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The homotrimeric structure of HtrA2 is indispensable for executing its serine protease activity

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Abbreviations: HAX-1, HS1-associated protein X-1; HtrA, high temperature requirement A; mnd2, motor neuron degeneration 2; XIAP, X-linked inhibitor of apoptosis protein

Abstract

Serine protease activity of high temperature requrement 2 (HtrA2) is essential for promoting cell death, as well as for protecting against cellular stresses. An X-ray crystallographic study described the formation of a pyramid shaped homotrimer that is a proteolytically competent form of HtrA2; however, little is known about effects of the trimeric structure of HtrA2 on the natural substrates. In this study, we generated the HtrA2 protein that has a single point mutation at the homotrimerization motif to assess relationship between structure and the proteolytic activity of HtrA2 on its substrates. Using gel filtration, a native gel electrophoresis system, and a co-precipitation assay, we confirm that phenylalanine 149 in HtrA2 is a crucial determinant for the formation of the HtrA2 homotrimeric structure. Moreover, we described that the HtrA2 monomeric form abolished not only autoproteolytic activity, but also the proteolytic activity against XIAP (X-linked inhibitor of apoptosis protein) known as the HtrA2 substrate. Taken together, the results indicate that the homotrimeric structure of HtrA2 is required for executing its serine protease activity.

Keywords: Omi serine protease; protein structure, tertiary; serine endopeptidases; structure-activity relationships; X-linked inhibitor of apoptosis protein

Introduction

An *Escherichia coli* (*E. coli*) HtrA (high temperature requirement A, also known as DegP) plays a role as a molecular chaperone at temperatures below 30°C, and its proteolytic activity rapidly increases at temperatures above 30°C (Lipinska *et al*., 1989). HtrA is indispensable for *E. coli* cell survival by controlling its proteolytic activity to reduce the levels of unfolded or aggregated proteins at elevated temperatures (Lipinska *et al*., 1990; Skorko-Glonek *et al*., 1995; Spiess *et al*., 1999; Krojer *et al*., 2002).

The recent crystal structures of two members of the HtrA protein family, human HtrA2 and *E. coli* HtrA, provide insights into their structure-function relationship (Gray *et al*., 2000; Li *et al*., 2002; Maurizi, 2002; Kim and Kim, 2005). A functional HtrA protein forms a hexamer molecule assembled by staggered association of two trimeric rings. Each monomer of HtrA consists of three functionally distinct domains, a conserved catalytic domain of serine proteases and two C-terminal PDZ domains (Faccio *et al*., 2000; Suzuki *et al*., 2001). HtrA2 (also known as Omi), one of human homologues of HtrA, has initially been identified as a stress-activated protease (Schlieker *et al*., 2004). A monomeric structure of mature HtrA2 (amino acid residues 134-458) consists of functionally defined structures, a trimerization motif (aa 146-151), a C-terminal PDZ domain (aa 364-445), and a central serine protease domain (aa 150-343) that contains the His-198, Asp 228, and Ser-306 catalytic triad in its conserved active site (Faccio *et al*., 2000; Gray *et al*., 2000; Suzuki *et al*., 2001; Li *et al*., 2002). Each of serine protease domains in HtrA2 is involved in mediating the formation of a pyramid-shaped homotrimer. The PDZ domains in *E. coli* HtrA appear to facilitate entry of the damaged or denatured proteins into the proteolytic active sites (Maurizi, 2002; Schlieker *et al*., 2004; Kim and Kim, 2005). In contrast, the PDZ domains in HtrA2 are hindered substrate access to the proteolytic active sites, as supported by evi-

dence that deletion of the PDZ domain from HtrA2 relieves inhibition and thus increasing the proteolytic activity (Martins *et al.*, 2003; Gupta *et al*., 2004).

