sorkin, 1997). FeSOD is a dimer of identical 21 kDa monomers, each containing a single high-spin non-heme non-sulfur Fe in the active site. This Fe alternates between the Fe^{3+} and Fe^{2+} states in the accepted catalytic cycle (Miller and Sorkin, 1997), but is always high spin and thus paramagnetic. Proton transfer is believed to be the rate-limiting step of FeSOD catalysis (Bull and Fee, 1985), and hydrogen bond networks are proposed to support the active site and connect it to solvent. Thus, we wish to directly observe hydrogen bonding protons in FeSOD. We have begun by assigning the backbone $^1H^N$, ^{15}N, $^{13}C'$, $^{13}C^\alpha$ and $^{13}C^\beta$ resonances of perdeuterated Fe^{3+}SOD suspended in ^1H_2O solution. We have obtained assignments for 61% of FeSOD's 192 residues per monomer, which represents all observable backbone NH resonances. This is a significant achievement considering FeSOD's size and the severe paramagnetic relaxivity of the active site high-spin Fe^{3+}, and constitutes the first substantial backbone assignment of a member of the growing class of non-heme, non-sulfur Fe enzymes.

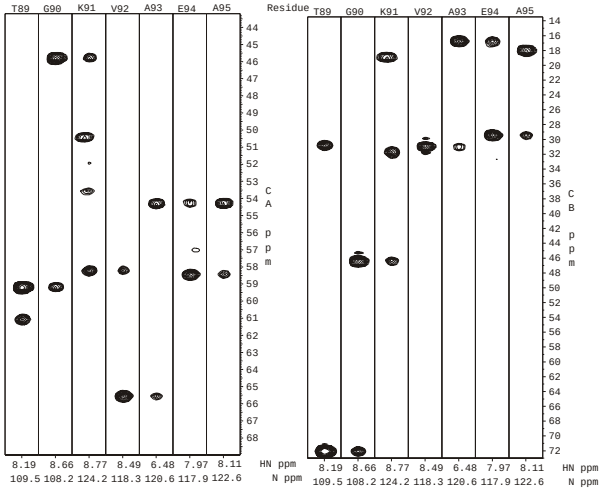

Figure 1. Representative strips from HNCA (left) and HN(CA)CB spectra (right) used to make backbone assignments. Each strip is labeled on top by the amino acid whose NH was detected and at the bottom with the $^1H^N$ chemical shift as well as the ^{15}N chemical shift. Light (dark) contours are positive (negative) and indicate an even (odd) number of attached aliphatic carbons.

Methods and results

Uniformly ^2H,^{13}C,^{15}N-labeled FeSOD was overexpressed from *E. coli* BL21 harbouring the plasmid pRLK3, a *sodB*-bearing derivative of pET-24a constructed by R.L. Koder, Jr. in this laboratory. Two 1 L cultures of bacteria were grown in 100% ^2H, ^{15}N,^{13}C-M9 (containing 100 μm $FeCl_2$ and [^2H,^{13}C] glucose), supplemented with 0.05% bioexpress™ (fully ^2H, ^{13}C and ^{15}N labeled, from C.I.L.). Fifty mg of pure FeSOD was purified as previously described (Sorkin and

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Miller, 1997) with a specific activity of 5900 units/mg protein, which is comparable to the usual activity of $\approx 6000 \pm 100$ u/mg.

Perdeuterated FeSOD was isolated and stored in solutions made up of ^1H_2O, so most of the backbone amides exchanged the ^2H incorporated biosynthetically for ^1H in the course of purification. Experiments were conducted on 17 mg of (oxidized) Fe^{3+}SOD in 0.25 ml 10 mM MES, 10 mM NaCl, pH 6.0, in 90% H_2O/10% D_2O in a 5 mm Shigemi^{TM} tube. The sample was stored at 4 °C and NMR experiments were conducted at 25 °C.

The backbone H^N, N, C^α, C^β and C' were assigned based on constant-time 3D NMR experiments incorporating sensitivity enhancement and deuterium decoupling (Yamazaki et al., 1994a, b). 3D NMR experiments were conducted on Unity^{plus} NMR spectrometers operating at 500 MHz for ^1H using a 5 mm Nalorac or Varian triple resonance probe with Z-axis PFG and a home-built deuterium decoupling channel (ABL-BRP, NCI-FCRDC). Figure 1 provides examples of spectra acquired.

NMR data were processed using NMRPipe (Delaglio et al., 1995) and analyzed using ANSIG (Kraulis, 1994). Chemical shifts are relative to (indirect) DSS (Wishart et al., 1995).

Extent of assignment and data deposition

Our assignments account for all the 95 backbone resonances visible in the HSQC. In addition, ^{13}C resonances for 23 residues were assigned via the H^N of the following residue, providing at least partial assignments for 118 of the total of 192 residues in the Fe^{3+}SOD monomer and extending from the first to the last residue. Most of the missing resonances can be accounted for by paramagnetic relaxation of H^Ns within 14 Å of Fe^{3+}. In addition, the H^Ns of some residues appear to have exchanged extremely slowly with solvent and so retained the deuteron incorporated biosynthetically at that position. This probably explains our inability to observe and assign residues in FeSOD's central three-strand β sheet. Nonetheless, our assignments encompass all the backbone NH resonances visible in the HSQC, they include Cs as close as 12 Å from Fe^{3+} and some of the residues at the interface between the two monomers of FeSOD as well as a couple at the interface between subdomains. These are expected to be useful probes of any rigid-domain reorientations that might occur in connection with ligand binding to Fe^{3+}.

Based on the number of resonances observed and the fact that no string of amino acids appeared twice in our assignments, the two monomers of Fe^{3+}SOD appear to be spectroscopically identical. The fact that we were able to completely assign all the surface loops and the termini, including residues 1 and 192, is consistent with a rigid structure, which in turn is consistent with FeSOD's excellent stability and regrettable resistance to H/D exchange at some positions. Chemical shift assignments have been deposited in the BioMagResBank under accession number 4315.

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