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ARTICLE

Backbone assignments of the 26 kDa neuron-specific ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)

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Abstract UCH-L1 is a member of the family of ubiquitin C-terminal hydrolases whose primary role is to hydrolyze small C-terminal adducts of ubiquitin to generate free ubiquitin monomers. Expression of UCH-L1 is highly specific to neurons and point mutations in this enzyme are associated with a hereditary form of Parkinson's disease. Herein, we present the NMR backbone assignments of human UCH-L1, thus enabling future solution-state NMR spectroscopic studies on the structure and function of this important protein.

Keywords De-ubiquitination · Ubiquitin C-terminal hydrolase · Knotted proteins · Parkinson's disease

Biological context

UCH-L1 is a member of the group of ubiquitin C-terminal hydrolases (UCHs), which are a small family of cysteine proteases consisting of three mammalian isoenzymes (Wilkinson et al. 1989) and close homologues in *Saccharomyces cerevisiae* and *Drosophila melanogaster* (Liu et al. 1989; Zhang et al. 1993). The expression of UCH-L1 is specifically located to neurons and it constitutes up to 1–2% of the total protein content in human brain (Wilkinson et al. 1989). As of yet, its function in vivo remains poorly understood. However, in vitro, UCH-L1 has been reported to hydrolyze C-terminal ubiquityl esters and amides (Larsen et al. 1996). Mutations in UCH-L1, such as I93 M, have been reported to be associated with Parkinson's disease (PD) (the UCH-L1 gene is also referred to as PARK5). The I93 M mutation has been associated with early onset PD (Carmine Belin et al. 2007). In addition, there is significant evidence of oxidative/carbonyl stress damage to UCH-L1 in the brains of patients with sporadic PD (Choi et al. 2004). Apart from its medical importance, UCH-L1 is also a structurally complex enzyme which has a knotted topology (Virnau et al. 2006). The polypeptide chain of UCH-L1, and its close homologue UCH-L3, is threaded five times through loops formed by other parts of the polypeptide backbone chain and adopts a so-called 5_2-knotted topology (Virnau et al. 2006). Until recently, such structures were thought to be unfeasible and hence avoided by Nature, however, an increasing number of knotted proteins are being identified (Virnau et al. 2006). UCH-L3 has recently been shown to reversibly unfold in vitro without the aid of molecular chaperones (Andersson et al. 2009).

Given the medical importance and structural interest in UCH-L1, we set out to determine the polypeptide backbone assignments which will be essential for future studies on the structure and function of this protein using NMR spectroscopy.

Methods and experiments

Sample preparation

The expression plasmid (pGEX) encoding human UCH-L1 was obtained from Dr. Chittaranja Das, Purdue University, USA. ^{1}H, ^{15}N (>95%), ^{13}C (>95%) labelled protein was expressed in the *E. coli* strain Rosetta BL21 (DE3) and purified using a glutathione-column (GE Healthcare). The GST-UCH-L1 fusion protein was then cleaved with

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Precision protease (GE Healthcare) and further purified using gel-filtration chromatography (Superdex 75; GE Healthcare) and glutathione columns. The purified proteins were then concentrated to ca. 250 μM and buffer exchanged into 50 mM Tris pH 7.6, 0.5 mM EDTA and 5 mM DTT.

NMR spectroscopy

Most NMR data were recorded on $^{13}C/^{15}N$ labelled UCH-L1 samples (250 μM protein buffered in 50 mM Tris, pH 7.6, 0.5 mM EDTA 5 mM DTT) at 25°C using either Bruker AVANCE spectrometers (500 and 700 MHz) or Varian INOVA spectrometers (600 and 800 MHz), all of which are equipped with cryogenic triple resonance probes. [^{15}N–^{1}H] HSQC, HNCA, CBCA(CO)NH, HNCACB, HNCO and HN(CA)CO spectra (Sattler et al. 1999) were recorded for backbone assignments. Additional HNCO, CBCA(CO)NH and HNCO spectra were recorded at 37°C and at 800 MHz. Furthermore, a 3D ^{15}N-edited NOESY-HSQC spectrum was recorded at 25°C and at 700 MHz to assist resonance assignments in conjunction with the crystal structure of UCH-L1 (PDB entry 2ETL). All NMR data were processed and analysed by TopSpin (Bruker BioSpin), NMRPipe (Delaglio et al. 1995) and Sparky (Goddard and Kneller) software packages. The resonance assignments have been achieved following a computer-aided procedure as described previously (Hsu and Dobson 2009).

Extent of assignment and data deposition

We have assigned 95% of the expected backbone ^{1}H–^{15}N correlations (202 out of 213; UCH-L1 contains 10 proline residues) and 92% of all ^{13}CO, $^{13}C\alpha$ and $^{13}C\beta$ (602 out of 654; Fig. 1). The eleven missing assignments of the backbone ^{1}H–^{15}N correlations include Q25, A44, Q84-S89, T205, S208 and Q209:Q25 is a loop residue; A44 is flanked by two proline residues; Q84-S89 are located in a loop close to the active site; T205, E208 and Q209 are located in the helix α7 at the C-terminus. All these missing residues are partially solvent-exposed in the crystal structure (Das et al. 2006), which may contribute to the loss of sequential correlations originating from the corresponding amide protons. UCH-L1 and UCH-L3 share a sequence identity of 55% and essentially identical folding topology (Das et al. 2006; Johnston et al. 1997). Comparing the current assignments of UCH-L1 and the previous reported assignments of UCH-L3 (Harris et al. 2007) showed that the secondary chemical shifts of Cα and CO are in general agreements as expected from the secondary structures found in the crystal structures (Das et al. 2006; Johnston et al. 1997) except for residues that are located in β2 and α6 which exhibit larger secondary chemical shifts in UCH-L1 suggesting higher structural orderness in these regions (Fig. 2). The assignments have been deposited in the BMRB under the accession number 16537.