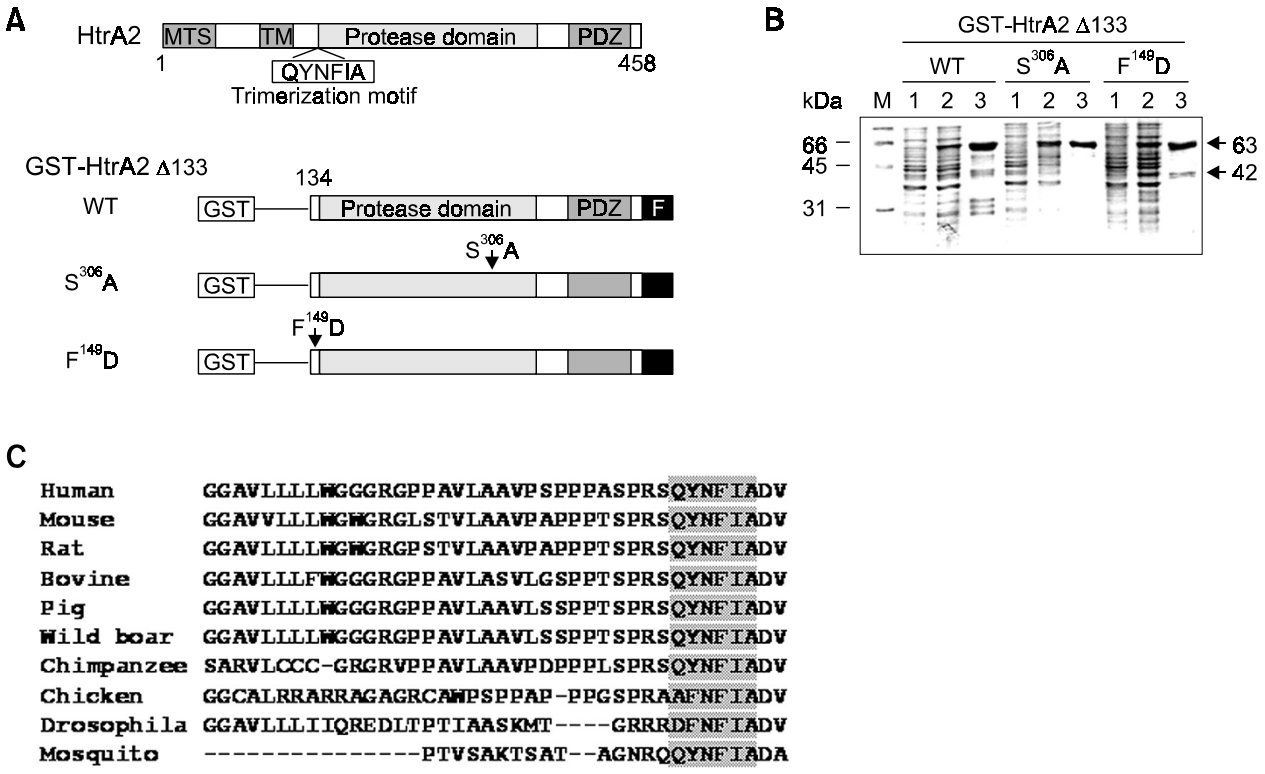
Previous biochemical study exhibited that HtrA2 has proteolytic activity on α-casein, β-casein, hyaluronidase, and glycoprotein α1 acid that were identified by screening 40 proteins (Gray *et al.*, 2000). Unlike bacterial HtrA, several lines of evidences describe a regulatory function of mature HtrA2 in either caspase-dependent or -independent apoptotic cell death (Suzuki *et al.*, 2001; Hegde *et al*., 2002; van Loo *et al.*, 2002; Blink *et al.*, 2004; Suzuki *et al.*, 2004). In conjunction with these results, IAP (inhibitor of apoptosis), apollon, HAX-1 (HS1-associated protein X-1), and PED/PEA-15, known as anti-apoptotic proteins that regulate programmed cell death, were reported as HtrA2 substrates (Srinivasula *et al.*, 2003; Cilenti *et al*., 2004; Trencia *et al.*, 2004; Sekine *et al*., 2005).

Structural and biochemical studies showed that the monomeric HtrA2 form generated by converting phenylalanine 149 to aspartic acid in the trimerization motif disrupted its endoproteolytic activity (Li *et al.*, 2002). To date, however, no in depth studies have examined whether this HtrA2 mutant virtually forms a monomeric structure, and thereby influencing the proteolytic activity on the substrates. Here we showed detailed experimental evidences that phenylalanine at residue 149 is important in mediating trimeric assembly of HtrA2, by using gel filtration and native gel electrophoretic analyses as well as GST- and FLAG-tagged HtrA2 co-precipitation assay. In addition, we demonstrate that homotrimerization of HtrA2 plays a critical role in its serine proteolysis activity.

