Biochimica et Biophysica Acta 1864 (2016) 283–296

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Intra- and intersubunit changes accompanying thermal activation of the HtrA2(Omi) protease homotrimer

Miroslaw Jarzab [a,1], Tomasz Wenta [a,1], Dorota Zurawa-Janicka [a], Agnieszka Polit [b], Artur J. Gieldon [c], Magdalena Wysocka [c], Przemyslaw Glaza [a], Joanna Skorko-Glonek [a], Jerzy Ciarkowski [c], Adam Lesner [c], Barbara Lipinska [a,*]

[a] Department of Biochemistry, Faculty of Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland
[b] Department of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland
[c] Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-952 Gdansk, Poland

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 26 November 2015
Accepted 14 December 2015
Available online 15 December 2015

Keywords:
Proteolytic enzyme
Allosteric regulation
HtrA2(Omi)
HtrA2 activation cascade
PDZ domain
TrIQ analysis

ABSTRACT

HtrA2(Omi) protease is involved in the maintenance of mitochondrial homeostasis and stimulation of apoptosis as well as in development of cancer and neurodegenerative disorders. The protein is a homotrimer whose subunits comprise serine protease domain (PD) and PDZ regulatory domain. In the basal, inactive state, a tight interdomain interface limits access both to the PDZ peptide (carboxylate) binding site and to the PD catalytic center. The molecular mechanism of activation is not well understood. To further the knowledge of HtrA2 thermal activation we monitored the dynamics of the PDZ–PD interactions during temperature increase using tryptophan-induced quenching (TrIQ) method. The TrIQ results suggested that during activation the PDZ domain changed its position versus PD inside a subunit, including a prominent change affecting the L3 regulatory loop of PD, and also changed its interactions with the PD of the adjacent subunit (PD*), specifically with its L1* regulatory loop containing the active site serine. The α5 helix of PDZ was involved in both, the intra- and intersubunit changes of interactions and thus seems to play an important role in HtrA2 activation. The amino acid substitutions designed to decrease the PDZ interactions with the PD or PD* promoted protease activity at a wide range of temperatures, which supports the conclusions based on the TrIQ analysis. The model presented in this work describes PDZ movement in relation to PD and PD*, resulting in an increased access to the peptide binding and active sites, and conformational changes of the L3 and L1* loops.

1. Introduction

All living organisms possess a complex system of molecular chaperones and proteases to defend a cell against accumulation of misfolded and aggregated proteins. The HtrA (high temperature requirement A) proteases, very well conserved in evolution, play an important role in reduction of the amount of aberrant proteins, by recognizing their exposed hydrophobic stretches and degrading them. They also participate in regulation of many cellular processes by proteolysis of specific target proteins [1–3].

The HtrAs can be distinguished from other serine proteases by sequence homology, and also by the presence of at least one C-terminal PDZ domain (postsynaptic density of 95 kDa, disc large and zonula occludens 1 domain). The protease domain (PD) is of the chymotrypsin type, and is composed of two 6-stranded β-barrels, the N-terminal one with His and Asp of the active site and the C-terminal, with active Ser; the active site triad His-Asp-Ser is located at the interface of these two perpendicularly arranged β-barrels. The β strands are connected by loops which are named, according to the chymotrypsin nomenclature, LA, LB, LC, LD, L1, L2 and L3 [4], and which are important for proteolytic activity and its regulation. The proper organization and interaction between these loops is crucial to the enzymatic activity of the protease (reviewed in [3,5]). The PDZ domains recognize and bind specific hydrophobic sequences in the C-termini of substrates or regulatory peptides and thus participate in the allosteric regulation of HtrA catalytic activity. The common structural unit is a pyramid-shaped homotrimer consisting of protease domains, which form the central core, and outward-protruding PDZ domains. At low temperatures or in the absence of substrate these proteins adopt inactive conformations which, according to the crystal structures, are characterized by the improper

Abbreviations: PD, protease domain; PD*, protease domain of the neighboring subunit; PDZ domain, domain with homology to postsynaptic density of 95 kDa, disc large and zonula occludens 1 proteins; F_w, fluorescence intensity of a labeled protein with tryptophan residue; F_0, fluorescence intensity of a labeled protein without tryptophan residue; τ_w, fluorescence lifetime of a labeled protein with tryptophan residue; τ_0, fluorescence lifetime of a labeled protein without tryptophan residue; mBBr, monobromobimane; T_m, melting point temperature.

