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Main-chain NMR assignments for AsiA

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Biological context

Following infection of *E. coli* by bacteriophage T4, the host RNA polymerase is recruited to transcribe T4-encoded genes. Much of the course of T4 development is regulated at the transcriptional level, in many cases by the reversible interaction of T4-encoded proteins with the host RNA polymerase. In the early stages of T4 transcription (early mode, σ^{70}-dependent transcription initiation), the T4-encoded gene designated *asi*A is transcribed (Orsini et al., 1993). The product of this gene, the AsiA (*A*nti-*SI*gma *A*) protein (Orsini et al., 1993), binds very tightly to the major sigma factor of the *E. coli* polymerase, σ^{70}. This tight interaction between AsiA and the σ^{70} subunit of the *E. coli* RNA polymerase contributes to the termination of early mode transcription (Stevens, 1977). In addition, the presence of AsiA is essential for activation of transcription at T4 middle mode promoters (Ouhammouch et al., 1994,1995). AsiA, through its interplay with σ^{70}, and the T4-encoded protein MotA, which binds to the −30 region of middle promoters, are necessary and sufficient for switching RNA synthesis from early to middle mode (Ouhammouch et al., 1995).

In order to understand the remarkable regulatory properties of this small protein, we have begun studies to characterize structurally and dynamically the interaction between AsiA and σ^{70}. Initial studies are aimed at determining high-resolution structural models for the AsiA protein alone in solution using NMR spectroscopy. Towards this end, herein we report main-chain (and $^{13}C^{\beta}$) NMR assignments for the AsiA protein.

The sequence of the T4-encoded AsiA protein (90 amino acids, 10.59 kDa) is as follows:

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1
MNKNIDTVRE IITVASILIK FSREDIVENR
                        51
ANFIAFLNEI GVTHEGRKLN QNSFRKIVSE
LTQEDKKTLI DEFNEGFEGV YRYLEMYTNK
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The AsiA protein is devoid of proline, cysteine, and tryptophan residues. The description of the cloning and sequencing of the *asi*A gene, and the overproduction and purification of the AsiA protein have been described recently (Orsini et al., 1993; Ouhammouch et al., 1994,1995).

Methods and Results

Because of the poor expression of the AsiA protein on minimal media, uniformly isotopically labeled protein (^{15}N- or ^{13}C,^{15}N-labeled) was prepared by growth on 'rich media' using ^{15}N- or ^{13}C,^{15}N-labeled 'Isogro' (Isotec Inc., Miamisburg, OH, U.S.A.) media. ^{15}N- and ^{13}C,^{15}N-labeled samples were prepared and used for the reported NMR assignment experiments. The samples contained, in a final volume of 600 µl, ~1.5 mM ^{13}C,^{15}N-labeled AsiA protein or ~2.0 mM ^{15}N-labeled AsiA protein, 50 mM sodium acetate-d_3, and 10% D_2O, pH 6.0 (uncorrected for the isotope effect).

All NMR assignment experiments were performed at 25 °C using a Varian INOVA 600 MHz spectrometer, with the exception of the ^{15}N-resolved TOCSY experiment which was recorded using a Varian INOVA 750 MHz spectrometer. The assignment experiments were recorded in the normal manner using pulsed field gradients for artifact removal and as part of the sensitivity enhancement scheme where appropriate (see for instance Muhan-

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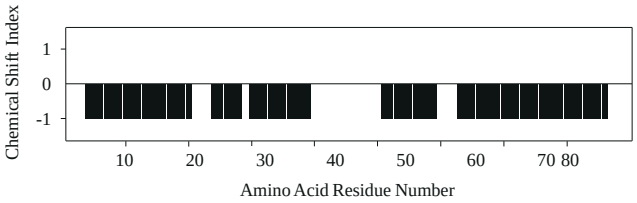

Fig. 1. Consensus ($^1H^\alpha$, $^{13}C^\alpha$, $^{13}C^\beta$, and $^{13}C'$) Chemical Shift Index (CSI) analysis results for the AsiA protein. An index of –1 indicates helical structure, an index of 0 indicates coil, and an index of 1 indicates β-sheet/strand structure.

