ELSEVIER

Biophysical Chemistry 101–102 (2002) 145–153

Biophysical Chemistry

www.elsevier.com/locate/bpc

Structure and zymogen activation of caspases

Mrudula Donepudi, Markus G. Grütter*

Institute of Biochemistry, University of Zürich, Winterthurerstr. 190, 8057 Zurich, Switzerland

Abstract

Apoptosis is primarily executed by active caspases, which are derived from the inactive zymogens. Structural and biochemical studies of caspases-1, -3, -7, -8 and -9 have greatly enhanced our understanding of the structure, function, and specificity of the active form of these enzymes. Only recently, the structures of procaspase-7 and biochemical studies of procaspase-9 and -8 have provided insight into the process of procaspase activation. The mechanism of zymogen activation requires limited proteolysis as for many other proteases. In addition, self-activation through oligomerization has been demonstrated for the initiator caspases-8, -9 and -10. These studies provide a structural mechanism for caspase activation, substrate/inhibitor binding, and contribute to the understanding of the biological role of caspases in the processes of apoptosis.

Keywords: Caspases; Inhibitor binding; Structure comparison; X-ray structure; Apoptosis; Zymogen activation

1. Structural overview of active caspases

Programmed cell death (apoptosis) is an essential mechanism in the development and homeostasis of multicellular organisms to eliminate unwanted cells [1,2]. Dys-regulated cell death is implicated in a growing number of clinical disorders. Excessive apoptosis can lead to ischemic damage and neurodegenerative diseases whereas conditions such as cancer or autoimmune diseases result from insufficient apoptosis [3].

Whilst controlled cell death pathways still need to be fully investigated, biochemical and genetic have established that caspases (cysteine aspartatic acid proteases) play an essential role at various stages of the apoptotic process [4–6]. The highly regulated apoptotic process involves an intricate cascade of events (Fig. 1a). Currently two major extrinsic pathways are known, which involve apical caspases; one of the extrinsic pathways relies on a cell surface stimulus. Here, the death signal is transmitted through binding of an extracellular death ligand such as tumor necrosis factor (TNF) to its cognate receptor, the TNF receptor. For both ligands and receptors alike, a number of different homologous proteins are already known, which act on cells that must die during tissue development, immunological development, and in response to viral infection. The death receptors transmit signals to the interior of the cells, where the apical proteases of the extrinsic pathway, caspases-2, -8 and -10 are recruited [7]. The other pathway occurs

*Corresponding author. Tel.: +41-1-635-5580; fax: +41-1-635-6834.

E-mail address: gruetter@bioc.unizh.ch (M.G. Grütter).

0301-4622/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0301-4622(02)00151-5

