BBA - Proteins and Proteomics 1865 (2017) 1141–1151

Contents lists available at ScienceDirect

BBA - Proteins and Proteomics

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

The role of the LB structural loop and its interactions with the PDZ domain of the human HtrA3 protease

Tomasz Wenta[a,1], Przemysław Glaza[a,1], Mirosław Jarząb[a], Urszula Zarzecka[a], Dorota Żurawa-Janicka[a], Adam Lesner[b], Joanna Skórko-Glonek[a], Barbara Lipińska[a,*]

[a] Department of General and Medical Biochemistry, Faculty of Biology, University of Gdansk, Poland
[b] Department of Molecular Biochemistry, Faculty of Chemistry, University of Gdansk, Poland

ARTICLE INFO

Keywords:
HtrA proteins
Human HtrA3 protease
LB loop
Human HtrA3 activity
XIAP proteolysis

ABSTRACT

Human HtrA3 protease is a proapoptotic protein, involved in embryo implantation and oncogenesis. In stress conditions the protease is activated by removal of its N-terminal domain. The activated form, ΔN-HtrA3L is a homotrimer composed of the protease (PD) and PDZ domains. The LB structural loop of the PD is longer by six amino acid residues than its counterparts of other human HtrA proteins and interacts with the PDZ in a way not observed in other known HtrA structures. By size exclusion chromatography of the ΔN-HtrA3L mutated variants we found that removal of the additional LB loop residues caused a complete loss of the proper trimeric structure while impairing their interactions with the PDZ domain decreased the amount of the trimers. This indicates that the LB loop participates in stabilization of the ΔN-HtrA3L oligomer structure and suggests involvement of the LB-PDZ interactions in the stabilization. Removal of the additional LB loop residues impaired the ΔN-HtrA3L activity against the peptide and protein substrates, including the antiapoptotic XIAP protein, while a decrease in the LB-PDZ interaction caused a diminished efficiency of the peptide cleavage. These results indicate that the additional LB residues are important for the ΔN-HtrA3L proteolytic activity. Furthermore, a monomeric form of the ΔN-HtrA3L is proteolytically inactive. In conclusion, our results suggest that the expanded LB loop promotes the ΔN-HtrA3L activity by stabilizing the protease native trimeric structure.

1. Introduction

Human HtrA3 belongs to the family of evolutionarily conserved HtrA (high temperature requirement A) stress proteins widely present in both prokaryotic and eukaryotic organisms. HtrAs are serine proteases which degrade proteins with aberrant structure and also specific native proteins, and thus function as protein quality controllers and regulators of many cellular pathways (reviewed by [1–3]).

The HtrAs are homo-oligomeric proteins whose basal units are trimers which may form higher order oligomeric structures. Their characteristic feature is the presence of a protease domain (PD) with catalytic triad formed by the His, Asp and Ser residues, followed by at least one C-terminal PDZ (PSD-95 (mammalian postsynaptic density protein of 95 kDa), DLG (*Drosophila* disc large tumor suppressor), ZO-1 (zonula occludens 1)) domain. The PD is structurally well conserved and adopts a canonical chymotrypsin-like fold, composed of two six-stranded β-barrels. The β-strands are connected by the loops, named according to the chymotrypsin nomenclature as LA, LB, LC, LD, L1, L2 and L3 [4], which are important for proteolytic activity and its regulation (reviewed by [1,2,5]). The catalytic triad residues are positioned within loops: LB (His), LC (Asp) and L1 (Ser). In their resting state the HtrA proteases are inactive due to an improper conformation of the active center; also, substrate access to catalytic site may be restricted. Their reversible transition to the active state (induced by ligand binding or temperature increase) involves conformational changes of the PD loops and results in formation of the proper geometry of the catalytic site and/or better substrate access. The PDZ domain of HtrA proteases mediates protein-protein interactions; it recognizes and binds substrate or regulatory peptides and thus acts as regulatory module. The HtrAs also possess a variable N-terminal domain, targeting the proteases to specific cellular compartments (reviewed by [1–3,5]).

There are four human HtrAs, HtrA1–4, which play important functions in cellular physiology and are involved in several pathological processes (reviewed in [3,6,7]).