Materials and Methods

Plasmid construction

The QuickChange Site-Directed Mutagenesis Kit



C

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Human       GGAVLLLLWGGGRGPPAVLAAVPSPPPASPRSQYNFIADV
Mouse       GGAVVLLLWGWGRGLSTVLAAVPAPPPTSPRSQYNFIADV
Rat         GGAVLLLLWGWGRGPSTVLAAVPAPPPTSPRSQYNFIADV
Bovine      GGAVLLLFWGGGRGPPAVLASVLGSPPTSPRSQYNFIADV
Pig         GGAVLLLLWGGGRGPPAVLAAVLSSPPTSPRSQYNFIADV
Wild boar   GGAVLLLLWGGGRGPPAVLAAVLSSPPTSPRSQYNFIADV
Chimpanzee  SARVLCCC-GRGRVPPAVLAAVPDPPPLSPRSQYNFIADV
Chicken     GGCALRRARRAGAGRCAWPSPPAP-PPGSPRAAFNFIADV
Drosophila  GGAVLLLIIQREDLTPTIAASKMT----GRRRDFNFIADV
Mosquito    ---------------PTVSAKTSAT--AGNRQQYNFIADA
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Figure 1. Expression and purification of the GST-HtrA2 proteins (A) Schematic representation of the GST-HtrA2 constructs. Human HtrA2 consists of functional domains: MTS, mitochondrial target sequence (aa 1-40), TM, transmembrane domain (aa 105-123), trimerization motif (aa 146-151), protease domain (aa 150-343), and PDZ domain (aa 364-445). GST-HtrA2 Δ133: wild-type (WT), a catalytic mutant ($S^{306}A$) by replacing the catalytic serine residue with alanine at residue 306, and a structural mutant ($F^{149}D$) by substitution of phenylalanine to aspartate at residue 149 in the trimerization motif. (B) Expression and purification of HtrA2 Δ133 as GST fusion proteins. WT, $S^{306}A$, and $F^{149}D$ expressed in *E. coli* were purified by specific binding to glutathione-Sepharose 4B beads. Proteins were resolved by 15% SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. Lane M is molecular weight standard; lanes 1 shows cell lysates of *E. coli* cultures before IPTG induction; lanes 2 shows cell lysates of *E. coli* cultures after IPTG induction; and lanes 3 shows 63-kDa GST-HtrA2 proteins selectively bound to glutathione-Sepharose 4B beads. (C) Sequence alignment of the trimerization motif (shaded box) of the HtrA2 orthologs.