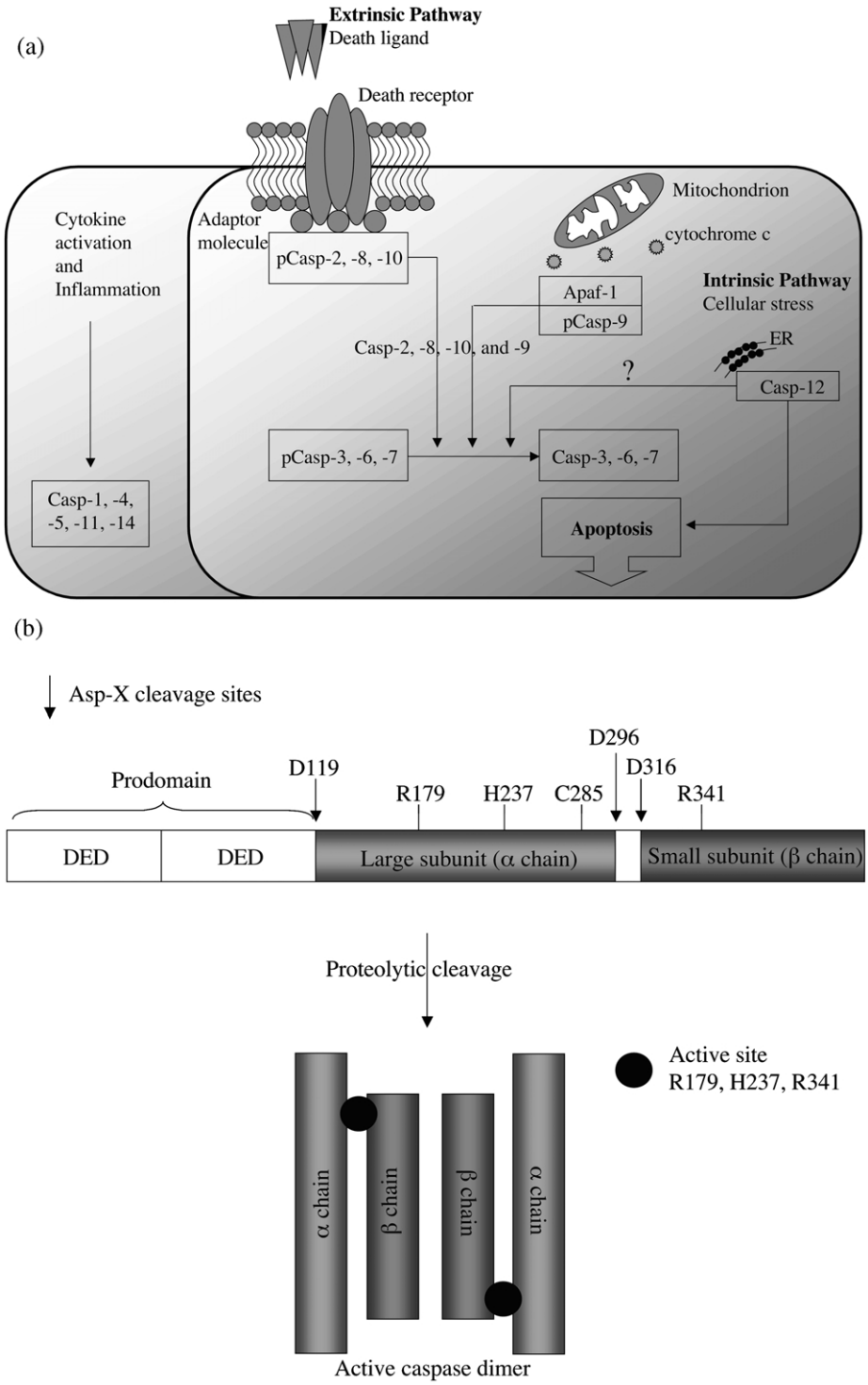
(a)
Extrinsic Pathway
Death ligand
Death receptor
Mitochondrion
cytochrome c
Cytokine activation and Inflammation
Adaptor molecule
pCasp-2, -8, -10
Apaf-1
pCasp-9
Intrinsic Pathway
Cellular stress
Casp-2, -8, -10, and -9
ER
?
Casp-12
pCasp-3, -6, -7
Casp-3, -6, -7
Casp-1, -4, -5, -11, -14
Apoptosis
(b)
Asp-X cleavage sites
D296
Prodomain
D119
D316
R179
H237
C285
R341
DED
DED
Large subunit (α chain)
Small subunit (β chain)
Proteolytic cleavage
Active site
R179, H237, R341
α chain
β chain
β chain
α chain
Active caspase dimer

Fig. 1.

as a consequence of cellular stress, and is mediated by cytochrome *c* in the mitochondrion, which leads to caspase-9 activation [8]. Recently, a third, stress-induced apoptotic pathway via the endoplasmic reticulum was discovered in murine cells where caspase-12 is involved [9]. All these pathways lead to the activation of downstream executioner caspases, which activate or inactivate their specific cellular protein targets via limited proteolysis (Fig. 1a).

Based on phylogenetic analyses, caspases have been classified into two subfamilies: the interleukin-1β converting enzyme- (ICE-) and the *Caenorhabditis elegans* protein-like (CED-3-like) family, for which the prototypes are ICE (caspase-1) and CED-3 [10]. To date, 14 mammalian caspase sequences have been reported, of which 11 are of human origin and 3 of murine origin (reviewed in [4]).

All caspases recognize tetrapeptidic sequences, with caspase-2 being the exception, and have a stringent specificity for cleaving C-terminally of aspartic acid residues (Fig. 1b). Downstream caspases are targeted and cleaved in a specific fashion, enabling cellular disassembly, which is characteristic of apoptosis [11,12].