* Corresponding author.
E-mail address: barbara.lipinska@biol.ug.edu.pl (B. Lipinska).

[1] The authors contributed equally in this work.

http://dx.doi.org/10.1016/j.bbapap.2015.12.002
1570-9639/

organization of active site residues and/or a restricted access to the catalytic triad. Thus, these proteins must become activated to perform their functions (reviewed in [1–3,5]).

The regulation mechanisms are best understood for those HtrAs whose crystal structures of both, active and inactive forms have been solved, e.g. the *Escherichia coli* HtrA(DegP) [5–9] and DegS [10–16], *Legionella falloni* DegQ [17] and *Arabidopsis thaliana* Deg1 [18]. In these proteases, peptide binding to the allosteric site of the PDZ domain adjacent to the PD causes conformational changes of the L3 sensor loop within a monomer which are then transmitted to the LD*, L2* and L1* activation loops of PD* (the asterisk denotes structural element of a neighboring subunit). However, the activation strategies of the best known HtrA proteins differ in many points, including the PDZ role [3,19,20].

The human HtrA2(Omi) protease under physiological conditions is a mitochondrial protein control factor, important for maintenance of mitochondrial homeostasis while during cellular stress it may be processed (by cleavage of the N-terminal 133 amino acid fragment) and released to cytoplasm, where the mature form functions as a proapoptotic factor. HtrA2 binds and cleaves the inhibitor of apoptosis proteins (IAP), thus promoting activation of caspases and apoptosis (reviewed in [21]). There are many indications that HtrA2 is involved in development of several diseases such as cancer (reviewed in [22,23]), Parkinson's disease (reviewed in [24–26]) and Alzheimer's disease [27–30]. HtrA2 has been proposed as a novel target in therapy of cancer (reviewed in [22,31]) and neurodegenerative disorders (reviewed in [26]). Because of its essential physiological function as well as possible use as a therapeutic target, understanding of the molecular mechanism of HtrA2 action is important.

In the case of HtrA2, crystal structure of its resting, inactive form only has been solved [32] (Fig. 1A of the Results section). The members of catalytic triad His198-Asp228-Ser306 are located in the LB, LC and L1 loops, respectively. The PDZ domain is linked to the protease domain via a flexible linker sequence, whose structure is partially unknown, and has a peptide-binding groove formed by the β13 and β14 strands on one side and α7 helix on the opposite side. The peptide-binding pocket of PDZ domain is buried in the interface between the PDZ and PD domains, and the access to the active site of the protease is restricted by the PDZ domain. The PDZ domain packs against PD through van der Waals contacts involving hydrophobic residues, mainly in two regions: (i) the strands β11 and β12 of the PD C-terminal β-barrel interact with the strand β14 and helix α5 of PDZ, and (ii) the β5–LC–β6 region of the N-terminal β-barrel interacts with the β13 and α7 of PD [32] (best visible in Fig. 1B of the Results section).

Based on the structure of the resting form of HtrA2, and on the fact that HtrA2 with deleted PDZ domain (HtrA2ΔPDZ) is more active than the full-length mature protein, Li et al. [32] developed a model of HtrA2 activation. It predicts that binding of a peptide to the hydrophobic groove of PDZ domain leads to a significant conformational change at the PDZ–PD interface which removes the inhibitory effect of PDZ from the active site, thus enhancing its activity. It has been shown that temperature increase causes a very significant increase of the purified HtrA2 activity [34–36]. As the heat shock-treated HtrA2 could not be further stimulated by activating peptide, and HtrA2ΔPDZ could not be stimulated by heat shock, it has been proposed that both treatments (i. e. peptide binding and temperature increase) might have a similar effect on the structure of HtrA2, resulting in displacement of the PDZ domain from the active site [34]. Since HtrA2 is up-regulated in mammalian cells in response to heat shock-induced stress [37], thermal regulation of its activity may be physiologically important. In spite of the considerable amount of research, including molecular dynamics modeling [38,39], the molecular mechanism of HtrA2 activation is not well understood. Specifically, it is not clear how PDZ domain changes its interactions with PD.