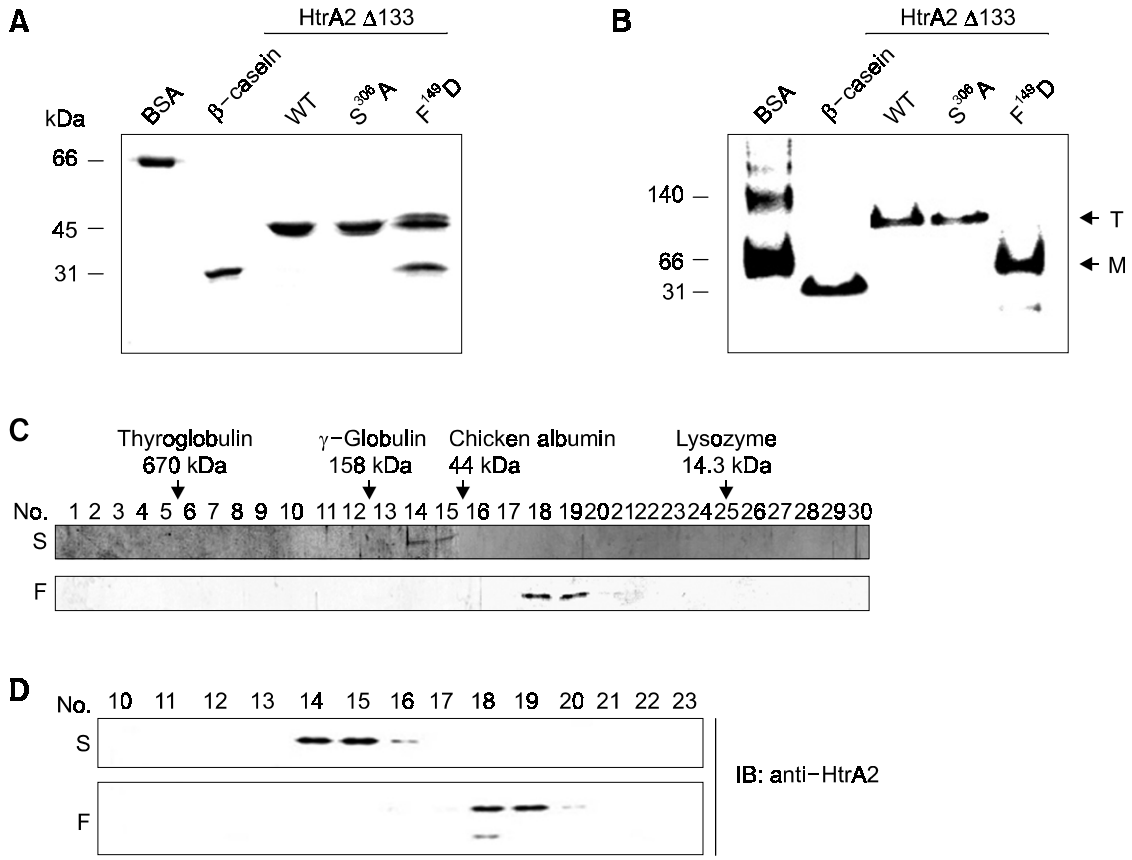


Figure 2. Effect of F^{149} on the homotrimerization of HtrA2. The thrombin cleaved proteins, WT, $S^{306}A$, and $F^{149}D$ were separated on a 15% SDS-polyacrylamide gel (A) and on a 10% native polyacrylamide gel (B) and visualized by staining with Coomassiae Brilliant Blue. T and M indicate the trimeric and monomeric form of HtrA2 Δ133, respectively. (C) Gel filtration analysis of the HtrA2 proteins. The protein samples were filtered through a Superose 12 gel filtration column. Fractions (1-30) were collected and analyzed on a 15% SDS-polyacrylamide gel, and the proteins were visualized by staining with Coomassie Brilliant Blue. (D) Eluted samples in fractions 10-23 were resolved by 15% SDS-PAGE, followed by immunoblot analysis with anti-HtrA2 antibody. No, S, and F indicate numbers of column fractions, $S^{306}A$, and $F^{149}D$, respectively.

(Stratagene, La Jolla, CA) was used according to manufacturer's instructions to generate a monomeric structural mutant ($F^{149}D$), with substitution of aspartic acid for phenylalanine at residue 149. The mutagenic oligonucleotides used in this study are 5'-CGGAGTCAGTACAAC**GA**CATCGCAGATG-3' and 5'-CATCTGCGATG**TC**GTTGTACTGACTCCG-3' (substitution of **TT** to **GA** in bold; TTC and GAC corresponding to phenylalanine and aspartic acid, respectively). For expression of HtrA2 Δ133 as GST fusion in mammalian cells, the HtrA2 (aa 134-358) fragment was removed from the pHtrA2-FLAG (WT) plasmid by digestion with *Bgl*II (5') and *Xho*I (3') restriction enzymes and inserted into the pcDNA3-GST tag plasmid (Lee *et al.*, 2005), designated pcGST-HtrA2 (Figure 2B). Details of all plasmid constructs are available upon request. The sequence integrity of all plasmid constructs was verified by DNA sequencing with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA).

Purification of recombinant HtrA2 expressed in *E. coli*

Expression of GST-HtrA2 Δ133 proteins in BL21 cells was induced with 0.1 mM isopropyl-1-thio-β-D-galactoside (IPTG) as described previously (Seong *et al.*, 2004). The cultures were collected by centrifugation, and the bacterial pellets were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, and 0.5% Nonidet P-40) containing 7 mM dithiothreitol (DTT) and 2 μg/ml of the protease inhibitors, aprotinin and leupeptin. The GST-HtrA2 Δ133 proteins were purified by selective binding to glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech., Piscataway, NJ) under non-denaturing condition and resolved by 15% SDS-PAGE.

For purification of HtrA2, the HtrA2 protein-bound beads were resuspended in 100 μl of cleavage buffer (50 mM Tris-HCl, pH 7.6, 20 mM KCl, and 1 mM DTT) and incubated with 5 U of human thrombin (Amersham Pharmacia Biotech.) for 1 h at room temperature. Protein purity and concentrations were estimated by comparison with BSA of known concentration in 15% SDS-PAGE followed by staining of the gel with Coomassie Brilliant Blue dye. Purified proteins were stored at -70°C in a final glycerol concentration of 20% and 7 mM DTT.