The global fold, the topology, and the quarternary structure of caspases define a new family of cysteine proteases. Three-dimensional X-ray crystallographic structures are available for caspase-1 [13,14], caspase-3 [15,16], caspase-8 [17,18], caspase-7 [19], and caspase-9 [20]. The caspase fold consists of a large, α subunit and a small, β subunit (Fig. 2). Each α/β subunit comprises six

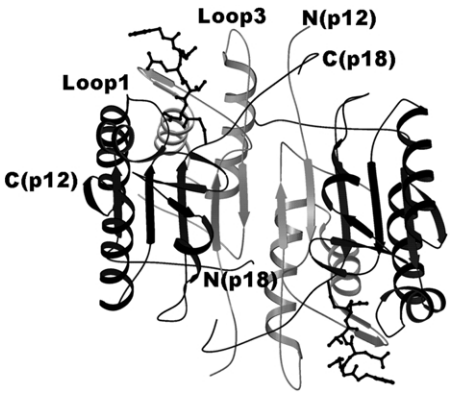

Fig. 2. Ribbon diagram of the caspase-fold represented by the caspase-8–Z-DEVD-CHO complex (1F9E.pdb) [30,31]. This figure was generated using the program SETOR [32].

β-strands, five parallel (a, b, c, d, e), and one antiparallel (f), which form a twisted β-sheet structure with two α-helices (H2, H3) on one side, and three α-helices (H1, H4, H5) on the other, running approximately parallel to the β-strands. The active site cavity is located at the C-terminal end of the parallel β-strands within each α/β heterodimer (Fig. 2). Two heterodimers associate and are related by a twofold axis. The entire quarternary arrangement can be described as an $(\alpha/\beta)_2$ tetramer, composed of two symmetry-related α/β heterodimers, each representing one folding unit (Fig. 2). Substrate recognition occurs in the active site cleft formed by loop regions from both the α and the β subunit. The cleft recognizes a tetrapeptide N-terminal of the canonical cleavage site Asp-X (Fig. 2). This review focuses on struc-

Fig. 1. (a) Schematic representation of the known apoptotic pathways, in which apical caspases are involved. In the death receptor-induced extrinsic pathway, the apoptotic signal is initiated by direct ligand-mediated trimerization of death receptors at the cell surface. This leads to the recruitment of adaptor proteins inside the cell, which is followed by the activation of the initiator caspases-2, -8 or -10. The executioner caspases-3, -6 -7 are cleaved and activated, which leads to the limited proteolysis events that are typical of programmed cell death. Irreparable genomic damage caused by mutagens, pharmaceuticals, or ionizing radiation, activates the intrinsic pathway in which cytochrome *c* is released from the mitochondria. This event triggers the formation of a complex between procaspase-9, Apaf-1, and cytochrome *c*. Caspase-9 subsequently activates the downstream caspases-3, -7, and possibly -6. Alternatively, procaspase-12, located in the ER, can be activated in the presence of Ca^{2+}. This leads to the activation of executioner caspases or to the limited proteolysis of substrates. The cytokine pathway leading to inflammation is activated by caspase-1. Caspases-4, -5, -11 and -14 are also involved in inflammatory processes. (b) Schematic representation of the proteolytic activation process of caspases. Caspases are synthesized as single chain precursors. Activation proceeds by cleavage of the N-terminal domain at Asp119, Asp296, and Asp316 (all caspase-1 numbering convention) leading to a large, α, and a small, β, subunit. The activity and specificity determining residues, R179, H237, C285 and R341 are brought into the necessary structural arrangement for catalysis. C285 is the catalytic nucleophile, H237 represents the general base. The crystal structures reveal that the active enzyme is a dimer in which one α/β unit that harbors the active site is related by a twofold axis with a second unit to form the active $(\alpha/\beta)_2$ dimer.

tural and functional research related to caspases within the last year. The recently published structures of procaspase-7 and studies regarding activation of procaspase-9 will specifically be reviewed. These experiments provide a preliminary picture of zymogen activation in caspases and further our understanding of the biological role of these proteolytic enzymes in programmed cell death.

2. Zymogen activation

Caspases are produced as inactive zymogens, which are activated by specific proteolytic cleavage. Initiator caspases activate downstream caspases-3, -6 and -7. However, the activation process for apical proteases is difficult to rationalize. Procaspase-8 possesses limited catalytic activity, which is sufficient to allow intramolecular processing of zymogen molecules at high concentrations [21,22]. The high concentration is achieved by zymogen clustering at the cytosolic part of the death receptor forming the death inducing signalling complex (DISC) (Fig. 1a) [23,24]. Activation is mediated by like–like protein interactions through adaptor proteins [22]. Activation of caspase-9 occurs in a similar manner by the formation of a complex between caspase-9, apoptotic protease activating factor-1 (Apaf-1) and cytochrome *c*: the apoptosome [25]. The adaptor protein, Apaf-1, acts as a cofactor, increasing the proteolytic activity by orders of magnitude [26] (Fig. 1a).

