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Molecules in focus

Tripeptidyl-peptidase II: A multi-purpose peptidase

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Abstract

Tripeptidyl-peptidase II is a high-molecular weight peptidase with a widespread distribution in eukaryotic cells. The enzyme sequentially removes tripeptides from a free N-terminus of longer peptides and also displays a low endopeptidase activity. A role for tripeptidyl-peptidase II in the formation of peptides for antigen presentation has recently become evident, and the enzyme also appears to be important for the degradation of some specific substrates, e.g. the neuropeptide cholecystokinin. However, it is likely that the main biological function of tripeptidyl-peptidase II is to participate in a general intracellular protein turnover. This peptidase may act on oligopeptides generated by the proteasome, or other endopeptidases, and the tripeptides formed would subsequently be good substrates for other exopeptidases. The fact that tripeptidyl-peptidase II activity is increased in sepsis-induced muscle wasting, a situation of enhanced protein turnover, corroborates this biological role.
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1. Introduction

Tripeptidyl-peptidase II (TPP II) was discovered in 1983 in a search for a peptidase specific for phosphorylated peptides. The characterization of the enzyme demonstrated that it was in fact an aminopeptidase which removed tripeptides sequentially from a free N-terminus of larger peptides (see Tomkinson (1999, 2004) for recent reviews). The dominant form of TPP II is extralysosomal, but a membrane-associated variant of the enzyme also exists (Rose et al., 1996). TPP II has a broad substrate specificity, considering that there is little apparent sequence similarity between the tripeptides removed. However, it is not indiscriminate, as the rate of cleavage varies more than 100-fold depending on the sequence of the substrate. The enzyme seems to have some preference for cleaving after hydrophobic residues, and cannot cleave before or after proline residues. Beside the predominant exopeptidase activity, TPP II also displays a low endopeptidase activity, cleaving preferentially after lysine-residues (Geier et al., 1999; Seifert et al., 2003). Interestingly enough, as an endopeptidase the enzyme can cleave after proline residues (Seifert et al., 2003). How the substrate specificity is deter-

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mined remains an intriguing question for future investigations.

2. Structure

Electron microscopic studies of TPP II have shown that the enzyme is a remarkably large proteolytic machine (Macpherson, Tomkinson, Bålöw, Höglund, & Zetterqvist, 1987) (Fig. 1A). Oligomeric complexes with a molecular weight of about 4 MDa are formed by 138 kDa subunits (Tomkinson, 2004). The complexes are required for full enzymatic activity, with about 10-fold higher activity than that of the dissociated form (Macpherson et al., 1987; Tomkinson, 2000). Association and dissociation could therefore be a way of regulating the enzymatic activity of TPP II (Tomkinson, 2000).

Baumeister and his co-workers have extended the investigation of the structure through high-resolution cryo-electron microscopy of the TPP II homologue from fruit fly (Rockel, Peters, Kuhlmorgen, Glaeser, & Baumeister, 2002). The 150 kDa subunits form a superstructure, composed of two segmented, twisted strands. Each strand consists of 11 segments which enclose a central channel (Rockel et al., 2002) (Fig. 1B). The location of the active site in this complex structure is not known. However, the synthetic substrates used are fairly small, and although TPP II appears to display endopeptidase activity towards certain polypeptides (Geier et al., 1999; Reits et al., 2004; Seifert et al., 2003), the largest substrate used is an ovalbumin fragment of 41 amino acids (Geier et al., 1999). There are no reports of proteins, native or denatured, being degraded by TPP II. This could indicate that access to the active site is limited. Therefore it is tempting to speculate that TPP II is a so-called "self-compartmentalizing" enzyme (Tomkinson, Ní Laoi, & Wellington, 2002).

