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Review

Structure and function of tripeptidyl peptidase II, a giant cytosolic protease ☆

Beate Rockel [a,*], Klaus O. Kopec [b], Andrei N. Lupas [b], Wolfgang Baumeister [a]

[a] Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany
[b] Department I — Protein Evolution, Max Planck Institute for Developmental Biology, Spemannstr. 35, D-72076 Tübingen, Germany

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ABSTRACT

Tripeptidyl peptidase II is the largest known eukaryotic peptidase. It has been described as a multi-purpose peptidase, which, in addition to its house-keeping function in intracellular protein degradation, plays a role in several vital cellular processes such as antigen processing, apoptosis, or cell division, and is involved in diseases like muscle wasting, obesity, and in cancer. Biochemical studies and bioinformatics have identified TPPII as a subtilase, but its structure is very unusual: it forms a large homooligomeric complex (6 MDa) with a spindle-like shape. Recently, the high-resolution structure of TPPII homodimers (300 kDa) was solved and a hybrid structure of the holocomplex built of 20 dimers was obtained by docking it into the EM-density. Here, we summarize our current knowledge about TPPII with a focus on structural aspects. This article is part of a Special Issue entitled: Proteolysis 50 years after the discovery of lysosome.

1. Introduction

The ubiquitin–proteasome system constitutes the main pathway for protein degradation in eukaryotic cells [1]. Its most downstream element, the 26S proteasome, has been studied in great detail even though a high resolution structure of the holocomplex is still not available. The structure of its proteolytic core complex, the 20S proteasome, has long been solved and in conjunction with mutagenesis has clarified the role of 14 α- and 14 β-subunits in protein breakdown [2]. In recent years, the group of giant post-proteasomal proteases and especially tripeptidyl peptidase II (TPPII) have also come into focus [3]. The proposed cellular role of the latter is in cytosolic protein degradation downstream of the proteasome in conjunction with other exo- and endopeptidases [4]. Under conditions where the function of the proteasome is compromised, e. g. by inhibitors, but also in certain diseases, TPPII is upregulated and a number of studies have been performed to reveal its role in health and disease states (see [5,6] for recent reviews). TPPII has been described as a 'multi-purpose peptidase' [7], and indeed, many functions have been ascribed to it, but in many cases its substrates or reaction partners have remained obscure. Bioinformatic and biochemical studies had suggested early on that the N-terminal part of TPPII is homologous to subtilisin [8] but the function of the larger part of the polypeptide chain remained enigmatic. Based on its similarity to subtilisin, homology models of the active site region of TPPII have been created [9,10] and sequence comparisons have been used to pinpoint potential functional regions [11]. New opportunities for the functional analysis of TPPII have been opened by the recent determination of a high resolution structure of TPPII using a hybrid EM-X-ray crystallography approach, where the crystal structure of TPPII dimers was docked into the structure of the TPPII holocomplex obtained by cryo-electron microscopy [12]. In this review we will summarize our current knowledge about this giant protease.

2. Cellular functions of TPPII

2.1. TPPII in cytosolic proteolysis

TPPII was discovered in 1983 in the extralysosomal fraction of rat liver during a search for peptidases with specificity to proteins phosphorylated by cyclic AMP-dependent protein kinase [13]. Subsequently it was found in many other tissues and also in red blood cells [14]. Its function – the removal of a tripeptide from the free N-terminus of longer peptides – had up to then only been observed for TPPI, a structurally unrelated lysosomal peptidase. In addition to exopeptidase activity, endopeptidase activity has also been ascribed to TPPII, but this activity is much lower than its exopeptidase activity [15,16].

