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Letter to the Editor: NMR assignment of HI1723 from *Haemophilus influenzae* – a sequence homologue from the iron sulfur cluster assembly (IscA) family

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Biological context

Iron-sulfur proteins are found throughout nature in a vast array of living systems and play an important role in a variety of processes such as electron transport, transcriptional regulation, and protein stabilization. The most common types of Fe-S clusters contain either [2Fe-2S], [3Fe-4S] or [4Fe-4S] arrangements although more than 100 different types of clusters are now known. While much work has been done on the biochemical function of these proteins, the assembly of Fe-S clusters has only recently begun to be understood. The first gene products found to be associated with this process were NifS and NifU from the nitrogen fixation (*nif*) operon of *Azotobacter vinelandii* (Jacobson et al., 1989). Further studies revealed an entire iron sulfur cluster assembly (*isc*) operon, originally detected in *A. vinelandii* and *E. coli*, but subsequently found to be widespread in other prokaryotes (Zheng et al., 1998). This operon contains a transcription factor IscR (Schwartz et al., 2001), a PLP-dependent cysteine desulfurase IscS (Zheng et al., 1998), scaffold proteins IscU (Agar et al., 2000) and IscA (Krebs et al., 2001; Ollagnier-de-Choudens et al., 2001), ferredoxin Fdx, and the molecular chaperones HscA and HscB (Hoff et al., 2000). The IscS and IscU proteins are homologous to the NifS and NifU gene products, respectively. With the exception of IscR, eukaryotic homologues have been found for all of the other *isc* operon proteins. It has been shown that both the IscU and IscA proteins assemble transient Fe-S clusters which can then be transferred to an apoferredoxin protein to form a fully functional [2Fe-2S] holoferredoxin (Ollagnier-de-Choudens et al., 2001). More recently, Fe-S cluster-containing IscA has also been shown to reactivate apo adenosine 5′-phosphosulfate reductase, an enzyme requiring a [4Fe-4S] cluster (Wollenberg et al., 2003).

Currently there are approximately 240 sequence relatives in the IscA protein family. A key feature of these proteins is the presence of three invariant cysteines, only two of which appear to play a role in transient Fe-S cluster assembly (Wollenberg et al., 2003). Little is known about the molecular details of this process although a number of models have been proposed (Krebs et al., 2001). To date, one solution structure has been deposited in the PDB (accession code 1nwb) for Aq_1857, a 124 residue IscA homologue from the hyperthermophile *Aquifex aeolicus.* No NMR assignments or other structures have yet been reported in the literature for these widely occurring proteins, however. Here, we describe ^{1}H, ^{13}C and ^{15}N chemical shift assignments for HI1723, a 114 residue *Haemophilus influenzae* homologue of IscA that has 42% sequence identity with Aq_1857. The assignments provide the basis for studying the structure and dynamics of IscA as well as its interaction with Fe-S cluster-containing proteins.

Methods and experiments

The gene for HI1723 was cloned into a pET-15b vector with an N-terminal His_6 tag using standard methods. Uniformly $^{13}C/^{15}N$-labeled samples were prepared by growing transformed *Escherichia coli* BL21 (DE3) cells (Novagen) in minimal media at 37 °C with $^{15}NH_4Cl$ and $^{13}C_6$-glucose as the sole nitrogen and carbon sources, respectively. *E. coli* cells were grown to an A_{600} of 1.0 and protein expression was induced with 1 mM IPTG. After an additional 3 h, the cells

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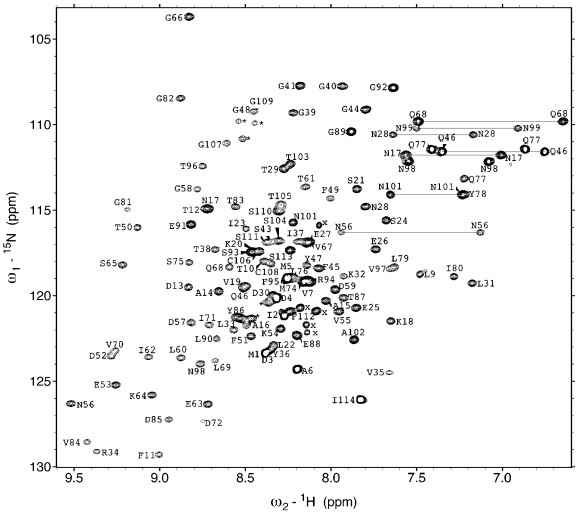