Even though the three-dimensional structure of TPP II has not yet been determined in atomic detail, some information about the structure is available. The enzyme has a catalytic domain with sequence similarity to serine peptidases of the subtilisin-type (Tomkinson, 2004). The catalytic residues have been identified through labelling with ^{3}H-DFP (Ser-449) (Tomkinson, 2004) or site-directed mutagenesis (Hilbi, Jozsa, & Tomkinson, 2002) (Fig. 1C). When compared with

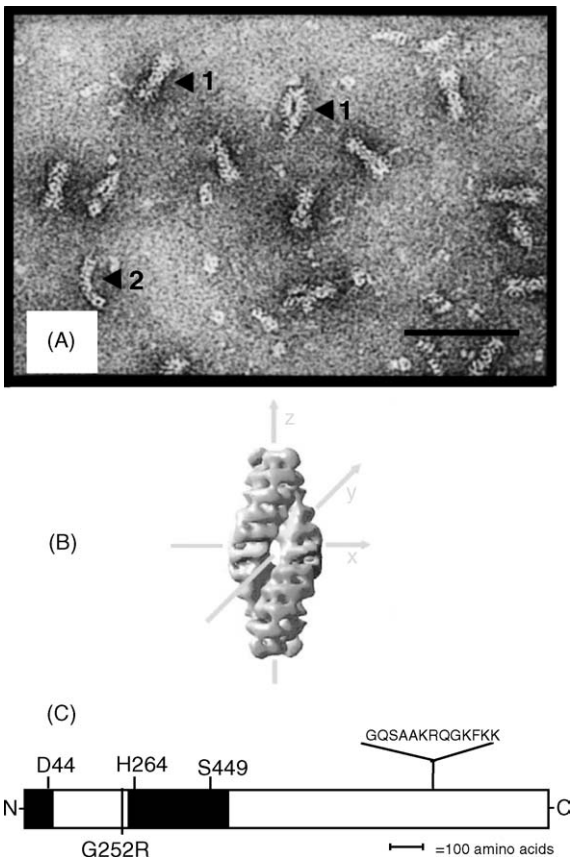

Fig. 1. Structure of TPP II. (A) Electron micrograph of human TPP II negatively stained by ammonium molybdate. Two different orientations of the complex (1) and a single strand (2) are indicated (reproduced with permission from Macpherson et al. (1987), ©The Biochemical Society). (B) A three-dimensional reconstruction of TPP II from fruit flies based on cryo-electron microscopic images (reproduced with permission from Rockel et al. (2002), ©Nature). (C) Outline of the domain structure of human TPP II. The subtilisin-like catalytic parts are black. D44, H264, and S449 indicate the catalytic amino acid residues and G252R the mutation that interferes with complex formation. The position of 13 extra amino acids, included as a result of alternative splicing, is indicated.

other subtilases, TPP II contains a 200 amino acid insert within the catalytic domain and a long C-terminal extension (Fig. 1C). These regions do not show any similarity to other sequences in current databases and their exact function is not known. However, it appears that at least the insert within the catalytic domain could be of importance for complex formation, since a variant enzyme with a single mutation in this region, G252R (Fig. 1C), cannot form the complexes which are es-

sential for full enzymatic activity (Tomkinson et al., 2002).

A splicing variant of TPP II has been identified. It contains an extra 39 bp exon encoding 13 amino acids in the C-terminal part of the protein (Fig. 1C). This splicing variant appears to form an even larger complex, but the exact physiological effect of this, if any, has not been shown (Tomkinson, 2004). Nevertheless, these data indicate that also the C-terminal part of the protein is important for complex formation.

3. Expression, activation and turnover

TPP II is expressed in a wide range of eukaryotes. The cDNA has been cloned from man, mouse, rat and fruit fly (Tomkinson, 2004) and genome sequencing projects reveal potential homologues also in plants (*A. thaliana*), fish (*F. rubripes*) and yeast (*S. pombe*). The enzyme is present in varying amounts in a number of different cells and tissues (Tomkinson, 1999, 2004). The half-life of TPP II has not been reported, but it appears to be fairly stable since it can be purified from outdated human red blood cells (Tomkinson, 1999, 2004).

