1-28-2010

NMR Assignments of a Stable Processing Intermediate of Human Frataxin

Kalyan C. Kondapalli
Wayne State University

Krisztina Z. Bencze
Wayne State University

Eric Dizin
Ohio State University

James A. Cowan
Ohio State University

Timothy L. Stemmler
Wayne State University, tstemmle@med.wayne.edu

NMR assignments of a stable processing intermediate of human frataxin

Kalyan C. Kondapalli[1], Krisztina Z. Bencze[1], Eric Dizin[2], James A. Cowan[2]

and Timothy L. Stemmler[1, §]

[1] Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI 48201, USA

[2] Evans Laboratory of Chemistry, Ohio State University, 100 West 18th Avenue, Columbus, OH, 43210, USA

[§] To whom correspondence should be addressed. E-mail: tstemmle@med.wayne.edu; Phone: 313 577 5712; Fax: 313 57 2765

Abstract

Frataxin, a nuclear encoded protein targeted to the mitochondrial matrix, has recently been implicated as an iron chaperone that delivers ferrous iron to the iron-sulfur assembly enzyme IscU. 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 in the matrix, however N-terminal extended forms of the protein have also been observed *in vivo*. The recent structural characterization studies of the human frataxin ortholog were performed on a truncated variant of the protein. Here we report the NMR spectral assignment of an extended form of the mature human frataxin ortholog as the basis for understanding the role of the N-terminal domain in protein function.

Keywords

NMR assignments, arsenic, ARS Operon, metallochaperone

Biological Context

Frataxin, a highly conserved protein found in prokaryotes and eukaryotes, is essential for cellular iron homeostasis (Babcock, 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, 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, 1996). At a cellular level, the effects of frataxin deficiency include an increase mitochondrial iron levels and a decrease in activity of iron-sulfur (ISC)-containing proteins (Babcock, 1997; Rotig, 1997; Pandolfo, 2002). Accumulating evidence suggests frataxin participates directly in Fe-S cluster bioassembly, either by serving as an iron chaperone that delivers Fe(II) to the cluster assembly enzyme IscU (Yoon, 2003; Kondapalli, 2008). An alternative hypothesis, with frataxin serving as a regulatory protein that controls assembly by interacting with partners within the pathway, has also been reported (Adinolfi, 2009). Frataxin's globular domain structure is conserved between prokaryotes and eukaryotes (see review (Bencze, 2006)) suggesting a conserved function.

In eukaryotes, frataxin maturation occurs following a two-step proteolytic processing of the protein's N-terminus (Condo, 2007). Processing of human frataxin's N-terminus involves cleaving residues 1-41 in the initial step and residues 42-80 in the final step (Condo, 2007; Schmucker, 2008). Additional extended variants of the "mature" (residues 81-210) human frataxin have also been found *in vivo* when maturation is impaired, including example constructs that span residues 56-210 and 78-210 (Schmucker, 2008). The structure of human frataxin has been 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, 1999; Dhe-Paganon, 2000; Musco, 2000). Preliminary biochemical data suggests the region N-terminal to residue 88 in human frataxin may be important in regulating the interaction between the protein and its partner IscU (Yoon, 2007). A section of the N-terminal region in mature yeast frataxin is structured, suggesting this region that is extended beyond the globular domain of the protein may play a functional role (He, 2004). In order to provide a structural basis for understanding the significance of the N-terminal region in full-length human frataxin, and the N-terminal extension section in processing variants, towards iron and protein partner binding, we characterized a stable extended full-length human frataxin construct spanning residues 45-210 by NMR spectroscopy and obtained nearly complete resonance assignments of these residues. These assignments set the basis for solving the complete solution structure of full-length human frataxin and for mapping intermolecular interactions between full-length human frataxin and binding partners.

