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# Letter to the Editor: Assignment of $^{1}H$, $^{13}C$ and $^{15}N$ NMR signals in the toluene 4-monooxygenase effector protein

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## Biological context

Toluene-4-monooxygenase (T4MO) catalyzes the NADH- and $O_2$-dependent hydroxylation of toluene to form *p*-cresol (Fox, 1997). T4MO belongs to an evolutionarily related family including methane monooxygenase, other aromatic monooxygenases, and alkene epoxidases. In all of these complexes, a small protein is obligately required to effect catalysis. It is reasonable to assume that the role of a diiron effector protein is to produce essential conformational changes by formation of specific protein–protein complexes.

Recently, NMR structures for the effector proteins from the methane monooxygenase (MMOB) and phenol hydroxylase (P2) complexes have been reported. While MMOB has a compact fold with βαββ and βααββ domains (Chang et al., 1999; Walters et al., 1999), multiple configurations of P2 were detected in solution. This heterogeneity was postulated to reflect conformational flexibility possibly associated with catalytic function (Qian et al., 1997).

Here we report chemical shift assignments for T4MOD. The solution structure of T4MOD arising from further analysis of these NMR assignments will provide a useful comparison to the NMR structures of other members of the effector protein family. Furthermore, this work provides the basis for more detailed study of the protein surface and specific residue contacts required for catalytic protein–protein interactions in the T4MO complex.

## Methods and results

[*U*-$^{13}$C, *U*-$^{15}$N] T4MOD was expressed in *Escherichia coli* BL21(DE3) grown on a medium containing [*U*-$^{13}$C]-D-glucose and $^{15}NH_4Cl$. T4MOD activity was measured before and after NMR data collection (Studts and Fox, 1999).

NMR samples contained 1.1 mM T4MOD in 50 mM phosphate buffer (pH 7.0, 90:10 v/v $H_2O/D_2O$), 0.5 μM sodium azide, and a protease inhibitor cocktail (Product No. P 27124, Sigma). 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 CCONH, HNCACB, HCCONH, HCCH-COSY, and HCCH-TOCSY spectra. Aromatic side chain resonances (2 Tyr, 4 Phe, and 1 His) were assigned from 2D $^{1}H$-NOESY and TOCSY, CT-$^{13}$C-HSQC, and 3D NOESY-CT-HSQC and TOCSY-CT-HSQC spectra recorded on natural abundance and [*U*-$^{13}$C, *U*-$^{15}$N]-labeled samples in the aromatic carbon region. Side-chain atom assignments in the Asn and Gln residues (6 Asn and 6 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.

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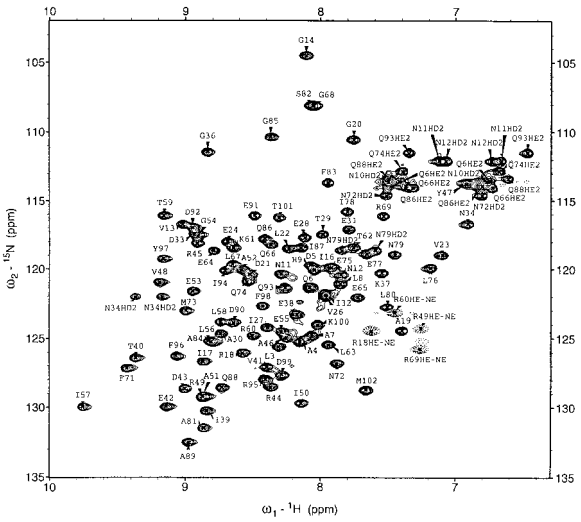


*Figure 1.* A 500 MHz 2D $^1$H-$^{15}$N HSQC spectrum of 1.1 mM T4MOD at pH 7.0 and 298 K. Cross peaks are labeled upon the basis of an analysis of through-bond connectivities. The spectrum was obtained with $^1$H and $^{15}$N carrier frequencies positioned at 4.7 and 118 ppm, respectively. A total of 4 scans of 128(t1) $\times$ 1024(t2) complex data points were collected with spectral widths of 20 ppm for $^1$H and 79 ppm for $^{15}$N.

## Extent of assignments and data deposition

The gene for T4MOD encodes 103 amino acids including 7 Arg, 6 Asn, 6 Gln, 1 His, 3 Lys, and 4 Pro residues (Yen et al., 1991). LC-EIMS analysis of NMR samples revealed that the amino-terminal Met residue was completely removed (Studts and Fox, 1999), yielding a mature form of the protein where 129 $^1$H-$^{15}$N cross peaks could be expected. Figure 1 shows the $^1$H-$^{15}$N-HSQC spectrum for T4MOD and reveals 123 assigned $^1$H-$^{15}$N cross peaks (95% complete).

Of the 400 backbone resonance signals expected from T4MOD, a total of 386 were observed and assigned. Backbone atoms that were not observed were the N, NH, C-$\alpha$, and carbonyl carbon signals from S1, the N and NH signals from T2, the N and NH signals of N10, and the six carbonyl carbons signals from H9, G14, E24, N34, R69, and M102. With the exception of S1, T2, and M102, the resonance assignments include the entire amino and carboxyl regions of the protein. Resonance assignments were also made for 789 of the 901 expected side-chain atoms (88% complete), with Arg side-chain atoms (H, C, and N) and Asp and Glu carboxylate carbons accounting for 70 of the 112 (63%) unassigned atoms.

Primary sequence analyses have revealed residues conserved among the 16 known effector proteins. In T4MOD, these residues correspond to V13, E28, N34, I50, E65, L67, G68, and G85. The side-chain $NH_2$ of N34 (9.29 and 9.49 ppm) showed unique downfield chemical shifts (http://www.bmrb.wisc.edu/data_access/outlier_selection_grid.html). These resonances were also observed in the $^1$H-$^{15}$N-HSQC spectrum 2 h after resuspension of a lyophilized T4MOD sample in $D_2O$, which suggests that this side-chain may form a structurally relevant hydrogen bond. Furthermore, in MMOB and P2, the Gly residues corresponding to T4MOD G68 and G85 adopt $\phi$ and $\psi$ angles required for $\beta$-sheet assembly. Comparison of the $^{15}$N-amide chemical shifts (T4MOD G68, 108.15 ppm; MMOB G97, 107.83 ppm; and P2 G58, 107.5 ppm; average $107.83 \pm 0.33$ ppm, 1$\sigma$ deviation) and (T4MOD G85, 110.35 ppm; MMOB G113, 111.6 ppm; and P2 G75, 110.9 ppm; average $110.95 \pm 0.63$ ppm, 1$\sigma$ deviation) suggests that these backbone atoms will occupy similar secondary structural environments.

The chemical shifts for T4MOD have been deposited in the BioMagResBank database (accession no. 4560).

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