Binding Specificity and Regulation of the Serine Protease and PDZ Domains of HtrA2/Omi*

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Inhibitor of apoptosis proteins (IAPs) prevent apoptosis through direct inhibition of caspases. The serine protease HtrA2/Omi has an amino-terminal IAP interaction motif like that found in Reaper, which displaces IAPs from caspases, leading to enhanced caspase activity. The cell death-promoting properties of HtrA2/Omi are not only exerted through its capacity to oppose IAP inhibition of caspases but also through its integral serine protease activity. We have used peptide libraries to determine the optimal substrate sequence for cleavage by HtrA2 and also the preferred binding sequence for its PDZ domain. Using these peptides, we show that the PDZ domain of HtrA2/Omi suppresses the proteolytic activity unless it is engaged by a binding partner. Subjecting HtrA2/Omi to heat shock treatment also increases its protease activity. Unexpectedly, binding of X-linked inhibitor of apoptosis protein (XIAP) to the Reaper motif of HtrA2/Omi results in a marked increase in proteolytic activity, suggesting a new role for IAPs. When HtrA2/Omi is released from mitochondria following an apoptotic stimulus, binding to IAPs may switch their function from caspase inhibition to serine protease activation. Thus although IAP overexpression can suppress caspase activation, it could have the opposite effect on HtrA2/Omi-dependent cell death. This, together with the ability of HtrA2/Omi to degrade IAPs, may limit the overall cellular protection that can be provided by these proteins.

Inhibitor of apoptosis proteins (IAPs) antagonize cell death by acting as inhibitors of activated caspases (1). In Drosophila, Reaper family members promote apoptosis by interacting with IAPs through amino-terminal conserved motifs (2). Two mammalian proteins have been found with amino-terminal Reaper-like motifs: Smac/DIABLO and HtrA2/Omi (3–9). They appear to be able to act through a similar mechanism to the fly Reaper proteins by interacting with IAPs through their Reaper motifs, which are critical for the interaction with IAPs.

In mammalian cells, apoptotic stimuli result in the release of Smac and HtrA2 from the intermembrane space of the mitochondria into the cytosol, along with other apoptosis regulatory molecules such as cytochrome c, apoptosis-inducing factor, and endonuclease G (10). Since IAPs are localized in the cytosol, the release of Smac and HtrA2 will result in displacement of IAPs from caspases and loss of their suppressive effect on caspase activity. Despite the general acceptance of this model, the physiological importance of these Reaper motif proteins in the regulation of mammalian cell death is still unclear. For example, deletion of the Smac gene in mice has no detectable phenotype (11). Little else is known about the function of Smac, and clear homologues have not been characterized in invertebrate species.

However, HtrA2 belongs to the HtrA family serine proteases, which is found across eukaryotic and prokaryotic species (12). The Escherichia coli periplasmic enzyme DegP/HtrA has chaperone function at low temperature and protease function at elevated temperature (13). It appears that the enzyme acts to chaperone unfolded proteins, possibly contributing to their refolding, and degrades them only at high temperatures, when the amount of unfolded protein in the cell is greatly increased. HtrA2 is the only member of the family of four mammalian HtrA proteases that has been found in the mitochondria and the only one that has been shown to be processed to reveal a Reaper-like amino-terminal motif. All HtrA family members have one or more PDZ motifs, domains that bind to carboxyl-terminal sequences of partner proteins.

