Journal of Biomolecular NMR, **11:** 233–234, 1998.
KLUWER/ESCOM

Backbone NMR assignments and secondary structure of the N-terminal domain of DnaB helicase from *E. coli*

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Received 8 December 1997; Accepted 6 January 1998

Key words: DnaB helicase, NMR assignments

Biological context

DnaB is a key protein in *E. coli* chromosomal replication, playing an important role during both the initiation and elongation stages. The functional protein is a hexamer of identical 52.3 kDa subunits. To date, no structure at atomic resolution has been determined for DnaB or any homologous protein. A 12 kDa N-terminal tryptic fragment has been shown to be essential for helicase activity, formation of the hexamer and interactions with other proteins (Nakayama et al., 1984; Biswas et al., 1994). The structurally well-defined core of the N-terminal domain was identified to comprise residues 24–136 through sequential resonance assignments of the flexible N- and C-terminal residues in two samples of unlabelled DnaB (Miles et al., 1997).

Methods and results

Two different N-terminal constructs of DnaB were prepared using *E. coli* strain BL21(DE3) transformed with plasmids pCM865 [DnaBΔC(143–470), comprising residues 1 to 142] (Miles et al., 1997) and pCM861 [DnaBΔC(162–470), comprising residues 1 to 161]. Both plasmids were constructed by insertion of appropriate fragments of the *dnaB* gene into the T7 promoter vectors pETMCSI and pETMCSII (for pCM865 and pCM861, respectively), which are derivatives of pET3c (Studier et al., 1990). [u-^{2}H/^{15}N/^{13}C]-labelled DnaBΔC(162–470) and a partially deuterated protein sample, [70%-^{2}H/u-^{15}N/^{13}C]-labelled DnaBΔC(162–470), were prepared following the protocol of Venters et al. (1995). Approximately 30 mg of purified protein was obtained per litre of culture. For preparation of [u-^{13}C/^{15}N]-labelled DnaBΔC(143–470), cells were grown in minimal medium at 37 °C. The yield was about 4 mg of purified protein per litre of culture. Purification of both proteins followed the protocol of Miles et al. (1997).

Deuteration of the larger fragment was motivated by substantial linebroadening caused by self-aggregation of the domain. In addition, the chemical shift dispersion is poor due to mostly α-helical secondary structure and large segments of residues with random coil conformation (Miles et al., 1997).

All NMR experiments were acquired on a Bruker DMX 600 NMR spectrometer at 32 °C. NMR samples were prepared in 90% H_2O/10% D_2O, containing 20 mM sodium phosphate, 1 mM EDTA, 1 mM Pefabloc protease inhibitor (Boehringer Mannheim) and 0.02% (w/v) NaN_3, and were about 1.2 mM in [u-^{2}H/^{13}C/^{15}N]-labelled DnaBΔC(162–470) at pH 6.7, 1.7 mM in [70%-^{2}H/u-^{13}C/^{15}N]-labelled DnaBΔC(162–470) at pH 6.5, and 1.8 mM in [u-^{13}C/^{15}N]-labelled DnaBΔC(143–470) at pH 6.5.

NMR spectra recorded for resonance assignment were: 3D HNCACB, HN(CO)CACB, HNCO, 3D HN(CA)CO, HNCA, HN(CA)H, H(CACO)NH, ^{15}N-^{1}H NOESY-HSQC and a 4D ^{15}N-^{1}H HSQC-NOESY-HSQC. 2D ^{15}N-^{1}H HSQC spectra are shown in Figure 1.

