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Solution structure of UIM and interaction of tandem ubiquitin binding domains in STAM1 with ubiquitin

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ABSTRACT

STAM1 and Hrs are the components of ESCRT-0 complex for lysosomal degradation of membrane proteins is composed of STAM1 Hrs and has multiple ubiquitin binding domains. Here, the solution structure of STAM1 UIM, one of the ubiquitin binding motif, was determined by NMR spectroscopy. The structure of UIM adopts an α-helix with amphipathic nature. The central hydrophobic residues in UIM provides the binding surface for ubiquitin binding and are flanked with positively and negatively charged residues on both sides. The docking model of STAM1 UIM-ubiquitin complex is suggested. In NMR and ITC experiments with the specifically designed mutant proteins, we investigated the ubiquitin interaction of tandem ubiquitin binding domains from STAM1. The ubiquitin binding affinity of the VHS domain and UIM in STAM1 was 52.4 and 94.9 μM, and 1.5 and 2.2 fold increased, respectively, than the value obtained from the isolated domain or peptide. The binding affinities here would be more physiologically relevant and provide more precise understanding in ESCRT pathway of lysosomal degradation.

1. Introduction

The ubiquitination of proteins is one of the widespread post-translational modifications and regulates a variety of cellular processes [1]. Protein modification by ubiquitin can be achieved through the formation of an isopeptide bond between the carboxyl group of the C-terminal glycine of ubiquitin and an ε–amino group of a lysine residue from a target protein [2]. The lysine residues on ubiquitin also can be modified by other ubiquitin, resulting in the formation of the polyubiquitin chains. Thus, the ubiquitinated target proteins are found in several ways, such as monoubiquitinated, multiple monoubiquitinated, and polyubiquitinated forms. The type of ubiquitination of target protein is related to the fate of the protein in various cellular processes. For example, it was reported that monoubiquitination is involved in endocytosis [3–5]. K48-linked polyubiquitination is very well characterized and the signal for the proteasomal degradation pathway [reviewed in Ref. 6], whereas recent evidences revealed that multiple monoubiquitinantion or K63-linked polyubiquitination is the sorting signal in the lysosomal degradation of membrane proteins [7,8].

Ubiquitin-mediated selective trafficking of membrane proteins for lysosomal degradation is crucial for quality control in the cell and proper cell signaling. Ubiquitinated membrane proteins should be delivered inside of the cell via multivesicular bodies (MVBs) by endocytosis, and finally to lysosomes. The endosomal sorting complex required for transport (ESCRT) complexes sort the ubiquitinated membrane proteins for lysosomal degradation [reviewed in Ref. 9]. The signal transducing adaptor molecule (STAM) and hepatocyte growth factor-regulated substrate (Hrs) constitute the ESCRT-0 complex to sort the ubiquitinated cargo proteins from the early endosomes to the ESCRT-1 complex [10,11]. Interestingly, the ESCRT-0 complex contains multiple ubiquitin binding domains, a Vps27/ Hrs/Stam (VHS) domain and a ubiquitin binding motif (UIM) for STAM, and a VHS domain and a double-sided ubiquitin interacting motif (DUIM) for Hrs, respectively. Via those multiple ubiquitin binding domains, ESCRT-0 recognizes ubiquitinated cargo proteins, in which the VHS domain of STAM has higher binding affinity to K63-linked diubiquitin than to K48-linked diubuquitin

Abbreviations: MVB, multivesicular body; ESCRT, endosomal sorting complex required for transport; STAM, signal transducing adaptor molecule; Hrs, hepatocyte growth factor-regulated substrate; VHS, Vps7/Hrs/Stam; UIM, ubiquitin interacting motif; DUIM, double-sided ubiquitin interacting motif; $STAM1^{N191}$, N-terminal 191 amino acids of STAM1; NOESY, nuclear Overhause effect spectroscopy; TOCSY, total correlation spectroscopy; DQF-COSY, double quantum filtered correlation spectroscopy; HSQC, heteronuclear single quantum correlation; CSP, chemical shift perturbation; ITC, isothermal titration calorimetry.

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[12,13]. Recently, Ren and Hurley suggested that the preference of ESCRT-0 to K63-linked tetraubiquitin over monoubiquitin should be attributed to the cooperation of flexibly connected VHS and UIM motifs of ESCRT-0 [12].

To investigate the binding preference of ESCRT-0 to K63-linked di-, or poly-ubiquitin, it is pre-requisite to understand both individual VHS-ubiquitin and UIM-ubiquitin interactions. Previously, we determined a novel ubiquitin binding site on STAM1 VHS and suggested the mode of VHS-ubiquitin interaction by using NMR spectroscopy [14]. We also reported the result of backbone resonance assignments for N-terminal 191 amino acids of STAM1 ($STAM1^{N191}$) which contain both VHS and UIM [15]. In this work, we present the solution structure of STAM1 UIM and the interaction between the UIM motif and ubiquitin. By using the mutant protein of $STAM1^{N191}$, we evaluated the affinities for ubiquitin of the VHS domain and UIM individually.

2. Materials and methods

2.1. Peptide synthesis and protein preparation

The synthetic peptide, KEEEDLAKAIELSLKEQRQQS, corresponding to UIM of STAM1 (Fig. 1A), was purchased from Anygen (Gwangju, Korea). The UIM peptide was dissolved in 20 mM sodium phosphate buffer, pH 6.8, with 1 mM DTT, 0.1 M NaCl, 0.5 mM PMSF, 0.05 mM NaN_3 and 5% (v/v) D_2O for NMR experiments. The concentration of UIM peptide was 2 mM.

