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Letter to the Editor: Chemical shift assignments of the (poly)ubiquitin-binding region of the proteasome subunit S5a

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Biological context

Ubiquitin recognition is essential for numerous cellular processes including protein degradation, endocytic sorting, transcriptional control, intranuclear localization, and DNA repair. Indeed the biological impact of ubiquitylation has recently been realized to be comparable to phosphorylation. Ubiquitylation as a signal for targeted protein degradation by the proteasome has been well studied due to its biological prominence. Among the duties of the ubiquitin-proteasome pathway is controlling the lifespan of regulatory proteins, removing improperly folded proteins that would otherwise aggregate, and producing immunocompetent peptides. Indeed failure of proteasome-mediated protein degradation is associated with numerous neurodegenerative diseases, including Parkinson's disease, Huntington's disease, Alzheimer's disease, and polyglutamine disease. To date two proteasome components have been reported to bind polyubiquitylated substrates: the ATPase S6′ (Lam et al., 2002) and S5a (Deveraux et al., 1994). S5a contains two ubiquitin recognition segments, the second of which also recognizes type-2 ubiquitin family members (Hiyama et al., 1999). The structure of the type-2 ubiquitin-like domain of the DNA repair protein hHR23a was recently solved in complex with a 45-residue S5a peptide (Mueller and Feigon, 2003). In addition S5a and hHR23a can form a ternary complex with ubiquitin and hHR23a undergoes structural changes to bind either S5a (Walters et al., 2003) or ubiquitin (Wang et al., 2003). Interestingly a recent study in yeast indicates that the homologues of these two proteins (Rad23 and Rpn10) serve as alternative ubiquitin receptors for the proteasome (Elsasser et al., 2004). We have applied multi-dimensional NMR techniques to study the ubiquitin binding mechanisms of S5a (196-307). Here we report the chemical shift assignments of S5a (196-307).

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Methods and experiments

For ease of purification, S5a (196-307) was produced as a recombinant protein from a pET vector in frame with an N-terminal histidine tag that comprises 21 amino acid residues with the sequence of MGHHHHHHHHHHSSGHIEGRH (Young et al., 1998). ^{13}C- and ^{15}N-labeled protein was produced from BL21 (DE3) cells grown in M9 minimal media supplemented with ^{13}C-labeled glucose and ^{15}N-labeled NH_4Cl, respectively. Purification was achieved by using affinity chromatography followed by FPLC. The protein sample used for the triple resonance experiments contained approximately 0.35 mM uniformly ^{13}C and ^{15}N labeled protein in 20 mM sodium phosphate buffer at pH 6.5, 100 mM NaCl, and 10% D_2O (v/v). All NMR measurements were performed at 25 °C on Varian INOVA 600 and 800 spectrometers.

Backbone assignments were obtained by using two pairs of triple resonance experiments: 3D HNCO, 3D HN(CA)CO, 3D HNCA, and 3D HN(CO)CA. Residual ambiguities were resolved by using sequential NOEs measured in 3D heteronuclear-resolved [^{1}H, ^{1}H]- NOESY spectra. Side-chain ^{1}H and ^{13}C assignments of the non-aromatic side-chain CH_n moieties, including all prolines, were obtained by using 2D [^{1}H, ^{13}C]-HSQC, 3D ^{13}C-dispersed NOESY, 3D HNHB, and 3D HN-TOCSY experiments. The NMR spectra

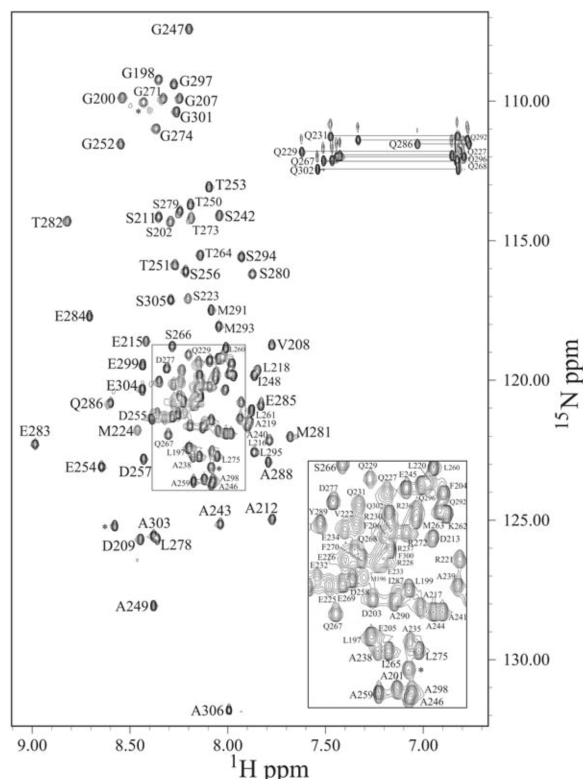

Figure 1. [^{1}H, ^{15}N]-HSQC spectrum of S5a (196-307) recorded at 800 MHz ^{1}H resonance frequency and 25 °C on 0.7 mM uniformly ^{15}N labeled protein in 20 mM sodium phosphate buffer, pH 6.5, 100 mM NaCl, 0.1% NaN_3 and 10% D_2O (v/v). Resonances are labeled according to their assignments and Gln side-chain NH_2 groups are included. Those resonances that originate from non-native residues are indicated with an asterisk. The boxed region of the spectrum is expanded and displayed in the right corner.

were processed by using NMRPipe (Delaglio et al., 1995) and visualized and analyzed with the software package XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

Figure 1 contains a [^{1}H, ^{15}N] HSQC spectrum of S5a (196–307). The numbering of the full-length protein was preserved and our construct begins with M196 and ends with D307. All ^{1}H, ^{15}N and ^{13}C polypeptide backbone resonances were assigned except for D307. The side-chain ^{13}C and ^{1}H assignments are nearly complete. The labile side-chain protons of Gln were completely assigned (this protein lacks Asn residues). Among the unassigned side-chain CH_n moieties of the native protein are $C^{\gamma}H^{\gamma}$ of L197, L199, L216, L218, L220, L260, L261 and L295, C^{δ} of L261, $C^{\varepsilon}H^{\varepsilon}$ of M263, and the side-chain carbon atoms of the aromatic residues. The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the BMRB accession number 6233.

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