The activation of proteases, generally, proceeds by limited proteolysis and removal of an N-terminal peptide or an entire N-terminal domain (Fig. 1b). Caspase zymogens possess an N-terminal prodomain, and a linker peptide within the protease domain, which are cleaved to render an active caspase [27]. Internal cleavage sites are consistent with the ability to auto-activate or to activate other caspases as part of an amplification cascade. The sequences of the prodomains vary considerably among caspase family members. There are small N-terminal peptides (e.g. caspases-3, -6, -7) and large N-terminal domains that are involved in recruitment and/or activation (e.g. caspases-2, -8, -9, -10 and the cytokine processing caspases-1, -4, -5, -11, -12, -13) [4]. The recent procaspase-7 structures and caspase-9 structure, along with biochemical data, provide insights into the molecular mechanism of caspase activation [28,29].

3. Structure and activation mechanism of procaspase-7

The structures of procaspase-7 (a caspase zymogen), and an active caspase-7 without ligand, display significant structural differences in the active site cleft region [28]. As in the bound caspase-7, the zymogen is composed of two catalytic domains. Each domain contains a central six-stranded β-sheet and five α-helices (Fig. 3). Four surface loops emerge from the core structure to form the potential active site. Comparison of the loop positions between the zymogen and the active enzyme shows large structural differences (Fig. 3) [28,29].

The procaspase-7 zymogen is a single continuous polypeptide chain. It possesses an intersubunit linker (called L2), which forms a loop between the large and the small subunits of the mature caspase. This intersubunit segment contains the cleavage site (Ile195–Gln196–Ala197–Asp198) for the activation of procaspase-7. Part of this loop (residues 190–203) was not visible in the structure, due to flexibility in the active site region, which is probably required for binding to the active site of an activator caspase (Fig. 3). In the crystal structure, the N-terminus of the small subunit in one heterodimer traverses the dimeric interface, and is oriented towards the C-terminus of the large subunit. This indicates that the heterodimer of procaspase-7 is built from a single polypeptide chain.

A second procaspase-7 structure was published towards the end of last year [29] describing similar findings as in the earlier structure. Continuous electron density for the intersubunit linkers was not observed, however, the direction which the C-terminus of the large subunit follows supports the idea that two monomers are brought together in close association, rather than monomer interdigitation. Unlike in the active caspase structures,

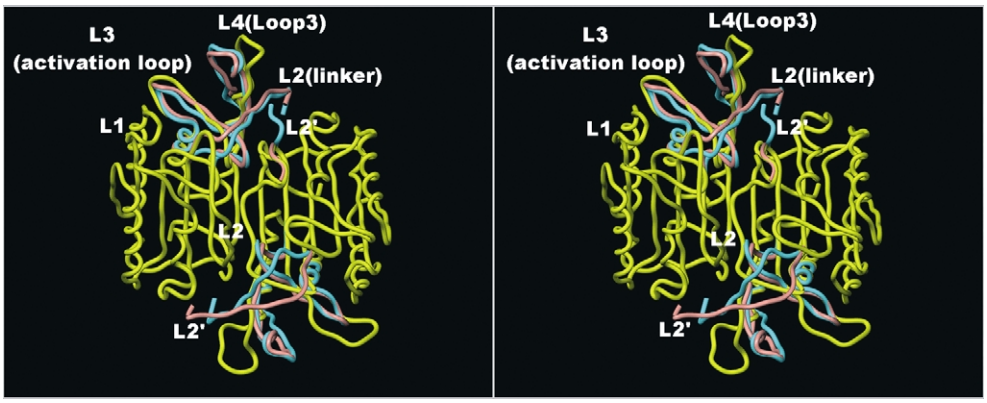

Fig. 3. A superposition of caspase-7 structures are shown here, in stereo, as a ribbon representation. Procaspase-7, shown in yellow (1K88.pdb), wild-type caspase-7, shown in pink (1K86.pdb) [28], and caspase-7–Ac-DEVD-CHO, shown in cyan (1F1J.pdb) [19] were superimposed in O [33]. For clarity, the structure of procaspase-7 has been shown in full and only loop regions from the other two structures have been shown, to highlight the differences. Labels written in parentheses represent alternative nomenclature, which agrees with caspase-1 nomenclature [14,34]. This figure was produced in SETOR [32].

where the chain extends straight on after Cys 285, the zymogen chain turns 90°, and travels upwards toward the activation loop. Linkers in both structures insert themselves into the central cavity and the dimer interface but in an asymmetric manner (Fig. 3) [28,29].