In contrast to other serine peptidases of the subtilisin-type, TPP II does not appear to be synthesized as a pre-pro-protein (Tomkinson et al., 2002) and no post-translational processing has been reported. Thus, the only method of activation of the enzyme demonstrated so far is through complex formation (Macpherson et al., 1987; Tomkinson, 2000), even though it remains to be shown that this is the case in vivo. There have not been any reports of natural inhibitors or other potential regulatory proteins interacting with TPP II in vivo or in vitro. However, an experiment using anti-FLAG agarose and a FLAG-tagged S8 subunit of the 19S regulatory complex of the proteasome shows that TPP II co-precipitates with the 19S regulatory complex (C. Gorbea, B. Tomkinson, M. Rechsteiner, unpublished observation), suggesting that the proteasome and TPP II are physically connected in vivo.

4. Biological function

The widespread distribution and broad substrate specificity suggests that TPP II takes part in the extensive turnover of intracellular proteins (Fig. 2). For example, defective ribosomal products (DRiPs) constitute 25–30% of all proteins synthesized, and they are rapidly recognized and degraded by the ubiquitin/proteasome system. The proteasome can cleave at least 150 peptide bonds per minute, and produces mainly oligopeptides (Princiotta et al., 2003). TPP II could attack these oligopeptides, and cut them into tripeptides which are good substrates for other exopeptidases. This would be an efficient way to increase

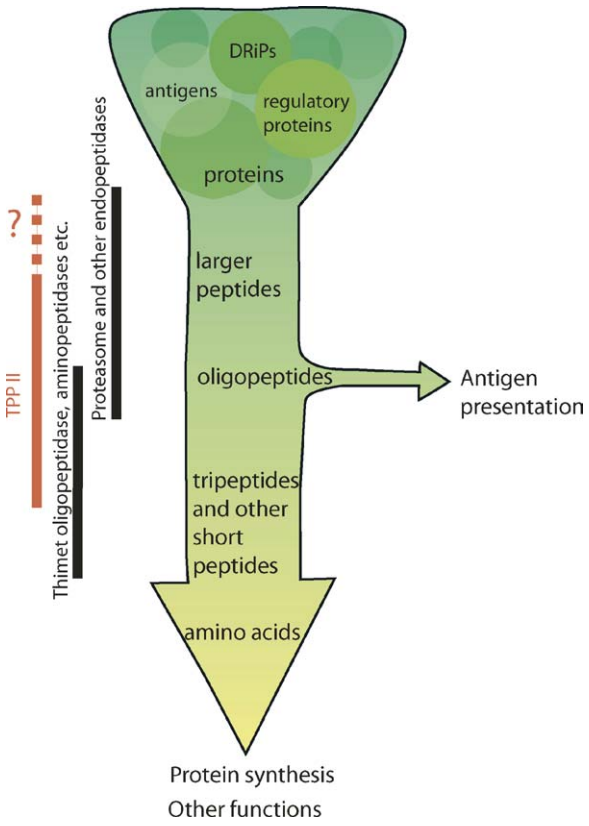

Fig. 2. Illustration of the proposed role of TPP II in cytosolic protein degradation. Pools of proteins are degraded by cytosolic peptidases to produce free amino acids. A small fraction of peptides are "rescued" and used for antigen presentation. DRiPs (defective ribosomal products), potential antigens and some rapidly degraded regulatory proteins (e.g. cyclins) are included in the protein pool. Proteasomes and other endopeptidases initiate degradation, while the role of TPP II in this process remains obscure. TPP II may act downstream of the proteasome and upstream of other exopeptidases. However, the specificities of the enzymes involved overlap and some substrates could cycle between peptidases rather than encountering them sequentially.

the substrate concentration and thereby accelerate the overall rate of degradation (Tomkinson, 1999, 2004) (Fig. 2). Although small peptides can be hydrolysed by many peptidases beside TPP II, the degradation of peptides >15 amino acids is exclusively dependent on TPP II (Reits et al., 2004).

