Mechanism for Selective Cleavage of Lys63-Linked Polyubiquitin Chains

Deubiquitinating enzymes (DUBs) remove ubiquitin from conjugated substrates to control various cellular processes. The Zn^{2+}-dependent DUBs AMSH (associated molecule with SH3 domain of STAM) and AMSH-LP (AMSH like protein) regulate receptor trafficking by selectively removing Lys63-linked polyubiquitin chains from internalized receptors. Using the AR-NW12A beamline, we have determined the crystal structures of the AMSH-LP DUB domain both alone and in complex with a Lys63-linked di-ubiquitin at spatial resolutions of 1.2 and 1.6 Å, respectively. This is the first reported structure of a DUB in complex with an isopeptide linked ubiquitin chain, and it reveals the mechanism for Lys63-linkage selective deubiquitination by AMSH family members.

Ubiquitin is a 76-residue conserved protein that can attach to substrate proteins via the covalent linkage between the carboxyl group of the terminal glycine residue in ubiquitin, and typically the ε-amino group of the target lysine residue in the substrate proteins. In many cases, additional ubiquitin molecules can be attached to the lysine residues in ubiquitin itself, producing polyubiquitin chains. The most abundant ubiquitin chains in cells are Lys48-linked ubiquitin chains, which serve as the canonical signals for degradation by proteasome. In contrast, Lys63-linked ubiquitin chains function in proteasome-independent processes such as DNA repair, ribosomal protein synthesis, inflammatory signaling, endocytosis, and vesicular trafficking. For instance, Lys63 polyubiquitinated receptors on the cell surface are internalized and delivered to sorting endosomes, where the receptors can be routed back to the cell surface or directed to lysosomes to be degraded.

AMSH family members are Zn^{2+}-dependent deubiquitinating enzymes (DUBs) that selectively cleave Lys63-linked polyubiquitin chains. AMSH-family members belong to a group termed JAMM. All DUB groups except JAMM are cysteine proteases, while JAMM is a zinc metalloprotease. AMSH proteins facilitate the recycling of receptors by removing Lys63-linked polyubiquitin chains. To date, the mechanism of linkage-specific polyubiquitin cleavage has remained unknown for all DUBs due to the lack of knowledge of their structures in complex with isopeptide linked ubiquitin chains. In this study, we have determined the crystal structures of the human AMSH-LP JAMM DUB domain both alone and in complex with a Lys63-linked di-ubiquitin (K63-Ub_2) at spatial resolutions of 1.2 and 1.6 Å, respectively, using high-quality diffraction data sets collected at AR-NW12A. This is the first crystallographic study that reveals the structural basis for the selective cleavage of Lys63-linked ubiquitin chains by AMSH family members [1].

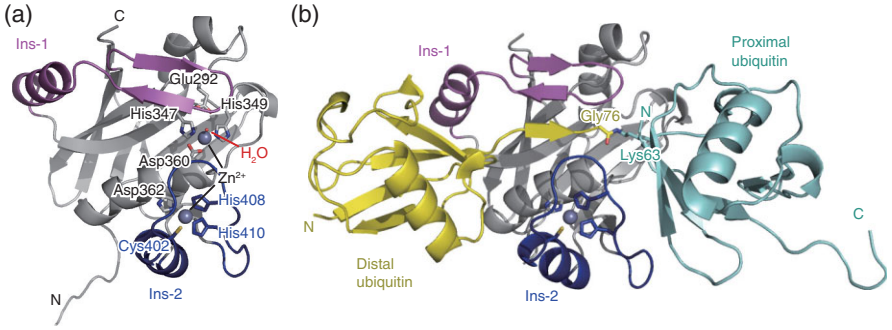

Figure 1
Overall structure of AMSH-LP. (a) Crystal structure of AMSH-LP. (b) Crystal structure of AMSH-LP in complex with K63-Ub_2.