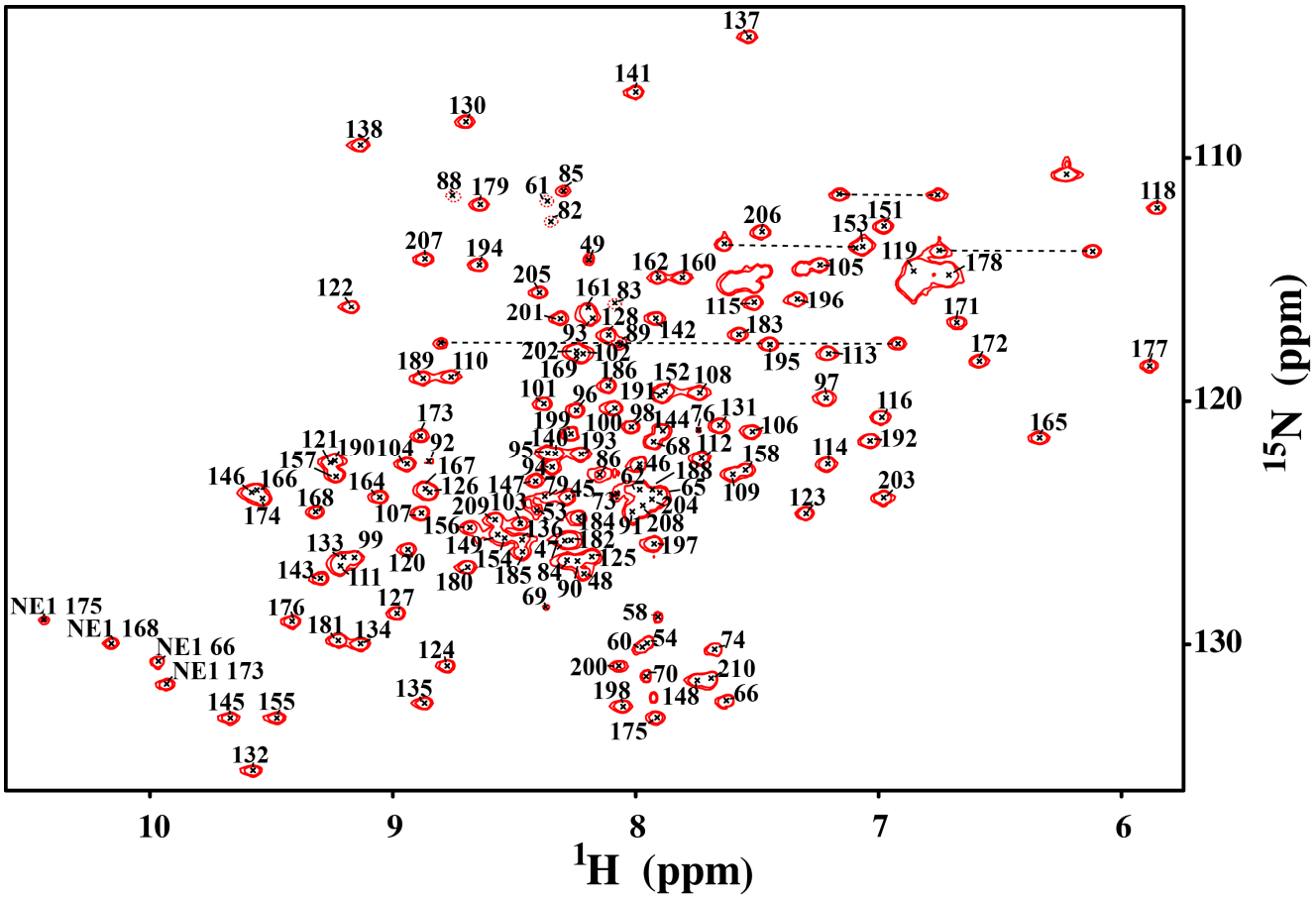

Figure 1. ^{15}N-HSQC spectrum of human frataxin (residues 45-210) in NMR buffer. Data were collected at a temperature of 300° K on a 600 MHz Varian INOVA with a cold probe. Dashed red lines indicate weak peaks (amide resonance for residues 61, 82 and 89) just below the threshold level of the plot. NH_2 side chain resonances are connected by black dashed lines.

Preliminary data suggest a high structural similarity between our 45-210 construct and the truncated forms of the proteins that have been solved previously by NMR (Musco, 2000). Dispersion of amide resonances in the ^{15}N HSQC spectra of labeled human frataxin (Figure 1) indicates the protein is well folded. Overlays of ^{15}N HSQC spectra for truncated human frataxin (residues 91-210) (Musco, 1999) and our N-terminally extended construct indicate the general structure of the previously characterized globular domain of the protein is maintained. A subset of amide resonances in the additional N-terminal region of our 45-210 construct are well dispersed, suggesting a possible partial fold for this N-terminal domain beyond the globular domain of the protein. Analysis of chemical shift values for these N-terminal residues (45-90) following chemical shift indices (CSI) protocols (Wishart, 1991) suggests that while a subset of residues in this region

(residues 54, 58, 60, 62, 66, 69 and 70) show real propensity for helix formation based on H_N, N, H_α, C_α and C_β chemical shifts, there is no real pattern to suggest a fully formed secondary structural unit in this region (data not shown). Based on CSI analysis, residues in the region between 81-90 have chemical shifts consistent with this region being unfolded.