Previous studies (5–8, 14) suggest that interaction with IAPs is not the only way that HtrA2 induces cell death. The serine protease activity of exogenously expressed HtrA2 when localized in the cytosol is sufficient to cause an "atypical" caspase-independent cell death. It is therefore possible that the protease activity of HtrA2 plays a significant, perhaps even dominant, role in its regulation of cell death. In this work, we have characterized the specificity of the HtrA2 protease activity with a view to generating reagents that will enable investigation of the molecular details of the regulation of this enzyme. We have also investigated the specificity of the PDZ domain of HtrA2 and shown that binding of peptides to this region leads to activation of the protease. In addition, binding of XIAP to HtrA2 is unexpectedly shown to activate its protease activity. It appears that although IAPs inhibit the activity of cysteine protease caspases, they have the opposite effect on this serine protease implicated in caspase-independent cell death. The ability of IAPs to block cell death may therefore be limited, even at high levels of expression.
Fig. 1. Determination of optimal cleavage site motif for HtrA2/Omi. A, purification of mature HtrA2/Omi (Δ133HtrA2/Omi-His6). Proteins were resolved by SDS-PAGE and stained with Coomassie Blue. M, markers; I, bacterial lysate; FT, column flow-through; EL, eluted purified HtrA2/Omi. B, zymogram analysis of purified mature HtrA2/Omi. C, cleavage specificity of HtrA2/Omi determined using peptide libraries. Values...
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MATERIALS AND METHODS

Reagents—Recombinant full-length XIAP, Bir domains, and Smac protein were expressed from pET-20b and pET-24b (Novagen) were expressed in BL21(DE3)pLysS (Novagen). The PDZ domain was deleted by removing the carboxyl terminus of HtrA2, amino acids 350–445. Protein expression was induced by culturing cells at 37 °C and GST-PDZ were expressed in human embryonic kidney cells (HEK293) were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Cells were transiently transfected using FuGENE (Roche Applied Science) according to the manufacturer’s instructions. 24 h after transfection, cells were lysed in PBS containing 0.1% Triton X-100, 1 µg/ml each of chymostatin, leupeptin, antipain, and pepstatin A. Cell lysates were incubated in the presence of either anti-HtrA2 antibodies or preimmune serum precoupled to protein A-Sepharose beads. Antigen-antibody complexes were allowed to form at 4 °C for 1 h. Beads were washed three times with PBS and resuspended in tobacco etch virus cleavage buffer containing 10 µM H-2-Opt fluorescent substrate for activity assays.

RESULTS

Determination of the Optimal Peptide Cleavage Site Motif for HtrA2/Omi—To establish the cleavage site motif for HtrA2/Omi, we have employed a recently described methodology involving the use of degenerate peptide libraries. This approach allows for the determination of the preferred amino acid residues both amino-terminal and carboxyl-terminal to the peptide bond cleaved by a given protease. We began by purifying the mature form of HtrA2/Omi from a bacterial expression system (Fig. 1A). Activity of the purified HtrA2/Omi was confirmed by zymogram analysis against casein (Fig. 1B). The only proteolytic activity of HtrA2/Omi that could be detected was toward casein, with the exception of auto-processing of the full-length protein in vitro transcription/translation system (data not shown). We therefore decided to test the activity of the purified enzyme as an endopeptidase toward a peptide containing the sequence in HtrA2/Omi that is auto-processed (VTLCAVPS). We were able to confirm that HtrA2/Omi can cleave the peptide Mca-VTLCAVPS(Dnp)/KK (termed self-cleavage peptide) between cysteine and alanine but not a control peptide (Mca-KPNNLRM(Dnp)) (data not shown).

Since cleavage of the peptide mimicking the auto-processing site was slow, we proceeded to determine the optimal motif for HtrA2/Omi by incubating the enzyme in the presence of a random peptide library mixture. The selectivities determined through this approach are shown in Fig. 1C. The major selectivity at position P1 (immediately amino-terminal to the cleaved peptide bond) is for non-polar aliphatic amino acids, in particular valine, isoleucine, and methionine. At the P2 and P3 positions, arginine is selected most strongly with a secondary activity at position P1 (immediately amino-terminal to the cleavage site). The selection for alanine and serine at P1, P2, and P4 shows that the library does not have a strong bias towards any given amino acid. The relative molar amounts of a given amino acid selected at positions carboxyl-terminal (P1′, P2′, P3′, P4′) and amino-terminal (P1, P2, P3, P4) to the HtrA2/Omi cleavage site and are normalized to an average value of 1. Primed positions were determined using the peptide library Ac-XXX, the P1 position was determined with the library XXXXSSYFPPKK(biotin), and the P2–P4 positions were determined with the library GXXXVSYPFFKK(biotin). D, kinetics of cleavage of HtrA2/Omi optimal peptide motif (H2-Opt, Mca-IRRVSYSF/Dnp/KK) and self-cleavage motif (Mca-AVLAAVPS/Dnp/KK). AFU, arbitrary fluorescence units.