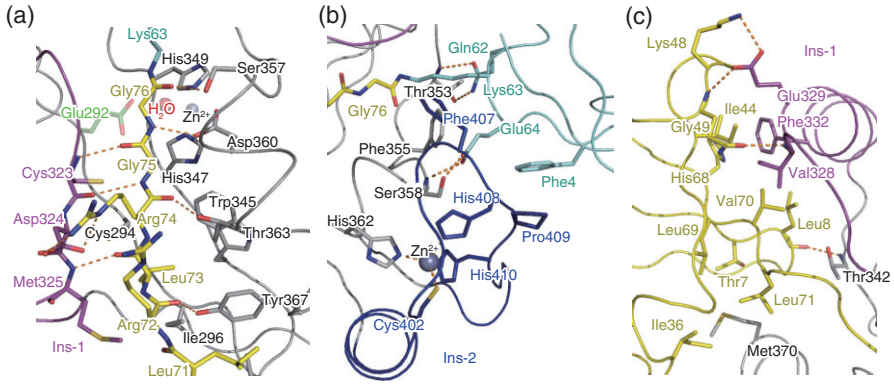

Figure 2
Recognition of K63-Ub_2 by AMSH-LP. (a) Recognition of the C-terminal tail of the distal ubiquitin. (b) Recognition of the proximal ubiquitin. (c) Recognition of the body of the distal ubiquitin.

The crystal structure of the AMSH-LP DUB domain consists of the so-called "JAMM core" and two AMSH-family specific insertions, Ins-1 and Ins-2 [Fig. 1(a)]. In the JAMM core of AMSH-LP, Zn^{2+} is coordinated by His347, His349, Asp360, and a water molecule hydrogen bonded to Glu292. Two important and unique features of the AMSH-LP DUB domain are the insertions Ins-1 and Ins-2. Ins-1 consists of a pair of antiparallel β-sheets and the following α-helix. Ins-2 is composed of one α-helix and the following long loop harbouring Cys402, His408, and His410, which coordinate the second Zn^{2+}, together with His362 in the JAMM core. In the AMSH-LP•K63-Ub_2 complex, K63-Ub_2 adopts an extended conformation [Fig. 1(b)]. The β-stranded C-terminal tail of the distal ubiquitin and the conjugated Lys63 of the proximal ubiquitin are accommodated in a 20-Å-long catalytic groove formed by the JAMM core and Ins-1 in AMSH-LP with an extensive hydrogen bonding network [Fig 2(a)]. This extensive interaction may guarantee accurate positioning of the scissile isopeptide bond in the Zn^{2+}-coordinating active site. The proximal ubiquitin is recognized by the Zn^{2+}-coordinating loop of Ins-2 and the loop connecting β6 and α3 in the JAMM core [Fig. 2(b)]. These two regions form a concave surface that binds the molecular surface around Lys63 of the proximal ubiquitin, and ensures that only Lys63-linked ubiquitin can bind in the proper orientation for catalysis. Hydrogen bonds formed between AMSH-LP and the proximal ubiquitin allow the tri-peptide sequence -Gln62-Lys63-Glu64- in the proximal ubiquitin to align correctly for Lys63-linkage selective deubiquitination. For distal ubiquitin recognition, the hydrophobic patch of the distal ubiquitin around Ile44 interacts with aliphatic side chains of Val328 and Phe332 in Ins-1 of AMSH-LP [Fig. 2(c)]. In addition, the hydrophobic pocket located on the face of the distal ubiquitin opposite the Ile44-centered hydrophobic patch accommodates the side chain of Met370 in the JAMM core of AMSH-LP. Finally, the functional importance of these interactions between AMSH-LP and K63-Ub_2 was confirmed from studies of their kinetics using site-directed AMSH-LP mutants designed on the basis of the complex structure.

REFERENCE

[1] Y. Sato, A. Yoshikawa, A. Yamagata, H. Mimura, M. Yamashita, K. Ookata, O. Nureki, K. Iwai, M. Komada and S. Fukai, *Nature*, **455** (2008) 358.

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