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Letter to the Editor: ^{1}H, ^{15}N, ^{13}C, and ^{13}CO assignments and secondary structure determination of ZipA*

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Biological context

ZipA is an essential component of cell division in *E. coli* and is required for viability of the cell (Hale and de Boer, 1997). ZipA, FtsZ and seven other proteins are integral components of the septal ring that directs the formation of a septum across the middle of the cell during cell division (for review see Rothfield and Justice, 1997). The initial stage for the formation of the septal ring involves the localization of FtsZ from the cytoplasm to the middle of the cell where it self-assembles into a ring-like structure. The FtsZ ring is associated with the inner surface of the cytoplasmic membrane where it acts as a scaffold to recruit other members of the septal ring. ZipA is recruited early to the division site and has been shown to directly bind FtsZ.

ZipA is a multidomain protein consisting of a hydrophobic N-terminus which forms the transmembrane domain that anchors the protein to the cytoplasmic membrane while the C-terminal domain has been shown to be sufficient for binding to FtsZ (Hale and de Boer, 1997; Liu et al., 1999). The exact nature of the septal ring is unknown, but data indicate that ZipA is involved in the assembly of the ring by linking FtsZs to the cytoplasmic membrane and that the ZipA–FtsZ interaction is mediated by their carboxy-terminal domains (Hale and de Boer, 1997, 1999; Liu et al., 1999). Filamentation is observed when the relative abundance of ZipA in the cell is changed either by depletion or overexpression, suggesting that disruption of the ZipA–FtsZ interaction would impair cell division and lead to cell lysis (Hale and de Boer, 1997). This indicates that the ZipA–FtsZ interaction may be a viable therapeutic target for drug development. Here we report the near complete ^{1}H, ^{15}N, ^{13}CO, and ^{13}C NMR assignments and secondary structure of the C-terminal domain of ZipA ($ZipA_{185-328}$, where M185 corresponds to M1 in the NMR structure). These data provide a basis for determining the solution structure of the C-terminal domain of ZipA and for further investigation in the design of novel inhibitors of the ZipA–FtsZ interaction.

Methods and results

The uniformly ^{15}N- and ^{13}C-labeled 144 amino acid C-terminal domain of ZipA was expressed from the plasmid pEG041 in the *E. coli* strain BL21(λDE3) plysS. pEG041 is a derivative of pET29 (Novagen, Madison, WI) with a gene insert coding for Met185 through Ala328 of *E. coli* ZipA. Cells were grown in M63 minimal media supplemented with 1 mM $MgSO_4$, 100 mg/L thiamine, and 2 g/L of ^{13}C-glucose and/or $(^{15}NH_4)_2SO_4$. Cells were grown at 37 °C to an OD_{600} of 0.6 to 1.0 and induced with 2 mM IPTG. Two hours after induction, the cells were harvested and resuspended in 50 mM Tris, pH 8.0, 50 mM KCl, 10% glycerol. After addition of 1 mM EDTA and 0.1 mM PMSF, cells were lysed in a French Press at 16 000 psi and the cell extract was clarified by centrifugation at 100 000 × g for 1 h. The supernatant was fractionated by a 50% ammonium sulfate cut and the pellet was resuspended in 50 mM Tris, pH 8.0, 10 mM NaCl, 10% glycerol, and dialyzed against the same buffer overnight. The sample was subsequently purified on a Mono Q column using a

*Supplementary material available from the authors: table containing the ^{1}H, ^{15}N, ^{13}CO, and ^{13}C resonance assignments for ZipA.
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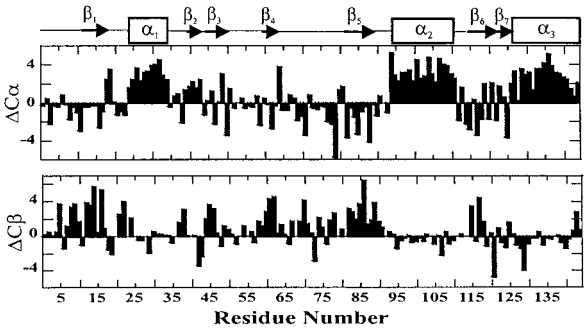

Figure 1. Cα/Cβ secondary chemical shifts plotted as a function of residue number with a cartoon of the observed secondary structure elements above the graph.

NaCl gradient followed by a Superose12 size exclusion column where both columns are equilibrated in 50 mM Tris, pH 8.0. The NMR samples contained 1 mM of $ZipA_{185-328}$ in a buffer containing 50 mM potassium phosphate, 2 mM NaN_3, 50 mM deuterated DTT, in either 90% H_2O/10% D_2O or 100% D_2O at pH 6.0.

All NMR spectra were recorded at 25 °C on a Bruker DRX 600 spectrometer equipped with a triple-resonance gradient probe. Spectra were processed using the NMRPipe software package (Delaglio et al., 1995) and analyzed with PIPP (Garrett et al., 1991), NMRPipe and PEAK-SORT, an in-house software package. The assignments of the ^{1}H, ^{15}N, ^{13}CO, and ^{13}C resonances were based on the following experiments: CBCA(CO)NH, CBCANH, C(CO)NH, HC(CO)NH, HNHB, HNCO, HNHA, HNCA and HCCH-COSY (for reviews see Bax et al., 1994; Clore and Gronenborn, 1994). The accuracy of the $ZipA_{185-328}$ NMR assignments was further confirmed by sequential NOEs in the ^{15}N-edited NOESY-HMQC spectra and by NOEs between the β-strands observed in the ^{13}C-edited NOESY-HMQC and ^{15}N-edited NOESY-HMQC spectra.

The secondary structure of $ZipA_{185-328}$ is based on characteristic NOE data involving the NH, Hα and Hβ protons from ^{15}N-edited NOESY-HMQC and ^{13}C-edited NOESY-HMQC spectra, $^{3}J_{HN\alpha}$ coupling constants from HNHA, slowly exchanging NH protons and $^{13}C\alpha$ and $^{13}C\beta$ secondary chemical shifts (for reviews see Wüthrich, 1986; Wishart and Sykes, 1994). It was determined that the $ZipA_{185-328}$ NMR structure is composed of three helical regions corresponding to residues 24–34 (α_1); 94–111 (α_2) and 126–144 (α_3); and a seven-stranded β-sheet region corresponding to residues 11–17 (β_1); 38–40 (β_2); 44–47 (β_3); 59–64 (β_4); 81–86 (β_5); 114–119 (β_6) and 122–124 (β_7).

Extent of assignments and data deposition

The $ZipA_{185-328}$ protein was extremely well behaved and provided high-quality NMR data resulting in the complete assignment of the backbone resonances for the C-terminal domain of ZipA. There were no observable regions of the protein with significantly sharper or broader linewidths or missing resonances. This observation, along with the complete assignments for $ZipA_{185-328}$, implies a well-packed ordered structure and the lack of disordered loops, N- or C-terminal regions. Similarly, the side-chain assignments are essentially complete (>95%) where the few missing assignments occur in residues with long side chains which are potentially solvent exposed. Figure 1 shows the Cα/Cβ secondary chemical shifts plotted as a function of residue number and the corresponding secondary structure assignments for the C-terminal domain of ZipA. A table of chemical shifts has been deposited in the BioMagResBank Database (accession code 4717).

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