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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the ribosome-associated cold shock response protein Yfia of *Escherichia coli*

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Biological context

Temperature changes are the most common stress factors that living organisms constantly confront in nature. All living organisms react on a sudden in- or decrease of temperature with the production of heat shock or cold shock proteins to adapt to the given environmental condition. Whereas heat shock response is triggered by the expression of a special sigma factor, RNA-binding proteins play a major role in cold shock response.

Recently, a novel ribosome-associated protein of *Escherichia Coli*, denoted protein Yfia or RaiA (**r**ibosome-**a**ssociated **i**nhibitor **A**), was discovered by tritium bombardment technique and was shown to be bound to the 30S subunit and located at the subunit interface in the 70S ribosome (Agafonov et al., 1999). It was shown that synthesis of this protein or its binding to ribosomes was induced by environmental stress (Agafonov et al., 2001). Homologues sequences of protein Yfia are found in genomes of many bacteria, suggesting that it might play an important role for survival under stress conditions. Very recently, the structure of the related protein HI0257 from *Haemophilus influenzae*, which is 64% identical to protein Yfia, was solved (Parsons et al., 2001). Yfia is involved in low temperature adaptation of *Escherichia coli* (Agafonov et al., 2001). In *E. coli*, the protein inhibits translation at the elongation stage by blocking the binding of aminoacyl-tRNA to the ribosomal A site. The appearance of the protein at the ribosome leads to a decline of protein synthesis. Yfia is important for growth arrest as a result of cold shock response, but is also found in the stationary phase of a cell culture. It is therefore not only a potential drug target, but also an interesting knockout factor for recombinant protein production in biotechnology.

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Methods and experiments

Sample preparation

A fragment of *E. coli* DNA encoding Yfia was cloned into the vector pET11c and expressed in *E. coli* strain BL21(DE3). Unlabeled NMR samples were purified from cells grown in LB medium at 30 °C; ^{15}N-labeled and ^{15}N-,^{13}C-labeled samples were purified from cells grown in rich growth media (E.Coli-OD2-N, E.Coli-OD2-C,N) purchased from Silantes (Munich, Germany). The supernatant of the cell lysate was heated to 60 °C for 10 min and the precipitated *E. coli* proteins were spun down. The protein was purified from the supernatant using ion exchange chromatography followed by gel-filtration. Yfia was dialyzed against a 50 mM KH_2PO_4 buffer at pH 6.5 containing 50 mM LiCl and was subsequently concentrated. A typical NMR sample contained NMR buffer and 10% (v/v) D_2O, with a pH ranging from 6.5 to 6.8 and a protein concentration between 0.5 mM and 0.8 mM.

NMR experiments

All NMR data were acquired with Varian Inova 600-MHz and Bruker DRX 500-MHz spectrometers at a

temperature of 27 °C. ^{1}H, ^{13}C and ^{15}N chemical shifts are referenced to TSP according to IUPAC recommendations (Markley et al., 1998). Data were processed and displayed by the program packages Xwinnmr 3.2 (Bruker, Rheinstetten, Germany) and Ndee (Spin-Up, Dortmund, Germany) on a SGI Octane workstation. The program Ndee was used for data analysis and assignment of all 2D spectra and the program Aurelia (Bruker, Rheinstetten, Germany) for analysis of all 3D spectra. Backbone resonances of Yfia were assigned on the basis of 3D HNCA, HNCACB and HNCO spectra, whereas aliphatic side-chain resonances were assigned using HC(CO)NH, C(CO)NH, 3D ^{15}N- and ^{13}C-edited HSQC-TOCSY and HCCH-TOCSY spectra. Aromatic side-chain resonances were assigned with the help of a 3D ^{13}C-edited HSQC-NOESY and 2D ^{13}C-HSQC, NOESY, TOCSY and COSY spectra recorded in a D_2O sample. Residual gaps and ambiguities were resolved by using sequential NOEs measured in 3D ^{15}N- and ^{13}C-edited HSQC-NOESYs.

The secondary structure of the protein could be predicted (Figure 1) using the chemical shift index method developed by Wishart and coworkers (1992, 1994) for Hα, Cα, Cβ and carbonyl atoms. Corrections for chemical shift values of residues preceding proline (Wishart et al., 1995) were done.

Extent of assignments and data deposition

Ninety-six percent of the backbone ^{15}N, $^{1}H_N$, ^{1}Hα, ^{13}Cα and ^{13}C′ resonances of all non-Pro residues were assigned. Residues with missing assignments are Met1, Thr2, Gln31, Thr32, His33 and Leu34, where no corresponding cross-peaks in the ^{15}N-HSQC spectra were found. The amide protons of these residues were not observed, presumably due to the fast exchange with bulk water. The ^{15}N assignment of Ile35 is uncertain. The assignment of the resonances of the four Pro residues could be facilitated by the use of HCCONH, CCONH and 3D-HCCH-TOCSY spectra. In total, 85% of the ^{13}C and 87% of the ^{1}H resonances of the protein could be assigned from the 2D and 3D experiments. The chemical shift assignment has been deposited in the BioMagResBank database (accession number 5389).

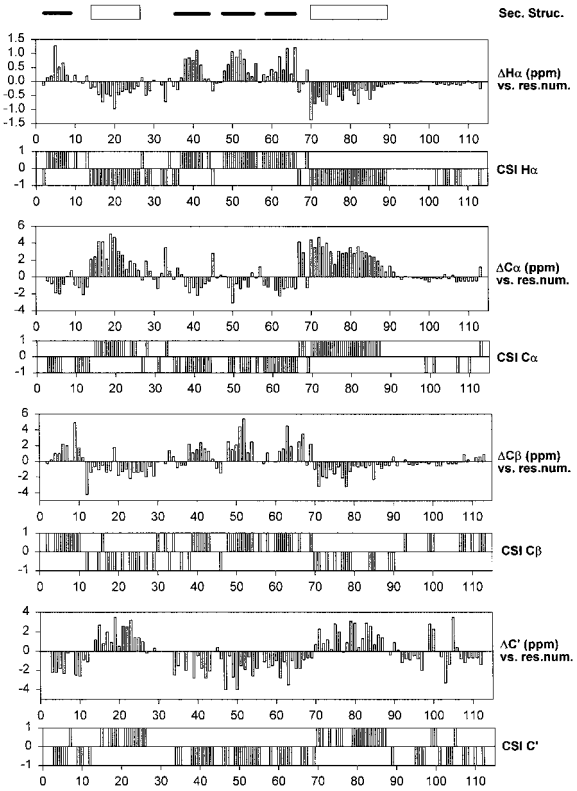

Figure 1. Deviations of chemical shifts of ^{1}Hα, ^{13}Cα, ^{13}Cβ and ^{13}C′ from the corresponding random coil values and chemical shift indices according to Wishart et al. (1992, 1994). The first line represents the secondary structure predicted from shift indices. Helices are drawn as rectangles and β-strands as black lines. The sequence of the protein is available in the swissprot databank (entry code: YFIA_ECOLI; accession number P11285).

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