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Crystallization and preliminary X-ray analysis of the tripeptidyl-peptidase from *Streptomyces herbaricolor*

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Introduction

Protein degradation is an essential process in cells of all organisms. The process consists of multiple steps catalyzed by various peptidases. Tripeptidyl-peptidases (TPP) are responsible for the middle of the process: they cleave oligopeptides produced by the proteasome in the cytosol or by cathepsins in the lysosome to form tripeptides, which will be processed further by dipeptidyl-peptidases and other exopeptidases down to amino acids. The formation of tripeptides develops efficient protein degradation. Ekino and Shin [1] has isolated a TPP from *Streptomyces herbaricolor*. It is a serine protease with a limited sequence identity (31%) to subtilisin. A 38-kDa active mature form is produced from a 51-kDa inactive nascent polypeptide by removal of the N-terminal signal- and pro-peptides in the same way as reported for subtilisin. The enzyme successively cleaves tripeptides from the N-terminus of a substrate. The mechanism of this substrate recognition is of structural interest and it would be a key driver for industrial application of the enzyme. We have crystallized the enzyme and performed preliminary analysis of the crystal.

Materials and Methods

The mature 38-kDa form of the enzyme was purified from cell extracts of *Streptomyces herbaricolor* strain TY-21 through ammonium sulfate precipitation and three steps of column chromatography. The final product was lyophilized and stored at 4 °C. For crystallization experiments, the lyophilized enzyme was dissolved in 10 mM Tris-HCl, pH 8.0, 1 M NaCl, and dialyzed against the same buffer overnight.

Thin square plate crystals were obtained by the hanging-drop vapor-diffusion method using a reservoir solution of 100 mM MES, pH 5.8, 2.3 M ammonium sulfate; drops were prepared by mixing protein and reservoir solutions at a 1:1 ratio. The crystals grew to 0.2x0.2x0.08 mm within a month.

X-ray diffraction data from flash-frozen crystals were collected at the BL6A beamline (λ=1.0000 Å). Prior to freezing, the crystals were briefly dipped in 2.3 M Na-malonate, pH 7.0 for cryo-protection. Collected images were processed with HKL2000 and the CCP4 suite. Molecular replacement (MR) and structure refinement were performed with PHASER and REFMAC5, respectively.

Results and Discussion

We found that the purified mature enzyme is accompanied by an 8-kDa polypeptide. N-terminal amino acid sequencing of the 8-kDa polypeptide revealed that it is a part of the N-terminal propeptide removed from a premature form of the enzyme. Since the mature enzyme and the 8-kDa polypeptide eluted as a single peak from a gel-filtration column, they are supposed to retain a tight complex form after cleavage of their covalent linkage. It is contrasted to the case of subtilisin, where maturation of the enzyme includes auto-proteolytic cleavage and following auto-degradation of the propeptide. A few structures of the subtilisin-propeptide complex have been reported but there included are genetically inactivated enzymes.

The best dataset collected from the TPP crystal contains reflection intensities between 29.7 Å and 1.98 Å with a completeness of 83.2% (I>2σ) and an overall R-merge of 6.1%. The crystal was identified as the tetragonal lattice belonging to space group $P4_12_12$ or $P4_32_12$ with unit cell dimensions of a=b=91.4 Å and c=272.5 Å.

MR was attempted on data in the resolution range 15–3.0 Å. A significant solution was obtained by assuming space group $P4_12_12$ and employing a mutant subtilisin E-propeptide complex structure (PDB ID: 1SCJ) as a search model. The solution indicates two molecules in the asymmetric unit, leading to a Matthews parameter V_M of 3.1 $Å^3Da^{-1}$ or a solvent content of 60%. The two molecules are related by the non-crystallographic two-fold symmetry, of which axis is almost parallel to the crystallographic c-axis. Rigid-body refinement was applied to the two molecules, each one of which was divided into propeptide and enzyme domains, resulting in an R factor of 54.1% and a correlation coefficient 0.42 in the resolution range 15–2.5 Å. These scores are not convincing but the provided electron density map looks promising. Pseudo-merohedral twinning was suspected but disclaimed by the Padilla-Yeates test using DATAMAT from the RAVE suite. Model building starting from the solution is under way.

References

[1] K. Ekino and T. Shin, *Kagaku to Seibutsu*, **41** (10) 687-689 (2003).

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