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Letter to the Editor: Expression and assignment of the ^{1}H, ^{15}N, and ^{13}C resonances of the C-terminal domain of the diphtheria toxin repressor

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Biological context

The diphtheria toxin repressor (DtxR) is an iron-activated protein that functions as a global regulator of iron-sensitive genes in *Corynebacterium diphtheriae*. DtxR exists as an inactive, monomeric apo-protein incapable of binding DNA in the absence of iron but binds metal, dimerizes, and forms a sequence-specific DNA-binding element in the presence of Fe^{2+} (Tao et al., 1994). The structure and function of DtxR is of interest because it is the best-characterized member of a family of functionally homologous proteins that regulate iron uptake and virulence gene expression in the gram-positive bacteria.

DtxR contains 226 amino acid residues per monomeric unit that fold into two structural domains separated by a linker (Schiering et al., 1995; Qiu et al., 1996). The N-terminal domain provides the DNA binding and dimer interface. Divalent metals are bound at two distinct sites within the N-terminal domain.

In contrast, the structure and function of the C-terminal domain are less well defined. We are using multi-dimensional heteronuclear NMR to determine the structure of the C-terminal domain in solution as a prelude to functional studies. Here we report the sequential ^{1}H, ^{15}N, and ^{13}C resonance assignments for the C-terminal domain (residues N130–L226) of DtxR.

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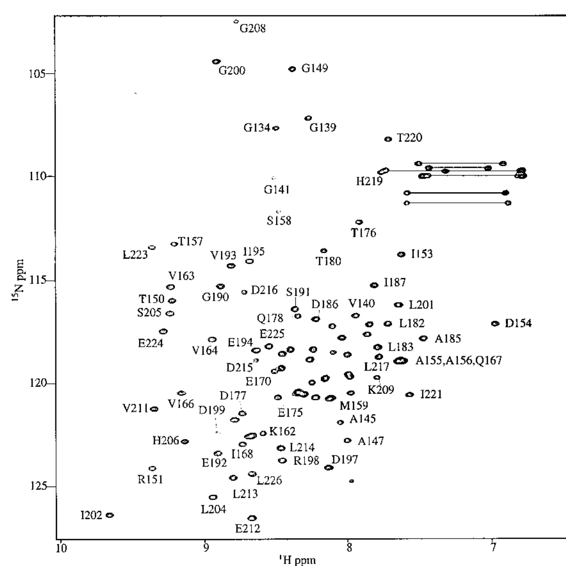

Figure 1. 2D ^{1}H, ^{15}N HSQC spectrum of 2 mM [U-^{15}N]DtxR(130–226) in 50 mM potassium phosphate buffer, at pH 6.5, 30 °C, indicating the backbone resonance assignments. Assignments of the backbone resonances for some residues are indicated in the figure; others are omitted for clarity. The amide side-chain resonances are connected by horizontal bars.

Methods and results

The cDNA coding for residues N130–L226 of DtxR was amplified by PCR from the full-length *dtx*R gene, ligated into the pQE-31 expression vector (Qiagen), and transformed into *E. coli* strain HMS-174. This construct contains 12 additional residues at the N-terminus, making the final amino acid sequence MRGSHHHHHHGS-DtxR(130–226), which is ab-

breviated as DtxR(130–226). Bacteria were grown in M9 medium and labelled following standard procedures. The cells were grown to an OD_{600} of ~0.6, induced by addition of 0.4 mM IPTG, and grown for an additional 3 h before harvesting by centrifugation. The cell pellet was resuspended in 20 mL of lysis buffer (50 mM potassium phosphate, pH 7.5, containing 0.5 M NaCl, 8 M urea, 5 mM imidazole, and 1 mM phenylmethylsulfonyl fluoride (PMSF)) and lysed by French press. The clarified lysate was chromatographed over a Ni^{2+}-chelating Sepharose Fast Flow column (Pharmacia) and eluted with a linear gradient of 10 to 600 mM imidazole. Fractions containing DtxR(130–226) were dialyzed and concentrated in a Centriprep 3 (Amicon) prior to exchange into 50 mM potassium phosphate, pH 6.5, containing 0.4% NaN_3 and 10% D_2O, for NMR analysis.

All NMR spectra were collected at 30 °C on a 500 MHz three-channel Varian Unity*plus* instrument equipped with waveform generators and pulsed field gradient accessories. The primary experiments used for assignments (HNCACB, CBCA(CO)NH, and H(C)(CO)NH-TOCSY) (Muhandiram and Kay, 1994) were collected with sweep widths and digital resolutions of 8333.33 Hz and 512 points, 1650 Hz and 40 points, 7649 Hz and 64 points in the ^{1}H (ω_3), ^{15}N (ω_2), and ^{13}C (ω_1) dimensions, respectively. The HNCO spectrum was collected using 1760 Hz sweep width in ω_1. 3D ^{15}N-separated TOCSY-HSQC and NOESY-HSQC spectra were collected using a ^{1}H (ω_3) sweep width of 6250 Hz with 64 or 96 points, and 30 ms and 120 ms mixing times, respectively. The HCCH-TOCSY spectrum ($\tau_m = 21$ ms) was collected using 6250 Hz and 64 points in the ^{1}H (ω_1) dimension. ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced indirectly to DSS according to Wishart et al. (1995).

Extent of assignments and data deposition

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum collected on a 2 mM sample of ^{15}N-labeled DtxR(130–226). Except for N207, all resonances have been identified in this spectrum, accounting for all the backbone and side-chain amides of DtxR(130–226). Backbone $^1H^N$, ^{15}N, C^α, and C^β resonances for individual spin systems of DtxR(130–226) were assigned using a combination of the 3D HNCACB and CBCA(CO)NH spectra starting from Ala, Ser, Thr, or Gly residues. The favorable relaxation properties of this domain resulted in complete sets of i and $i - 1$ correlations in the HNCACB spectrum for nearly all residues. Backbone carbonyl assignments were obtained from analysis of a 3D HNCO spectrum. Assignments for aliphatic side-chain proton resonances were obtained from H(C)(CO)NH-TOCSY and 3D HCCH-TOCSY spectra. The proton assignments were further substantiated by analysis of 3D ^{15}N-edited TOCSY-HSQC and NOESY-HSQC spectra (Wüthrich, 1986). Aromatic assignments were obtained using a combination of heteronuclear (Yamazaki et al., 1993) and homonuclear (NOESY) 2D experiments. Stereospecific assignments for 33% of the Val and Leu methyl groups were obtained using the method of Neri et al. (1989).

In conclusion, essentially complete assignments were achieved for DtxR(130–226) except the signals for the histidine tag. The assignments have been deposited with the BioMagResBank (accession code BMRB-4183). Chemical shift index analysis (Wishart and Sykes, 1994) indicates the presence of five β-strands and three short helical regions, consistent with the available low resolution crystal structure.

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