Crystallography

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Crystal structure analysis of the UCH37 catalytic domain

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Introduction

Ubiquitin is a highly conserved protein in eukaryotes and it is used as a label of the target protein for a proteolysis with 26S proteasome in ubiquitin-proteasome system. After degradation of the target protein, the ubiquitin chain is removed from target protein by deubiquitinating enzymes (DUBs) and recycled. The DUBs are cystein proteases and classified into two groups: ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases. The UCHs catalyze hydrolysis of the isopeptide bond between the C-terminus of ubiquitin and a lysine side chain on the adduct. UCH37 belongs to the UCHs family and it is composed two domains. The N-terminal domain is called UCH-domain including a catalytic site, the C-terminal domain is able to interact to the hRPn13 of 26S base subunit via KEKE motif. The C-terminal domain is not conserved in UCHs and depress the deubiqutinating activity of UCH-domain [1]. The structure known UCHs disassemble only a small adduct from ubiquitin (such as an oligopeptide). Whereas, UCH37 can disassemble a large adduct (ubiquitin). To elucidate the mechanism of deubiquitin activity by based on the structure, we have determined the structure of UCH-domain of UCH37 using X-ray crystallography [2].

Materials and Methods

The native and Se-Met substituted UCH-domain were crystallized in almost same conditions (20-30% PEG4000, 0.1M Tris-HCl pH8.5 and 0.2M $MgCl_2$ and any additives). However, both crystals belong to different space groups. The native crystal belongs to space group *C*2, with unit cell dimensions *a*=67.58 Å, *b*=57.06 Å, *c*=48.74 Å, β=100.91°. The Se-Met crystal belongs to space group $C222_1$, with unit cell dimensions *a*=62.77 Å, *b*=69.43 Å, *c*=96.19 Å.

We had harvested and processed the native and Se-Met MAD datasets up to the 2.2 Å and 3.2 Å resolution, respectively. To obtain the phase information, we had processed the Se-Met MAD data sets by the program Phenix. The initial model from MAD data was used for to determined the native structure using molecular replacement method by the program MolRep. Refinement for the native structure was carried out using the programs CNS and Lafire.

Results and Discussion

The crystal structure of the UCH-domain shows it is composed of a central six-stranded anti-parallel β-sheet, with seven α-helices on either side of the sheet causing it to form a bilobal structure (Fig.1). The catalytic nucleophile Cys88, the general base His164, Asp179 and the oxyanion hole residue Gln82 are completely conserved in the UCHs.

The putative substrate-binding site (P'-site) of UCH-L3 forms a V-shaped trough with helix-3 and a loop (Fig.2B). Helix-3 comprises a wall on the edge of the P'-site. Comparison of the four UCHs indicates that the shape and electrostatic surface potential of the P'-site differ. Helix-3 is collapsed in UCH-domain (long-loop in Fig.1), resulting in a broader V-shaped trough compared to other UCHs (Fig.2A). These observations suggest that UCH37 can recognize substrates with different properties, such as larger substrates than those recognized by other UCHs.

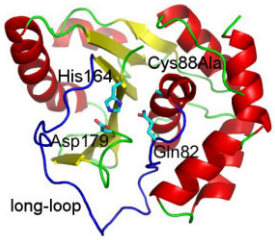

Fig.1: Overall structure of the UCH-domain of UCH37

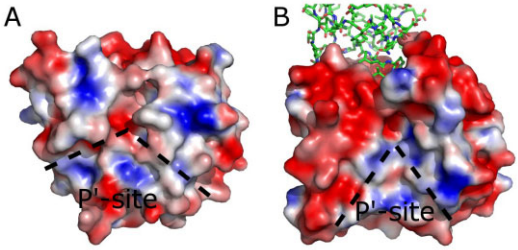

Fig.2: The shape and electrostatic potential of the UCH surfaces.

References

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