Journal of Biomolecular NMR **30:** 457–458, 2004.

Letter to the Editor: Assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the Josephin domain of human ataxin-3

Giuseppe Nicastro, Laura Masino, Thomas A. Frenkiel, Geoff Kelly, John McCormick, Rajesh P. Menon & Annalisa Pastore*
National Institute for Medical Research, The Ridgeway, London NW71AA, U.K.

Received 8 July 2004; Accepted 28 September 2004

Key words: aggregation, ataxin-3, josephin, polyglutamine disease, SCA3

Biological context

Machado-Joseph disease (MJD), also called spinocerebellar ataxia type-3 (SCA3), is an inherited progressive neurodegenerative disorder characterized by clumsiness and weakness in the limbs, spasticity, and difficulties in various muscle movements. The progressive neurological deficits result in death from debilitation within 10–30 years from onset. MJD was initially identified among families of Portuguese-Azorean descent.

We now know that MJD is caused by the expansion, within a gene located on chromosome 14, of a CAG trinucleotide repeat that codes for polyglutamine (polyQ) in the protein product, ataxin-3, a member of the family of polyglutamine disorders (Kawagushi et al., 1994).

Ataxin-3 is a ubiquitous 41 kDa protein present both in the cytoplasm and in the nucleus (Cumming and Zoghbi, 2000). The protein is widely expressed in neurons and outside the central nervous system, but the mutation ultimately leads to selective neuronal loss in restricted brain regions. The domain architecture of ataxin-3 comprises an N-terminal folded globular domain, named josephin (from Joseph-Machado syndrome) that spans residues 1–182 (Masino et al., 2003), and a more flexible C-terminal region, containing two ubiquitin-interacting motifs (UIMs) and the polyQ tract. The josephin domain seems to play an important role in determining the stability and aggregation properties of non-expanded full-length ataxin-3 (Masino et al., 2004). Structure determination of the josephin domain is therefore expected to provide a better understanding of the molecular and cellular mechanisms that underlie this triplet repeat expansion disease. As a first step towards solving the structure, we present here the virtually complete NMR chemical shift assignment of the josephin domain from ataxin-3.

*To whom correspondence should be addressed: E-mail: apastor@nimr.mrc.ac.uk

Methods and experiments

The josephin domain (fragment 1–182 of human ataxin-3) cDNA was subcloned into a modified pET plasmid vector (Stratagene) to produce a glutathione S-transferase (GST) fusion protein with a cleavage site for the recombinant Tobacco Etch Virus (rTEV) protease. The protein was over-expressed in *E. coli* strain BL21(DE3) cells at 37 °C and purified using a Glutathione Sepharose affinity matrix (Amersham Biosciences). The josephin domain was cleaved from GST using a hexahistidine-tagged rTEV protease and separated from rTEV protease using Ni-NTA agarose (QIAGEN). The final product has two non-native residues (GA) at the N-terminus of the protein sequence. Isotopically ^{15}N-labeled and $^{13}C/^{15}N$ double labelled samples were over-expressed in *E. coli* by growing it on minimal media containing 1 g l^{-1} ^{15}N-ammonium sulphate and 2 g l^{-1} ^{13}C-glucose and purified as described.

The sample purity was assessed by SDS-PAGE and mass spectrometry. Protein concentration was determined using UV absorption, with a calculated extinction coefficient at 280 nm of 24750 M^{-1} cm^{-1}. Protein purification and all NMR experiments were performed in the presence of β-mercaptoethanol to prevent cysteine oxidation. Protein samples were concentrated typically to reach concentrations of 0.4 mM and

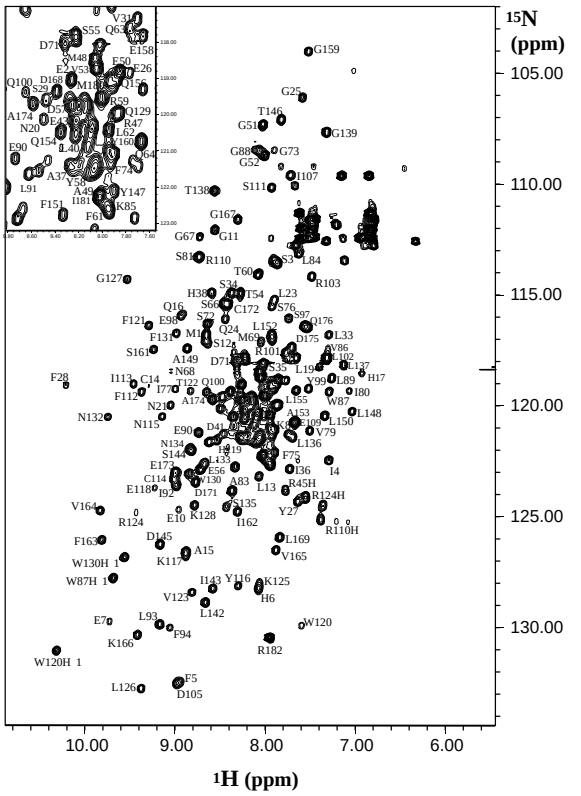

Figure 1. Assignments of some of the backbone and sidechain resonances were omitted for sake of clarity.