So far only unfolded peptides have been reported to be cleaved by TPPII, the longest one, with a length of 41-residues, being $Ova_{37–77}$ [15]. Based on the type of substrates degraded and in analogy to Tricorn protease [17], TPPII was assigned a role downstream of the proteasome in cellular protein degradation [4]; however, direct experimental evidence for this disassembly line is still lacking. In fact, the processing of proteasomal products is something TPPII has in common with other peptidases like leucine aminopeptidase LAP [18], thimet oligopeptidase TOP [19], bleomycin hydrolase BH [20,21], or

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* Corresponding author at: Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Am Klopferspitz 18, D-82152 Martinsried, Germany. Tel.: +49 89 8578 2698; fax: +49 89 8578 2641.
E-mail addresses: rockel@biochem.mpg.de (B. Rockel), klaus.kopec@tuebingen.mpg.de (K.O. Kopec), andrei.lupas@tuebingen.mpg.de (A.N. Lupas), baumeist@biochem.mpg.de (W. Baumeister).

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puromycin-sensitive aminopeptidase PSA [21,22]. Nonetheless, TPPII appears to be the only peptidase capable of degrading peptides that are longer than 15 residues [23,24].

Among the cellular functions attributed to TPPII, its function as a neuropeptidase inactivating the satiety hormone CCK8 is probably characterized best [25]. The cleavage of CCK8 to CCK5 in rat brain is carried out by a membrane-associated version of TPPII, which was suggested to be anchored in the lipid bilayer by a covalent glycosyl phosphatidyl inositol link [25]. Its influence on satiety has made TPPII an interesting target for obesity-treatment and, indeed, TPPII-inhibition by treatment with the specific inhibitor butabindide was shown to reduce the food intake in rats [25]. A role in fat metabolism was also proposed for *C. elegans* TPPII, which surprisingly appeared to be independent of the presence of a functional proteolytic domain [26].

TPPII has been implicated in antigen processing, however the extent to which it is essential for the processing of antigenic peptides has remained controversial (for reviews see [5,6,27]). TPPII was reported to be involved in the generation of two viral epitopes [16,28], although a proteasome with an altered specificity could also be responsible for the creation of these MHC class-I peptides [29]. Altogether, the generation of most MHC class I-bound peptides appears to be independent of TPPII [27]. Nevertheless, the processing of peptides longer than 15 residues requires TPPII [23,24], but only a small fraction of the peptides released by the proteasome falls into that size range [24,30,31].

2.2. TPPII and its role in diseases

TPPII-activity is increased in skeletal muscle during sepsis-induced muscle wasting [32] as well as during cancer cachexia [33]. Responsible for the accelerated proteolysis under such catabolic conditions is the ubiquitin proteasome-system [34,35], supporting the notion that TPPII functions downstream of the proteasome and is co-induced with it. TPPII is also upregulated in tumor cells, a finding that might have an impact on cancer therapy: EL4 thymoma or EL4 lymphoma cells adapted to proteasome-inhibition as well as Burkitt's lymphoma cells, where the proteasome appears to be functionally impaired, show increased TPPII activity [15,36,37]. From such observations it was concluded that TPPII may allow survival of these cells by compensating for a loss of proteasome function [15,36]. Indeed, it was shown that in Burkitt's lymphoma cells, protein turnover is unaffected and that ubiquitinated proteins do not accumulate [37] unless the cells are treated with the covalent serine protease inhibitor AAF-CMK, which inhibits TPPII [37,38]. However, AAF-CMK is not specific for TPPII, since it affects also other proteases like the proteasome [39]. In the presence of the specific TPPII inhibitor butabindide or siRNA against TPPII no such accumulation occurs, implying that TPPII cannot substitute for the proteasome in the cleavage of ubiquitinated proteins [38].

Burkitt's lymphoma cells are apoptosis-resistant, but apoptosis can be induced by TPPII-inhibition with AAF-CMK [37]. For EL-4 lymphoma cells adapted to proteasome-inhibition apoptosis-resistance and increased growth-rate was ascribed to an impaired degradation of inhibitors of apoptosis (IAP) and both features could be induced by TPPII-upregulation after TPPII-transfection [40]. HEK293 cells are yet another cell line for which apoptosis-resistance and accelerated growth upon overexpression of TPPII were shown. Such TPPII-overexpressing HEK293-cells could survive the effect of the spindle poison nocodazole and showed a higher degree of aneuploidy as well as more structural and numerical centrosome abnormalities than control cells [41,42]. Also the cell-division errors observed in Burkitt's lymphoma cells seem to depend on TPPII, since the observed c-MYC induced centriole overduplication can be avoided by TPPII inhibitors like butabindide or by siRNA-mediated protein knock-down [43]. A participation of TPPII in cell division, as suggested by these experiments, might be the reason for its observed localization in the vicinity of daughter centrioles in late mitosis and between daughter and mother centrioles during G2 phase [43].