4. The loop bundle

The loop bundle, termed by Chai et al. [28], comprises L2 (intersubunit linker), L4 (Loop-3), and L2′ (symmetry-related intersubunit linker) (Fig. 3) and is present in all structures of active caspases. Conversely, the procaspase-7 structures both have disseminated loop bundles; the required structural arrangement, which favours catalysis, is not present. L2, which in active caspases extends outwards to the bulk solvent, is turned 90° such that the catalytic cysteine is completely turned away from the active site [29,28]. L4, which normally lines one side of the active site, now turns by 60° away from the active site. The C-terminus of L2′ in procaspase-7 is actually observed as the N-terminus of the small subunit, in active caspases. This loop flips 180° to point downwards towards its active site rather than upwards into the bulk solvent to interact with L2 and L4 like in the active caspases (Fig. 3).

5. Substrate-induced fit

A structure of an unbound active caspase was also determined as mentioned above [28]. Surprisingly, a few notable structural differences do exist between bound active caspase-7 and active caspase alone. The active site cleft is more defined than in procaspase-7, but less defined than in an inhibited caspase-7, indicating that active caspase-7 adopts an active site conformation, which is intermediary between the two extremes. L3 (activation loop) and L4 (L3, caspase-1 nomenclature) are in similar positions as that of an inhibited caspase. However, L2′ is still in a procaspase conformation; it is still flipped 180° and points downwards towards the N-terminal side of L2′. Therefore, the loop bundle is not yet fully assembled in unbound, active caspase-7, and binding of a substrate/inhibitor is the catalyst for achieving a correctly assembled active caspase (Fig. 3) [28]. The flipping of L2′ cannot occur prior to intersubunit cleavage in procaspase-7, thus deeming procaspase-7 as incapable of substrate/inhibitor interaction.

6. Procaspase-9

Renatus et al. [20] have shown that caspase-9 exists primarily as a monomer, under normal phys-

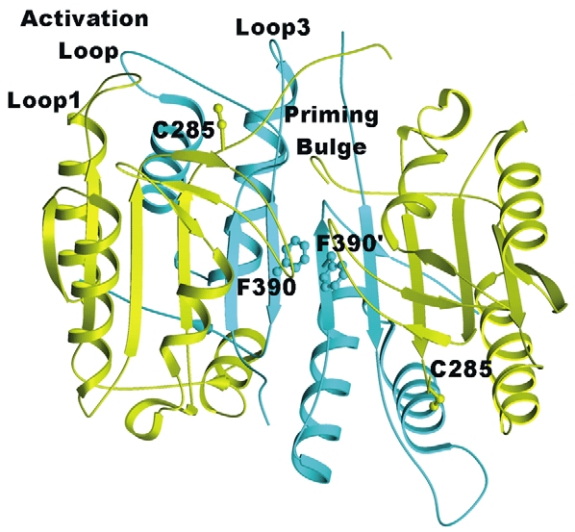

Fig. 4. Secondary structure ribbon representation of caspase-9 showing the two different active site conformations and resultant dimeric asymmetry. The α subunits are coloured in yellow and the β subunits are coloured in cyan. Nomenclature used in this figure are in accordance with caspase-1 nomenclature (see Fig. 3 references). This figure was generated in SETOR [32].

iological concentrations. They used gel filtration chromatography coupled with activity assays, and glutaraldehyde crosslinking experiments followed by SDS-PAGE, to demonstrate that the majority of the enzyme exists in an inactive monomeric form, which is in equilibrium with an active dimeric form. The crosslinking experiment followed by SDS-PAGE conducted with caspase-9, in the presence of the inhibitor Z-VAD-FMK, drives the equilibrium towards the dimeric form. At high concentrations, as used in crystallization, the dimeric form is the predominant species. The idea of a concentration dependent dimer formation preceding activation [22] is supported by these findings. The structure of a caspase-9 inhibitor complex was also published [20]. The unique feature in this caspase structure is the presence of two different active site conformations, one for the inhibited form, and the second for the uninhibited (Fig. 4). The active site of the inhibited active catalytic domain is very similar to other inhibited caspase structures. The loops forming the active site are the shortest in caspase-9, resulting in a shallower binding groove and wider substrate specificity. Large structural differences are visible in the region of the catalytic site of the uninhibited domain, rendering it inactive. The greatest differences exist in the activation loop (Fig. 4).