Gel electrophoresis under a non-denaturing condition

Laemmli discontinuos system without SDS was used for a native gel system (Bollag *et al.*, 1996). Protein concentrations were adjusted to 1 μg/ml with loading buffer (12 mM Tris-HCl, pH 6.8, 5% glycerol, 0.02% bromophenol blue), and the proteins were separated by running a 10% native gel for 2 h at 10 mA. The protein bands were detected by staining of the gel with Coomassie Brilliant Blue dye. The electrophoretic mobility of proteins was analyzed by comparison with proteins with known molecular masses (Tanaka *et al.*, 2001).

Gel filtration chromatography

The HtrA2 proteins purified by selective binding to glutathione-4B beads were applied to a Superose 12 HR 10/30 column in a Pharmacia FPLC System. The column was equilibrated and run in PBS at a flow rate of 0.5 ml/min at 25°C, and 400 μl fractions were collected and resolved by 15% SDS-PAGE. The size difference between the monomeric and trimeric HtrA2 was assessed by the elution profile of molecular mass standards on the gel filtration.

Cell culture and transfection

Human embryonic kidney 293 cells (American Type Culture Collection, Manassas, VA), which yield a high transfection efficiency, were maintained at 37°C in a humidified 5% CO_2 incubator in Dulbecco's modified Eagle minimum essential medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen.), 50 U/ml penicillin and 50 μg/ml streptomycin (Invitrogen). For all transfections into 293 cells, the LipofectAMINE reagent (Invitrogen) was used according to the manufacturer's instructions. Cell extracts were prepared at 24 h post-transfection, and the HtrA2 trimeric assembly was analyzed by co-precipitation assay.

Immunoblot (IB) and co-precipitation assays

For co-precipitation, the transfected cells were homogenized in lysis buffer [0.2% digitonin, 20 mM HEPES, 100 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$] containing protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, and 0.1 mM PMSF) and incubated for 1 h on ice. Protein extracts (1 mg) were incubated with glutathione-sepharose 4B beads for 1 h at 4°C. The resulting HtrA2 protein bound beads were resolved by 15% PAGE, immunoblotted with anti-HtrA2 antibody, and then detected with the Enhanced Chemiluminescent (ECL) immunoblotting system as described by the manufacturer (Amersham Pharmacia Biotech.).

Endoproteolytic cleavage assays

For cleavage of β-casein as an exogenous substrate, 10 μM β-casein was incubated with the indicated GST-HtrA2 bound to beads to final concentrations of 100 nM in a total 100 μl of cleavage buffer (50 mM Tris-HCl, pH 7.5, 1 mM DTT) for the indicated time at 37°C. The reaction mixtures were resolved by 15% SDS-PAGE, and the cleavage of β-casein was visualized by staining with Coomassie Brilliant Blue dye.

[^{35}S] Methionine-labeled XIAP and full-length HtrA2 S^{306}A were generated with the TNT T7 coupled reticulocyte lysate system following the manufacturer's instructions (Promega, Madison, WI). Cleavage reactions were initiated by adding wild-type or mutant of HtrA2 Δ133 to a final concentration of 0.5 μM in 30 μl of cleavage buffer (50 mM Tris-HCl, pH 7.5, 1 mM DTT) for the indicated time at 37°C. The reaction products were analyzed by 15% SDS-PAGE, and gels were dried and exposed to X-ray film.

Results and Discussion

Rapid purification of human HtrA2 recombinant proteins expressed in *E. coli* as GST-fusion

To characterize the structure-activity relationship of HtrA2, we used to GST-HtrA2 Δ133 WT, S^{306}A, and F^{149}D constructs (Figure 1A). The recombinant GST-HtrA2 Δ133 proteins expressed from these constructs were purified with glutathione-Sepharose 4B beads, in a rapid method that has been previously described (Figure 1B) (Seong *et al.*, 2004). Most of the GST-HtrA2 Δ133 proteins purified had molecular mass of approximately 63 kDa. Several distinct fragments of lower molecular masses were also detected in WT containing the trimerization motif and the catalytic serine residue; whereas, these fragments were barely detected in both S^{306}A and

