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REVIEW

Interleukin-1β converting enzyme: A novel cysteine protease required for IL-1β production and implicated in programmed cell death

NANCY A. THORNBERRY[1] AND SUSAN M. MOLINEAUX[2,3]

[1] Department of Enzymology, Merck Research Laboratories, Rahway, New Jersey 07065
[2] Department of Molecular Immunology, Merck Research Laboratories, Rahway, New Jersey 07065

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Abstract

Interleukin-1β converting enzyme is the first member of a new class of cysteine proteases. The most distinguishing feature of this family is a nearly absolute specificity for cleavage at aspartic acid. This enzyme has been the subject of intense research because of its role in the production of IL-1β, a key mediator of inflammation. These studies have culminated in the design of potent inhibitors and determination of its crystal structure. The structure secures the relationship of the enzyme to CED-3, the product of a gene required for programmed cell death in *Caenorhabditis elegans*, suggesting that members of this family function in cell death in vertebrates.

Keywords: apoptosis; cysteine protease; enzyme inhibition; inflammation; interleukin-1

Interleukin-1 (IL-1) is the general term for 2 proteins, IL-1β and IL-1α, that are encoded by separate genes but bind to the same receptors with comparable affinity (Dinarello, 1991). IL-1 has long been considered an attractive target for therapeutic intervention in chronic and acute inflammation. Support for this view has been provided by a naturally occurring receptor antagonist protein (IL-1ra) (Hannum et al., 1990) which is effective in several clinical models, including rheumatoid arthritis, inflammatory bowel disease, and graft vs. host disease (Dinarello & Thompson, 1991). However, efforts to find low molecular weight receptor antagonists have proven difficult, prompting the search for alternative strategies.

IL-1α and IL-1β are both synthesized in monocytes as 31-kDa precursors (Auron et al., 1984; March et al., 1985), but only IL-1β requires proteolytic processing to produce a biologically active form of the cytokine (Mosley et al., 1987). The protease responsible for this cleavage, interleukin-1β converting enzyme (ICE, EC 3.4.22.36), was identified in 1989 (Black et al., 1989; Kostura et al., 1989). Upon its discovery, the enzyme immediately presented itself as an attractive therapeutic target for 2 principal reasons. First, as a protease, it was thought to be more amenable than the receptor to discovery of a small molecule therapeutic agent. Second, it was shown to have a highly unusual substrate specificity, cleaving after an aspartic acid in pro-IL-1β, suggesting that selective inhibitors could be developed.

The enzyme has been purified, cloned, and its structure determined by X-ray diffraction (Cerretti et al., 1992; Thornberry et al., 1992; Walker et al., 1994; Wilson et al., 1994). It is a cysteine protease composed of 2 subunits of 10 and 20 kDa, both of which are derived from a 45-kDa proenzyme. Potent and selective inhibitors have been developed that inhibit the production of IL-1β in monocytes (Thornberry et al., 1992). More recently, the enzyme has been shown to be the first identified member of a new cysteine protease family that includes the product of the *ced-3* gene, known to be required for programmed cell death in *Caenorhabditis elegans* (Yuan et al., 1993). This finding has led to a flurry of research aimed at investigating the putative role of ICE and its homologs in programmed cell death.

Catalytic reaction

Substrate specificity

ICE catalyzes the cleavage of the inactive 31-kDa human pro-IL-1β at Asp^{116}-Ala^{117} to generate the 17.5-kDa mature, biologically active cytokine (Black et al., 1989; Kostura et al., 1989). The enzyme also cleaves the native substrate at Asp^{27}-Gly^{28}, although the physiological relevance of this catalytic event is not known because proteolysis at this upstream site is clearly not re-

Reprint requests to: Nancy A. Thornberry, Merck Research Laboratories, R80W-243, P.O. Box 2000, Rahway, New Jersey 07065; e-mail: nancy_thornberry@merck.com.

[3] Present address: Pharmaceutical Peptides, Inc., Hampshire Street, Cambridge, Massachusetts 02139.

quired for generation of mature IL-1β (Howard et al., 1991). Both cleavage sites are evolutionarily conserved in mammalian species. The only other macromolecular substrate that has been identified is the proenzyme (Thornberry et al., 1992), although it is a poor substrate ($k_{cat}/K_m < 1 \times 10^4$ M^{-1} s^{-1}) relative to pro-IL-1β ($k_{cat}/K_m = 1.5 \times 10^5$ M^{-1} s^{-1}).