Figure 1. Two-dimensional ^{15}N-HSQC spectrum of HI1723 at 298 K and pH 7.0. Peaks marked with an x are from the His tag while those with an asterisk remain unassigned.

were harvested, suspended in lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 8.0), and lysed by sonication. The lysed cells were centrifuged and the supernatant was loaded onto a Ni-NTA column (Qiagen) and washed with 20 column volumes of lysis buffer followed by 20 column volumes of lysis buffer with 20 mM imidazole. HI1723 was eluted with 3 column volumes of lysis buffer containing 250 mM imidazole. Fractions were tested for purity by SDS-PAGE and those containing pure HI1723 were combined, dialysed against NMR buffer (50 mM potassium phosphate, 100 mM sodium chloride, 4 mM DTT, pH 7.0) and concentrated. For reasons of improved solubility and stability the 20 residue N-terminal His tag was not removed by thrombin cleavage and all NMR experiments were done on the 134 residue construct. Sequence numbering starts at the first residue in the native sequence, however.

NMR samples of ^{13}C/^{15}N-labeled HI1723 were at a concentration of approximately 1.5 mM in 450 μL of NMR buffer containing 90% H_2O/10% D_2O. Two-dimensional ^{15}N-HSQC, ^{13}C-HSQC, and (HB)CB(CGCD)HD/(HB)CB(CGCDCE)HE, and three-dimensional HNCACB, CBCA(CO)NH, HNCO, HNHA, HNCA, HBHA(CO)NH, H(CCO)NH-TOCSY, (H)C(CO)NH-TOCSY, HCCH-TOCSY, HCCH-COSY, ^{15}N-NOESY HSQC, and ^{13}C-NOESY HSQC were acquired at 25^{o}C on Bruker DRX-500 and DRX-600 spectrometers. Constant time ^{13}C-HSQC spectra were also recorded on a 15% uniformly ^{13}C-labeled sample (1.0 mM) for stereospecific assignments of methyl groups in Leu and Val residues (Neri et al., 1989). The methyl groups of 7 out of 8 Val residues and all 9 Leu residues were stereospecifically assigned in this way. Spectra were processed using nmrPipe (Delaglio et al., 1995) and analyzed with Sparky (Goddard and Kneller, UCSF).

Extent of assignments and data deposition

Assignments were made for 109 of the 111 backbone amide proton resonances (114 residues minus 3 prolines) in the IscA-relevant part of the polypeptide chain (Figure 1). Residues with missing H_N assignments were C42 and N99. Secondary structure analysis based on CSI suggests the presence of 7 beta strands and 2 helices. The position and type of these secondary structure elements is in good agreement with predictions from PSIPRED. The extent of assignment for non-labile protons (H_α and side chain) is 93.6% for residues 1-114. In addition, all of the labile protons of side chain amides were identified. Assignments have also been made for many of the ^{13}C and ^{15}N resonances. The chemical shifts of HI1723 have been deposited in the BioMagResBank (accession number BMRB-5963).

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References

Agar, J.N., Zheng, L., Cash, V.L., Dean, D.R. and Johnson, M.K. (2000) *J. Am. Chem. Soc.*, **122**, 2136–2137.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Hoff, K.G., Silberg, J.J. and Vickery, L.E. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 7790–7795.

Jacobson, M.R., Brigle, K.E., Bennett, L.T., Setterquist, R.A., Wilson, M.S., Cash, V.L., Beynon, J., Newton, W.E. and Dean, D.R. (1989) *J. Bacteriol.*, **171**, 1017–1027.

Krebs, C., Agar, J.N., Smith, A.D., Frazzon, J., Dean, D.R., Huynh, B.H. and Johnson, M.K. (2001) *Biochemistry*, **40**, 14069–14080.

Neri, D., Szyperski, T., Otting, G., Senn, H. and Wüthrich, K. (1989) *Biochemistry*, **28**, 7510–7516.

Ollagnier-de-Choudens, S., Mattioli, T., Takahashi, Y. and Fontecave, M. (2001) *J. Biol. Chem.*, **276**, 22604–22607.

Schwartz, C.J., Giel, J.L., Patschkowski, T., Luther, C., Ruzicka, F.J., Beinert, H. and Kiley, P.J. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 14895–14900.

Wollenberg, M., Berndt, C., Bill, E., Schwenn, J.D. and Seidler, A. (2003) *Eur. J. Biochem.*, **270**, 1662–1671.

Zheng, L., Cash, V.L., Flint, D.H. and Dean, D.R. (1998) *J. Biol. Chem.*, **273**, 13264–13272.