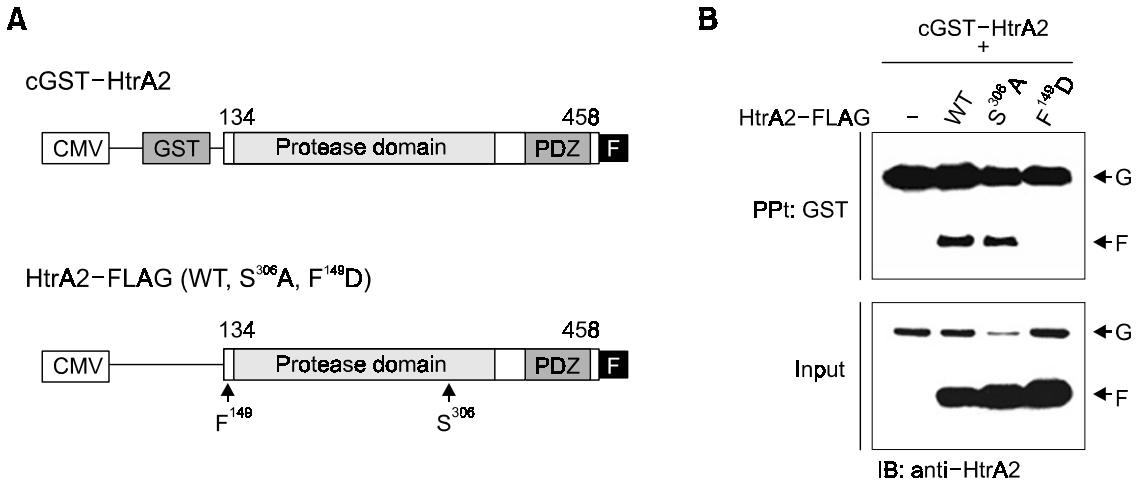


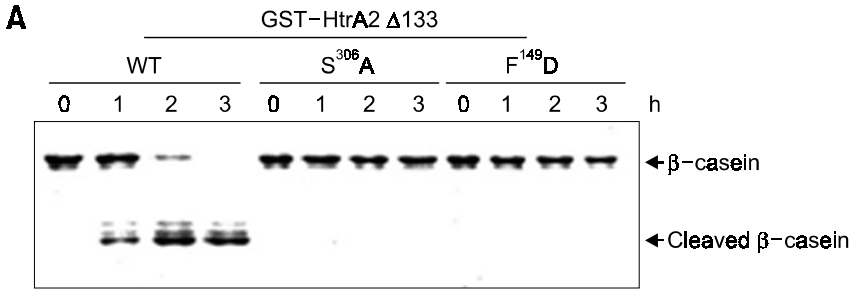
Figure 3. F^{149} is essential for the HtrA2 trimeric assembly *in vivo*. (A) Schematic representation of HtrA2 Δ133 constructs for a mammalian expression system. The cGST-HtrA2 and HtrA2-FLAG constructs were tagged with the GST- or FLAG- epitopes at the N- or C-termini, respectively. (B) The F^{149}D mutant forms a monomric structure. A plasmid encoding the GST-HtrA2 protein was co-transfected into 293 cells with the indicated plasmid encoding the C-terminal FLAG tagged HtrA2, WT, S^{306}A, or F^{149}D. The levels of HtrA2 proteins were determined by IB analyses of whole cell extracts with anti-HtrA2 antibody (input). The GST-HtrA2 protein was precipitated with glutathione-Sepharose 4B beads (PPt), and the resulting precipitates were immunoblotted with anti-HtrA2 antibody (upper panel). G and F indicate GST-HtrA2 and HtrA2-FLAG, respectively.

F^{149}D, in which the catalytic site and the trimerization motif were disrupted, respectively. The result indicates that the phenylalanine residue in the trimerization motif is as important as the catalytic serine residue for self-proteolytic processing activity of HtrA2. Nonetheless, some differently migrating band with molecular mass of approximately 42 kDa appeared in F^{149}D, probably due to the presence of the catalytic triad of serine proteases.