The most distinguishing catalytic feature of this protease is its unusual requirement for aspartic acid in the P_1 position (Sleath et al., 1990; Howard et al., 1991; Thornberry et al., 1992). Substitution of this residue with any other amino acid in pro-IL-1β and peptide substrates leads to >100-fold reduction in k_{cat}/K_m. The only other mammalian protease described with a similar specificity is the T-cell serine protease Granzyme B, although it is more permissive than ICE to substitutions in P_1 (Odake et al., 1991; Poe et al., 1991).

Regarding other features required for catalytic recognition by the enzyme, peptide substrates must contain at least 4 amino acids on the N-terminal side of the cleavage site, in that removal of the P_4 residue results in >100-fold reduction in k_{cat}/K_m (Thornberry et al., 1992). In contrast, there do not appear to be any functionally important specificity subsites binding C-terminal residues, typical of other cysteine proteases. The best peptide substrate yet identified for the enzyme contains methylamine in P_1' ($k_{cat}/K_m = 4 \times 10^5$ M^{-1} s^{-1}), indicating that an amino acid is not even desirable in this position (Thornberry et al., 1992). Consequently, peptides with the general structure Ac-Tyr-Val-Ala-Asp-X, where X is a photometric leaving group, are excellent substrates for the enzyme and have been used to develop continuous fluorometric (X = amino-4-methylcoumarin [Thornberry et al., 1992]), and spectrophotometric (X = *p*-nitroanilide [Reiter, 1994; Thornberry, 1994]), assays. A continuous fluorometric assay has also been developed based on resonance energy transfer with the substrate DABCYL-Tyr-Val-Ala-Asp-Ala-Pro-Val-EDANS (Pennington & Thornberry, 1994). Further details of the enzyme's substrate specificity are summarized in Figure 1.

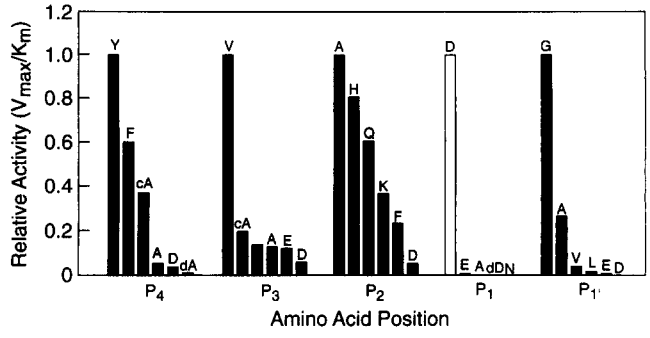


Fig. 1. Peptide substrate specificity. In peptide substrates, ICE has a stringent requirement for a tetrapeptide on the N-terminal side of the cleavage site (Thornberry et al., 1992). The enzyme's substrate specificity was further investigated using a series of peptides containing single amino acid substitutions in the peptide Ac-Tyr-Val-Ala-Asp-Gly-Trp-NH_2. The results indicate that Asp is required in P_1, liberal substitutions are tolerated in P_2, Val is preferred in P_3, and hydrophobic amino acids are favored in P_4. The finding that liberal substitutions are tolerated in the P_2 position has been exploited in the design of the affinity matrix and affinity labels described in the text. The results also confirm the finding that small hydrophobic amino acids are preferred in the P_1' position (Sleath et al., 1990; Howard et al., 1991), although the enzyme clearly tolerates other residues in macromolecular substrates, as exemplified by the broad substitution found in nature (e.g., Gly, Pro, Asn). Not surprisingly, the optimal sequence on the N-terminal side of the cleavage site, Ac-Tyr-Val-Ala-Asp, closely matches the corresponding sequence in human pro-IL-1β, Tyr^{113}-Val^{114}-Cys^{115}-Asp^{116}. This sequence has been employed in the development of peptide substrates and the potent, selective inhibitors described in this review. Reprinted with permission from *Annals of The New York Academy of Sciences* (Miller et al., 1993b).