The STAM1 protein containing both the VHS domain and UIM was prepared as previously described [15]. The single and double mutant STAM1 proteins, $STAM1^{N191}$W26A, I179G, W26AL176A, W26AI179G, and W26AS183A were prepared by site-directed mutagenesis.

Ubiquitin for NMR and ITC experiments was expressed and purified as described previously [14].

2.2. NMR measurements

All NMR experiments were carried out at 298 K on Varian VNMRS 900 MHz spectrometer with z-axis gradient equipment. For STAM1 UIM, two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY) (mixing times of 120 and 200 ms), total correlation spectroscopy (TOCSY) (mixing times of 60 ms), and double quantum filtered correlation spectroscopy (DQF-COSY) were performed using presaturation of the H_2O resonance by continuous irradiation.

For NMR titration experiments, increasing amount of unlabeled STAM1 UIM peptides, the wild-type $STAM1^{N191}$ were added to ^{15}N-labeled ubiquitin, respectively, and series of 2D $^{1}H–^{15}N$ heteronuclear single quantum correlation (HSQC) spectra were recorded. Reciprocally, unlabeled ubiquitin were titrated into ^{15}N-labeled $STAM1^{N191}$W26A and series of 2D $^{1}H–^{15}N$ HSQC spectra were recorded at various molar ratios. From the NMR titration data, the sites of protein–protein interaction were determined based on the chemical shift perturbation (CSP) plot versus the protein sequences, where CSP was calculated by using the formula, $CSP = [(\Delta H)^2 + (\Delta N/6.51)^2]^{1/2}$.

All NMR data were processed using NMRPipe software [16] and were analyzed using NMRView software [17].

2.3. Structure calculation of STAM1 UIM

The spin systems of STAM1 UIM were identified from the TOCSY and DQF-COSY spectra, and the sequential assignments were unambiguously accomplished from the analysis of the NOESY spectra by the conventional method [18]. The NOE-based distance restraints were derived based on the peak volume by using the program ARIA 2.0 [19]. The backbone dihedral angle restraints were obtained from the analysis of DQF-COSY spectrum. The peptide structures were calculated by using the program ARIA 2.0 and CNS 1.2 [20] with 160 distance restraints and 30 dihedral angle restraints (Table 1). Twenty-six hydrogen bond restraints were incorporated into the final calculation of the peptide structures based on the secondary structure of UIM. In the final ARIA run, 100 structures were calculated and the 20 lowest energy structures were selected for statistical analysis by using PROCHECK-NMR 3.4 [21].

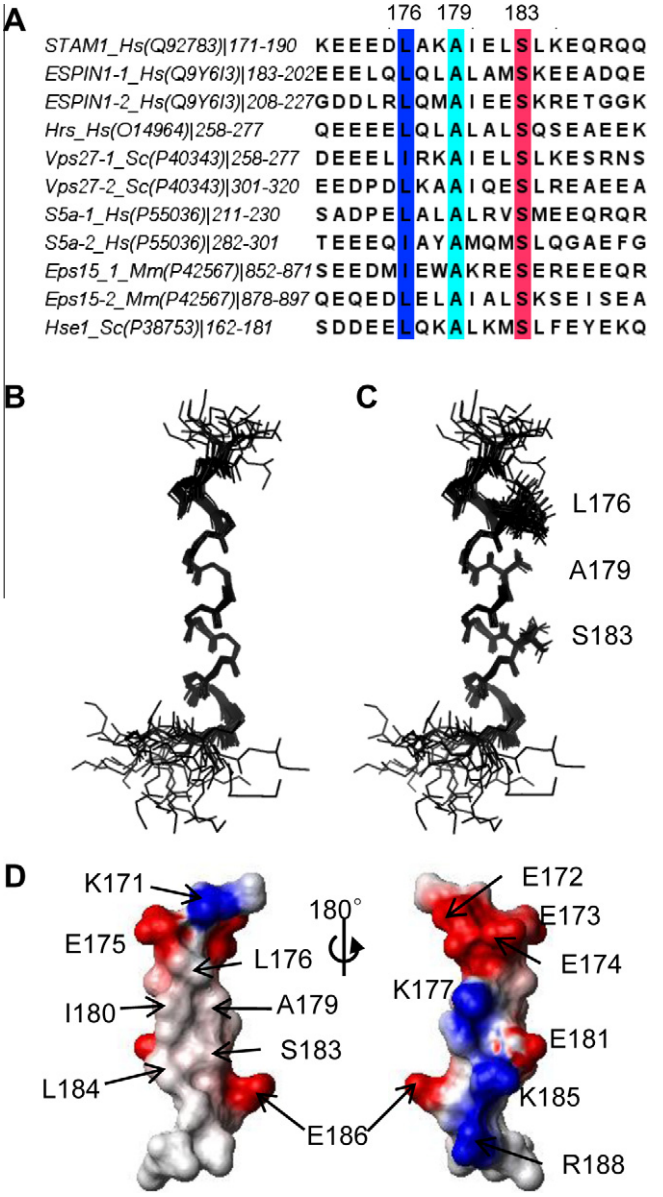

Fig. 1. (A) Sequence alignment of UIM. The highly conserved hydrophobic residues are indicated in color. (B) Energy lowest 20 ensemble structures of STAM1 UIM. (C) Conformation of side-chains from the conserved hydrophobic residues. (D) Electrostatic potential presentation of STAM1 UIM. The hydrophobic surfaces are colored in gray, and the positively and negatively charged surfaces are shown in blue and red, respectively. Throughout the figure, one-letter amino acid codes are used with the sequence number of STAM1. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Isothermal titration calorimetry

ITC measurements were carried out by using ITC-200 microcalorimeter (MicroCal, Northampton, MA). The same buffer for NMR