Published in final edited form as:

Biomol NMR Assign. 2010 April ; 4(1): 61–64. doi:10.1007/s12104-010-9209-x.

NMR assignments of a stable processing intermediate of human frataxin

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Abstract

Frataxin, a nuclear encoded protein targeted to the mitochondrial matrix, has recently been implicated as an iron chaperone that delivers Fe(II) to the iron-sulfur assembly enzyme ISU. During transport across the mitochondrial membrane, the *N*-terminal mitochondrial targeting sequence of frataxin is cleaved in a two-step process to produce the “mature” protein found within the matrix; however, *N*-terminally extended forms of the protein have also been observed in vivo as a result of processing deficiencies. Structural characterization studies of the mature human frataxin ortholog suggest the protein’s *N*-terminus is predominately unfolded, in contrast to what has been observed for the yeast ortholog. Here we report the NMR assignments of a stable intermediate in the processing of human frataxin. These studies were completed to provide structural insight into editing events that lead to mature protein formation. This report also provides structural details of frataxin editing anomalies produced in vivo during altered protein processing events.

Keywords

Friedreich’s ataxia; Iron chaperone; Frataxin; Iron-sulfur cluster biosynthesis; ISU

Biological context

Frataxin, a highly conserved protein found in prokaryotes and eukaryotes, is essential for cellular iron homeostasis (Babcock et al. 1997). Protein deficiency in humans is the cause of the cardio- and neurodegenerative disorder Friedreich's ataxia (FRDA), which affects 1 in 50,000 (Delatycki et al. 2000). Although expressed ubiquitously in humans, tissues with high metabolic rates like those in cardio-muscle, nerve, kidney and liver are most affected by frataxin deficiency (Campuzano et al. 1996). At a cellular level, the effects of frataxin deficiency include an increase in mitochondrial iron levels and a decrease in activity of iron-sulfur (ISC)-containing proteins (Babcock et al. 1997; Rotig et al. 1997; Pandolfo 2002). Accumulating evidence suggests frataxin participates directly in Fe-S cluster bioassembly, most likely by serving as an iron chaperone that delivers Fe(II) to the cluster assembly enzyme ISU (Yoon and Cowan 2003; Kondapalli et al. 2008). An alternative hypothesis, with frataxin serving as a regulatory protein for cluster assembly, has also recently been reported (Adinolfi et al. 2009). The C-terminal globular domain structure is conserved between prokaryotes and eukaryotes (see review Bencze et al. 2006) suggesting a conserved function, while differences in the ortholog's N-terminal structure brings into question the conserved functional properties of the protein.

In eukaryotes, frataxin maturation occurs following a two-step proteolytic processing of the protein's N-terminus (Condo et al. 2007). Processing of human frataxin's N-terminus involves the cleavage of residues 1–41 in the initial step and residues 42–80 in the final step (Condo et al. 2007; Schmucker et al. 2008). While mature human frataxin spans residues 81–210, extended variants of the protein have been found in vivo when maturation is impaired (Schmucker et al. 2008). Examples of these extended variants include constructs that span residues 56–210 and residues 78–210. The initial structures of human frataxin were solved both by NMR and X-ray crystallography, however complications from proteolysis and degradation of the protein's N-terminus hindered the characterization beyond residues 88–210 (Musco et al. 1999; Dhe-Paganon et al. 2000; Musco et al. 2000). Recent solution structural data indicate that the N-terminus of mature human frataxin, starting at residue 61, is unfolded and highly dynamic (Prischi et al. 2009). Biochemical data however indicates the region N-terminal to residue 88 may be important in regulating the interaction between frataxin and ISU (Yoon et al. 2007). This region in the mature yeast frataxin ortholog is structured, suggesting key differences between the frataxin orthologs (He et al. 2004) (Karlberg et al. 2006). In order to provide a structural basis for understanding the significance of the human frataxin N-terminus, specifically in the N-terminal extension region found in frataxin processing variants, we characterized a stable human frataxin intermediate spanning residues 45–210 by NMR spectroscopy and obtained nearly complete resonance assignments of these residues. These assignments provide an initial structural basis for understanding processing events that lead to the formation of mature human frataxin. In addition, they provide structural details of frataxin processing intermediates formed following disruption of normal editing events.

Assignment data for the 45–210 human frataxin construct suggests a high similarity for the bulk of the C-terminal region of the protein when compared to the previously reported truncated form of human frataxin starting at residue 91 (Musco et al. 2000). Dispersion of amide resonances in the ¹⁵N HSQC spectra of the 45–210 human frataxin construct (Fig. 1) confirm the majority of residues are folded in the extended construct. Overlays between the spectrum of the construct covering residues 45–210 and the truncated construct covering residues 91–210 indicate the globular domain structure of human frataxin remains intact regardless of the presence of the extra N-terminal residues (data not shown).