7. Caspase-9 dimeric interface

Two features distinguish caspase-9 from other caspases regarding the dimer interface. A β-sheet between the C-terminus of the large subunit and the N-terminus of the small subunit is absent when compared to other caspases. Additionally, a seven-residue loop in the large subunit juts across the dimer interface and is proximal to its symmetry-related loop. These two features could be the reason for the weak dimer interaction in caspase-9. The dimer asymmetry is necessary and inherent to caspase-9, however, it could also be a contributing factor to its instability. Residues Phe-390 and Phe-390′ are symmetry-related residues located on β-strand-8, which is the main β-strand involved in the dimeric interface. If the two residues were to have the equivalent orientations in their respective molecules, they would collide into each other (Fig. 4). Therefore, caspase-9 resolves the problem by having an inactive, asymmetrically related monomer [20].

8. Caspase-9 activation

Renatus et al. [20] show that proteolytic cleavage between the caspase-9 subunits is not necessary for proteolytic activity. Moreover, catalytic activity is only present in dimeric caspase-9. Based on their findings, a novel ‘self-priming’ mechanism for procaspase-9 activation is proposed. Activation is achieved when two inactive monomers pair up, the priming bulge is inserted into the dimer interface, and the activation loop is properly orientated in its active conformation. The above sequence of events permits the alignment of the catalytic residues [20].

It appears that caspase-8 follows a similar sequence of events leading to activation as caspase-9. Using gel filtration chromatography, we have observed that caspase-8 exists primarily as a monomer in solution, but shifts to a dimer when bound to an inhibitor. However, procaspase-8 stays mono-

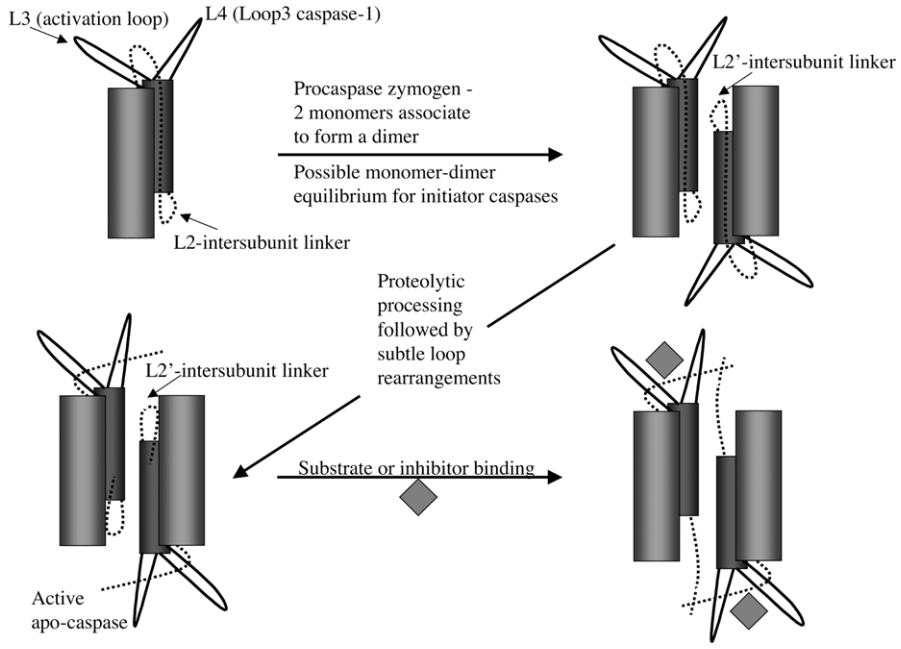

Fig. 5. A schematic representation of the events leading up to caspase activation. A monomeric procaspase molecule dimerizes; this event is concentration-dependent for the initiator caspases. Once dimerized, proteolytic processing occurs, which causes some structural rearrangements in the active site, thus enabling the cleaved caspase to optimally bind its substrates and inhibitors.

meric even in the presence of an inhibitor, suggesting that cleavage is necessary for optimal substrate/inhibitor binding, and that dimerization precedes cleavage (unpublished results). A model of procaspase-8 shows that the intersubunit linker could contribute to the sub-optimal catalytic arrangements in the active site, thus preventing optimal substrate/inhibitor binding (Fig. 5, general caspase activation scheme).