Cysteine protease mechanism

ICE is not only unusual with regard to substrate specificity, but also has a sequence and topology unlike members of known protease families (see below). Nonetheless, based on its chemical and catalytic properties, the enzyme appears to be a typical cysteine protease. First, nonspecific alkylating agents, such as N-ethylmaleimide and iodoacetate, are competitive inactivators and preferentially react with a single cysteine (Cys^{285}) (Thornberry et al., 1992). Second, this cysteine has enhanced nucleophilicity relative to ordinary thiols, such as glutathione (N.A. Thornberry, unpubl. obs.). Finally, as described below, acyloxymethylketones, considered selective for cysteine proteases, are potent inactivators that covalently modify the same cysteine (Thornberry et al., 1994). The only anomaly in the inhibition pattern is with the irreversible cysteine protease inhibitor E-64, which is inactive against ICE (Black et al., 1989), presumably because of the enzyme's unusual specificity relative to other cysteine proteases.

The recently published crystal structures secure the relationship of ICE to known cysteine proteases (Walker et al., 1994; Wilson et al., 1994). The active site contains a catalytic diad composed of a cysteine sulfhydryl group in close proximity to a histidine imidazole group, which is presumed to function as a general acid–base in catalysis. In analogy with other cysteine proteases, the enzyme appears to stabilize the oxyanion of the tetrahedral transition state through hydrogen bonding interactions.

Selective reversible and irreversible inhibitors

Two general strategies, summarized in Figure 2, have been successfully employed in the development of cysteine protease inhibitors (for reviews, see Rich, 1986; Shaw, 1990). Peptide aldehydes (VI), nitriles (VIII), and ketones (X) are reversible inhibitors that undergo nucleophilic addition of the catalytic cysteine to form thiohemiacetals (VII), thioimidates (IX), and thiohemiketals (XI), respectively. The potency of these compounds has been attributed to structural similarity between these thiol adducts and the tetrahedral (II and IV) and acyl-enzyme (III) intermediates formed during substrate hydrolysis. The second approach involves peptide α-substituted ketones of the general structure R-CO-CH_2-X (XII), where X is a halogen (halomethylketone), diazonium ion (diazomethylketone), or carboxylate (acyloxymethylketone) leaving group. These inhibitors irreversibly inactivate cysteine proteases through expulsion of the leaving group (X^-) to form a thiomethylketone (XIV) with the active site cysteine. Although the details of the chemical mechanism have not been established, inactivation is presumed to proceed through formation of a thiohemiketal as shown (XIII).

Both strategies have been applied to the development of ICE inhibitors with remarkable success. The most potent and selective inhibitors contain the tetrapeptide sequence, Ac-Tyr-Val-