In this study, we aimed at a deeper understanding of molecular events leading to HtrA2 thermal activation with a focus on the PDZ–PD intra- and intersubunit interactions, and the role of the L3, LD and L1 regulatory loops in this process. To achieve this goal, we monitored the dynamics of the PDZ–PD interactions during thermal activation using a tryptophan-induced quenching (TrIQ) method. We

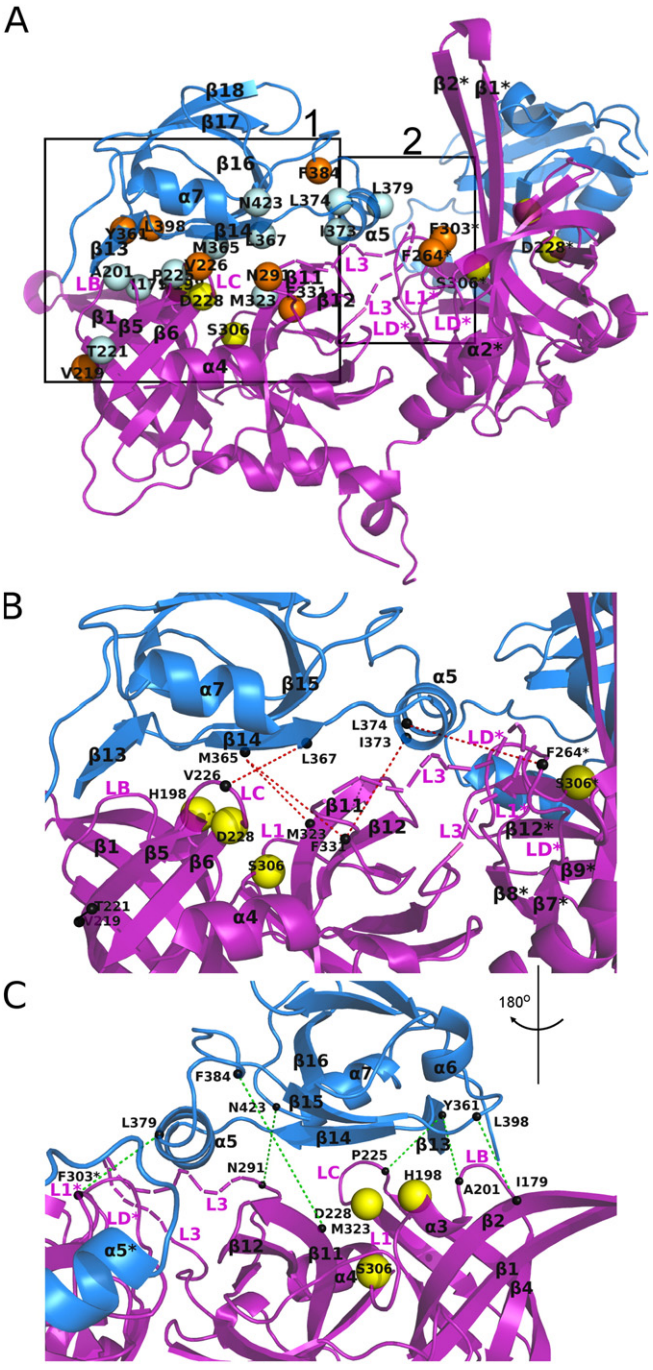

Fig. 1. The changes of distances between the HtrA2 residues located in the PDZ and PD domains during temperature increase, as indicated by TrIQ analysis. In (A) the HtrA2 residues replaced with Trp and Cys are marked in orange and blue, respectively. The PDZ domains are blue, the PDs—purple. The catalytic triad residues (His198, Asp228 and Ser306) are depicted as yellow balls. For clarity, only two subunits of a trimer are shown. The regions of the intrasubunit (1) and intersubunit (2) PDZ–PD interactions are marked by the rectangles. In (B) and (C) the dotted lines indicate respectively a decrease (red) and increase (green) of distance between the residues. The structural elements of the neighboring subunit are marked with asterisks. The (A) and (B) show the side of the trimer opposite to the side where the active sites are located (i.e. the "back" of the trimer); the side with the active sites is shown in (C). The diagrams are based on the HtrA2 crystal structure (PDB: 1LCY) [32]. The modeled part of L3 loop is marked with a dashed line.