Abbreviations: HtrA, high temperature requirement A; PD, protease domain; PDZ domain, Postsynaptic density protein 95, *Drosophila* disc large tumor suppressor and Zonula occludens-1 protein domain; XIAP, X-linked Inhibitor of Apoptosis Protein

* Corresponding author at: Department of General and Medical Biochemistry, Faculty of Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland.
E-mail address: barbara.lipinska@biol.ug.edu.pl (B. Lipińska).

[1] The authors contributed equally in this work.

http://dx.doi.org/10.1016/j.bbapap.2017.06.013
Received 27 February 2017; Received in revised form 29 May 2017; Accepted 17 June 2017
Available online 20 June 2017

HtrA3 is associated with pregnancy [8,9], involved in embryo implantation and development of placenta [10–12]. HtrA3 is also implicated in oncogenesis [13–18]. It is suggested that HtrA3 functions as a tumor suppressor and is a prospective target in cancer therapy [3,17]. HtrA3 involvement in oncogenesis is connected to its proapoptotic activity. Upon treatment with cytotoxic agents, HtrA3 residing in the mitochondrium is released to the cytosol where it triggers apoptosis in a manner dependent upon its protease activity [17]. The molecular target of this activity is not known. However, since the HtrA1 and HtrA2 promote apoptosis by cleavage of the X-linked inhibitor of apoptosis protein (XIAP) which contributes to activation of caspases [19–22], XIAP is a possible HtrA3 cellular substrate.

HtrA3 has two isoforms: the long 49 kDa one (HtrA3L) and the short 36 kDa (HtrA3S) one, produced by an alternative RNA splicing. HtrA3L consists of a signal secretory peptide at the N-terminus, an N-terminal domain possessing a motif with homology to the insulin-like growth factor binding proteins (IGFBD) and a Kazal-type inhibitor motif (KI), followed by PD with the catalytic triad composed of His191, Asp227 and Ser305, and one C-terminal PDZ domain. In contrast, HtrA3S lacks the PDZ domain [9]. The HtrA3 N-terminal domain is not required for the protease activity [23] and its autocatalytic cleavage occurs upon induction of apoptosis; the cleavage is necessary for mitochondrial to cytoplasmic translocation of the protease and increased cell death [17].

Recently, a crystal structure of the HtrA3 trimer composed of the protease and PDZ domains, deprived of the N-terminal domain (ΔN-HtrA3L) has been solved [24]. The ΔN-HtrA3L is an equivalent of the naturally existing HtrA3 form active in apoptosis [17]. In terms of general architecture it closely resembles the structures of two other human HtrA proteins: HtrA1 and HtrA2 [25–27]. However, delving into the details revealed that there are clearly visible differences. The main structural difference in the ΔN-HtrA3L PD compared to the domain structures of HtrA1 and HtrA2 is the LB loop (residues 189–203), which is six residues longer than its counterparts. Interestingly, these additional six residues are conserved among HtrA3 proteins of various species and not present in other members of the HtrA family (Fig. 1). The elongation of the LB loop allows it to interacts with the PDZ domain forming a unique ring-like structure (Fig. 2A) not found in other HtrAs of known crystal structures. The structure is stabilized by a hydrogen bonds network between the LB loop and the PDZ domain, including five bonds in the 3–3.2 Å bond-distance range. The main-chain nitrogen and oxygen atoms of the loop form multiple hydrogen bonds with the PDZ residues R362, E371, and Q389. The LB loop amino acid residues engaged in the bonding belong to the stretch of the six residues (196–201) unique for the HtrA3 proteins (Fig. 2B,C). Such LB-PDZ interactions are not present in the human HtrA1 [28] and HtrA2 [24,27], and other HtrA proteins of the plant or prokaryotic origin (Table S1).

