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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the catalytic domain of E6-associated protein (E6AP)

Yu-Sung Wu & Peter J. Domaille*
DuPont Pharmaceuticals Company, Research and Development, Wilmington, DE 19880-0353, U.S.A.

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Biological context

Ubiquitin (ub)-mediated degradation is a major regulatory mechanism for selective protein turnover in eukaryotic cells. Various cellular processes, including cell cycle progression, signal transduction, antigen presentation, and protein homeostasis are all tightly regulated and temporally controlled by ubiquitin dependent degradation. In this proteolytic pathway, ubiquitin successively forms a transient thioester linkage with the ubiquitin-activating enzyme (E1) and then a ubiquitin-conjugating enzyme (E2). Ub-E2 alone, or in conjunction with an ubiquitin-protein ligase (E3), then binds to a substrate protein and catalyzes the formation of a polyubiquitin chain that targets the substrate for destruction by the 26S proteasome (Ciechanover, 1998). During this cascade of enzymatic reactions, E3s play pivotal roles to provide key determinants for substrate recognition and specificity. All ubiquitin ligases identified thus far are either large proteins or protein complexes.

The ubiquitin protein ligase E6AP is involved in the human papillomavirus (HPV) E6-dependent p53 degradation pathway (Scheffner, 1998). Infection by high risk type HPV correlates closely with the development of cervical carcinoma. E6AP is a ∼100 kDa cellular protein with a conserved HECT (Homologous to E6AP C Terminus) domain that forms a transient thioester linkage with ubiquitin and subsequently transfers multiple ubiquitin molecules to the substrate. In HPV infected cell lines E6AP, in combination with the E6 oncoprotein from HPV, catalyzes the polyubiquitination of p53 and commits it to resultant degradation by the proteasome. Although the structure of the HECT domain of E6AP was characterized recently by crystallography (Huang et al., 1999), it remains unclear how ubiquitin is transferred from an E2 enzyme to the catalytic cysteine of E6AP. We report here the ^{1}H, ^{15}N and ^{13}C sequence-specific resonance assignments for a 13.8 kDa domain of E6AP (cat-E6AP$_{733-852}$). The chemical shift assignments constitute the basis for characterization the solution structure of this essential domain and the information obtained from the structural study will provide a basis to develop potential therapeutic agents for cervical cancer and related diseases.

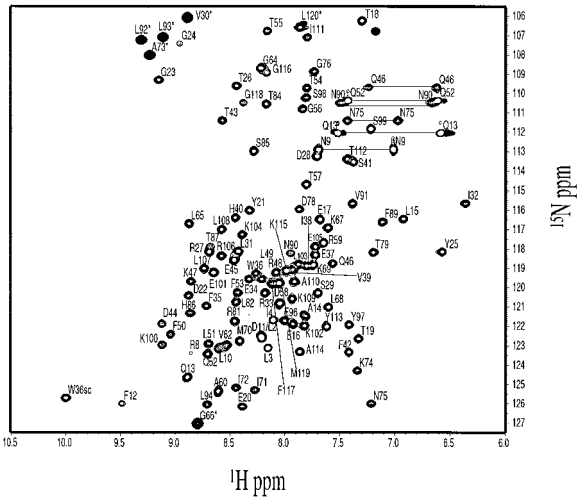

Figure 1. 600 MHz ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled cat-E6AP$_{733-852}$ recorded at pH 7.5 and 298 K. The ^{15}N spectral width was set to 22.16ppm, with aliasing optimized to reduce the number of time increments necessary to achieve the desired digital resolution. Side chains of NH_2 resonances of asparagine and glutamine are connected with a line. The side chain of Trp36 is denoted by sc. Aliased resonances are marked with an asterisk.

*To whom correspondence should be addressed. E-mail: Peter.J.Domaille@dupontpharma.com

Methods and experiments

Cat-E6AP$_{733-852}$ was expressed in *E. coli* strain BL21 (DE3) pLysS cells transformed with the plasmid pGEX-4T. The coding sequence of the cloned gene was verified by DNA sequencing. The molecular weight of ^{15}N-labeled cat-E6AP$_{733-852}$ was confirmed by electrospray mass spectrometry. Note that our amino acid numbering starts with Glu1 which corresponds Glu733 in the full length E6AP. For the preparation of uniformly ^{15}N-labeled and ^{15}N, ^{13}C-labeled cat-E6AP$_{733-852}$, cells were grown at room temperature in a minimum medium supplemented with 1 g/l $(^{15}NH_4)_2SO_4$ or 1 g/l $(^{15}NH_4)_2SO_4$ and 2g/l ^{13}C-glucose, respectively. Protein expression was induced by 0.2 mM IPTG when the optical density at 596 nm reached a value of 0.6–0.7. Cat-E6AP$_{733-852}$ was purified by glutathione affinity chromatography, thrombin digestion to remove the fusion protein, followed by gel filtration chromatography. The purified fractions containing cat-E6AP$_{733-852}$ were pooled and concentrated to 0.8–1.2 mM and the NMR samples were in a buffer solution of 20 mM sodium phosphate, 150 mM NaCl, 1 mM ^{12}D-EDTA, 0.05% NaN_3, 10 mM deuterated DTT in either 93% H_2O/7% D_2O or 100% D_2O at pH 7.5.

All NMR spectra were recorded at 298 K on a Bruker Avance 600 spectrometer equipped with a triple-resonance, 3-axis gradient probe. Spectra were processed using AZARA and analyzed with ANSIG (Kraulis et al., 1994). ^{1}H-^{15}N HSQC, CBCACONH and HNCACB spectra provided the basis for the majority of the sequential assignments for ^{1}H, ^{15}N, $^{13}C_\alpha$, and $^{13}C_\beta$ resonances. 4D HBCB/HACACONH, and 3D HNCO spectra were also acquired for $^1H_\alpha$, $^1H_\beta$, and ^{13}CO assignments respectively to complete the backbone sequential assignment. Further ^{1}H and ^{13}C side chain assignments were completed from the HCCH-TOCSY experiment with 21 ms mixing time and ^{1}H-^{13}C HSQC, CBHD and CBHE experiments (for a review see Sattler et al., 1999).

Extent of assignments and data deposition

The backbone resonances assignments were essentially complete for most residues observed in the ^{1}H-^{15}N HSQC spectrum (Figure 1). Excluding the four proline residues, five amide resonances (Glu1, Gly6, Ser7, Arg8 and Cys88) were also unassigned in the ^{1}H-^{15}N HSQC spectrum. In total, 97% of $^{13}C_\alpha$, $^1H_\alpha$ and $^{13}C_\beta$, 90% of ^{13}CO and 93% of $^1H_\beta$ resonances were determined from 3D and 4D triple resonance experiments. A table of chemical shifts has been deposited in the BioMagResBank Database (accession code 5013).

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