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Letter to the Editor: Assignment of the ^{1}H, ^{15}N and ^{13}C resonances of SufA from *Escherichia coli* involved in Fe-S cluster biosynthesis

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Biological context

Iron–sulfur (Fe–S) proteins play an important role in electron transfer, redox and non-redox catalysis, regulation, and as sensors within all living organisms (Beinert et al., 1997). Although the chemical reactivity and spectroscopic properties of biological (Fe–S) clusters have been characterized extensively over the past years, the mechanisms underlying their biosynthesis are in an early phase of exploration. Three bacterial operons that encode critical proteins for (Fe–S) assembly have been identified, namely the *nif*, *isc* and *suf* operons (Frazzon and Dean, 2003). Key proteins include sulfur donor proteins (IscS, SufS/E), chaperones (HscA/B, SufBCD), electron transport protein (Fdx), and scaffold proteins (IscA/B, SufA) for transient (Fe–S) cluster assembly and delivery to apotarget proteins. The latter are divided into two groups (IscU and IscA/SufA) based on their amino acid sequence. Both scaffold proteins are able to assemble (2Fe–2S) and (4Fe–4S) clusters and to transfer them to the same target apoprotein. Recently structural informations were obtained for IscU (NMR spectroscopy) and for IscA (Cristallographic studies) proteins (Bertini et al., 2003 ; Bilder et al., 2004 ; Cupp-Vickery et al., 2004). In contrast, no structural information is available regarding SufA scaffold protein.

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In order to better understand the molecular mechanism of (Fe–S) cluster transfer of scaffold proteins to target apoproteins, a structural investigation by NMR spectroscopy was initiated on SufA protein from *Escherichia coli*. In this note we report the first assignment of the ^{1}H, ^{15}N and ^{13}C resonances of apoSufA.

Methods and experiments

An isotopically $^{15}N/^{13}C$ double-labelled sample of SufA from *Escherichia coli* (122 residues, Swissprot accession number P77667) was obtained from 1.5 l *E. coli* BL21(DE3) culture in M9 minimal medium containing 1 g/l $^{15}NH_4Cl$ and 2 g/l $^{13}C_6$-Glucose as sole nitrogen and carbon sources, respectively. For purification purposes, the construct contained additional residues at the C-terminus including 6 histidine residues (LEH_6). Plasmid pET/SufA encoding *E. coli* SufA was derived from pET22b(+) plasmid (Novagen). The *sufA* insert, obtained by PCR amplification using the oligonucleotides described below and *Nde*I/*Xho*I digestion, was introduced into the pET22b(+) digested by the same restriction enzymes, yielding pET/SufA. The oligonucleotides are: *Nde*I, 5′-CCG**CATATG**GACATGCATTCAGGAACC-3′; *Xho*I, 3′-GTT**CTCGAG**TACCCCAAAGCTTTCGCCAC-AGCC-5′. Expression and purification procedures were identical to that *E. chrysanthemi* SufA protein (Ollagnier-de Choudens et al., 2003).

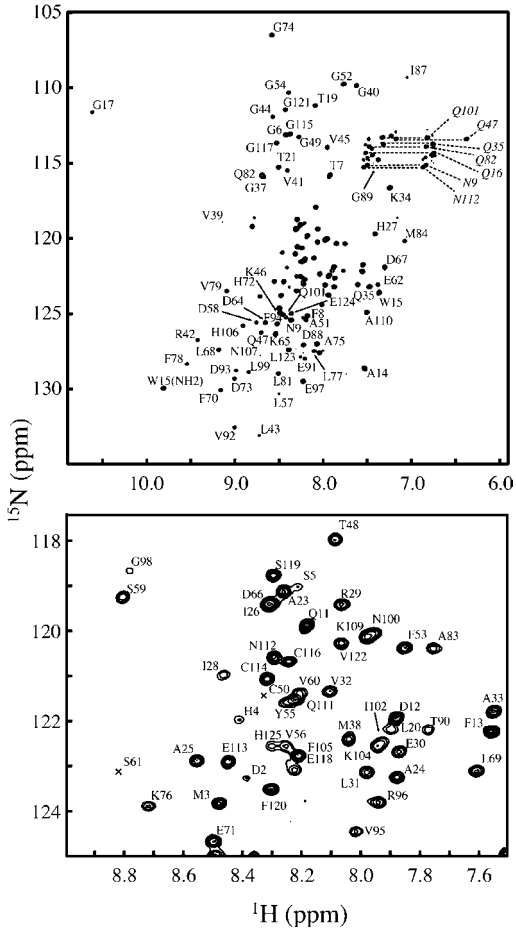

Figure 1. 2D ^{1}H–^{15}N HSQC spectrum of a 1 mM uniformly ^{15}N/^{13}C-enriched SufA from *E. coli* recorded on a 600 MHz Varian Inova spectrometer at 298 K. The side chain NH_2 resonances of asparagine and glutamine residues are connected by horizontal bars. The full spectrum is shown on the left while the crowded central area is expanded on the right.

The sample used for resonance assignments (protein concentration ≈ 1 mM) was prepared in potassium phosphate buffer (50 mM pH 7) containing 5 mM DTT, protease inhibitor cocktail without EDTA (Complete, Boehringer Mannheim), 0.02% NaN_3, and 10% D_2O. The NMR tube was then sealed under argon gas. The lack of degradation of the protein was monitored by comparing ^{1}H-^{15}N HSQC spectra recorded over a 6-month period.

NMR spectra were acquired at 298 K on a 600 MHz Varian Inova spectrometer equipped with a triple resonance (^{1}H, ^{13}C, ^{15}N) probe including shielded *z*-gradients. Spectra used for sequential backbone assignment were as follows : 2D ^{1}H-^{15}N HSQC, 3D HNCA, 3D CBCANH, 3D CBCA(CO)NH, 3D HNCO, 3D HNCACO experiments. The sequential connectivities were built using the in-house program ALPS (Assignment Of Labelled Protein Spectra, Morelle et al., 1995). Aliphatic ^{1}H and ^{13}C sidechain assignment was performed using the following experiments: 3D H(CC)(CO)NH-TOCSY, 3D (H)CC(CO)NH-TOCSY and 3D HC(C)H-TOCSY. The (H)C(CO)NH-TOCSY spectrum also allowed unambiguous assignment of the $^{1}H^{N}$ and ^{15}N side chain resonances of 2 Asn and 5 Gln residues. All triple resonance experiments used the pulse sequences provided by the Varian protein pack (available at ftp site: http://www.varianinc.com/). Parameters for all experiments can be obtained from the authors. All data were processed with Felix 2000 (Accelrys) using 90° shifted sine-bell squared apodizing functions. Residual water suppression was achieved using a sinebell convolution. Proton chemical shifts were reported with respect to the H_2O signal (4.773 ppm at 25 °C). The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the ^{1}H/X frequency ratios of the zero-point : 0.101329118 (^{15}N) and 0.251449530 (^{13}C) (Wishart et al., 1995).

Extent of assignments and data deposition

With the exception of residues Q16, L18, F86, F103 and H126 to H130 (poly-histidine tag), 92.7% of non-prolyl backbone ^{15}N and backbone amide ^{1}H, 65.1 % of non-labile ^{1}H (with 82.4% of ^{1}Hα, 67.8 % of ^{1}Hβ and 50.3% of other side chain protons), 85.3% of protonated ^{13}C (the aromatic ring atoms of aromatic residues are not included) and 90.8% of backbone carbonyl ^{13}C have been assigned. The chemical shift values of ^{1}H, ^{15}N and ^{13}C of SufA from *E. coli* have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-6224.

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