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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the class II E2 conjugating enzyme, Ubc1

Nadine Merkley & Gary S. Shaw*
Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada, N6A 5C1

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Biological context

Ubiquitin-dependent proteolysis is an important cell regulatory mechanism, which is responsible for turnover of damaged or misfolded proteins in the cell. The ubiquitination pathway is a cascade of events in which the protein ubiquitin (Ub) is passed from one enzyme to another until it ultimately labels the target for degradation (Scheffner et al., 1998). A key enzyme in this pathway is the E2 or ubiquitin-conjugating enzyme.

E2 proteins are structurally conserved through many species including yeast, *S. cerevisiae* (Scheffner et al., 1998). All E2 proteins contain a 150-residue catalytic domain, and there is > 25% sequence identity between the 13 yeast E2 proteins identified. Several E2 proteins have a C-terminal extension or tail and are classified as Class II E2 proteins (Scheffner et al., 1998). The C-terminal tails of these Class II E2 proteins range from 45 - 100 residues and have very little sequence similarity. A variety of distinct functions have been identified for these C-terminal extensions including, substrate specificity (Haldeman et al., 1997), dimerization (Leggett and Candido, 1997) and a change in the pattern of ubiquitination (Hodgins et al., 1996). To date the three-dimensional structure of a Class II E2 protein has not been reported. Here we report the NMR assignment of *S. cerevisiae* Ubc1, a 215 residue E2 protein with a 65 residue C-terminal tail as a first step towards its three-dimensional structure.

*To whom correspondence should be addressed. E-mail: gshaw1@uwo.ca

Methods and experiments

Ubc1(R48K) was over expressed in *Escherichia coli* BL21(DE3)pLysS strain and purified as previously described (Merkley and Shaw, 2003; Hodgins et al., 1996). Isotopic labelling was achieved using M9 minimal media containing 1.0 g/L 99% $^{15}NH_4Cl$ for uniformly ^{15}N-labelled Ubc1 or both 2.0 g/L 99% [$^{13}C_6$]-glucose and 1.0 g/L 99% $^{15}NH_4Cl$ for uniformly ^{15}N,^{13}C-labelled Ubc1. Uniformly ^{2}H, ^{15}N and ^{13}C labelled protein was produced by growing cells M9 media in 100% D_2O supplemented with 2.0 g/L 99% [$^{13}C_6$; 1,2,3,4,5,6,6-$^{2}H_7$]-glucose and 1.0 g/L 99% $^{15}NH_4Cl$ (Gardner and Kay, 1998). Fractionally ^{13}C-labelled Ubc1 was prepared by growing the cells in minimal M9 media with a 1:10 mixture of 99% [$^{13}C_6$]-glucose and unlabelled glucose (Neri et al., 1989). Cell cultures were grown at 37 °C and induced with 0.4 mM IPTG. Proteins were purified by anion exchange chromatography followed by gel filtration chromatography. Fractions containing pure Ubc1 were pooled and concentrated. NMR samples were prepared at pH 7.5 in 90% H_2O/10% D_2O (v/v) to a final concentration of 0.48 mM. NMR experiments were acquired at 35 °C on Varian INOVA 600 and 800 MHz spectrometers using pulse field gradient triple resonance probes. Sequential assignments for the backbone residues were made from HNCA, HNCACB, CBCA(CO)NH, and ^{1}H-^{15}N HSQC experiments (Bax and Grzesiek, 1993). Sidechain assignments were made from ^{15}N-edited TOCSY, C(CO)NH, HC(CO)NH, HCCH-TOCSY and ^{1}H-^{13}C HSQC experiments. Stereospecific assignment of the prochiral methyl groups from valine and leucine were achieved from analysis of a CT-^{1}H-^{13}C HSQC experiment using a fractionally ^{13}C-labelled Ubc1 sample. NMR spectra were processed using

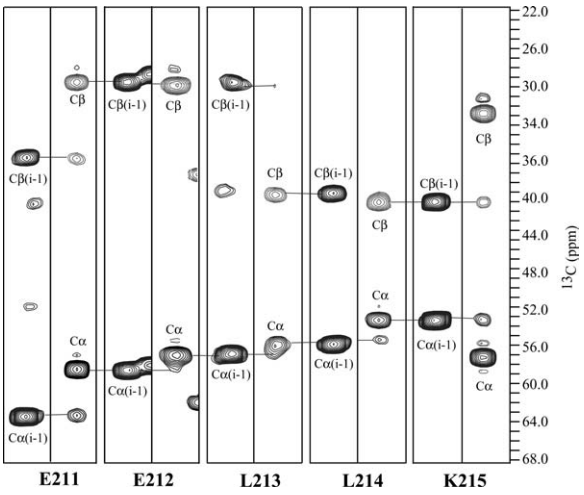

Figure 1. Selected regions from the 600 MHz NMR Spectra used for the backbone assignment of yeast Ubc1. Regions from the F3-F1 planes from HNCACB and CBCA(CO)NH were extracted for residues E211-K215. For each pair of planes, the CBCA(CO)NH is shown on the left and the HNCACB is on the right. Cα and Cβ are labeled on the HNCACB spectra and the corresponding Cα and Cβ from the $(i-1)$ residue are indicated on the CBCA(CO)NH spectra.

NMRPipe and NMRDraw (Delaglio et al., 1995) and analyzed using Pipp and Stapp (Garrett et al., 1991) software using a Sun Ultra 10 workstation.

Extent of assignments and data deposition

Figure 1 depicts the regions of the HNCACB and CBCA(CO)NH spectra used for sequential backbone assignment of Ubc1 collected on a Varian INOVA 600 MHz spectrometer. Excluding proline residues, 195 out of 201 expected backbone amide nitrogen and proton resonances have been assigned. Many of the peaks are well resolved except residues R93 and R192, R132 and Q170, A20 and H34, I11 and I186, V53 and E182, I7 and Q156, which had degenerate amide proton and nitrogen chemical shifts. Extent of assignments are 97% Cα, 94% Hα, 83% C′, 78% sidechain ^{13}C, and 81% sidechain ^{1}H resonances. The ^{1}H, ^{13}C, and ^{15}N resonance assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-6202.

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