Methods and Experiments

Labeling, Expression and Purification of Human Frataxin - ^{15}N-labeled and ^{13}C/^{15}N doubly labeled human frataxin spanning amino acids 45 to 210 were obtained from bacteria grown in M9 minimal medium with $^{15}NH_4Cl$ and U-$^{13}C_6$ 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, 2007). The constructs were expressed in *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 hours by addition of 0.3 mM IPTG after the cultures reached an optical density between 0.4 – 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 (ε_{280} = 33920 M^{-1} 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}H/^{13}C/^{15}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 1996). Side chain atom assignments were made using ^{1}H-^{13}CHSQC, H(CCO)NH-TOCSY, C(CO)NHTOCSY and ^{15}N-filtered TOCSY experiments. All spectra were analyzed according to established lab protocols (He, Alam et al. 2004)

using the processing programs NMRPipe (Delaglio, Grzesiek et al. 1995) and Sparky (Goddard and Kneller 2001).

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 (Figure 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.

Acknowledgements

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

References

Adinolfi, S., C. Iannuzzi, et al. (2009). Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. *Nat Struct Mol Biol,* **16**:390-6.

Babcock, M., D. de Silva, et al. (1997). Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science,* **276**:1709-12.

Bencze, K. Z., K. C. Kondapalli, et al. (2006). The structure and function of frataxin. *Crit Rev Biochem Mol Biol,* **41**:269-91.

Campuzano, V., L. Montermini, et al. (1996). Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science,* **271**:1423-7.

Cavanagh, J., Fairbrother,W.J., Palmer III, A.G., Skelton,N.J. (1996). Protein NMR Spectroscopy: Principles and Practice. San Diego, Academic Press.

Condo, I., N. Ventura, et al. (2007). *In vivo* maturation of human frataxin. *Hum Mol Genet,* **16**:1534-40.

Delaglio, F., S. Grzesiek, et al. (1995). NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR,* **6**:277-93.

Delatycki, M. B., R. Williamson, et al. (2000). Friedreich ataxia: an overview. *J Med Genet,* **37**:1-8.

Dhe-Paganon, S., R. Shigeta, et al. (2000). Crystal structure of human frataxin. *J Biol Chem,* **275**:30753-6.

Goddard, T. D. and D. G. Kneller (2001). SPARKY 3. University of California, San Francisco.

He, Y., S. L. Alam, et al. (2004).Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. *Biochemistry,* **43**:16254-62.

Kondapalli, K. C., N. M. Kok, et al. (2008). Drosophila frataxin: an iron chaperone during cellular Fe-S cluster bioassembly. *Biochemistry,* **47**:6917-27.

Musco, G., T. de Tommasi, et al. (1999). Assignment of the ^{1}H, ^{15}N, and ^{13}C resonances of the C-terminal domain of frataxin, the protein responsible for Friedreich ataxia. *J Biomol NMR,* **15**:87-8.

Musco, G., G. Stier, et al. (2000). Towards a Structural Understanding of Friedreich's Ataxia: The Solution Structure of Frataxin. *Structure*, **8**:695-707.

Pandolfo, M. (2002). Iron metabolism and mitochondrial abnormalities in Friedreich ataxia. *Blood Cells Mol Dis,* **29**:536-47.

Rotig, A., P. de Lonlay, et al. (1997). Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet*, **17**:215-7.

Schmucker, S., M. Argentini, et al. (2008). The *in vivo* mitochondrial two-step maturation of human frataxin. *Hum Mol Genet,* **17**:3521-31.

Wishart, D. S., B. D. Sykes, et al. (1991). Relationship between Nuclear Magnetic Resonance Chemical Shift and Protein Secondary Structure. *J. Mol. Bio.,* **222**:311-333.

Yoon, T. and J. A. Cowan (2003). Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. *J. Am. Chem. Soc.,* **125**:6078-84.

Yoon, T., E. Dizin, et al. (2007). N-terminal iron-mediated self-cleavage of human frataxin: regulation of iron binding and complex formation with target proteins. *J Biol Inorg Chem*, **12**:535-42.