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Structure of yeast N-terminal amidase in the N-end rule pathway

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1 Introduction

The N-end rule pathway is a universally present ubiquitin (Ub)-dependent proteolytic system that mediates and regulates the degradation of intracellular proteins through the recognition of their (destabilizing) N-terminal residues. [1] It is conserved among both prokaryotes and eukaryotes. In this hierarchically organized pathway, N-terminal amidase converts N-terminal asparagine (Asn) and N-terminal glutamine (Gln), the tertiary destabilizing residues of N-end rule substrates, to the secondary destabilizing N-terminal residues aspartic acid and glutamic acid, respectively. The yeast (*Saccharomyces cerevisiae*) N-terminal amidase Nta1 was identified as a component of the N-end rule pathway more than 20 years ago [2] but its dual specificity for N-terminal Asn and Gln residues remains uncharacterized, owing the absence of structural and biochemical data. The present structures of yeast Nta1 with various N-degron peptides, and biochemical analyses with Nta1 mutants, illuminate specific mechanisms of the first step in the N-end rule pathway.

2 Experiment

yNta1 and mutants were expressed with modified pET vector containing a N-terminal 6×His-bRIP (ribosome in-activation protein from barley) vector. [3] The over-expressed proteins were purified by Ni-NTA affinity chromatography followed by anion exchange and additional Ni-NTA column and size exclusion chromatography. yNta1 was crystallized using the hanging drop method by vapor-diffusion at 20 ºC. To cocrystallized purified yNta1 mutants was incubated with ~ 25 fold molar excesses of each peptide for 24 h. yNta1 and substrate peptide complex crystal diffracted up to 2.0 ~3.0 Å. The space group and unit cell parameters were $I4_122$ with a=b=134.1 Å and c=119.5 Å. Diffraction data were collected using an ADSC quantum CCD detector at the NW12 beamline of Photon Factory. The diffraction data were processed and scaled using the HKL2000 software package. Phases were obtained by Selenomethionin derivative crystal using Autosol according to the PHENIX program suite. The initial model was manually built and refined using COOT and PHENIX.

3 Results

yNta1 has an α-β-β-α sandwich fold, which is commonly found in members of the nitrilase superfamily. [4] yNta1 exist a monomer containing 14 β-strands, 11 α-helices, and three 3_{10}-helices. The core region of the enzyme shows antiparallel and parallel mixed β-sheets surrounded by helices, and these six stranded β-sheets face each other (Fig. 1A). yNta1 makes cleft to interact with different N-degron peptides. The cleft of the active site could be tightly fitted to N-degrons and no complex structures showed significant conformational changes, in comparison with the structures of free yNta1 (Fig. 1B).

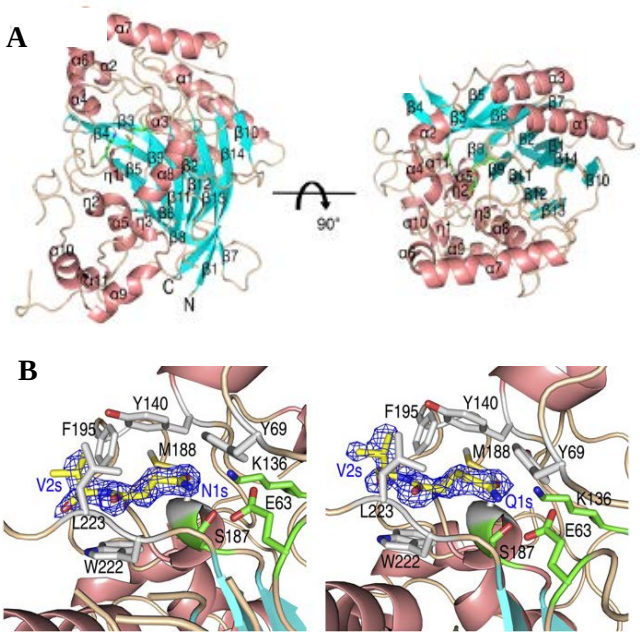

Fig. 1: (A) Overall structures of yeast Nta1. (B) Close-up view of N-degron binding site and electron-density map for the bound N-degrons Asn–Val and Gln–Val dipeptide

References

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