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FIG. 2. Determination and characterization of peptide motifs that interact with the PDZ domain of HtrA2/Omi. A, SDS-PAGE analysis of purified GST and GST-PDZ. Proteins were stained with Coomassie Blue. B, peptide binding selectivity of the HtrA2/Omi PDZ domain. Relative distribution of amino acids selected from the library KNxxxxx by the immobilized PDZ domain of HtrA2/Omi. The positions are...
labeled largely unselective carboxyl-terminal to the P2' position. Based on the information obtained from the digestion of the oriented peptide library mixture, we designed a fluorescent peptide substrate, named H2-Opt, that accommodates optimal HtrA2/Omi amino acid preferences at each position: Mca-IRRV5SYF-KK. Mature HtrA2/Omi cleaves the H2-Opt substrate very efficiently ($k_{cat}/K_m = 1250 \text{ s}^{-1} \text{ M}^{-1}$), even under conditions where activity toward the self-cleavage peptide substrate is undetectable (Fig. 1D). This value was estimated at a substrate concentration (10 µM) that was well below the $K_m$ (estimated at >100 µM).

The PDZ Domain of HtrA2/Omi Regulates Its Proteolytic Activity—To address directly the role of the PDZ domain in the regulation of HtrA2/Omi activity, we first decided to determine the preferred carboxyl-terminal-binding sequence selected by the PDZ domain of HtrA2/Omi (H2-PDZ). Peptides with high affinity toward H2-PDZ fused to GST (Fig. 2A) were purified from a random mixture and sequenced. We found that peptides terminating in the hydrophobic amino acids valine, isoleucine, or alanine were highly represented in the bound peptide pool (Fig. 2B). We observed further selectivity at positions −1, −2, −3, and −5 for the aromatic side chain-containing amino acids phenylalanine and tyrosine. To test the ability of PDZ-interacting peptides to influence the activity of HtrA2/Omi, we preincubated the enzyme in the presence of candidate H2-PDZ-interacting peptides. The optimal HtrA2/Omi PDZ domain-binding peptide, b-GQYYFV-COOH (termed PDZ-Opt), caused a major increase in the reaction rate and loss of the initial lag for cleavage of H2-Opt substrate peptide by HtrA2/Omi. The peptide based on the carboxyl-terminal sequence generated by HtrA2/Omi auto-proteolysis, b-GQYYFV-COOH (termed PDZ-Sub), also activated the protease, but significantly less efficiently than PDZ-Opt. Substitution of the carboxyl-terminal group with an amide residue abrogates this effect (Fig. 2C and D), consistent with requirement for a free COOH terminus in PDZ-binding peptides (16). A protein lacking the PDZ domain, HtrA2/OmiΔPDZ, showed rapid hydrolysis of H2-Opt peptide without a lag phase, confirming that the PDZ domain normally restrains the protease activity of the catalytic site (Fig. 2C and D). None of the peptides used produced an increase in the reaction rate when incubated with HtrA2/OmiΔPDZ (data not shown). These data therefore suggest that the PDZ domain of HtrA2/Omi usually restrains the protease activity, possibly through restricting access to the catalytic site. Binding of peptides to the PDZ domain results in a conformational change that improves substrate access to the proteolytic site. Since we are unable to measure the $K_m$ due to its high value, we cannot conclude whether these effects are on the $k_{cat}$ or $K_m$ of the enzyme.

