Journal of Biomolecular NMR, **13:** 89–90, 1999.
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Letter to the Editor: Backbone resonance assignments of human UBC9

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Received 26 June 1998; Accepted 8 July 1998

Key words: UBC9, heteronuclear NMR, resonance assignments, ubiquitination

Biological context

Human UBC9, which contains 158 amino acid residues, is a member of the family of ubiquitin-conjugating enzymes (UBC or E2) (For a review on ubiquitination, see Jentsch, 1992). In the ubiquitination pathway, E1 activates ubiquitin by hydrolysing ATP to form a high energy bond with ubiquitin. Ubiquitin is then transferred to a UBC (E2). Interactions between E2 and substrate proteins resulted in transferring ubiquitin to the substrate proteins. This process requires the participation of ubiquitin-protein ligase (E3), in some cases. Many crucial proteins are degraded by the ubiquitination pathway. These include some cyclins, cyclin-dependent kinase inhibitors and histones (Seufert et al., 1995; Barinaga, 1995; Pagano et al., 1995). Therefore, ubiquitination plays important roles in a variety of biological processes. UBC9 has been shown to be involved in DNA-repair, cell-cycle regulation, and p53 dependent processes (Seufert et al., 1995).

Despite the intensive studies in ubiquitination, the mechanism of protein-protein recognition in ubiquitination is still not well understood. Although each UBC protein has specificity for substrate proteins, the proteins recognized by each UBC are highly diverse. The substrate proteins of UBC9 include several important proteins, such as RAD51, RAD52, and P53 (Shen et al., 1996). These interactions are specific for UBC9, since the homologous UBC2 does not interact with these proteins. The structures of several UBC proteins including UBC9 have been determined by X-ray crystallography (Tong et al., 1997; Cook et al., 1993, 1997). Comparison of these structures reveals that these UBC proteins have highly conserved tertiary structures with RMSD of C^{α} atoms less than 2 Å, excluding a couple of surface loops. Despite intensive structural and biochemical studies, the binding epitopes of E1, ubiquitin, and substrate proteins on E2 are still controversal. In addition, it is not clear how E2 proteins confer both substrate specificity and diversity. The substrate diversity is likely to be correlated to conformational flexibility of the UBC molecules.

NMR methods have played important roles in characterizing the internal mobilities of proteins. In addition, NMR methods can be used to map binding epitopes of protein-protein complexes. As a step towards understanding mechanisms of protein-protein recognition in ubiquitination at the molecular details using NMR methods, we report the backbone and some sidechain ^{1}H, ^{13}C, and ^{15}N NMR assignments of human UBC9.

Methods and results

Uniformly labeled hUBC9 with 6xHis tag at the N-terminus was expressed to a high level (30 mg/L) in *E. coli*. Heteronuclear enriched samples were obtained by growing the cells in minimal medium supplimented with ^{13}C-glucose and $(^{15}NH_4)_2SO_4$. The protein was purified on a Ni-NTA column. ^{15}N-, $^{13}C/^{15}N$-, and $^{2}H/^{13}C/^{15}N$-labeled samples were prepared. The samples for NMR experiments contained about 0.7 mM of hUBC9 in the buffer (100 mM sodium phosphate buffer, pH 6.0) containing 90% H_2O/10% D_2O. Higher concentration of protein resulted in aggregation as evident by much broader resonance lines.

All NMR experiments were acquired at 30° on a Varian Unity-plus 500 NMR spectrometer.

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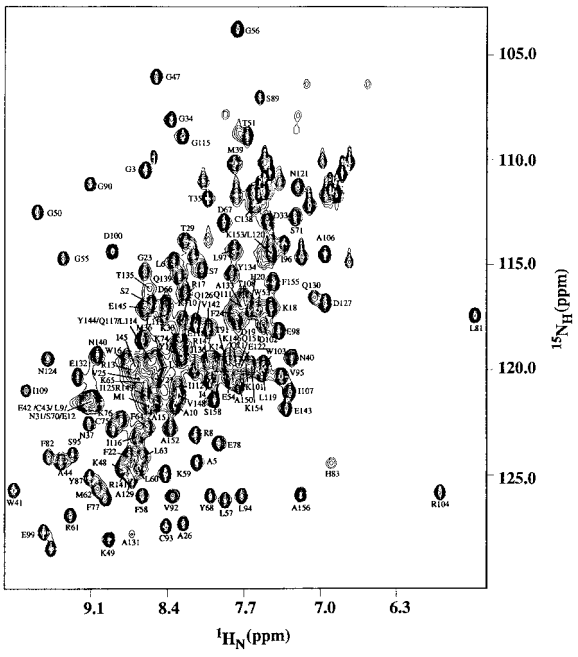

Figure 1. ^{1}H-^{15}N HSQC spectrum of hUBC9 at 30 °C showing the sequence-specific assignments. The samples contains 0.7 mM protein and 100 mM sodium phosphate buffer, pH 6.0, in 90% H_2O/110% D_2O.

The following experiments were recorded: 2D ^{1}H-^{15}N HSQC, 3D ^{15}N-resolved-NOESY-HSQC, ^{15}N-resolved-TOCSY-HSQC, HBHA(CBCACO)NH, HNCA, HNCB, CBCA(CO)NH, HNCO, HCCH-TOCSY (for reviews, see Bax et al., 1994; Clore and Gronenborn, 1994). Fourier transformation was accomplished using the FELIX software. Data from ^{15}N-TOCSY-HSQC, HBHA(CACBCO)NH, HNCA, HNCB, CBCA(CO)NH were used to establish the chemical shift correlation of $^1H^N$(i), ^{15}N(i), $^1H^\alpha$(i), $^{13}C^\alpha$(i), $^{13}C^\beta$(i), $^1H^\alpha$(i−1), $^{13}C^\alpha$(i−1), $^{13}C^\beta$(i−1) for each ^{15}N(i)/^{1}H(i) chemical shift pair. The connectivity was further confirmed by d_{NN} and $d_{\alpha N}$ NOEs in ^{15}N-edited NOESY. One large peak in ^{1}H-^{15}N HSQC clearly contains 6 backbone amide ^{1}H-^{15}N HSQC peaks. The assignments are indicated in the HSQC spectrum in Figure 1. The HNCO experiment had the best resolution for this region. Based on the $^{13}C^\alpha$ and $^{13}C^\beta$ chemical shifts and characteristic NOEs, hUBC9 in solution has similar secondary structure as that determined by X-ray crystallography (Tong et al., 1997).

Extent of assignments and data deposition

Sequence-specific backbone assignments of $^1H^N$, ^{15}N and $^{13}C^\alpha$ resonances have been obtained for all residues. $^1H^\alpha$ resonances have also been assigned for all residues except for Pro^{79} which is followed by Pro^{80}. In addition, approximately 92% $^{13}C^\beta$ and many side chain protons and ^{13}C resonances have been assigned. The assignments have been deposited in the BioMagResBank database (accession code 4132).

Acknowledgements

We thank Dr Benoit Boulat for computer programs for assignments. This work has been supported in part by NIH GM54190. Y.C. is a recipient of the American Cancer Society Junior Faculty Research Award (JFRA-646).

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