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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N NMR signals from toluene 4-monooxygenase Rieske ferredoxin in its oxidized state

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Biological context

Rieske [2Fe-2S] centers are found in membrane ubiquinone cytochrome oxidase complexes (Trumpower and Gennis, 1994), as integral parts of the active site in the *cis*-dihydrodiol forming aromatic dioxygenases (Mason and Cammack, 1992), and as soluble electron carriers in bacterial dioxygenase and monooxygenase complexes (Harayama et al., 1992).

X-ray crystal structures of the Rieske domains from the bovine bc_1 (Iwata et al., 1996; Link and Iwata, 1996) and chloroplastic b_6f oxidase (Carrell et al., 1997) complexes, naphthalene dioxygenase (Kauppi et al., 1998), and the soluble electron carrier ferredoxin of the *Burkholderia* sp. strain LB400 biphenyl dioxygenase (Colbert et al., 2000) have been reported. A more comprehensive understanding of the functional specialization of the Rieske-type ferredoxins would be advanced by the availability of additional structural and functional information.

Toluene 4-monooxygenase (T4MO) from *Pseudomonas mendocina* is a soluble bacterial monooxygenase complex (Fox, 1998), consisting of an NADH oxidoreductase (T4moF), a diiron hydroxylase [T4moH, $(\alpha\beta\gamma)_2$ quaternary structure (Pikus et al., 1996)], a catalytic effector protein [T4moD (Hemmi et al., 2001)], and a Rieske ferredoxin (T4moC, 12 195 Da after removal of N-terminal Met). T4moC acts as an obligate electron carrier between T4moF and T4moH. Here we report the assignment and deposition of diamagnetic chemical shifts for oxidized T4moC. The solution structure of T4moC arising from these NMR assignments can provide further insight into this ubiquitous class of ferredoxins.

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Methods and results

[U-^{13}C, U-^{15}N] T4moC was expressed in *Escherichia coli* BL21(DE3) grown on a minimal medium containing [U-^{13}C]-D-glucose (Isotec, Inc., Miamisburg, OH) and $^{15}NH_4Cl$ (Cambridge Isotope Labs, Andover, MA). The methods for fed-batch fermentation, expression, and purification are reported elsewhere (Studts and Fox, 1999; Xia et al., 1998).

NMR samples contained ~1–2 mM T4moC in 20 mM phosphate buffer, pH 6.4. NMR experiments were recorded at 298 K with Bruker DMX-500 and DMX-600 spectrometers (http://www.nmrfam.wisc.edu). Sequence-specific assignments of the polypeptide backbone resonances were made from ^{1}H-^{15}N HSQC, HNCA, HNCO, and HN(CO)CA spectra, while assignments of the side chain resonances were made from C(CO)NH, HNCACB, HC(CO)NH, HCCH-COSY, and HCCH-TOCSY spectra. Side-chain atom assignments in the Asn and Gln residues (4 Asn and 2 Gln) were confirmed using HNCO and HNCACB measurements with side-chain-optimized delay values (Wittekind and Mueller, 1993).

NMR data were processed using Felix95 and analyzed using Sparky (http://www.cgl.ucsf.edu/home/Sparky). All ^{1}H dimensions were referenced to internal 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), and ^{13}C and ^{15}N were indirectly referenced to DSS.

Extent of assignments and data deposition

The gene for T4moC encodes 112 amino acids. including 1 Arg, 4 Asn, 2 Gln, 5 His, 5 Lys, and 4

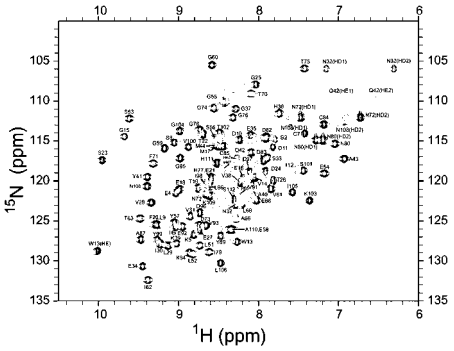

Figure 1. A 500 MHz 2D ^{1}H-^{15}N HSQC spectrum of 1.1 mM T4moC at pH 6.4 and 298 K. Labeling of cross peaks was based on analysis of through-bond connectivities from protein labeled with >98% ^{13}C and >98% ^{15}N. The spectrum was obtained with the ^{1}H and ^{15}N carrier frequencies positioned at 4.7 and 118 ppm, respectively. A total of 4 transients of $128(t_1) \times 1024(t_2)$ complex data points were collected, with spectral widths of 20 ppm for ^{1}H and 39.5 ppm for ^{15}N.

Pro residues (Yen et al., 1991). LC-EIMS analysis of the [U-^{13}C, U-^{15}N]-enriched NMR samples revealed >98% enrichment by the fed-batch labeling method (Studts and Fox, 1999), closely matching the isotopic content of the precursors used for bacterial growth. Mass spectral analysis also revealed that the N-terminal Met was completely removed, yielding a mature protein for which 133 ^{1}H-^{15}N cross peaks could be expected in the absence of hyperfine broadening caused by the [2Fe-2S] center. Figure 1 shows that 120 ^{1}H-^{15}N cross peaks (90% complete) have been assigned in the ^{1}H-^{15}N-HSQC spectrum of T4moC.

In summary, assignments have been made to a total of 1015 ^{1}H, ^{13}C, and ^{15}N resonances from T4moC (from the 1446 expected if this were a diamagnetic protein, ∼70% complete). Of the 551 expected backbone resonance signals, a total of 518 resonances were observed and assigned (∼94% complete). Backbone atoms that were not assigned to NMR resonances included the ^{15}N, $^{1}H^{N}$, ^{13}C, $^{13}C^{\alpha}$ and H^{α} of C45, Q48 and E49, the ^{15}N and $^{1}H^{N}$ of F3, I12, C64, the H^{α} and ^{13}C from L68, the ^{15}N, $^{1}H^{N}$, and H^{α} from I50 and W69, the $^{13}C^{\alpha}$ and ^{13}C of 52, the ^{13}C of H47 and H67, and the ^{15}N of P46 P81, P90, and P107.

Previous paramagnetic NMR studies suggested that C45, H47, Q48, I50, C64, H67 and A66 contain backbone atoms whose resonances are hyperfine-shifted; these residues account for the majority of unassigned signals from the peptide backbone. Thus far, resonance assignments have been made for 497 of the 895 expected side-chain atoms, with ∼30% of the unassigned atoms associated with the amino acids previously identified to contain hyperfine-shifted nuclei or with amino acids immediately adjacent in the primary sequence. Another 30% of the currently unassigned atoms are associated with aromatic side chains. A total of 1188 ^{1}H-^{15}N NOE cross-peaks have also been identified. Further analysis of the NOE data will permit assignments of aromatic side-chain resonances. In addition, these data are being used to initiate calculation of the three-dimensional structure.

The chemical shifts for oxidized T4moC have been deposited in the BioMagResBank http://www.bmrb.wisc.edu (accession no. 4992).

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