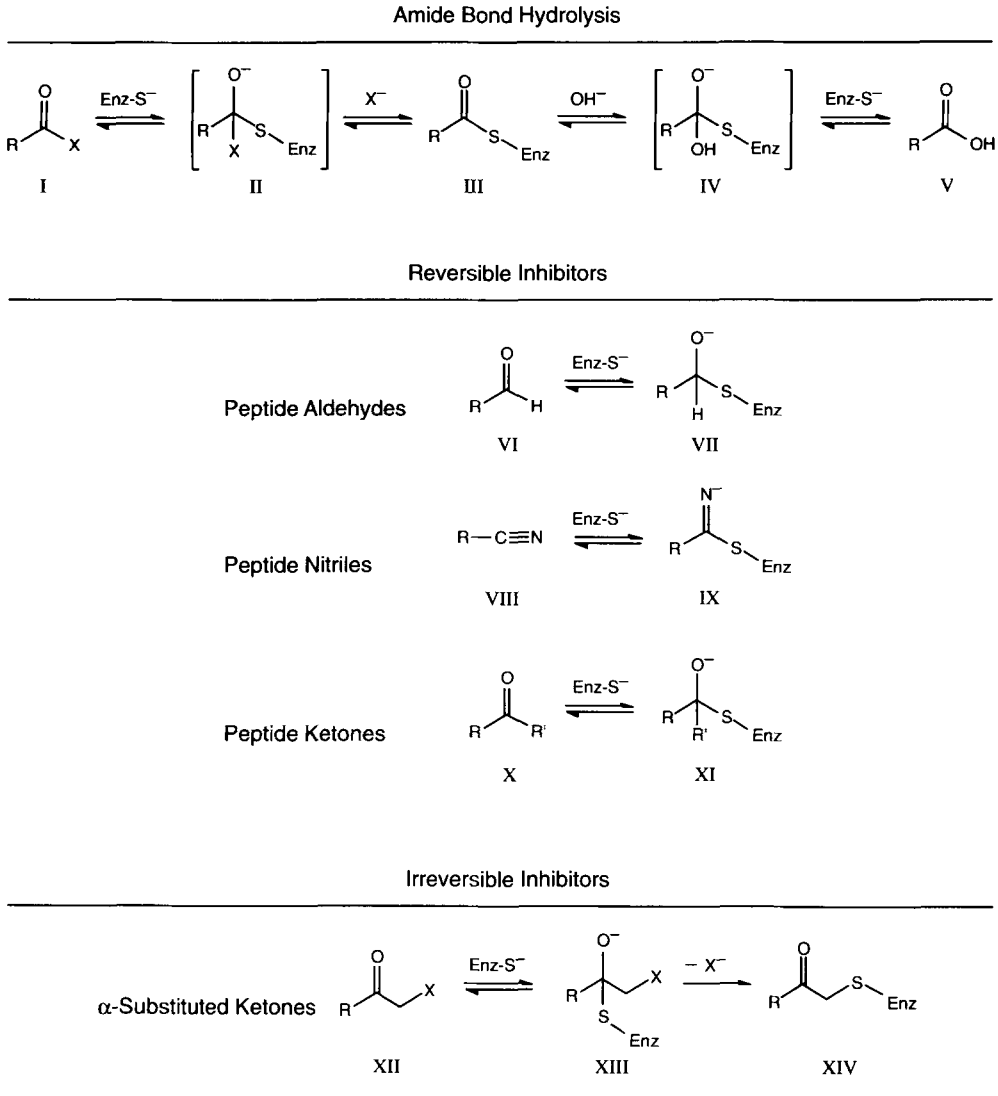


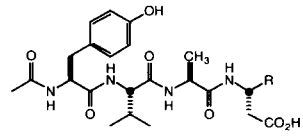
Fig. 2. Strategies used in the development of cysteine protease inhibitors.

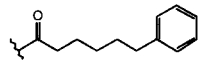
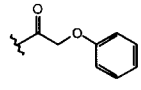
Ala-Asp, consistent with the substrate specificity of the enzyme. These inhibitors are described in detail below, and their kinetic parameters are summarized in Figure 3. Although the peptide nature of these compounds limits their utility for therapeutic applications, they have proven to be valuable tools for studies of catalytic mechanism, structure, and cell biology.

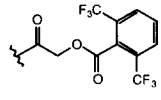
Reversible inhibitors

The most potent reversible inhibitor developed for ICE to date is the tetrapeptide aldehyde, Ac-Tyr-Val-Ala-Asp-CHO (**1**) (Chapman, 1992; Thornberry et al., 1992). This competitive inhibitor displays slow formation ($k_{on} = 3.8 \times 10^5$ M^{-1} s^{-1}) and slow dissociation ($k_{off} = 2.9 \times 10^{-4}$ s^{-1}, $t_{1/2} = 40$ min) of enzyme-inhibitor complex, with an apparent overall dissociation constant of 0.76 nM. The intrinsic K_i, which corrects for the extent to which the aldehyde exists as the inactive hydrate under the reaction conditions (86%), is 0.1 nM, making this compound one of the most potent peptide aldehydes described for a cysteine protease. As a measure of the selectivity of this inhibitor for ICE in monocytic cells, when employed as an affinity chromatography ligand it achieves a 100,000-fold purification of the enzyme directly to homogeneity from crude cell homogenates (Thornberry et al., 1992). The ability of this compound to inhibit other members of this emerging cysteine protease family (see below) has yet to be determined. Replacement of the aldehyde with a cyano group to form the corresponding nitrile (**2**) results in an inhibitor with a K_i of 60 nM, consistent with its lower intrinsic electrophilicity (K. Chapman, pers. comm.).

