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

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Biological context

DivIB is a component of the septasome, a macromolecular protein assembly that is responsible for coordinating bacterial cytokinesis (Robson et al., 2002). DivIB localizes to the incipient division site in *Bacillus subtilis* and appears to stabilize the septasome (Beall and Lutkenhaus, 1989; Harry et al. 1993), but its specific role in bacterial cell division remains elusive. The *Escherichia coli* ortholog (FtsQ) is essential, whereas a *divIB* null mutant is only inviable at high growth temperatures in *B. subtilis*. Orthologs of DivIB and several other membrane-associated components of the division apparatus, namely FtsL, DivIC (FtsB in *E. coli*), and PBP2B (FtsI in *E. coli*), appear to be completely absent from wall-less bacteria (Katis and Wake, 1999; Margolin, 2000), suggesting that they may play a role in the synthesis or placement of peptidoglycan during septation.

DivIB is a bitopic membrane-bound protein that contains a short N-terminal cytoplasmic portion, a single-pass transmembrane segment, and a larger extracellular C-terminal region. Recent work in our laboratory (S.A.R. and G.F.K., unpublished data) revealed that the extracellular region of *Bacillus stearothermophilus* DivIB comprises two autonomously folded N-terminal domains (α and β) and an unstructured C-terminal segment (the γ 'tail') (see Figure 1A). The absence of a clear understanding of how DivIB facilitates bacterial cytokinesis prompted us to pursue determination of the structure of the α and β domains. Here we present near-complete ^{1}H, ^{15}N, and ^{13}C chemical shift assignments for the β domain of DivIB from *B. stearothermophilus*, hereafter referred to as *Bste*-DivIBβ.

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Methods and results

Bste-DivIBβ was expressed in *E. coli* BL21 cells as a translational fusion to the C-terminus of glutathione-S-transferase (GST), with an intervening thrombin recognition site. The GST:*Bste*-DivIBβ fusion protein was purified from the soluble cell fraction using glutathione affinity chromatography and cleaved with thrombin as described previously (Tedford etal., 2001). The protease-liberated *Bste*-DivIBβ was subsequently purified to >98% homogeneity using anion exchange chromatography. NMR samples were prepared by dialyzing *Bste*-DivIBβ against NMR buffer (10 mM NaPi, 150 mM NaCl, 10 μM EDTA, 10 μM 4-(2-aminoethyl)benzenesulfonyl fluoride, 0.02% NaN_3, pH 6.0) before concentrating the protein to a final concentration of ~1 mM. Expression of *Bste*-DivIBβ as a thrombin-cleavable GST-fusion appends two non-native residues (Gly-Ser) to the N-terminus; the recombinant *Bste*-DivIBβ thus contains 119 residues (M_w = 13,349 Da).

NMR experiments were performed at 308 K on Varian INOVA 500 and 600 MHz spectrometers. Data were processed with NMRPipe (Delaglio et al., 1995) using our web-based script generator (http://sbtools.uchc.edu/nmr/). Spectra were analyzed using XEASY (Bartels et al., 1995). Most

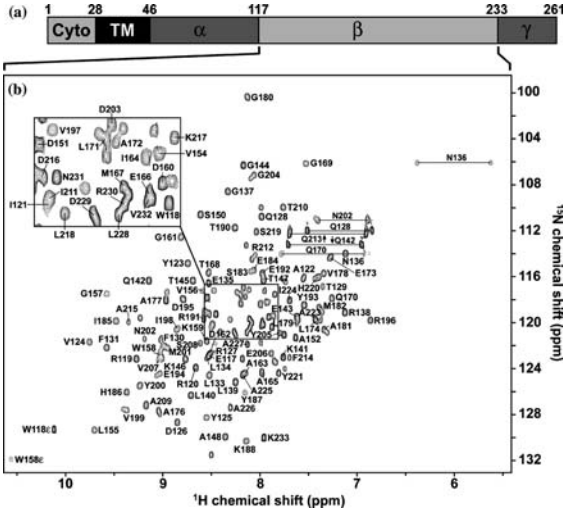

Figure 1. (A) Domain architecture of *B.stearothermophilus* DivIB. The cytoplasmic and transmembrane regions are labeled "Cyto" and "TM", respectively. (B) Assigned ^{1}H–^{15}N HSQC spectrum of *Bste*-DivIBβ at 308 K, pH 6.0. The insert shows an expansion of the most-crowded region. Pairs of Gln/Asn sidechain cross peaks are connected by horizontal lines. Unlabeled peaks correspond to the minor *cis* conformer.

$^{1}H_N$, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$, and $^{13}C'$ resonance assignments were obtained from analysis of 3D HNCACB, CBCA(CO)NH, HN(CA)CO, and HNCO spectra. Side-chain ^{1}H, ^{15}N, and ^{13}C assignments were obtained from analysis of H(CC)(CO)NH-TOCSY, (H)CC(CO)NH-TOCSY, and HCCH-TOCSY experiments. Aromatic side-chain ^{1}H assignments were obtained using 2D homonuclear TOCSY and NOESY experiments as well as ^{15}N- and ^{13}C-edited 3D NOESY-HSQC spectra. The HCCH- TOCSY and NOESY data were acquired at 600 MHz; all other data were acquired at 500 MHz.

Extent of assignments and data deposition

Chemical shift assignments were complicated by the presence of an equilibrium between two conformers caused by *cis–trans* isomerization of the Y221–P222 peptide bond. This resulted in the presence of ~20 additional peaks in the ^{1}H–^{15}N HSQC spectrum of *Bste*-DivIBβ (see Figure1B). Mass spectrometric analysis of the recombinant protein confirmed that it was of the correct molecular weight and that the additional peaks result from multiple solution conformations. The two structural isomers gave rise to duplicate sets of resonances for two separate sections of the protein backbone (residues 176–182 and 202–220); these two regions of the protein are spatially proximal to the aromatic ring of Y221, whose conformation is significantly perturbed by isomerism of the Y221–P222 peptide bond. We were able to perform two parallel sequential assignments using the HNCACB/CBCA(CO)HN experiments that corresponded to the same series of amino acids for each isomer. ^{15}N and $^{1}H_N$ chemical shifts differed by as much as 6 and 1 ppm, respectively, between the two isomers.

Despite the additional spectral complexity caused by isomerism of the Y221–P222 peptide bond, we were able to assign virtually all ^{1}H, ^{15}N, and ^{13}C resonances for the dominant *trans* isomer of *Bste*-DivIBβ, which comprised ~60% of the sample population. Complete backbone and side-chain ^{1}H, ^{15}N, and ^{13}C assignments were obtained for all 119 residues with the exception of $^{1}H_N$ and ^{15}N of residue Q213, $^{13}C'$ of residues G157, W158, H186, Y187, K188 and A225, aromatic ^{13}C resonances, and the labile protons of lysine and arginine residues and their associated side-chain ^{15}N.

In summary, we have obtained almost complete ^{1}H, ^{15}N and ^{13}C chemical shift assignments for the dominant *trans* conformation of the β domain of *B. stearothermophilus* DivIB; these assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6395.

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