Crystallography

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The crystallographic study of the deubiquitinating enzyme UCH37 N-terminal 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 enzyme and recycled. The deubiquitinating enzymes are cystein proteases. They are classified into two groups: ubiquitin carboxyl-terminal hydrolases and ubiquitin-specific proteases. UCH37 belongs to the ubiquitin C-terminal hydrolases family and it is composed two domains. The N-terminal domain of UCH37 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 ubiquitin carboxyl-terminal hydrolase family and depress the deubiqutinating activity of UCH-domain[1]. Therefore, this domain would be expected to form a unique conformation to UCH37. To elucidate the mechanism of deubiquitin activity, inactivation of UCH-domain and interaction with 26S by based on the structure, we have tried to determine the structure of UCH37 using X-ray crystallography.

Results and Discussion

The native and Se-Met substituted N-terminal domain of UCH37 were crystallized in almost same conditions. However, both crystals belong to different space groups, native and Se-Met crystals with space group of *C*2 and *C*2221, respectively (fig. 1).

We had harvested the native dataset over the 2.4 Å resolution at NW12A in PF-AR and diffraction images were processed using program *Mosflm*. The data processing statistics are described bellow (table 1). The calculated Matthews coefficient was approximately 1.6 $Å^3$ Da^{-1} assuming the presence of one molecule in the asymmetric unit. To obtain the phase information, we had harvested the Se-Met MAD data sets, too. The Se-Met crystal diffract by the about 3.2Å resolution and the phase was calculated successfully by the program *Phenix*.

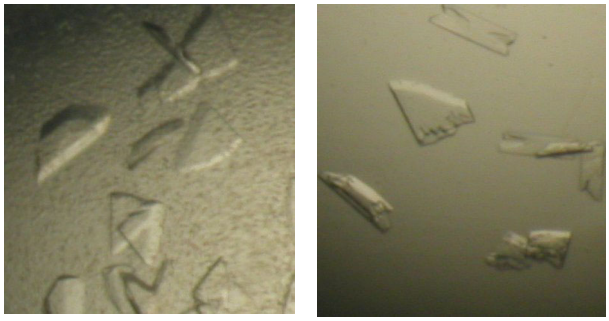

Fig.1: Crystals of the N-terminal domain of UCH37 (left ;native crystal, right; Se-Met crystal)

Table 1: The statistics of the native data set

X-ray source	NW12A
Space group	*C*2
Unit cell parameters (Å)	*a*=66.5, *b*=56.8, *c*=45.3, beta=99.9
Wavelength (Å)	1.00000
Resolution range (Å)	32.84-2.40 (2.53-2.40)
No. of total reflections	29402 (4360)
No. of unique reflections	6542 (950)
Completeness (%)	99.2 (99.4)
R_{merge} (%)	7.5 (48.7)
I/sigma(I)	11.5 (2.9)
Multiplicity	4.5 (4.6)

Values in parentheses are for the outer shell.

References

[1] T. Yao et al., Nat. Cell. Biol. 8 (2006).

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