Heat Shock Activation of HtrA2/Omi—The proteolytic activity of bacterial HtrA (DegP) was shown previously to increase rapidly upon a rise in temperature from 30 to 42 °C (13). We were interested to determine whether the same is true for HtrA2/Omi, and if so, whether heat shock led to alteration of the ability of the PDZ domain to suppress the activity of the protease domain. We observed that preincubation of HtrA2/Omi at 42 °C for 30 min resulted in increased proteolytic activity toward H2-Opt (measured after return to 30 °C) and loss of the initial lag phase in substrate cleavage. We could not observe a similar effect using the enzyme lacking the PDZ domain (Fig. 3A and C). This truncated enzyme had increased activity relative to full-length HtrA2/Omi as discussed above but was actually inhibited by heat shock, possibly reflecting some denaturation of the catalytic site.

It is possible that elevated temperature activates the protease by promoting a rearrangement of the PDZ domain, so we determined whether the PDZ-Opt peptide was able to activate HtrA2/Omi further following heat shock. Our results show that the presence of PDZ-Opt peptide is incapable of promoting further activation of heat-shocked HtrA2/Omi (Fig. 3B and D). The addition of PDZ-Opt, resulting in peptide engagement of the PDZ domain, caused the same degree of activation of HtrA2/Omi protease activity as did heat shock. It is possible that the two treatments have a similar effect on the structure of HtrA2/Omi, resulting in displacement of the PDZ domain from the active site. On the other hand, it is also a possibility that the heat-treated enzyme may no longer be able to bind PDZ-Opt peptide. Heat shock could conceivably lead to selective unfolding of the PDZ domain, resulting in it being unable either to bind PDZ-Opt peptides or to interfere with the catalytic site.

Interaction with XIAP Activates HtrA2/Omi Protease—Upon translocation to the cytosol following mitochondrial damage due to apoptotic stress, HtrA2/Omi binds to BIR domains of IAPs through its amino-terminal Reaper-like motif. Previous reports indicated that IAPs had little or no effect on the activity of HtrA2/Omi toward the substrate β-casein (5, 7). Since bovine β-casein has a carboxyl-terminal sequence similar to a putative PDZ-interacting peptide (FPIIV, which fits the PDZ-binding consensus better than IRRV), we hypothesized that this carboxyl-terminal sequence could lead to HtrA2/Omi activation masking any effect of other activators. We therefore decided to address the effect of XIAP on the cleavage of H2-Opt peptide by HtrA2/Omi. We observed a dramatic increase in the proteolytic reaction rate when XIAP was incubated with HtrA2/Omi (Fig. 4, A and C). Preincubation of XIAP with either Smac or peptides mimicking the amino-terminal Reaper-like motif of processed HtrA2/Omi reversed the effect of XIAP on HtrA2/Omi. As a control, a peptide with a substitution in the amino-terminal alanine critical for interaction with XIAP (6) failed to abrogate the effect of XIAP on HtrA2/Omi. This indicates that interaction of XIAP with the amino-terminal Reaper motif of HtrA2/Omi results in the activation of the proteolytic activity of the enzyme.

It was recently reported that HtrA2 is able to cleave XIAP in vitro (18, 19). It is therefore possible that XIAP is cleaved by HtrA2 to generate fragments that could interact with the PDZ domain of HtrA2/Omi and activate the protease in this manner. We observed that XIAP was unable to alter the activity of HtrA2/Omi lacking the PDZ domain (Fig. 4, B and D). The activity of this truncated enzyme is already maximal, so XIAP binding may be unable to promote it further. Alternatively, some cooperativity might occur such that XIAP interaction with HtrA2/Omi amino-terminal Reaper-like motif may be needed to promote a weaker interaction of the carboxyl-terminal sequence of XIAP with the PDZ domain, resulting in activation of the protease.