dialyzed. The final NMR protein samples were in 20 mM sodium phosphate buffer (pH 6.5), containing 0.05% sodium azide (w/v), 2 mM β-mercaptoethanol in 90% H_2O/10% D_2O. To circumvent the intrinsic tendency of josephin to aggregate, freshly purified samples were used for each experiment. All NMR experiments were acquired at 25 °C on Varian Inova spectrometers operating either at 14.1 T or at 18.8 T.

HNCA, HN(CO)CA, HNCO, HNCACB, CBCA(CO)NH experiments were recorded and analysed to obtain sequence specific ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}C′ backbone assignments (Muhandiram and Kay, 1994). Side chain aliphatic proton and carbon assignments were achieved by a combination of 3D ^{15}N-edited TOCSY- and NOESY-HSQC (Fesik and Zuiderweg, 1988), HNHA, HNHB, C(CO)NH, H(CCO)NH and HCCH-TOCSY (Kay et al., 1993) experiments. For the assignment of the large number (24) of aromatic side chains present in the josephin domain, (Hβ)Cβ(CγCδ)Hδ and (Hβ)Cβ (CγCδ) Hε experiments were used in combination with ^{13}C-HSQC and HCCH-TOCSY experiments tuned for the aromatic resonances.

All spectra were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analysed by the XEASY software (Bartels et al., 1995).

Extent of assignment and data deposition

The 2D ^{1}H, ^{15}N HSQC spectrum of the josephin domain of human ataxin-3 (Figure 1) exhibits a good dispersion of both the proton and nitrogen resonances that is characteristic of a globular protein. The measurement of ^{15}N spin relaxation parameters revealed that the josephin domain reorients with a correlation time of 11.75 ns at 25 °C, confirming that, immediately after purification, the protein is mainly monomeric in solution. From the above mentioned triple-resonance experiments, a virtually complete assignment of ^{1}H, ^{13}C and ^{15}N backbone resonances could be achieved, with the exception of a few C′ atoms that were not assigned either because of resonance overlap or because belonging to residues followed by prolines. All the H^{α}, H^{β}, C^{α}, and C^{β} chemical shifts could be assigned. Side chain aliphatic ^{1}H and ^{13}C resonances, $^{15}NH_2$ of asparagine and glutamine residues and the $N^{\varepsilon}H^{\varepsilon}$ and guanidinium groups of arginines could be assigned to an extent of >98%. Amongst the aromatic residues, the three tryptophan residues were completely assigned and of the 17 tyrosines and phenylalanines, 16 H^{δ}/C^{δ} resonances were unambiguously assigned. However, because of spectral overlap and ambiguities, only 13 $H^{\varepsilon}/C^{\varepsilon}$ and 2 H^{ζ}/C^{ζ} resonances were assigned.

The assigned ^{1}H, ^{13}C and ^{15}N chemical shifts of the Josephin domain of human ataxin-3 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-6241.

Acknowledgements

The authors are grateful to C. de Chiara for helpful discussion. The work was funded by the European EUROSCA grant.

References

Bartels, C. et al. (1995) *J. Biomol. NMR*, **6**, 1–10.
Cumming, C.J. and Zoghbi, H.Y. (2000) *Hum. Mol. Genet.*, **9**, 909–916.
Delaglio, F. et al. (1995) *J. Biomol. NMR*, **6**, 277–293.
Fesik, S.W. and Zuiderweg, E.R.P. (1988) *J. Magn. Reson.*, **78**, 588–593.
Masino, L. et al. (2003) *FEBS Lett.*, **549**, 21–25.
Masino, L. et al. (2004) *J. Mol. Biol.*, Doi 10.1016/j.jmb.2004.09.065.
Muhandiram, D.R. and Kay, L.E. (1994) *J. Magn. Reson.*, **B103**, 203–216.
Kawagushi, Y. et al. (1994) *Nat. Genet.*, **8**, 221–228.
Kay, L.E. et al. (1993) *J. Magn. Reson.*, **B101**, 203–216.
Zoghbi, H.Y. and Orr, H.T. (2000) *Annu. Rev. Neurosci.*, **23**, 217–247.