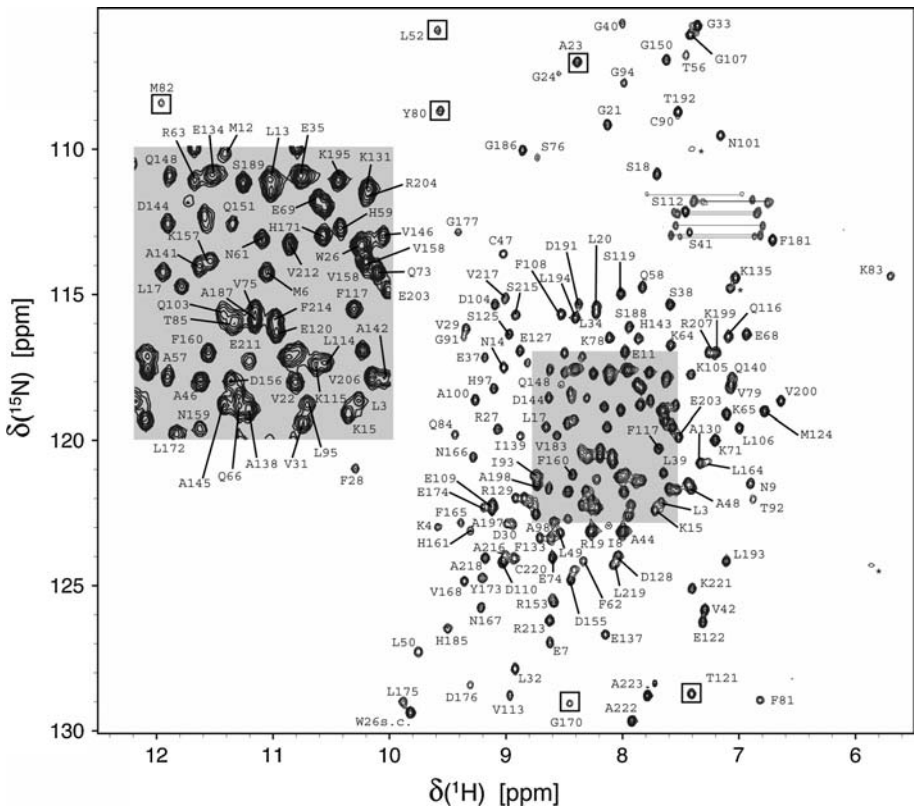

Fig. 1 Assigned 2D [^{15}N–^{1}H] HSQC spectrum of UCH-L1 recorded at 25°C and at a ^{1}H frequency of 600 MHz. Aliased crosspeaks are *boxed*, unassigned correlations are indicated by *asterisks*, and paired amino side-chain NH_2 correlations are linked by *horizontal lines*. The central area of the spectrum where crowded crosspeaks are present (shown in *grey*) is expanded and also shown as an *inset* for clarity

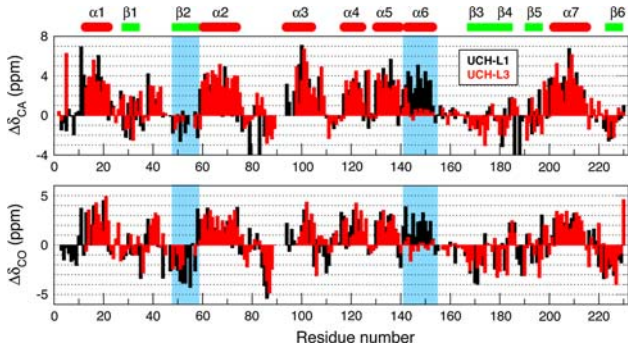

Fig. 2 Comparison of secondary chemical shifts between UCH-L1 (*black bars*) and UCH-L3 (*red bars*). The secondary chemical shifts of Cα (*upper panel*) and CO (*lower panel*) are derived by subtracting the random coil shifts from the observed ones ($\Delta\delta = \delta_{observed} - \delta_{random\ coil}$). The sequence-dependent random coil shift values are calculated using CamCoil (De Simone et al. 2009) (http://www-vendruscolo.ch.cam.ac.uk/camcoil.php). The sets of residues that form the α-helices and β-strands in the crystal structure are indicated by *red* and *green boxes*, respectively, at the top of the diagram with labels that are consistent with the nomenclature used in the crystallographic study (Das et al. 2006). Note that UCH-L3 is seven-residue longer than UCH-L1 (230 versus 223) and that the residue numbering here follows that of UCH-L3. The regions that exhibit clear deviations between UCH-L1 and UCH-L3, namely β2 and α6, are indicated by *light blue boxes*

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Conflict of interest statement The authors declare that they have no conflicts of interest.

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