We therefore decided to examine the state of XIAP following incubation with HtrA2/Omi. Under the conditions used in these experiments, we were unable to detect the appearance of any 

numbered from the carboxyl terminus (position 0). Data were normalized as in Fig. 1. The table summarizes the most selected amino acids at each position. Selectivity values are given in parentheses. C, effect of PDZ-interacting peptides on the kinetics of H2-Opt cleavage by HtrA2/Omi and comparison with HtrA2/OmiΔPDZ. PDZ-interacting peptides, PDZ-Sub (biotin-GGIRRV-COOH), PDZ-sub-amide (biotin-GGIRRV-NH$_2$), and PDZ-Opt (biotin-GQYYFV-COOH), were preincubated with HtrA2/Omi for 1 h at room temperature before the addition of fluorescent substrate. Control was performed with an equivalent concentration of vehicle (Me$_2$SO). AFU, arbitrary fluorescence units. D, comparison of the reaction rates of HtrA2/Omi following incubation with PDZ-interacting peptides.
FIG. 3. Effect of heat shock on the activity of HtrA2/Omi and HtrA2/OmiPDZ. **A and C,** effect of heat shock (HS) on the kinetics of H2-Opt cleavage by HtrA2/Omi and HtrA2/OmiΔPDZ. HtrA2/Omi and HtrA2/OmiΔPDZ were preincubated at 42 °C for 30 min before the addition of fluorescent substrate peptide. AFU, arbitrary fluorescence units. **B and D,** effect of PDZ-interacting peptides (500 μM) on the kinetics of H2-Opt cleavage by heat-shocked (42 °C for 30 min) HtrA2/Omi and HtrA2/OmiΔPDZ.
XIAP cleavage fragments following incubation with full-length HtrA2/Omi (Fig. 4E). It is therefore unlikely that the activation of HtrA2 seen here is due to interaction of its PDZ domain with XIAP fragments generated following proteolysis, especially since the activation can be seen at similar molar concentrations of XIAP and HtrA2/Omi. The HtrA2/Omi protein was capable of cleaving XIAP in this system, but only when activated by PDZ domain deletion or PDZ-Opt peptide binding (data not shown). To explore this further, we tested whether either BIR2 or BIR3 domains of XIAP, which lack carboxy-terminal sequences that might be able to interact with HtrA2/Omi PDZ domain, are sufficient to activate HtrA2/Omi. Our results show that individual BIR2 or BIR3 domains are sufficient to promote the activation of HtrA2/Omi (Fig. 4, F and G). The ability of XIAP to promote the protease activity of HtrA2/Omi is thus most likely due to a conformational effect caused by engagement of the amino-terminal Reaper-like motif and not due to direct engagement of the PDZ domain. To determine whether the mechanism of HtrA2 activation via heating versus XIAP binding was different, we assessed whether they could synergize. We observed that incubation of XIAP with heat-shocked HtrA2 did not lead to an activation greater that that achieved at the standard reaction temperature of 30 °C (data not shown). We therefore propose that the activation mechanisms are likely to be similar.

The Activity of Cellular HtrA2/Omi Can Be Modulated by XIAP—To study whether XIAP can modulate the activity of endogenous cellular HtrA2/Omi, we decided to measure the effect of XIAP overexpression following transfection on the catalytic activity of HtrA2/Omi. Since other contaminating cellular proteolytic activities prevented us from assaying cell lysates directly, we immunoprecipitated the endogenous HtrA2/Omi prior to analysis of proteolytic activity in immunocomplexes. When looking at the activity of endogenous HtrA2/Omi, we observed that increased levels of XIAP expressed in HEK293 cells led to increased proteolytic activity (Fig. 5A). The amount of HtrA2/Omi protein present in the immunocomplexes was not affected by XIAP overexpression (data not shown).

DISCUSSION

The work that we report here was initiated to characterize the primary sequence specificity of the protease activity of HtrA2/Omi. One aim of this was to allow the development of a robust assay for HtrA2/Omi protease. This may have two benefits. Firstly, it would allow the design of high throughput screening approaches for the discovery of inhibitors of the protease. The use of a fluorogenic optimal substrate peptide along with recombinant HtrA2/Omi (Fig. 1) lays the basis for such an assay. Secondly, it would enable better study of the regulation, and ultimately function, of HtrA2/Omi. Despite the interest the mammalian enzyme has attracted since its interaction with IAPs was reported, its cellular function remains unclear.

