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Letter to the Editor: NMR assignment of the ^{1}H, ^{15}N and ^{13}C resonances of the *E. coli* frataxin orthologue, CyaY

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Biological context

The protein frataxin is localised in the cell mitochondria and is implicated in the progressive neurodegenerative disease, Friedreich's ataxia. Frataxin deficiency in Friedreich's ataxia patients leads to accumulation of iron in the mitochondria and impaired anti-oxidant defences. The function of human frataxin is not known but its absence, and the resulting accumulation of iron in mitochondria suggests a possible role in iron binding and storage (Pandolfo, 2002). Orthologues of frataxin include bacterial and yeast frataxin and these are currently in use as model systems for understanding the properties and iron-binding role of the frataxin family (Adinolfi et al., 2002). Studies on the knockout of the yeast homologue gene showed mitochondrial iron accumulation (Babcock et al., 1997) while similar studies for the CyaY gene in *E. coli* showed no effect on cellular iron content (Li et al., 1999). The bacterial frataxin protein, CyaY is a highly acidic protein comprising 106 residues (Mw 12231Da). NMR and crystallographic structural studies on human frataxin (PDB_ID: 1LY7 and 1EKG) and crystallographic studies on bacterial frataxin (PDB_ID: 1EW4) revealed a similar fold for both proteins. No similarity to known protein folds in the protein databases were found. Disease-related mutations and metal binding studies form the basis of our studies on the assignment and solution structure of the bacterial CyaY protein (Adinolfi et al., 2002).

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Methods and experiments

A CyaY construct (Swiss-Prot:P27838) was cloned into a pET derived plasmid vector as a His-tagged Glutathione-S-Transferase (GST) fusion protein comprising a GA tag at the N-terminus and glycine insert between residues M1 and N2. The protein was over-expressed in *E. coli* strain BL21(DE3) at 37 °C using minimal media supplemented with ^{13}C glucose and /or ^{15}N ammonium sulphate as carbon and nitrogen sources and purified using affinity chromatography (Gutathione-S-Sepharose). Protein purity was checked using SDS-PAGE and amino acid analysis. Uniformly labelled ^{15}N and ^{13}C,^{15}N NMR samples were prepared using 50 mM KH_2PO_4, 50 mM K_2HPO_4, 150 mM KCl, 1 mM DTT, and 0.05% sodium azide as sample buffer in 90% H_2O, 10% D_2O at pH 7. All spectra were recorded at 25 °C on a Varian Unity 600 MHz, UnityPlus 500 MHz, Inova 600 MHz and Inova 800 MHz spectrometers equipped with pulsed field gradients and triple resonance probes. All NMR spectra were processed using NMRPipe (Delaglio et al., 1995) and analysed using XEASY (Bartels et al., 1995). Sequence specific backbone assignments were obtained using the 3D triple resonance HNCA, CBCA(CO)NH, HN(CO)CA, HNCO and HCC(CO)NH experiments and additional assignments and chemical shift data were obtained from a 2D ^{15}N HSQC, 2D ^{13}C HSQC (aromatic and aliphatic), 3D ^{13}C HCCH-TOCSY (aliphatic and aromatic), ^{15}N TOCSY-HSQC, HNHA, HNHB and validated using data from ^{15}N NOESY-HSQC, and ^{13}C NOESY-HSQC spectra (Cavanagh et al., 1996). Sidechain aromatic resonances were assigned with the aid of a (Hβ)Cβ(CγCδ)Hδ experiment (Yamazaki et al., 1993).

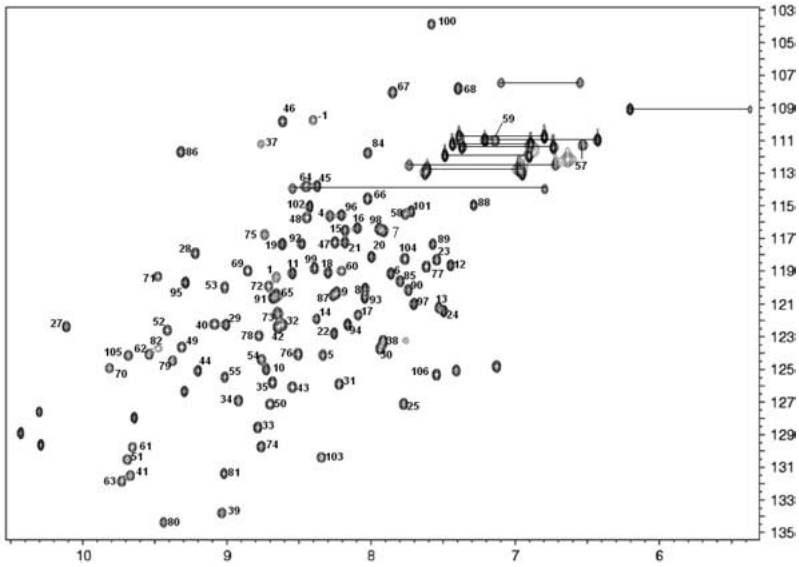

Figure 1. A 500 MHz ^{1}H-^{15}N spectrum of CyaY protein in 50 mM KH_2PO_4, 50mM K_2HPO_4, 150 mM KCl, 1 mM DTT, 0.05% sodium azide and 90% H_2O, 10% D_2O at pH 7, obtained at 298 K showing backbone resonance assignments (labelled peaks). Side chain peaks of W and R residues are not labelled. Side chain amide protons of N and Q residues are indicated by solid horizontal lines.

Extent of assignments and data deposition

The ^{15}N HSQC NMR spectrum for CyaY protein is shown in Figure 1. Backbone resonances were assigned for all non-proline residues except for N-terminal tagged residues and residues N2, D3, G26, G36 and S83 (93% completed). The extent of chemical shift assignment is as follows: 94% for Hα, 97% for $^{13}C\alpha$, 93% for $^{13}C\beta$, 94% for Hβ and 93% of $^{13}C'$ resonances. Chemical shift data for the majority of aliphatic side chain groups were determined using a combination of 3D spectra. Despite overlap and ambiguities in the spectra, aromatic side chain resonances were assigned for a total of 14 aromatic residues excluding the Hζ3/Cζ3 resonances of W24 and the Hη2/Cη2 resonances of W61. Only the W88 Hδ1/Cδ1, Hε1/Nε1 and Hε3/Cε3 aromatic chemical shifts were assigned for this residue. Side chain amide resonances were assigned for all asparagine (except N2) and glutamine residues. Secondary structure prediction based on ϕ and φ angle data obtained from TALOS calculations (Cornilescu et al., 1999) using available Hα, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$ and $^{13}C'$ chemical shift data indicate secondary structure elements that are in good agreement with crystallographic data (Cho et al., 2000). Available ^{1}H, ^{13}C and ^{15}N chemical shifts for the *E. coli* CyaY protein have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5792.

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