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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C, ^{15}N resonance assignments and secondary structure of the carboxyterminal domain of the *E. coli* transcription factor NusA

Anke Eisenmann, Sabine Schwarz, Paul Rösch & Kristian Schweimer*
Lehrstuhl für Biopolymere, Universität Bayreuth, Germany

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Biological context

E. coli NusA protein (*Eco*NusA) is involved in transcription pausing, termination, and antitermination by associating with the transcription complex (Mah et al., 2000). Sequence comparison among several bacterial species shows a common domain organization of NusA proteins, comprising an amino-terminal RNA-polymerase (RNAP) binding domain, a central RNA binding domain of one S1 and two KH modules, and an approximately 160 amino acid carboxy-terminal domain present in some NusA proteins. The function of the carboxyterminal domain is still not fully understood, however biological data suggest an autoinhibitory role. RNA binding could be observed either in EcoNusA mutants lacking the carboxy-terminal 79 amino acids or in full-length, 495 amino acid *Eco*NusA in the presence of the bacteriophage λ N gene product or the carboxy-terminal domain of RNAP subunit α (Mah et al., 2000). Profile based sequence analysis predicts a modified five helix fold of two consecutive helix-hairpin-helix (HhH) motifs linked by a connector helix (Aravind et al., 1999). In addition to participating in RNA binding, this $(HhH)_2$ fold is known to mediate protein-protein interactions (Shao et al., 2001), suggesting a major role for the carboxy-terminal domain of NusA in transcription modulation.

Whereas the structures of the amino-terminal domain and the central part of *T. maritima* and *M. tuberculosis* NusA have been elucidated by X-ray crystallography (Worbs et al., 2001; Gopal et al., 2001), three-dimensional structure information for the carboxy-terminal 160 amino acid domain of *Eco*NusA is unavailable. However, to address questions about the molecular basis of the interaction with the λ N protein or the RNAP α subunit and thus the basic principles governing processes such as transcription elongation, termination, and antitermination, a high-resolution structure of *Eco*NusA(339-495) is highly desirable. As an initial step, we virtually completely assigned the ^{1}H, ^{13}C, and ^{15}N resonances of *Eco*NusA(339-495) with multidimensional heteronuclear NMR and determined the secondary structure by chemical shift index analysis.

*To whom correspondence should be addressed. E-mail: kristian.schweimer@uni-bayreuth.de

Methods and results

*Eco*NusA(339-495) was expressed as a deka-histidine tagged protein in *E. coli* BL21 (DE3) and purified by immobilized nickel affinity chromatography under native conditions. The histidine-tag was cleaved off by rhinovirus protease 3C yielding *Eco*NusA(339-495) with two additional amino-terminal residues (Gly-Pro). For NMR studies, *Eco*NusA(339-495) was dissolved at ∼1.5 mM concentration in 50 mM potassium phosphate, pH 6.4, 50 mM NaCl, and 10% (v/v) D_2O.

All NMR spectra were acquired on Bruker DRX 600 and Avance 700 spectrometers at 25 °C. HNCO, HNCACB, CBCA(CO)NH, CCONH, HBHA(CO)NH, H(C)CH-COSY, HC(C)H-TOCSY, HNHA, and NNH-NOESY (Bax and Grzesiek, 1993; Sattler et al., 1999) 3D NMR experiments were recorded for backbone and aliphatic resonance assignment. Assignment of aromatic resonances was achieved with a [^{1}H, ^{13}C]-HSQC spectrum recorded in the aromatic region and 3D ^{13}C-edited NOESY experiments. The NMR data

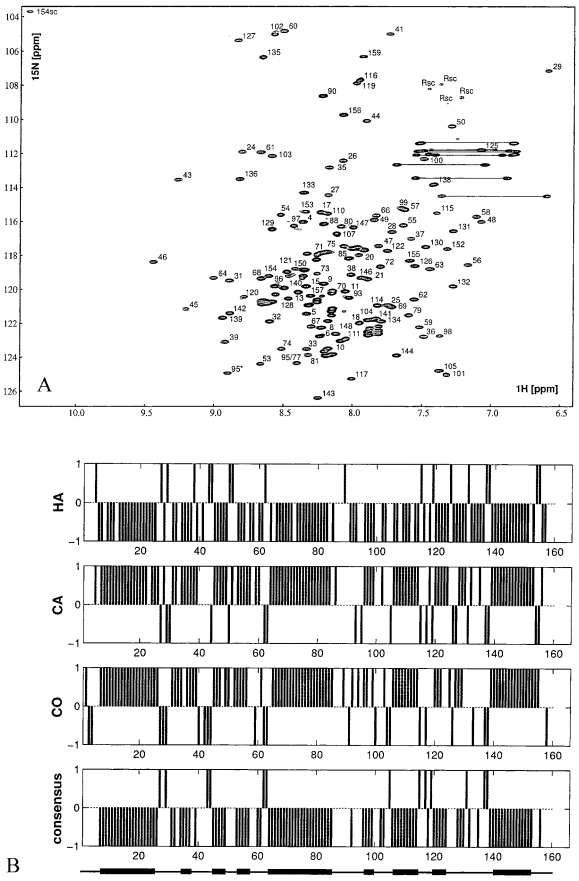

Figure 1. (A) [^{1}H, ^{15}N] spectrum of the uniformly ^{15}N/^{13}C labeled *Eco*NusA(339-495). NH_2 sidechains are connected by lines, the arginine εNH, and the tryptophane NH are marked by 'sc'. Asterisks indicate minor conformations. To avoid overlap, labels in crowded regions have been omitted. (B) Secondary chemical shift indices for H^α, C^α, CO nuclei, and the consensus of *Eco*NusA(339-495). Filled bars represent helical regions.

was processed using in-house written software and analyzed with the program package NMRview (B.A. Johnson, Merck, Whitehouse Station, NJ, U.S.A.).

Extent of assignment and data deposition

Analysis of triple resonance spectra allowed identification and sequential assignment of all 154 backbone NH resonances, and assignment of H^α, H^β, C^α and C^β resonances is complete with partial exception of L112, A159, and P160 (completeness > 99%). ^{1}H and ^{13}C assignments for longer amino acid side chains are complete to > 90%. An assigned [^{1}H, ^{15}N] HSQC spectrum is shown in Figure 1A.

Analysis of the H^α, C^α, and CO secondary chemical shifts (Figure 1B) indicates a mainly α-helical protein, with helices spanning residues 5–26, 31–39, 45–49, 53–57, 64–85, 96–99, 106–114, 120–124, and 139–153. The two HhH motifs predicted by profile based sequence analysis with hairpins at G61-L62-D63 and G136-L137-T138 could be confirmed.

The *Eco*NusA(339-495) assignments have been deposited in the BioMagResBank, accession code 5800.

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