The primary sequence specificity determined here for HtrA2/Omi indicates that the protease favors cleavage following aliphatic residues, similar to bacterial HtrA. Secondary selections include basic residues at the P2 and P3 positions as well as small non-polar residues at P1’ and aromatic residues at P2’. It is likely that this specificity at least partly determines the choice of sites selected by HtrA2/Omi within a target protein. However, the selection of target proteins may also be dependent on which proteins are bound by the PDZ domain of HtrA2/Omi and thus brought into proximity with the activated protease domain. Use of a similar peptide library approach allowed determination of the binding selectivity of the PDZ domain of HtrA2/Omi. This was shown to favor a carboxy-terminal valine or isoleucine, preceded by three residues rich in tyrosine and phenylalanine or large aliphatic groups.

Binding of a peptide, such as PDZ-Opt, to the PDZ domain of HtrA2/Omi stimulates its protease activity toward an optimal peptide substrate, H2-Opt (Fig. 2). In the x-ray crystal structure of the HtrA2/Omi trimer (14), the PDZ domains pack in such a way as to restrict access to the protease domains. A loop from the catalytic domain interacts with the peptide-binding groove in the PDZ domain via a non-canonical interaction, suggesting that there may be some sort of conformational change upon canonical engagement of the PDZ domain by a binding partner. Deletion of the PDZ domain of HtrA2/Omi results in increased protease activity against casein (14), a result that we also show here with peptide substrates. However, Li et al. (14) also found that mutation of residues at the interface of the PDZ and protease domains actually decreased protease activity, suggesting that the interaction of PDZ domain with the protease domain had a complex effect on activity. Based on the data of Li et al. (14) and what we report here, we suggest that the PDZ domains in the trimer of HtrA2/Omi normally act to keep the protease activity in check, limiting access to the catalytic site. Engagement of binding partners with the PDZ domains results in opening up of access to the catalytic site (Fig. 5E). Since short peptides binding to the PDZ domain can achieve this, it is likely that occupation of this binding site triggers a conformational change that results in realignment of the PDZ domains relative to the protease domains. In this scheme, binding of target proteins by the PDZ domain would also result in up-regulation of the protease activity of HtrA2/Omi toward the bound substrate. We would also speculate that the interaction of the PDZ with partner proteins was a major determinant of HtrA2/Omi protease specificity and that mutant forms lacking the PDZ were significantly more promiscuous in their target selection, as well as being more active.

The normal activity of HtrA2/Omi within the mitochondria of healthy cells is still not known. Comparison of the structure of mammalian HtrA2/Omi and bacterial DegP/HtrA has led to the suggestion that, unlike DegP/HtrA, HtrA2/Omi lacks chaperone activity (12, 14, 21). It may function normally to degrade misfolded proteins within the mitochondria or may have a more selective processing function. The protease activity of DegP/HtrA is induced at elevated temperatures (13); similarly, we show here that the protease activity of HtrA2/Omi is increased following transient exposure to heat shock (Fig. 3). Since this does not happen with HtrA2/Omi lacking the PDZ domain, it is plausible that this occurs as a result of displacement of the PDZ domain to improve access to the catalytic site, perhaps involving denaturation of the PDZ domain (Fig. 5B). HtrA2/Omi may play a significant role in removing denatured proteins from the mitochondria following exposure to heat shock or other stresses.