The aim of this study was to gain insight into the role of the elongated LB loop and its unique interactions with PDZ in stabilization of quaternary structure and proteolytic activity of the ΔN-HtrA3L protease. Towards this aim we constructed a set of ΔN-HtrA3L variants with a deletion of the additional six residues in the LB loop (ΔLB) or with the substitutions of the LB-interacting amino acid residues in the PDZ domain. Size exclusion chromatography (SEC) of the mutated proteins showed that the ΔLB mutation caused a loss of proper trimeric structure while mutations decreasing the LB-PDZ interactions resulted in a decrease of the amount of trimers, suggesting that the LB loop and its interactions with PDZ domain participate in stabilization of the ΔN-HtrA3L trimeric structure. The ΔLB mutation significantly impaired proteolytic activity of the ΔN-HtrA3L towards the peptide and protein substrates, including the potential physiological substrate, XIAP. Kinetic studies with a model peptide substrate showed that the LB-PDZ interactions promoted the peptide cleavage. These results together with the finding that a monomeric form of ΔN-HtrA3L is inactive suggest that the expanded LB loop promotes the ΔN-HtrA3L activity by stabilizing its trimeric structure.

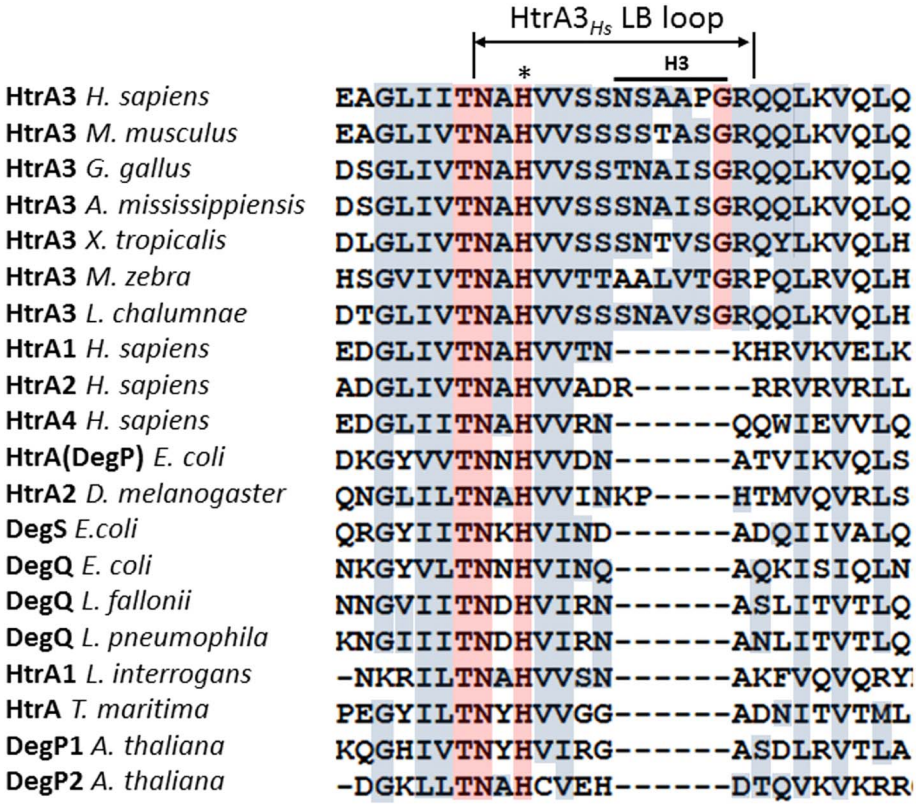

Fig. 1. Comparison of the amino acid sequence of the LB loops of selected HtrA proteases. The alignment of the amino acid sequences of the HtrA proteins was performed using ClustalX2 (http://clustal.org/). The fully conserved residues are marked in red; the residues of similar polarity are marked in blue. The horizontal arrow shows the extent of the LB loop of human HtrA3. The histidines of catalytic triads are indicated by an asterisk (*). The six residues unique for the LB loops of the HtrA3 proteins are marked above the sequences as H3. *H. sapiens - Homo sapiens; M. musculus - Mus musculus; G. gallus - Gallus gallus; A. mississippiensis - Alligator mississippiensis; X. tropicalis - Xenopus tropicalis; M. zebra - Maylandia zebra; L. chalumnae - Latimeria chalumnae; E. coli - Escherichia coli; D. melanogaster - Drosophila melanogaster; L. fallonii - Legionella fallonii; L. pneumophila - Legionella pneumophila; L. interrogans - Leptospira interrogans; T. maritima - Thermotoga maritima; A. thaliana – Arabidopsis thaliana.*