In accordance with the role of TPP II in general protein turnover, it is not surprising that TPP II appears to be involved in the substantial proteolytic degradation which occurs during muscle wasting. It has recently been found that there is an increased expression and activity of TPP II (Wray, Tomkinson, Robb, & Hasselgren, 2002) and increased proteasome activity (Hasselgren, Wray, & Mammen, 2002) in skeletal muscle during sepsis. The changes in TPP II activity correlated with an altered regulation of protein metabolism in different cells during sepsis. Thus, there was increased TPP II activity in catabolic white, fast-twitch muscle, no change in red, slow-twitch muscle and decreased TPP II activity in liver (Wray et al., 2002). During sepsis there is an anabolic response in liver with increased synthesis instead of degradation of proteins. In conformity with the ubiquitin-proteasome pathway, also TPP II expression appears to be regulated by glucocorticoids. The increase in TPP II expression during sepsis was inhibited by RU 38486, a glucocorticoid receptor antagonist (Wray et al., 2002). However, inhibition of the proteasome did not affect the TPP II activity, thereby demonstrating that the increased TPP II activity during sepsis is not a secondary effect of increased proteasome activity (Wray et al., 2002). Clearly, a concomitant up-regulation of these two systems would make a substantial contribution to the enhanced protein breakdown that takes place during this pathological situation.

A role of TPP II in the generation of peptides for antigen presentation by the MHC class I is becoming increasingly clear (recently reviewed by Kloetzel, 2004) (Fig. 2). Its main role appears to be N-terminal trimming of peptides generated by the proteasome (Kloetzel, 2004; Reits et al., 2004). Furthermore, the endopeptidase activity of TPP II can apparently create a specific epitope ($Nef_{73–82}$) of the human immunodeficiency virus (HIV) independently of the proteasome (Seifert et al., 2003). The systems available for intracellular protein degradation are evidently extremely efficient and only about 1 peptide out of 2000 potential targets can actually be presented by the MHC class I system (Princiotta et al., 2003). An intriguing possibility is that the selection of peptides for antigen presentation is more random than previously expected (Rock, York, & Goldberg, 2004). Maybe good epitopes are peptides which are poor substrates for TPP II and other aminopeptidases? For example, the $Nef_{73–82}$ peptide obtained from HIV, QVPLRPMTYK, would not be a good substrate for the exopeptidase activity of TPP II, since it is not expected to cleave the Pro-Leu or Pro-Met bonds in this peptide (Tomkinson, 1999, 2004).

An important question is if TPP II can recognize and degrade specific protein substrates independently of the proteasome. Since TPP II compensates for compromised proteasome activity in cells adapted to a high concentration of proteasome inhibitors (Geier et al., 1999; Wang et al., 2000) the specificity of the two proteolytic systems must overlap to some extent. However, TPP II does not appear to be involved in the degradation of the known proteasomal substrate cyclin D1 (Reits et al., 2004). Furthermore, there have been no reports that TPP II can degrade ubiquitinated proteins, even though ubiquitinated proteins are not accumulated in proteasome-deficient cells (Wang et al., 2000). Nevertheless, in cells with high TPP II activity and low proteasome activity there is an altered specificity of intracellular proteolysis that affect the stability of some proteins (Hong, Lei, & Glas, 2003) and this appears to give the cells a growth advantage. For example, a number of tumor cells have a high TPP II activity (Hong et al., 2003; Stavropoulou et al., 2005), and overexpression of TPP II in HEK 293 cells is also correlated with mitotic infidelity and genetic instability (Stavropoulou et al., 2005). It therefore appears that the degradation of some specific targets is dependent on the relative activity of the different proteolytic systems. The identification of these specific targets remains a challenging task for the future.

5. Possible medical applications

The membrane-associated variant of TPP II has been shown to degrade and inactivate cholecystokinin (Rose et al., 1996), a family of neuropeptides involved in regulation of food intake. In fact, a specific inhibitor of TPP II, butabindide, decreases food intake in rats (Rose et al., 1996). There is therefore an interest in the development of specific inhibitors of TPP II which could

be used for treatment of e.g. overeating syndromes (Breslin et al., 2003). However, in experiments with small interfering RNAs (siRNA), no more than 90% reduction in TPP II expression in Burkitt's lymphoma cells could be achieved, and it was accompanied by a significant slow-down of cell growth (Stavropoulou et al., 2005). Thus, as TPP II appears to be a multi-purpose proteolytic system involved in a number of processes, it could be essential for cell viability and it is important to gain further knowledge about possible targets for the enzyme in order to foresee side-effects of potential drugs.

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