Navigation inside a Protease: Substrate Selection and Product Exit in the Tricorn Protease from *Thermoplasma acidophilum*

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Initial intracellular protein degradation is carried out mostly by unspecific proteases with sieving mechanisms for substrate selection. Proteasomes are representatives of this class of proteases, which form large multi-subunit complexes reaching molecular weights of 2.0 MDa. Peptides, with a preferred length of between 7 to 9 amino acids, are the major products of the proteasome. For reuse in protein synthesis or energy production, these peptides must be further degraded into free amino acids. In the archaeon *Thermoplasma acidophilum*, the degradation of proteasomal products is performed by the tricorn protease to preferentially yield di- and tripeptides. The further and final degradations to free amino acids are accomplished by the tricorn interacting factors, a proline iminopeptidase termed F1 and two metalloproteases termed F2 and F3[1-3].

The proposed pathway and mechanism of substrate entry and product egress through both propeller structures[4] in the hexameric D3 symmetric tricorn protease from *Thermoplasma acidophilum* were explored by crystallographic studies of ligand complexes and by structure-based mutagenesis. Obstruction of the pore within the 7-bladed β-propeller (β7) domain by alkylation or oxidation of an engineered double cysteine mutant strongly decreased enzymatic activities. In line herewith, the crystal structure of the tricorn protease in complex with a tetrapeptide chloromethyl ketone with deca hydrocarbon chain at the N-terminus modifying the catalytic Ser965 indicated that the channel of the β7 domain could be a substrate route to the active site. The cysteine mutation widening the lumen of the 6-bladed β-propeller (β6) domain enhanced catalytic activity, which was restored to normal values after its alkylation. A charge reversal mutant at the putative anchor site of the substrate C-terminus, R131E-R132E, drastically reduced the proteolytic activity. However, a free carboxy terminus is not required and the tricorn protease processes also a substrate with an amidated C-terminus. These two contradictory experimental results were clarified by the complex crystal structure of a peptide inhibitor with a diketo group at the cleavage site, which mapped the substrate recognition site and confirmed the role of Arg131-Arg132 as an anchor site. Our results strongly suggest the wider β7 domain to serve as a selective filter and guide of the substrate to the sequestered active site, while the narrower β6 domain routes the product to the surface. Moreover, we identified the role of Arg131-Arg132 in anchoring the substrate C-terminus.

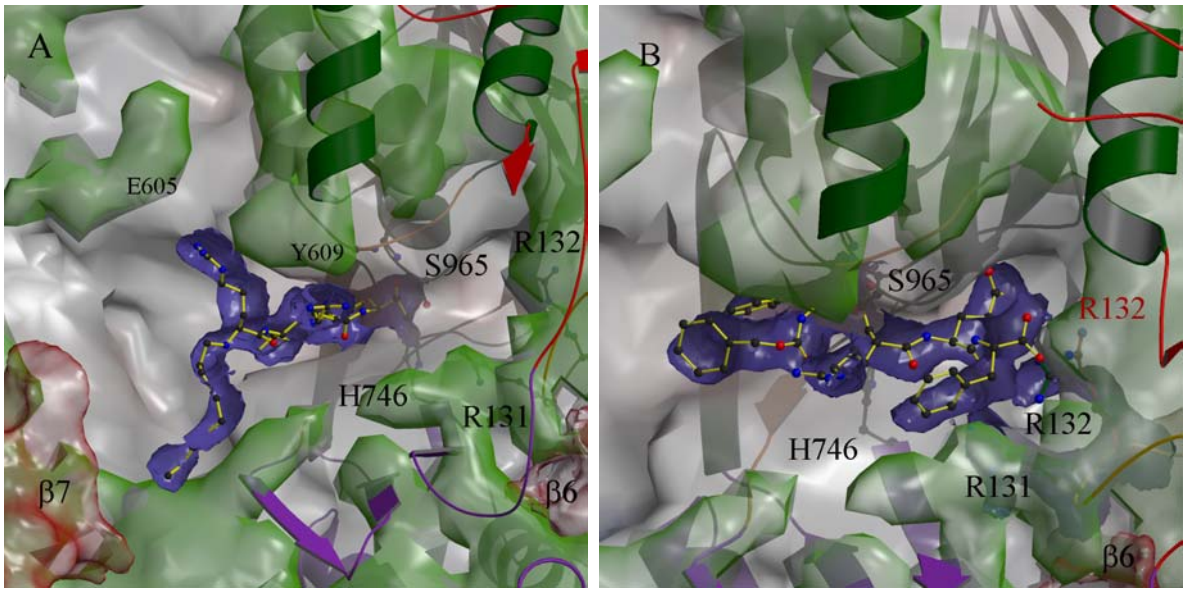

Figure 1, Tetrapeptide chloromethyl ketone derivative with deca carbon chain at the N-terminus at 2.8 Å resolution (left) and inhibitor complex structure of peptide derivative with a diketo group at the cleavage site at 2.7 Å resolution (right).

References

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