Phe-149 serves as the major determinant of formation of the HtrA2 homotrimer

The trimerization motif, QYN^{149}FIA, is identical in eight mammalian HtrA2 orthologs, and amino acid residues, N^{149}FIA, are conserved in even two invertebrates, *Drosophila* and mosquito (Figure 1C). To investigate role of the conserved phenylalanine 149 in the trimeric assembly of HtrA2, we analyzed the electrophoretic mobility of the HtrA2 proteins in both denaturing and non-denaturing gels (Figure 2). To exclude the possibility of self-oligomeric forms through the GST moiety of the fusions, the thrombin-cleaved HtrA2 proteins were collected by elution from the GST-bound beads and resolved by 15% SDS-PAGE (Figure 2A) and 10% non-denaturing gel (Figure 2B). All three HtrA2 proteins, WT, S^{306}A, and F^{149}D, migrated at an approximate molecular mass of 36 kDa through SDS-PAGE. In contrast, F^{149}D revealed molecular mass close to 30 kDa, and WT and S^{306}A appeared to an apparent molecular mass between those of already known 66- and 140 kDa proteins, in a native gel system. To further analyze the structural difference between S^{306}A and F^{149}D, the thrombin-cleaved HtrA2 proteins were filtered through a Superose 12 FPLC column, and eluted samples were resolved by 15% SDS-PAGE (Figure 2C and D). A significant portion of S^{306}A eluted in a trimer peak, whereas F^{149}D was detected in a monomer peak from gel filtration column, as compared the elution pattern of standard of known molecular mass: thyroglobulin (670 kDa), γ-globulin (158 kDa), chicken albumin (44 kDa), and lysozyme (14.3 kDa). The results indicate that HtrA2 exists as a homotrimeric structure, and this structure is disrupted by altering the trimerization motif of HtrA2.

To verify whether the trimerization motif plays a role in the formation of the HtrA2 homotrimeric complex *in vivo*, N-terminal GST- and C-terminal FLAG- tagged HtrA2 proteins were co-expressed in 293 cells (Figure 3). The cGST-HtrA2 protein was precipitated with glutathione-Sepharose 4B beads, and self-interaction of HtrA2 was analyzed by IB assay with anti-HtrA2 antibody (Figure 3B). Both the 36 kDa WT and S^{306}A proteins co-precipitated along with the 63-kDa cGST-HtrA2 protein; whereas, the 36-kDa F^{149}D protein was not detected. The replacement of aspartic acid residue at phenylalanine 149 in HtrA2 completely abolished the formation of a homotrimer, indicating that phenylalanine is also essential for the homotrimeric structure of HtrA2 *in vivo*.



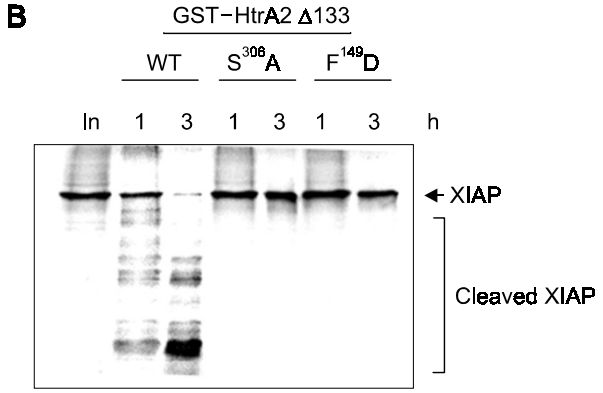


Figure 4. Trimer formation of HtrA2 is indispensable for its serine protease activity. (A) An analysis of endoproteolytic activity of $F^{149}D$. β-Casein was incubated with WT, $S^{306}A$, and $F^{149}D$ for the indicated times at 37°C. The reaction samples were resolved by 15% SDS-PAGE and visualized by staining with coomassie brilliant blue. (B) Effect of the HtrA2 trimer on proteolytic cleavage of XIAP. *In vitro* cleavage reaction, [^{35}S] Met-labeled XIAP was incubated without (In; Input) or with WT, $S^{306}A$, or $F^{149}D$ for the indicated times. The reaction products were analyzed by 15% SDS-PAGE and visualized by autoradiography.

Trimeric structure of HtrA2 is required for its serine protease activity

To assess functional significance of the trimeric form of HtrA2, we compared endoproteolytic activity of WT and $F^{149}D$ by using β-casein as an exogenous substrate (Figure 4A). WT exhibited endoproteolytic activity against β-casein, whereas the endoproteolytic activity of $F^{149}D$ against β-casein was completely abrogated as a comparable level of the catalytically inactive mutant $S^{306}A$. The result demonstrates that phenylalanine 149 is important not only for the formation of a homotrimer, but also for the HtrA2 serine protease activity.

To further analyze the homotrimeric structure of HtrA2 necessary to degrade XIAP as a natural substrate, [^{35}S] Met-labeled XIAP was incubated with WT, $S^{306}A$, or $F^{149}D$ for the indicated time at 37°C (Figure 4B). Several distinct protein fragments were detected only in WT, but not in the proteolytically inactive $S^{306}A$ and the monomeric $F^{149}D$. The results indicate that the formation of the homotrimer is the requisite for the XIAP processing by the serine protease activity of HtrA2.

