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Letter to the Editor: Assignment of the ^{1}H, ^{15}N, and ^{13}C resonances of the C-terminal domain of frataxin, the protein responsible for Friedreich ataxia

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Biological context

The Friedreich ataxia is an autosomal recessive inherited neurodegenerative disorder affecting 1 in 50000 individuals. It is the most common form of hereditary ataxia, leading to severe physical disabilities connected with cardiomyopathy which often leads to premature death. This relentlessly progressive neurological disease is caused by defects in the FRDA gene, which encodes a 210 amino acid mitochondrial protein called frataxin (Campuzano et al., 1996). The structure and the function of frataxin are unknown, although a role in iron homeostasis has been proposed (Babcock et al., 1997; Radisky et al., 1999).

Sequence analysis of the protein suggested the presence of a mitochondrial targeting peptide (Gibson et al., 1997). Multiple alignment of frataxin with the related protein sequences from eukaryotes and bacteria highlighted the presence of a conserved C-terminal domain which was predicted to form a β-sheet with two flanking α-helices. The knowledge of the three-dimensional structure of frataxin should give valuable insights into the function of the protein. No sequence similarities with proteins with known structure or functions have been detected.

We have undertaken the 3D solution structure determination of frataxin, in order to increase our understanding of the molecular basis of the disease and to elucidate the structure–function relationship of frataxin. Here we report the ^{1}H, ^{15}N, ^{13}C assignments for the conserved C-terminal domain of frataxin.

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Methods and results

Isotopically ^{15}N-labeled and ^{13}C/^{15}N double labeled samples of frataxin (accession number U43752), spanning amino acids 91 to 210 (corresponding respectively to residue 11 and 130 in our numbering scheme) were obtained from bacteria grown in minimal medium with $^{15}NH_4Cl$ and ^{13}C-glucose as the sole nitrogen and carbon sources. The construct contained 10 additional residues at the N-terminus (MKHHHHHHPM) included for purification purposes and not removed. Details on the expression and purification of the protein will be published elsewhere. NMR measurements were performed on a sample of 0.5 ml of 1 mM frataxin in sodium phosphate buffer (10 mM, pH 6.8) either in 99% D_2O or in a mixed solvent 90% H_2O/10% D_2O.

^{15}N-HSQC,^{13}C-HSQC, HNHA, HNHB, HNCA, HN(CO)CA, CBCA(CO)NH, HNCACB, C(CO)NH, H(CCO)NH, HCCH-TOCSY, ^{15}N-edited NOESY and ^{13}C-edited NOESY experiments were recorded at 300 K or 295 K on either Bruker DMX500 or Varian Unity 600 or Varian Unityplus 500 NMR spectrometers equipped with triple-resonance gradient probes. The spectra were processed using NMRPipe/NMRDraw (Delaglio et al., 1995). The time domain data were zero-filled to the next power of two and multiplied by a phase shifted sine-bell. Mirror-image linear predic-

tion was used to extend the time-domain data of the constant-time dimension when applicable. The spectra were analyzed with XEASY (Bartels et al., 1995).

105 out of the 120 expected backbone resonances (excluding the His-tag) were observed in a 2D ^{1}H-^{15}N-HSQC spectrum (Figure 1). Using the commonly followed protocol, sequential through-bond connectivities for the backbone HN, CA and CB resonances were then obtained from the HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH data (Muhandiram and Kay, 1994). Entrance points for the assignments were Ala, Ser, Thr and Gly residues. Side-chain chemical shift assignments were obtained by establishing partial connectivities with HC(CO)NH-TOCSY and C(CO)NH-TOCSY spectra (Grzesiek et al., 1993). Assignments of the aliphatic ^{1}H and ^{13}C side chains were completed by analysis of the HCCH-TOCSY spectra (Kay et al., 1993). The proton assignments were further substantiated by analysing the 3D ^{15}N-NOESY-HSQC, HNHA and HNHB experiments. The assignment of the aromatic side chains was particularly difficult because of the large number (16) of aromatic residues, most of which are overlapped both in the proton and carbon domain. However, nearly complete assignment of the side chains was achieved using a combination of (Hβ)Cβ(CγCδ)Hδ (Yamazaki et al., 1993) and ^{13}C-HSQC and HCCH-TOCSY tuned for the aromatic resonances.

Extent of assignments and data deposition

The backbone HN assignments were completed for all residues. Side chain ^{1}H, ^{13}C assignments and ^{15}N assignments for Asn and Gln residues and of the three indole groups Trp75, Trp88 and Trp93 are essentially complete with the exceptions of residue 110, the guanidinium groups and the Nϵ-Hϵ of arginines. Cδ signals of His97, His103, Trp75, Trp88, Trp93, Cϵ and Cζ of Phe 40 and Phe 47 could not be identified because of severe spectral superposition. It was possibile to assign the hydroxyl protons of residues Thr22, Tyr43, Tyr63 and Thr111.

The ^{1}H, ^{13}C, and ^{15}N chemical shifts of the C-terminal domain of frataxin have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4342.

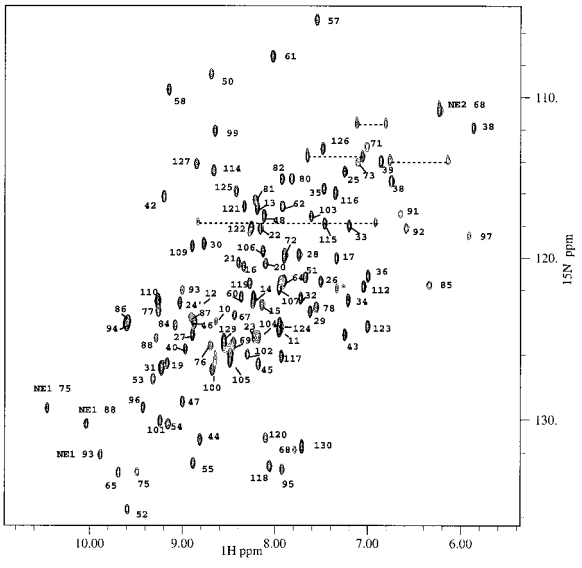

Figure 1. 2D ^{1}H,^{15}N-HSQC spectrum of 1 mM frataxin in 10 mM sodium phosphate buffer, at pH 6.8, 300 K, indicating the backbone assignments. Assignments of the backbone resonances for some residues are omitted for clarity. The NH2 side chain resonances are connected by dotted lines. The star indicates an impurity.

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