In addition to being activated by engagement of the PDZ domain and by heat shock, the protease activity of HtrA2/Omi is also stimulated by binding to XIAP. This requires interaction between the BIR domains of XIAP and the Reaper motif at the amino-terminal of HtrA2/Omi (Fig. 4). It does not appear that the engagement of the PDZ domain by the carboxy-terminal of XIAP is involved as isolated BIR2 and BIR3 domains with distinct carboxy-terminals lacking PDZ-binding residues have the same effect. In addition, despite the fact that HtrA2/Omi has been reported to degrade XIAP (18, 19), in the system described here, the activation of HtrA2/Omi by XIAP can occur under conditions in which no XIAP degradation is detectable, ruling out the possibility that the activation is caused by PDZ-binding peptides generated from XIAP. It is therefore likely that XIAP activates the protease activity in a distinct manner to that of PDZ-binding proteins, with a conformational change.
FIG. 4. Effect of XIAP on the activity of HtrA2/Omi. A and C, effect of XIAP and XIAP-interacting molecules on the kinetics of H2-Opt cleavage by HtrA2/Omi. HtrA2/Omi was incubated in the presence of the indicated molecules for 30 min at room temperature followed by the addition of H2-Opt. BSA, bovine serum albumin; AFU, arbitrary fluorescence units. B and D, effect of XIAP and XIAP-interacting molecules on the kinetics of H2-Opt cleavage by HtrA2/Omi/H9004 PDZ. HtrA2/Omi was incubated in the presence of the indicated molecules for 30 min at room temperature followed by the addition of H2-Opt. E, lack of cleavage of XIAP by HtrA2/Omi under the conditions used for the protease assays. XIAP was incubated in the presence of HtrA2/Omi and subjected to immunoblot analysis. F and G, effect of XIAP BIR2 and BIR3 on the kinetics of H2-Opt cleavage by HtrA2/Omi. HtrA2/Omi was incubated in the presence of the indicated molecules at the concentrations stated for 30 min at room temperature followed by the addition of H2-Opt.
induced from the amino-terminal, rather than the carboxyl-terminal, of HtrA2/Omi (Fig. 5B). The XIAP and PDZ-binding peptide-induced activation of HtrA2/Omi activity is non-additive, suggesting that the final conformational changes induced by each pathway are functionally similar, although the mechanism by which this might be achieved is unclear. It is possible that bind-
ing of XIAP to HtrA2/Omi may alter its substrate specificity by removing the requirement that the PDZ domain is engaged; thus, after interaction with XIAP, HtrA2/Omi may be able to proteolyze targets that are not substrates for PDZ binding.

The biological implications of the reported activation of HtrA2/Omi by XIAP could be significant. IAPs act to suppress the activation of caspases following release of cytochrome c from mitochondria and activation of the apoptosome (1). It has been proposed that the Reaper motifs of Smac and HtrA2/Omi act to suppress this function, although definitive proof of the physiological significance of this in the mammalian system is still lacking (3–8). A balance may therefore exist between IAPs blocking caspase activity and Reaper motifs on Smac and HtrA2/Omi relieving this and allowing caspase activation. This
would suggest that overexpression of IAPs would provide a very effective mechanism for the suppression of cell death and that this might be expected to be a common event in human tumors. However, although IAP overexpression has been reported in certain tumors (22, 23), extensive data implicating IAP overexpression in the majority of cancers have not been forthcoming. IAPs have not been found as naturally occurring oncogenes, even when defined in the loosest of senses.

The fact that XIAP, and possibly other IAPs, activate the protease activity of HtrA2/Omi indicates that there may be a down side to IAP overexpression. Although caspase activity is suppressed, HtrA2/Omi protease activity could be up-regulated in cells overexpressing IAPs on subject to an apoptotic stimulus. IAP overexpressing cells may therefore be subject to higher than normal levels of HtrA2/Omi serine protease activity released into the cytosol from the mitochondria following an apoptotic stimulus. In addition, this activity may have relaxed substrate specificity relative to that in the mitochondria as a result of loss of requirement for PDZ domain binding by substrate. Since HtrA2/Omi can also degrade IAPs, at least under some conditions (18, 19), the ability of IAP overexpression to suppress cell death and caspase-independent cell death following release from mitochondria. In addition to its various possible evolutionarily ancient roles as chaperone and heat shock-induced protease, in mammals HtrA2/Omi may act as a limiter of the ability of IAPs to prevent cell death, even when overexpressed. The full physiological significance of this may require the study of animals lacking HtrA2/Omi and overexpressing IAPs.

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