9. Conclusions

The recent structural and biochemical investigations of caspase-7 and -9 provide a more detailed picture of the activation mechanism of caspases. The structures of caspase-7 and procaspase-7 reveal that the active site cleft in procaspase-7 is deformed and is occluded by a linker peptide between the two domains that form the active site after the enzyme is activated. This represents a new mechanism of zymogen activation in proteases. The mechanism might hold true for executioner caspases-7, -3 and -6, which all have linker peptides that are comparable in length. The situation in initiator caspases such as caspase-2, -8, -9 and -10 is somewhat different. At least for caspase-9, its activation seems to be governed by proteolytic cleavage as well as a monomer–dimer equilibrium. These caspases are activated by a proximity-induced mechanism. In vivo, caspases are brought together in supramolecular complexes, such as the DISC for caspase-8, and the apoptosome for caspase-9, via their N-terminal domains. In vitro, the proximity can be obtained by the high concentration of the enzyme, e.g. as used in crystallization.

Although all aspects of substrate specificity are not yet clearly elucidated for the caspases, a plethora of structural information already exists. We have just begun to understand the molecular processes involved in activating this family of enzymes. What remains to be determined and discovered are the subtleties associated with each caspase's activation mechanism. Do the N-terminal

prodomains play a role in keeping the caspases dormant? Three-dimensional X-ray crystallographic structures of full-length caspases and complexes with interaction partners involved in the activation process are needed to answer these questions.

Acknowledgments

The financial support by the Swiss National Science foundation (grant number 3100-053957.98) and by the Baugartenstiftung, 8022 Zürich to M.G.G. gratefully acknowledged.

