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Letter to the Editor: Sequence-specific ^{1}H, ^{15}N, and ^{13}C resonance assignments for the whole region 4 of *Escherichia coli* RNA polymerase σ^{70} subunit

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Biological context

Transcription initiation is a major control step in the expression of bacterial genes. In *Escherichia coli*, σ^{70} is the primary sigma factor which first binds to the core RNA polymerase ($\alpha\beta\beta'$) and then helps to recognize promoters (Gross et al., 1998). The σ^{70} polypeptide consists of four (1–4) conserved domains, the 4.2 and 2.4 subdomains of which are thought, on the basis of genetic and biochemical evidence, to be involved in specific recognition of the −35 and −10 hexamer regions of promoter DNA, respectively. Some residues of a narrow region of the 4.2 domain (590 to 603) have also been identified as contact sites for transcription activation factors (Lonetto et al., 1998). So far, only the crystal structure of a proteolytically stable fragment including the conserved 2.4 region has been solved at 2.6 Å resolution (Malhotra et al., 1996). Alignment of the sequence of the 4.2 σ^{70} region with the HTH consensus sequence (Gardella et al., 1989) and more recent homology modeling studies (Reddy et al., 1997; Lonetto et al., 1998) suggested that this region contained a typical HTH DNA binding motif.

Here we report the ^{1}H, ^{15}N, and ^{13}C resonance assignments of 86 residues from a C-terminal recombinant polypeptide corresponding to the whole region 4 and a part of region 3.2 of σ^{70}, preceded by a 21-residue fragment carrying the thrombin cleavage site and N-His_6-Tag.

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Methods and experiments

The recombinant region 4 of *E. coli* σ^{70} was overexpressed in *E. coli* BL21(DE3) strain. The *Xho* I/*Hind* III fragment of the *rpoD* gene, containing 86 C-terminal residues from 528 to 613, was first cloned into pAED4 plasmid and then the *Xho* I/*BamH* I fragment thereof was recloned into pET-15b plasmid in the frame coding for N-His_6-Tag and the thrombin cleavage site. The protein, in the form of inclusion bodies, was dissolved in 6M urea and purified by metal-ion affinity column chromatography on Ni^{2+}-NTA agarose (Novagen), renatured by dialysis against 1 mM HCl and lyophilized. At a pH higher than ca. 4 the protein aggregated. Uniformly ^{13}C/^{15}N labeled protein was prepared by growing the bacteria in minimal media containing $(^{15}NH_4)_2SO_4$ and ^{13}C-D-glucose. The yield of unlabeled and labeled proteins was 15–20 mg/L.

For NMR studies, the protein was dissolved in 90% H_2O/10% D_2O at pH 2.8 to a final concentration of ca. 3 mM. The experiments were carried out at 298 K on a Varian Unity-Plus 500 MHz spectrometer using a 5 mm triple resonance probe with Z-gradients. The ^{1}H, ^{15}N, ^{13}C backbone and ^{13}C side-chain resonances were assigned using the gradient versions of C(CO)NH (Lohr and Rüterjans, 1995), HNCACB, CBCA(CO)NH, HNCO and (HCA)CO(CA)NH spectra. ^{1}H aliphatic side-chain resonances were assigned on the basis of a combination of HA(CO)NH, H(CCO)NH and ^{15}N-NOESY-HSQC (τ_m = 150 ms) spectra (Clore and Gronenborn, 1993). Resolution in the indirect dimensions was increased by linear predic-

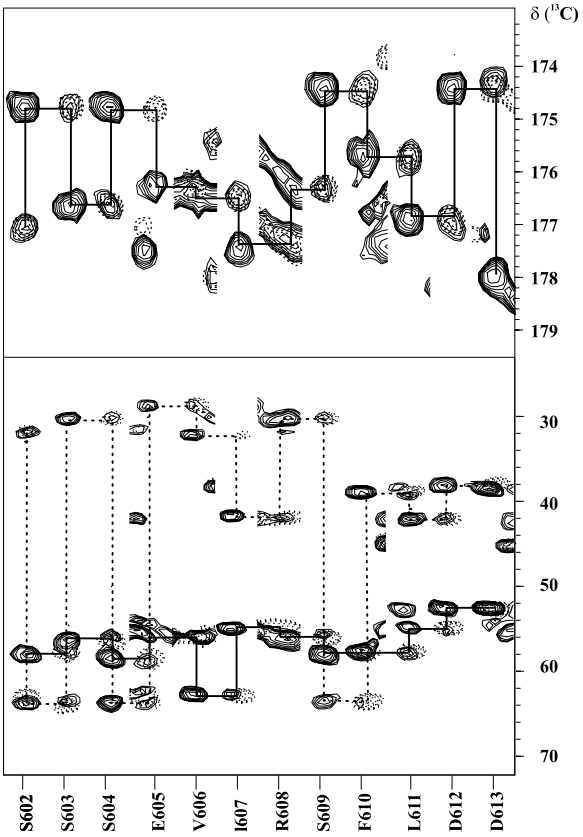

Figure 1. Representative strips from the (HCA)CO(CA)NH vs. HNCO (upper) and HNCACB vs. CBCA(CO)NH (lower, trace lines: CA – solid, CB – dotted) spectra demonstrating sequential assignment for the S602–D613 fragment (region 4.2) of *E. coli* σ^{70} factor.

tion and multiplication with a $\pi/6$ shifted squared sine-bell weight function followed by zero-filling. To avoid errors, the sequential assignment trace was followed simultaneously using both CBCA and CO resonances and additionally verified in the ^{15}N-NOESY-HSQC spectrum.

Extent of assignments and data deposition

A representative sequential assignment pattern for the C-terminal 602SRSEVLRSFLDD fragment is presented in Figure 1. Almost all the expected amide resonances were assigned with the exception of the N-terminal Met, found to be deleted in the expressed protein, and F580, which was not observed in the ^{1}H-^{15}N HSQC spectrum. Due to strong signal overlap three pairs of side-chain carbon resonances (559LR,573LE; 598LR,607LR; 560RM,562RF) could not be resolved. Consequently, the assignments for the 559LRMR and 573LEE fragments are ambiguous. All the carbonyl and aliphatic side-chain carbon ^{13}C resonances were assigned, including Asn/Gln HN amide groups, with the exception of Met methyl and Arg guanidinium groups.

The 529ELPLD fragment appeared to have a minor component corresponding to the alternative sequential pattern with P531 in the *cis* form. The population of the latter increased up to 10–15% after one month. Chemical shift analysis made by the Talos program (Cornilescu et al., 1999) showed the presence of five helical regions corresponding to the His_6-tag region, L532–S539, A542–F563, M567–R599 and R603–L611, respectively. The latter finding agrees with our CD data (not shown) indicating that 4-σ^{70} is almost fully helical at pH 2.8.

A table of the ^{1}H, ^{15}N and ^{13}C chemical shift assignments of the 4 region of *Escherichia coli* σ^{70} has been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4870.

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