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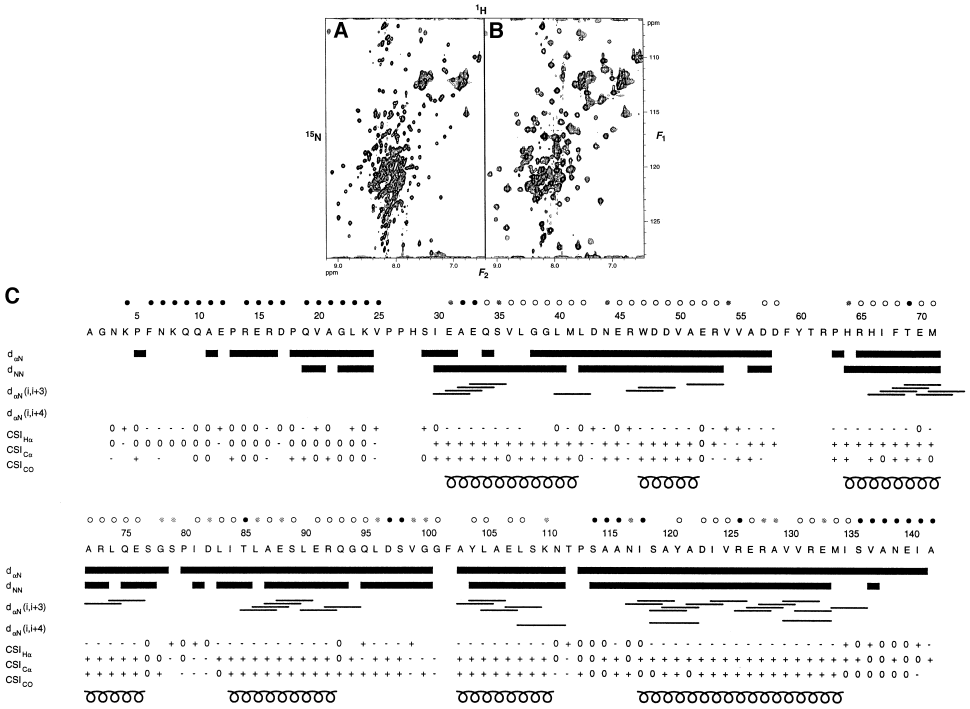

Figure 1. 2D ^{15}N-^{1}H HSQC spectra of [70%-^{2}H/u-^{15}N/^{13}C]-labelled DnaBΔC(162–470) (A) and [u-^{15}N/^{13}C]-labelled DnaBΔC(143–470) (B) recorded with identical parameters (see Supplementary Material). (c) Amino acid sequence, short-range NOEs, chemical shift indices (CSI) for $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and $^{13}C'$ spins (Wishart and Sykes, 1994), and amide proton exchange data for DnaBΔC(143–470). Black/grey shaded circles identify amide protons for which intense/weak cross peaks with the water resonance were observed in the 3D NOESY-HSQC. These cross peaks originate either from direct chemical exchange with water or from intramolecular NOEs with rapidly exchanging protons. Open circles identify amide protons for which exchange peaks were clearly absent. Six α-helices suggested by the CSIs and the short-range NOEs are indicated at the bottom.

Further details including acquisition parameters are compiled in the Supplementary Material (available from the authors).

Extent of assignments and data deposition

Sequence-specific assignments ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$, $^{13}C'$) for DnaBΔC(143–470) are given in Table 2 of the Supplementary Material and have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database (accession number 4075). Resonances remaining to be assigned include all those for A1, G2, P26, P27, H28, F59, Y60, T61, R62 and F102 as well as $^{1}H^{N}$, $^{13}C^{\alpha}$ and ^{15}N for S29; $^{1}H^{N}$ and ^{15}N for A103; and $^{13}C'$ for K4, F6, K8, Q9, E12, D17, D58, R65, S79 and A142. The assignments of the remaining backbone resonances were hampered by extensive overlap with the very narrow and intense resonances from the residues in random coil conformation (Miles et al., 1997; Figure 1) and by exchange broadening. Side-chain resonance assignments are not reported.

Acknowledgements

Financial support from the Swedish Natural Science Research Council (Project 10161) and from the Erik and Edith Fernström Foundation for Medical Research is gratefully acknowledged.

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