Journal of Biomolecular NMR, **20:** 187–188, 2001.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N signal assignments and secondary structure of *Archaeoglobus fulgidus* SRP19

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Received 4 January 2001; Accepted 13 March 2001

Key words: NMR assignments, secondary structure, SRP, SRP19

Biological context

The signal recognition particle (SRP) is an essential cytoplasmic ribonucleoprotein complex that is involved in targeting of signal-peptide-containing proteins to membranes. The 54 kDa protein component of the SRP, or SRP54, recognizes the signal peptides, interacts with a receptor on the membrane surface, and also binds to SRP RNA. Another SRP structural component, SRP19, which is the focus of this study, is the primary assembly protein of the mammalian SRP and obligatory for binding of SRP54 to the SRP RNA (Römisch et al., 1990). Eukaryotic polypeptides related to SRP19 have been identified in the genomes of *Archaea*, and recently the SRP of *Archaeoglobus fulgidus* has been sucessfully reconstituted from recombinant components (Bhuiyan et al., 2000). Site directed mutagenesis indicates that SRP19 has two binding sites in SRP RNA: a conserved tetraloop at the apex of helix 6, and three base pairs in the distal part of helix 8 (Zwieb, 1992, 1994). Both the N- and C-terminal regions of SRP19 are required for RNA binding (Chittenden et al., 1994). Since there is no evidence for interactions between SRP54 and SRP19 in the absence of RNA, binding of SRP19 to SRP RNA is believed to be accompanied by a conformational change in SRP RNA (Römisch et al., 1990). This model is supported by recent work which has shown that SRP19 induces conformational changes at an asymmetric bulge of helix 8 of SRP RNA located within the SRP54 binding site (Diener and Wilson, 2000). Thus, SRP19 may be involved in the induction of an RNA binding interface compatible with the subsequent binding of SRP54.

At present, there is no 3D structural information available for SRP19 or any of its homologues. Moreover, the structure–function relationships that govern its interaction with SRP RNA and the assembly with SRP54 are poorly understood. Here we present nearly complete ^{1}H, ^{13}C, ^{15}N backbone assignments and the secondary structure of the full-length 12.4-kDa SRP19 protein from the hyperthermophilic archaebacterium *Archaeoglobus fulgidus*.

Methods and experiments

The 104-residue recombinant *Archaeoglobus fulgidus* SRP19 protein, herein designated Af19, was expressed using a pET23d vector in *E. coli* strain BL21(DE3) (Novagen, Madison, WI). Cells were also co-transformed with plasmid pLysE to inhibit basal level transcription. The coding region for Af19 protein included cysteine to serine substitutions at positions 4 and 41, which did not affect binding to *A. fulgidus* SRP RNA in vitro, but prevented covalent dimer formation over the extended periods required for NMR data collection. The protein was purified from the soluble fraction on a BioRex 70 cation exchange column using a 0.3–2.5 M NaCl gradient in 100 mM KH_2PO_4, 1 mM EDTA, 10% glycerol buffer at pH 6.8. Other impurities were removed by reverse phase chromatography on a C4 column using a 10–50% gradient of acetoni-

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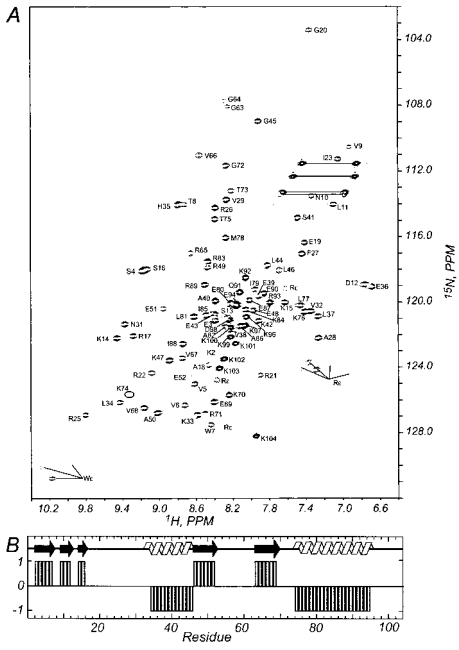

Figure 1. A summary of the backbone assignments and secondary structure of Af19. (A) The two-dimensional ^{1}H/^{15}N HSQC spectrum of Af19 recorded at 27 °C. The peaks are labeled according to their assignments with the one-letter amino acid code. (B) The predicted secondary structure of Af19 as determined by the consensus chemical shift index.

trile in 0.1% TFA/water. The Af19 protein obtained using this protocol appeared as a single band when examined with denaturing PAGE. Isotopically labeled forms of the protein for NMR spectroscopy were prepared by culturing the cells on M9 minimal medium containing $^{15}NH_4Cl$ alone or $^{15}NH_4Cl$ and D-[$^{13}C_6$]-glucose (Cambridge Isotope Laboratories, Cambridge, MA).

All NMR spectra were acquired at 27 °C on a Bruker AMX2-500 spectrometer equipped with a pulsed-field gradient unit and a triple-resonance 5 mm probe. The ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced to DSS according to the IUPAC recommendation (Markley et al., 1998). Spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed with PIPP (Garrett et al., 1991). Triple-resonance methodology was employed to obtain sequential backbone and side-chain assignments for 1.8–2.2 mM samples of ^{13}C/^{15}N uniformly labeled Af19 protein in 25 mM KH_2PO_4, 50 mM NaCl and 5% or 100% $^{2}H_2O$ at pH 6.0. The sequential connectivities were made using a CBCA(CO)NH and HNCACB pair of experiments, and verified using a HNCO and HN(CA)CO pair. Side-chain ^{13}C assignments were made using a C(CO)NH experiment. Backbone and side-chain ^{1}H resonance assignments were obtained by acquiring and analyzing HBHA(CO)NH and HCCH-TOCSY data sets.

Extent of assignments and data deposition

Nearly complete backbone and side-chain sequential resonance assignments were obtained for 92 of 104 amino acid residues. Partial assignments are available for the N-terminal methionine, E62 and Q95. The remainder of the residues forms a nine-residue cluster positioned between K53–E61 in the sequence that is completely unassigned. The temperature dependence of adjacent assigned amides indicates that this region may represent a mobile loop whose resonances are broadened by a chemical exchange mechanism involving *cis:trans* isomerization about the Y55–P56 peptide bond. The secondary structure of Af19, as determined by the consensus of the chemical shift index (Wishart and Sykes, 1994), comprises three long and two short β-strands and two α-helices. The unassigned flexible loop is located between the fourth and fifth β-strands. The assignments have been deposited in the BioMagResBank database with accession number 4935.

Acknowledgements

This work was supported by NIH grants GM-49034 to C.Z. and RR13879 to A.P.H.

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