In several malignant cell lines TPPII translocated into the nucleus upon γ-irradiation and the production of reactive oxygen species (ROS), which suggested a role for TPPII in DNA-repair [44,45]. However, this translocation as well as the accumulation of p53 remains controversial, since they were not observed in EL4 cells, COS cells, and transformed fibroblasts [46,47], a discrepancy that was attributed to different levels of ROS and sub-optimal cell densities [44].

2.3. TPPII-deficient species

In order to investigate the importance of TPPII for cell survival, a number of TPPII-deficient species have been created. A T-DNA mutant of *Arabidopsis* defective in TPPII expression showed no phenotypic abnormalities [48] and likewise, a TPPII-knockout strain of *S. pombe* was viable and did not have any obvious growth defects [11,49]. Suppressing TPPII expression by siRNA in *C. elegans* resulted in decreased fat stores in adult worms; however, reduced CCK8-degradation was not detectable and therefore no connection to satiety control could be established [26]. Divergent observations were reported for TPPII-deficient mice: McKay et al. [26] failed to obtain homozygotic TPPII-deficient mice due to early embryonic lethality. However, their *tpp2* heterozygous mutants were lean compared with wild-type littermates, while their food intake was normal. Kawahara et al. [50] produced gene-trapped mice with an expression level of TPPII reduced by >90% compared to wild-type. These mice with a gene-trap disrupting *tpp2* were viable, fertile, and normal in appearance and behavior. In contrast, Huai et al. [51] describe knockout mice homozygotic for *tpp2*−/−, which were viable but in which the TPPII-deficiency activated cell-type specific death programs. As a consequence the mice had a decreased life-span. Also, how TPPII-deficiency affects *Drosophila* is not clear. In a screen of lethal mutants on the second chromosome of *D. melanogaster* for those that could enhance a weak Ras1 eggshell phenotype, one insertion disrupted two genes, Nrk, a neurospecific receptor tyrosine kinase and TPPII. Whether the lethality is attributable to either of the two disrupted genes alone or to the additive effect of both remains unclear [52].

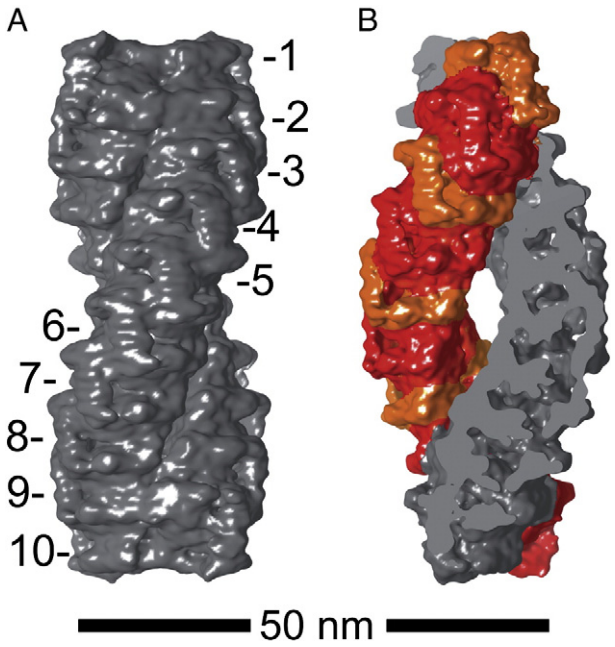

Fig. 1. 3D structure of the TPPII holocomplex. A) 3D-reconstruction of *Dm*TPPII, segment numbers are indicated for one strand. B) *Dm*TPPII rotated about 90° around the longitudinal axis. Dimers in the strand on the left are highlighted in orange and red to visualize their stacking; the strand on the right was cut open in order to show the internal cavity system of TPPII.