diram and Kay, 1994), and using minimal dephasing of the H_2O resonance and gradient and H_2O-flip-back pulses for H_2O suppression, again where appropriate (Grzesiek and Bax, 1993a,b). The following experiments were recorded with the indicated number of acquired complex points in the indicated dimensions: ^1H,^{15}N-HSQC (1024 (t_2,^1H) × 256 (t_1,^{15}N)), constant-time ^1H,^{13}C-HSQC (1024 (t_2,^1H) × 115 (t_1,^{13}C)), NOESY-^1H,^{15}N-HSQC (1024 (t_3,^1H) × 64 (t_2,^1H) × 32 (t_1,^{15}N)), ^1H,^{15}N-HSQC-TOCSY (1024 (t_3,^1H) × 60 (t_2,^1H) × 60 (t_1,^{15}N)), HCCH-TOCSY (512 (t_3,^1H) × 90 (t_2,^1H) × 50 (t_1,^{13}C)), HNCA (512 (t_3,^1H) × 64 (t_2,^{13}C) × 36 (t_1,^{15}N)), HN(CO)CA (512 (t_3,^1H) × 60 (t_2,^{13}C) × 34 (t_1,^{15}N)), CBCA(CO)NH (512 (t_3,^1H) × 53 (t_2,^{13}C) × 42 (t_1,^{15}N)), HNCACB (512 (t_3,^1H) × 53 (t_2,^{13}C) × 42 (t_1,^{15}N)), and HNCO (512 (t_3,^1H) × 64 (t_2,^{13}C) × 56 (t_1,^{15}N)). For each experiment, the data were acquired using the ^{13}C, ^{15}N-labeled AsiA sample, with the exception of the ^{15}N-resolved NOESY experiment, where the data were acquired using the ^{15}N-labeled sample. In all cases, the ^1H chemical shifts were referenced to external Na^+DSS^- in D_2O (0.00 ppm; Wishart et al., 1995) while ^{13}C and ^{15}N chemical shifts were referenced indirectly assuming the absolute frequency ratios $^{13}C/^1H = 0.251449530$ and $^{15}N/^1H = 0.101329118$ (Wishart et al., 1995). All data processing and visualization were accomplished using FELIX (Biosym Technologies, San Diego, CA, U.S.A.).

Data from the HNCA and HN(CO)CA spectra were used to correlate initially the $^1H_i^N$, $^{15}N_i$, $^{13}C_i^\alpha$, and $^{13}C_{i-1}^\alpha$ chemical shifts. The HNCACB and CBCA(CO)NH spectra then afforded correlation of the $^{13}C_i^\beta$ and $^{13}C_{i-1}^\beta$ chemical shifts with those already correlated. These correlated chemical shift units were then linked via the common $^{13}C^\alpha$ and $^{13}C^\beta$ chemical shifts. $^1H_i^N$-$^1H_{i+1}^N$ NOEs from the NOESY-^1H,^{15}N-HSQC spectrum were used to resolve any ambiguities and to verify the connectivities. Carbonyl carbon ($^{13}C'$) chemical shifts were assigned by correlation of $^1H_i^N$, $^{15}N_i$, and $^{13}C'$ chemical shifts using data from the HNCO spectrum. Subsequently, data from the ^{15}N-resolved TOCSY experiment, acquired with a short mixing time to maximize $^1H^\alpha$ resonance intensities, and data from the HCCH-TOCSY experiment were used to provide the $^1H^\alpha$ assignments.

It was initially suspected, based on the conspicuous lack of downfield-shifted $^1H^\alpha$ resonances in the constant-time ^1H,^{13}C-HSQC spectrum, that the AsiA protein is devoid of β-strand/sheet secondary structural elements. Assignment of the $^{13}C^\alpha$, $^{13}C^\beta$, $^{13}C'$ and $^1H^\alpha$ chemical shifts permits a preliminary explication of secondary structure based on chemical shifts (Spera and Bax, 1991; Wishart and Sykes, 1994). Based on the consensus chemical shift index (CSI; Wishart and Sykes, 1994) using $^{13}C^\alpha$, $^{13}C^\beta$, $^{13}C'$ and $^1H^\alpha$ chemical shifts, and a cursory examination of $^1H_i^N$-$^1H_{i+1}^N$ NOEs from the ^{15}N-resolved NOESY spectrum, a preponderance of helix and coil secondary structural elements in AsiA is detected. As shown in Fig. 1, the consensus CSI analysis predicts that AsiA is composed primarily of alternating regions of helix and coil secondary structure. The helices span residues 4–20, 24–28, 30–39, 51–59, and 63–86. A more thorough delineation of the secondary structural elements of AsiA based on NOE patterns is ongoing.

Extent of assignments and data deposition

Sequence-specific assignments ($^1H^N$, ^{15}N, $^{13}C^\alpha$, $^1H^\alpha$, $^{13}C'$, $^{13}C^\beta$) for the AsiA protein are presented in Table 1 of the Supplementary Material, and have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database (accession number 4040). Resonances remaining to be unambiguously assigned include all those for M1, N2, and K3, $^1H^N$ and ^{15}N Y81, $^{13}C'$ V80 and K90, $^{13}C^\beta$ T7, S16, F73, and F77, and $^1H^\alpha$ N4, R9, K20, F33, Q51, and F77. Side-chain assignments other than $^{13}C^\beta$ are not reported or deposited.

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