Trimeric structure of HtrA2 is essential for autocatalytic processing of HtrA2

Our previous study demonstrated that autocatalytic processing of HtrA2 is crucial for induction of apoptosis through the relief of XIAP-mediated caspase inhibition (Seong *et al.*, 2004). We assessed whether the HtrA2 trimeric structure is required for the autocatalytic processing of HtrA2 by using an *in vitro* cleavage assay. Full-length [^{35}S] Met-labeled HtrA2 $S^{306}A$ used as a substrate was incubated with the GST-HtrA2 Δ133 WT, $S^{306}A$, or $F^{149}D$ (Figure 5). We observed distinct fragment with molecular mass of approximately 36 kDa in WT. In contrast, production of the 36 kDa protein fragment was completely abolished in $F^{149}D$ as a comparable level in the catalytically inactive mutant $S^{306}A$, suggesting that the trimeric assembly of HtrA2 is necessary for its

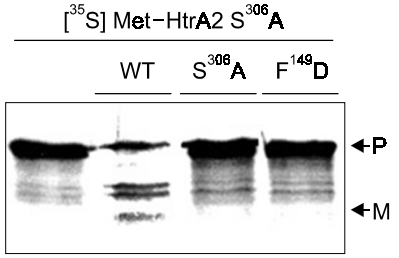


Figure 5. Trimerization of HtrA2 is essential for its autocatalytic processing activity. An [^{35}S] Met-labeled, proteolytically inactive ($S^{306}A$) form of full-length HtrA2 was incubated with WT, $S^{306}A$, or $F^{149}D$ for 1 and 3 h at 37 ºC. Input indicates HtrA2 ($S^{306}A$) only. The reaction mixtures were resolved on 15% SDS-PAGE, the cleavage patterns were visualized by autoradiograph. P and M indicate the precursor and mature form of HtrA2 ($S^{306}A$), respectively.

maturation in an intermolecular manner.

A previous X-ray crystallographic study described that the homotrimeric assembly of HtrA2 observed in the crystal structure is consistent with the structure in solution using gel filtration chromatography (Li *et al*., 2002). Here, we add more experimental evidence for the formation of HtrA2 homotrimer. Moreover, we demonstrate that the HtrA2 homotrimerization is critical in proteolytic cleavage of an anti-apoptotic protein, XIAP, as well as in the HtrA2 autocatalytic processing. We previously showed that production of mature HtrA2 by autocatlytic processing is essential for induction of caspase-dependent cell death through antagonizing XIAP activity (Seong *et al*., 2004). Therefore, the replacement of aspartic acid residue at phenylalanine 149 in HtrA2 eliminated not only production of mature HtrA2 to expose the IAP-binding motif, but also the serine protease activity, suggesting that the HtrA2 trimerization might be crucial for caspase-dependent and -independent cell death (Suzuki *et al.*, 2001; Hegde *et al.*, 2002; van Loo *et al.*, 2002; Blink *et al.*, 2004; Suzuki *et al.*, 2004). Our results would provide insight into understanding the relationship between structure and function of HtrA2, such as proteolytic and pro-apoptotic activities. Further work will be required to elucidate the molecular mechanisms in regulating the trimeric assembly of HtrA2 and in executing the HtrA2 function that is correlated with its structure.

Several lines of recent evidence suggest that HtrA2 might be associated with the pathogenesis of neurodegenerative disorders (Jones *et al.*, 2003; Martins *et al.*, 2004; Strauss *et al.*, 2005). Deletion of HtrA2 showed neurological phenotype with selective loss of striatal neurons (Jones *et al.*, 2003). Also, the missense mutation of the HtrA2 protease domain (S276C; substitution of cysteine for serine at residue 276) has been identified in the *mnd*2 mice (motor neuron degeneration 2), which exhibits progressive loss of striatal neurons, and eventually leading to motor dysfunction (Strauss *et al*., 2005). In addition, the missense mutation (G399S; substitution of serine for glycine at residue 399) has been recently identified in four patients with PD, but not in healthy controls (Martins *et al.*, 2004). Our study may, therefore, provide a basis for understanding the pathophysiological function of HtrA2 involved in neurodegenerative disorders and for developing a new strategy to treat neurodegenerative disorders by regulating the HtrA2 serine protease activity.

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