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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the catalytic domain of the yeast E2, UBC1

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Biological context

The covalent linkage of a multi-ubiquitin chain to a protein plays a well-established role in targeting proteins for degradation via the proteasome. Over the years, a great deal of research has focused on the conjugating or E2 enzymes of the ubiquitin system. This family of proteins is an essential component that accepts ubiquitin from the E1 activating enzyme, and delivers ubiquitin, most likely in association with an E3 ligase, to the target protein. Not only are the E2 enzymes central to the building of a multi-ubiquitin chain, they can also regulate the linkage configuration between ubiquitin molecules. Added to this, recent findings indicate that certain E2 enzymes are utilised by ubiquitin-like proteins, while others only by ubiquitin (Hochstrasser, 1996).

Thus, an essential component to deciphering the mechanism of regulation of the ubiquitin system is a detailed analysis, at the molecular level, of the E2 and ubiquitin protein–protein interactions. In order to do this we have utilised an in vitro conjugation system composed of ATP, Mg^{2+} and purified yeast proteins: ubiquitin (K48R), E1, and the catalytic domain of UBC1 (UBC1Δ450(K93R)). Under in vitro conditions, UBC1 will auto-ubiquitinate itself at residue K93 with a multi-ubiquitin chain which has a linkage configuration that is dependent upon the presence or absence of the C-terminal tail (Hodgins et al., 1996). Thus, the C-terminal deleted form used for these studies, UBC1Δ450(K93R), and a ubiquitin mutant which cannot form linkages via Lys-48 (Ub(K48R)) permits the sole production of an E2-Ub thiolester in greater than 90% yield. For our analysis, we have chosen to use NMR spectroscopy to provide detailed analysis of protein–protein interactions in the E2-Ub thiolester complex. Essential for this analysis has been the NMR assignment of the 151-residue UBC1 conjugating protein, since this is the first report of NMR studies of this yeast E2 enzyme.

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Methods and results

All UBC1Δ450(K93R) proteins were over-expressed using a pET 3a-based construct that has been previously reported (Hodgins et al., 1996). For the preparation of uniformly ^{15}N-labeled and ^{15}N,^{13}C-labeled UBC1 derivatives, the *E. coli* strain BL21DE3pLysS (Novagen) was used with a supplemented minimal medium containing 1 g/l 99% $^{15}NH_4Cl$ or 1 g/l 99% $^{15}NH_4Cl$ and 2 g/l ^{13}C-glucose, respectively (Muchmore et al., 1989). Cultures were grown at 37 °C and induced with 0.4 mM IPTG. Proteins were purified by anion exchange chromatography, ammonium sulphate precipitation of appropriate fractions, followed by gel filtration chromatography. Fractions containing UBC1 were pooled and concentrated to 0.7–0.9 mM. Samples for NMR were made to 10% (v/v) D_2O in 40 mM HEPES, 1 mM EDTA, 400 mM NaCl, 1 mM DTT.

NMR spectroscopy experiments were performed on Varian Unity 500, Inova 500 and Inova 800 MHz spectrometers equipped with pulse field gradient triple resonance probes. Sensitivity-enhanced ^{1}H-^{15}N HSQC spectra were acquired at 25 °C and 30 °C (Kay et al., 1992). For the resonance assignments of UBC1, several 3D experiments were acquired at

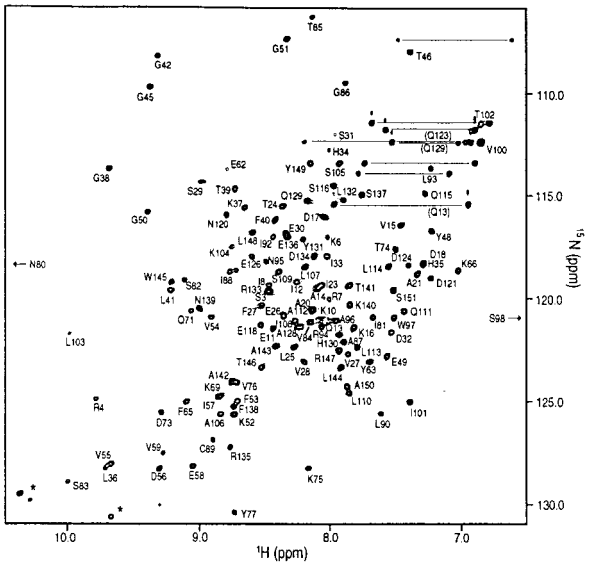

Figure 1. ^{1}H-^{15}N HSQC spectrum of 0.7 mM uniformly ^{13}C,^{15}N-labeled yeast UBC1Δ450(K93R) in 40 mM HEPES, 1 mM EDTA, 400 mM NaCl, pH 7.5 acquired at 25 °C on an Inova 800 MHz spectrometer. Backbone amide cross peaks are labelled accordingly. Asterisks denote cross peaks from side-chain resonances, other than Gln and Asn residues, not yet identified. Arrows indicate chemical shifts of N80 and S98 which are further downfield (11.49, 117.72 ppm) and upfield (5.62, 121.01) in F2, respectively, than the range plotted.

25 °C. Sensitivity-enhanced HNCACB and CBCA-CONH experiments (Muhandiram and Kay, 1994) provided the basis for the majority of the sequential assignment and ^{1}H, ^{15}N, ^{13}Cα, and ^{13}Cβ resonance assignments. The ^{15}N-edited NOESY (150 ms mixing time), ^{15}N-edited TOCSY (50.9 ms mixing time), HNCO and H(CA)CO(CA)NH spectra were also acquired for ^{1}Hα, ^{1}Hβ, and CO assignments, respectively, and to complete the sequential assignment (Muhandiram and Kay, 1994; Zhang et al., 1994; Löhr and Rüterjans, 1995). Further ^{1}H and ^{13}C side-chain assignments were obtained from the HCCH-TOCSY experiment with a 7.5 ms mixing time (Kay et al., 1993). Analysis of the ^{1}H-^{15}N HSQC spectra of the specifically labelled ^{15}N-leucine and ^{15}N-isoleucine proteins confirmed the assignments of these residues. Data was processed and analysed using the NMR-Draw, NMRPipe, Pipp and Stapp programs on an Indigo2 Silicon Graphics workstation (Garrett et al., 1991; Delaglio et al., 1995).

Extent of assignments and data deposition

Figure 1 depicts the ^{1}H-^{15}N HSQC spectrum, acquired at 25 °C on an Inova 800 MHz spectrometer, indicating assignment of backbone $^{1}H^{N}$ and ^{15}N resonances of the catalytic domain of UBC1 (UBC1Δ450(K93R)). Excluding the 12 proline residues, the $^{1}H^{N}$ and ^{15}N resonance assignments of the backbone amides for 128 out of 139 residues have been assigned. All backbone amide cross peaks that appear in the ^{1}H-^{15}N HSQC spectrum have been assigned, with 11 unobserved residues most likely absent due to rapid exchange with the solvent. Extents of assignments are: 94% of ^{13}Cα, 84% of ^{1}Hα, 92% of ^{13}Cβ, 70% of ^{1}Hβ, and 86% of CO resonances. The ^{1}H, ^{15}N and ^{13}C resonance assignments have been deposited in the BioMagResBank databank under accession number BMRB-4567.

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