References

[1] M. Raff, Cell suicide for beginners, Nature 396 (6707) (1998) 119–122.
[2] M.D. Jacobson, M. Weil, M.C. Raff, Programmed cell death in animal development, Cell 88 (3) (1997) 347–354.
[3] V.L. Cryns, J. Yuan, in: R.A. Lockshin, Z. Zakeri, J.L. Tilly (Eds.), The Cutting Edge: Caspases in Apoptosis and Disease. When cells die, 1998, pp. 177–210.
[4] B.B. Wolf, D.R. Green, Suicidal tendencies: apoptotic cell death by caspase family proteinases, J. Biol. Chem. 274 (29) (1999) 20049–20052.
[5] N.A. Thornberry, Y. Lazebnik, Caspases: enemies within, Science 281 (5381) (1998) 1312–1316.
[6] D.R. Green, Apoptotic pathways: the roads to ruin, Cell 94 (6) (1998) 695–698.
[7] A. Ashkenazi, V.M. Dixit, Death receptors: signaling and modulation, Science 281 (5381) (1998) 1305–1308.
[8] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (5381) (1998) 1309–1312.
[9] T. Nakagawa, H. Zhu, N. Morishima, E. Li, et al., Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 403 (6765) (2000) 98–103.
[10] D.W. Nicholson, Caspase structure, proteolytic substrates, and function during apoptotic cell death, Cell Death Differ. 6 (11) (1999) 1028–1042.
[11] N.A. Thornberry, T.A. Rano, E.P. Peterson, D.M. Rasper, et al., A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis, J. Biol. Chem. 272 (1997) 17907–17911.
[12] M. Garcia-Calvo, E.P. Peterson, B. Leiting, R. Ruel, et al., Inhibition of human caspases by peptide-based and macromolecular inhibitors, J. Biol. Chem. 273 (49) (1998) 32608–32613.
[13] K.P. Wilson, J.A. Black, J.A. Thomson, E.E. Kim, et al., Structure and mechanism of interleukin-1 beta converting enzyme, Nature 370 (6487) (1994) 270–275.
[14] N.P. Walker, R.V. Talanian, K.D. Brady, L.C. Dang, et al., Crystal structure of the cysteine protease interleukin-1 beta-converting enzyme: a $(p20/p10)_2$ homodimer, Cell 78 (2) (1994) 343–352.
[15] J. Rotonda, D.W. Nicholson, K.M. Fazil, M. Gallant, et al., The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis, Nat. Struct. Biol. 3 (7) (1996) 619–625.
[16] P.R. Mittl, S. Di Marco, J.F. Krebs, X. Bai, et al., Structure of recombinant human CPP32 in complex with the tetrapeptide acetyl–Asp–Val–Ala–Asp fluoromethyl ketone, J. Biol. Chem. 272 (10) (1997) 6539–6547.
[17] H. Blanchard, L. Kodandapani, P.R. Mittl, S.D. Marco, et al., The three-dimensional structure of caspase-8: an initiator enzyme in apoptosis, Structure 7 (9) (1999) 1125–1133.
[18] W. Watt, K.A. Koeplinger, A.M. Mildner, R.L. Heinrikson, et al., The atomic-resolution structure of human caspase-8, a key activator of apoptosis, Structure 7 (9) (1999) 1135–1143.
[19] Y. Wei, T. Fox, S.P. Chambers, J.A. Sintchack, et al., The structures of caspases-1, -3, -7 and -8 reveal the basis for substrate and inhibitor selectivity, Chem. Biol. 7 (6) (2000) 423–432.
[20] M. Renatus, H. Stennicke, F. Scott, R. Liddington, et al., Dimer formation drives the activation of the cell death protease caspase 9, PNAS 98 (25) (2001) 14250–14255.
[21] T.T. Yamin, J.M. Ayala, D.K. Miller, Activation of the native 45-kDa precursor form of interleukin-1-converting enzyme, J. Biol. Chem. 271 (22) (1996) 13273–13282.
[22] M. Muzio, B.R. Stockwell, H.R. Stennicke, G.S. Salvesen, et al., An induced proximity model for caspase-8 activation, J. Biol. Chem. 273 (5) (1998) 2926–2930.
[23] J.P. Medema, C. Scaffidi, F.C. Kischkel, A. Shevchenko, et al., FLICE is activated by association with the CD95 death-inducing signaling complex (DISC), EMBO J. 16 (10) (1997) 2794–2804.
[24] X. Yang, H.Y. Chang, D. Baltimore, Essential role of CED-4 oligomerization in CED-3 activation and apoptosis, Science 281 (5381) (1998) 1355–1357, See comments.
[25] S.M. Srinivasula, M. Ahmad, T. Fernandes-Alnemri, E.S. Alnemri, Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization, Mol. Cell 1 (7) (1998) 949–957.
[26] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, et al., Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (4) (1997) 479–489.
[27] H.R. Stennicke, G.S. Salvesen, Catalytic properties of the caspases, Cell Death Differ. 6 (11) (1999) 1054–1059.
[28] J. Chai, Q. Wu, E. Shiozaki, S. Srinivasula, et al., Crystal structure of a procaspase-7 zymogen: mecha-

nisms of activation and substrate binding, Cell 107 (2001) 399–407.
[29] S. Riedl, P. Fuentes-Prio, M. Renatus, N. Kairies, et al., Structural basis for the activation of human procaspase-7, PNAS 98 (26) (2001) 14790–14795.
[30] H. Blanchard, M. Donepudi, M. Tschopp, L. Kodandapani, et al., Caspase-8 specificity probed at subsite S4: crystal structure of the caspase-8–Z-DEVD-cho complex, J. Mol. Biol. 302 (2000) 9–16.
[31] H. Berman, J. Westbrook, Z. Feng, G. Gilliland, et al., The Protein Data Bank, Nucleic Acids Res. 28 (2000) 235–242.
[32] S.V. Evans, SETOR: hardware lighted three-dimensional solid model representations of macromolecules, J. Mol. Graphics 11 (1993) 134–138.
[33] T. Jones, M. Bergdoll, M. Kjeldgaard, in: C. Buggs, S. Ealick (Eds.), O: A Macromolecular Modeling Environment: An Overview Crystallographic Computing and Modeling Methods in Macromolecular Design, Springer, New York, 1989.
[34] K.P. Wilson, J.A. Black, J.A. Thomson, E.E. Kim, et al., Structure and mechanism of interleukin-1 beta converting enzyme, Nature 370 (6487) (1994) 270–275.