Crystal structure of TET protease reveals complementary protein degradation pathways in prokaryotes

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Protein synthesis and protein degradation are two universal complementary processes, permanently occurring in a living cell. Proteinolysis plays an important role in maintaining biological homeostasis and regulation of different cellular processes, such as cell differentiation, cell cycle control, antigen processing and hormone metabolism. Proteasomes represent the major protein degradation machinery, digesting proteins to short peptides of 7-15 residues (Groll and Clausen, 2003). It is remarkable, that despite the abundance of protein degradation systems, they are not able to complete the proteolytic process and to degrade proteins to single amino acids. They generate a pool of oligopeptides of different length, which need further processing. Tricorn protease (TRI), a 720 kDa hexameric complex discovered in Thermoplasma acidophilum, was the first identified protease, which performs degradation of oligopeptides produced by the proteasome (Tamura et al., 1996). It is able to digest oligomeric peptides to tri- and dipeptides, which are degraded sequentially to free amino acids by peptidases named tricorn interacting factors F1, F2 and F3 (Göttig et al., 2002; Tamura et al., 1998). TRI is only present in the genomes of some prokaryotes, and primary sequence alignment reveals no homologues in eukaryotes (Brandstetter et al., 2001). Recently, a novel energy independent protease complex from the archaeon Haloarcula marismortui has been described (Franzetti et al., 2002). It belongs to the M42 peptidase family and was named tetrahedral aminopeptidase (TET), due to its peculiar structure. This protease is a self-compartmentalised protein complex of 12 identical subunits, showing aminopeptidase activity with broad substrate specificity. Negative staining electron microscopy revealed that TET exists as a large tetrahedral complex having an edge of about 150Å, with several channels leading to the central cavity (Franzetti et al., 2002).

Primary sequence alignment revealed homologues of TET in different archaea and bacterial species (Franzetti et al., 2002). Using the beamline at the BW6, DESY, we determined the crystal structure of TET from the thermophilic archaeon Pyrococcus horikoshii. We solved the crystal structure of TET using anomalous multiple dispersion methods at 1.6Å resolution in its native form and in complex with the inhibitor amastatin. Structural data provided initial information for mutagenesis experiments, which revealed the mechanism of substrate entry and product release. Additionally, we have performed structure based functional analysis of purified TET to reveal its relation to proteasomes and its role in the protein disassembly line. We demonstrate, that PAN, proteasome and TET act sequentially in the protein degradation pathway, processing proteins from their folded state to single free amino acids.
Figure 1: Ribbon presentation of the TET viewed along the molecular two-fold (left) and three-fold symmetry axis (right). The domains of one subunit are coloured according to the colour code used above. The ball model illustrates the orientation of the molecule. The highlighted subunit is coloured in red.

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References