In the *N*-terminal region of the protein, a subset of amide resonances in the ^{15}N HSQC for the construct covering residues 45–210 are well dispersed, suggesting possible partial fold for residues in this region beyond the globular domain (*C*-terminal region) of the protein. However, analysis of chemical shift values for these *N*-terminal residues (45–90) using chemical shift indices (CSI) protocols (Wishart et al. 1991) indicates that while a subset of residues in this region appear to be dispersed in the HSQC spectrum (residues 54, 58, 60, 62, 66, 69 and 70), there is no real pattern to suggest a fully formed secondary structural unit in this region (Fig. 2). The fact that residues 81–90 are unfolded confirm the previous result indicating this region in the mature human protein is unfolded (Prischi et al. 2009); structural difference may provide insight into the differences in stability between the human and yeast orthologs in the *N*-terminal region. Regarding normal processing events, the unfolded nature of residues 45–81 present a structural template for the protein as a substrate to the processing by the proteases.

Methods and experiments

Labeling, expression and purification of human frataxin

^{15}N -labeled and $^{13}\text{C}/^{15}\text{N}$ doubly labeled human frataxin spanning amino acids 45–210 were obtained from bacteria grown in M9 minimal medium with $^{15}\text{NH}_4\text{Cl}$ and U- $^{13}\text{C}_6$ D-glucose as the sole nitrogen and carbon sources, respectively. The construct contained an *N*-terminal poly-His tag which was included for purification purposes and not removed (Yoon et al. 2007). The constructs were expressed in the *E. coli* BL21 (DE3) strain transformed with a modified pET 28b(?) vector DNA. For protein expression, the cells were inoculated into 4 liters of M9 medium with kanamycin (50 mg/L) induced for 16 h by addition of 0.3 mM IPTG after the cultures reached an optical density between 0.4 and 0.6 at 600 nm.

Cell pellets were resuspended in buffer A (50 mM sodium phosphate, 300 mM NaCl, 10% glycerol, pH 7.0) supplemented with 1 mM TCEP, 0.1% Triton X-100, 75 ng/mL lysozyme, DNase I. Cell lysis was achieved by sonication (10 s pulse/2 min) for 30 min at 4°C. Lysate was centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was loaded onto a TALON resin preequilibrated with buffer A. Subsequently, the loaded resin was washed with 10 bed volumes of buffer A, 5 bed volumes of buffer A with 5 mM imidazole and 5 bed volumes of buffer A with 10 mM imidazole. Protein was eluted with buffer A and 200 mM imidazole, pH 7.0. Protein purity was assessed by SDS–PAGE. For NMR experiments, buffer was exchanged by dialysis against 100 mM sodium phosphate, pH 7.5. Concentration was determined by absorbance at 280 nm ($\epsilon_{280} = 33920 \text{ M}^{-1} \text{ cm}^{-1}$).

Nuclear magnetic resonance spectroscopy (NMR)

Frataxin samples used in the structure determination were prepared at 1 mM concentration in NMR buffer (100 mM sodium phosphate, pH 7.5, 90% H_2O and 10% D_2O). NMR spectra were acquired at 300 K on an Varian INOVA 600 MHz spectrometer equipped with a triple resonance $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ Varian cold probe with *z*-axis pulsed field gradients. Sequence specific backbone assignments were done using the following experiments: ^{15}N -HSQC, HNCACB, CBCA(CO)NH and HNCO (Cavanagh et al. 1996). Side chain atom assignments were made using ^1H - ^{13}C HSQC, H(CCO)NH-TOCSY, C(CO)NHTOCSY and ^{15}N -filtered TOCSY experiments. All spectra were analyzed according to established lab protocols (He et al. 2004) using the processing programs NMRPipe (Delaglio et al. 1995) and Sparky (Goddard and Kneller 2001). CSI analysis was performed using a program provided by the Sykes laboratory (Wishart et al. 1995) using backbone and C_β residues only.

Assignments and data deposition

Residue assignments for the full-length human frataxin protein are provided in the high-resolution ^{15}N -HSQC spectrum for the protein (Fig. 1). Full backbone and side chain assignments was achieved for 160 out of 167 possible residues. For further reference, the residue sequence for full-length human frataxin can be found at <http://www.uniprot.org/uniprot/Q16595>. Chemical shift assignments are deposited in the Biological Magnetic Resonance Bank (<http://www.bmrb.wisc.edu/>) under the accession code BMRB-15736.

Acknowledgments

This work was supported by the American Heart Association for K.C.K. (0610139Z) and the National Institutes of Health for J.A.C (AI072443) and for T.L.S. (DK068139).

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Fig. 1.

^{15}N -HSQC spectrum of human frataxin covering residues 45–210. Data were collected at a temperature of 300° K on a 600 MHz Varian INOVA with a cold probe. Red lines represent residues 45–88 covering the *N*-terminal region of the protein. *Dashed red lines* indicate weak peaks (amide resonance for residues 61, 82 and 88) just below the threshold level of the plot. NH_2 side chain resonances are connected by *black dashed lines*

Fig. 2.

Summary of the proposed secondary structural elements for the extended human frataxin construct (residues 45–210) from CSI analysis using NMRView. Single letter residue designations are given along with their predicted secondary structure displayed in *red* (helix), *blue* (strand) or *grey* (unfolded). Residues with a *shaded circle* represent complete atomic residue assignments