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Letter to the Editor: Backbone and side-chain ^{1}H, ^{15}N, and ^{13}C assignments for the topological specificity domain of the MinE cell division protein

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Biological context

A key question with regard to cell division is: How do cells decide *where* to divide? This process is best understood in *E. coli*, where correct placement of the division septum requires the coordinated action of three proteins: MinC, MinD, and MinE (de Boer et al., 1989). MinC and MinD interact to form a nonspecific division inhibitor that blocks septation at *all* potential division sites. MinE carries out two functions. First, it is an antagonist of the MinCD division inhibitor. Second, it is a *topological sensor*, since it relieves the inhibitory action of MinCD at midcell only, thus restricting MinCD activity to the unwanted division sites at the cell poles.

The two functions of MinE reside in separate regions of the 88-residue protein. The domain responsible for counteracting MinCD division inhibition is located within the N-terminal 22 residues of the protein (Zhao et al., 1995), while the topological specificity function is located within the C-terminal region (Zhang et al., 1998; Pichoff et al., 1995). We recently demonstrated that the topological specificity function of MinE resides in a structurally autonomous C-terminal domain (residues 31–88) that folds into a 14-kDa dimer (King et al., 1999). Here we report ^{1}H, ^{15}N, and ^{13}C chemical shift assignments for this domain (hereafter referred to as MinE$^{31-88}$).

Methods and results

Samples of recombinant [*U*-^{15}N] and [*U*-^{15}N,^{13}C] MinE$^{31-88}$ were obtained as described previously (King et al., 1999). Expression of MinE$^{31-88}$ as a thrombin-cleavable His-tag fusion protein results in the purified protein having four non-native residues (Gly–Ser–His–Met, referred to hereafter as residues -4 to -1) appended to the N-terminus; the recombinant MinE$^{31-88}$ thus contains 62 residues (124-residue dimer). NMR samples contained 2.9–3.5 mM MinE$^{31-88}$ in 20 mM sodium phosphate, 50 mM NaCl, 15 mM dithiothreitol, 1 mM EDTA, 1 mM PMSF, 0.02% NaN_3, 7.5% D_2O, pH 5.7. All NMR experiments were performed at 25 °C on Varian INOVA 500 or 600 MHz spectrometers. The data were processed using Felix95 (MSI) and analysed using XEASY (Bartels et al., 1995).

The spectral widths, number of scans, and numbers of complex points acquired in the F3, F2, and F1 dimensions for the experiments used for resonance assignment were as follows: CBCA(CO)NH: 5498 × 9050 × 1333, 24 scans, 512 × 53 × 37; HNCACB: 5498 × 9050 × 1333, 24 scans, 512 × 64 × 32; HNCO: 5498 × 1600 × 1333, 4 scans, 512 × 64 × 32; TOCSY-HSQC: 6000 × 6000 × 1600, 16 scans, 512 × 128 × 32; HCCH-TOCSY: 6000 × 6000 × 12000, 16 scans, 512 × 64 × 64; C(CO)NH-TOCSY: 5498 × 8800 × 1333, 16 scans, 512 × 96 × 28; HC(CO)NH-TOCSY: 5498 × 5498 × 1333, 16 scans, 512 × 96 × 32. The mixing times for the TOCSY-HSQC, HCCH-TOCSY, C(CO)NH-TOCSY, and HC(CO)NH-TOCSY experiments were 50, 16, 22, and 22 ms, respectively. All experiments were acquired at 500 MHz except the TOCSY-HSQC and HCCH-TOCSY, which were acquired at 600 MHz.

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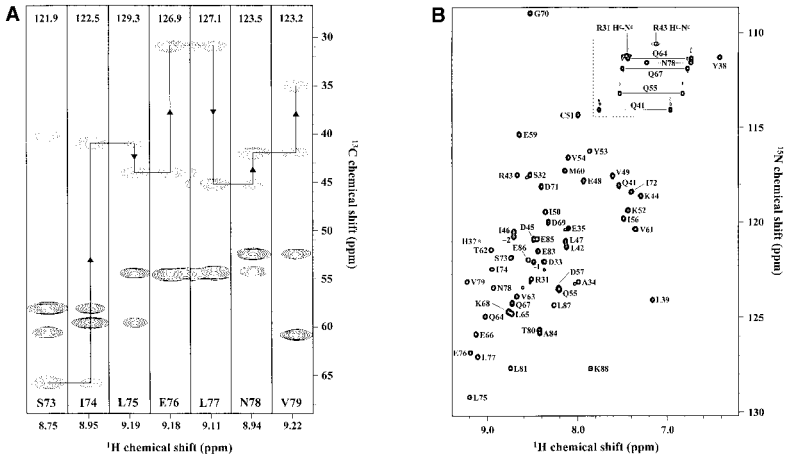

Figure 1. (A) Strips from the HNCACB experiment illustrating the sequential assignment of residues 73–79. Positive contours (C^{α} cross peaks) and negative contours (C^{β} cross peaks) are indicated by solid and dotted lines, respectively. The sequential connectivities between residues Ser^{73} and Val^{79} are illustrated by lines connecting the C^{β} cross peaks; similar connectivities (not shown) can be made using the C^{α} cross peaks. The numbers at the top of the figure indicate the chemical shift of each ^{15}N plane. (B) ^{1}H-^{15}N HSQC spectrum of $MinE^{31-88}$ at 298 K, pH 5.7. Peaks are labelled according to the $MinE^{31-88}$ sequence, except for the four non-native N-terminal residues which are labelled −4 to −1 (no cross peaks are visible for residues −4 and −3). The number of backbone amide cross peaks, side-chain Asn/Gln cross peaks (enclosed by the dashed lines), and side-chain Arg H^{ε}–N^{ε} cross peaks (which are aliased in this spectrum) is consistent with the primary structure of $MinE^{31-88}$, indicating that it forms a completely symmetric dimer. The five small unassigned cross peaks result from minor proteolytic degradation at the N-terminus of the domain. ^{13}C and ^{15}N chemical shifts were indirectly referenced as described previously (Wishart et al., 1995).

Extent of assignments and data deposition

Complete sequence-specific ^{1}H, ^{15}N, and ^{13}C backbone assignments for $MinE^{31-88}$ were obtained from concurrent analysis of the HNCACB and CBCA(CO)NH experiments (Figure 1A), plus a 3D HNCO experiment collected primarily to obtain the carbonyl carbon assignments for compilation of the chemical shift index. Virtually all predicted cross peaks were observed in these three spectra. Some side-chain assignments were made initially from the 3D TOCSY-HSQC spectrum, but the cross-peak intensities were weak and there was often very little coherence transfer beyond the H^{β} protons. Thus, most side-chain ^{1}H, ^{15}N, and ^{13}C assignments were obtained from the HC(CO)NH-TOCSY and C(CO)NH-TOCSY spectra. Additional side-chain assignments (particularly for residues preceding proline) were obtained from analysis of HCCH-TOCSY data.

In summary, we have obtained >97% complete ^{1}H, ^{15}N, and ^{13}C assignments for the $MinE^{31-88}$ topological specificity domain; these chemical shift assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4237. The 2D HSQC spectrum of $MinE^{31-88}$ (Figure 1B) indicates that this domain forms a symmetric dimer, and consequently the key to determining its three-dimensional structure will be unravelling intra- and intermolecular NOEs by the acquisition of heteronuclear edited/filtered NOESY experiments on mixtures of [*U*-^{15}N] and [*U*-^{15}N,^{13}C] samples.

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