As described above, it is generally accepted that peptide aldehydes inhibit cysteine proteases by forming a thiohemiacetal with the active site cysteine. However, it is a matter of some debate as to whether peptide aldehydes are transition-state analogs as initially proposed (Westerik & Wolfenden, 1972), or instead inhibit the enzyme by forming a thiohemiacetal bound in a nontransition state conformation (Frankfater & Kuppy, 1981; Mackenzie et al., 1986; Ménard et al., 1991; Schröder et al., 1993). The recently determined crystal structure clearly shows the tetrapeptide aldehyde (**1**) bound in a nontransition-



	Reversible Inhibitors	R	K_i (nM)	Monocyte IC_{50} (nM)
1	aldehyde		0.76	2,700
2	nitrile		57	1,300
3	ketone		19	6,000
4	phenoxymethylketone		3	N.D.[1]

	Irreversible Inhibitors		k_2/K_i ($M^{-1}s^{-1}$)	
5	diazomethylketone		1.6×10^4	N.D.[1]
6	acyloxymethylketone		9.6×10^5	3,000

[1] Not Determined

Fig. 3. Tetrapeptide inhibitors of ICE.

state conformation, with the oxyanion of the inhibitor being stabilized by the active site histidine (Wilson et al., 1994).

Unsubstituted phenyl-alkyl peptide ketones are surprising potent competitive, reversible inhibitors of ICE. This strategy has the advantage of potentially accommodating specificity and affinity enhancing substituents on both sides of the carbonyl group. The best inhibitor of this series developed to date, Ac-Tyr-Val-Ala-Asp-CO-$(CH_2)_5$-Ph (**3**), has a K_i of 18 nM (Mjalli et al., 1993b). Clearly the potency of these inhibitors is due, at least in part, to the enzyme's ability to accommodate hydrophobic residues in the S_1'-S_2' subsites. In an attempt to enhance the potency of these inhibitors by adding electron-withdrawing fluoro substituents, phenylpropionyl-Val-Ala-Asp-CO-CF_2-$(CH_2)_3$-Ph was prepared but found to be a weaker inhibitor ($K_i = 6$ μM) than its unsubstituted counterpart ($K_i = 42$ nM) (Mjalli et al., 1993a). This is consistent with previous studies demonstrating that cysteine proteases are curiously insensitive to polyfluoroketones (Smith et al., 1988b; Angelastro et al., 1990). Alternatively, activation of the carbonyl by replacement of the β-carbon with a heteroatom results in a significant improvement in potency. For example, the peptide phenoxymethylketone, Ac-Tyr-Val-Ala-Asp-CO-CH_2-O-Ph (**4**) has a K_i of 3 nM (Adnan Mjalli, pers. comm.). Although this inhibitor has the potential to modify the enzyme covalently, the rate of this process is relatively slow ($k_{inact}/K_i < 1{,}000$ M^{-1} s^{-1}), such that the half-life for irreversible inhibition at 50% saturation is >60 h.

Irreversible inhibitors

The most potent and selective α-substituted ketones described for ICE are peptide (acyloxy)methyl ketones with the general structure, Ac-Tyr-Val-Ala-Asp-CH2-OCOPh (e.g., **6**) (Dolle et al., 1994; Thornberry et al., 1994). Acyloxymethylketones were first developed by Krantz and his colleagues as potent, selective inactivators of cathepsin B (Smith et al., 1988a; Krantz et al., 1991). Inactivation of ICE proceeds through expulsion of the carboxylate leaving group to form a thiomethylketone with the active site Cys^{285}. The second-order inactivation rate is independent of leaving group pK_a with an approximate value of 1×10^6 M^{-1} s^{-1}, leading to the proposal that the rate-limiting step in inactivation is association of enzyme and inhibitor, rather than any bond-forming reactions. The selectivity of these inhibitors for ICE was demonstrated by affinity labeling a crude THP.1 cell cytosol, where ICE constitutes <0.001% of the total protein. At concentrations and time periods that inactivate >99.99% of the enzyme, there is no detectable labeling of any other protein. In addition, these inhibitors are relatively inert toward bionucleophiles such as glutathione ($< 5 \times 10^{-4}$ M^{-1} s^{-1}), making them excellent candidates for in vivo studies of enzyme inhibition.

The relatively slow rate of association observed with acyloxymethylketones (1×10^6 M^{-1} s^{-1}) appears to be characteristic of all tetrapeptide-based ICE inhibitors. This rate constant is di-

rectly proportional to reaction macroviscosity (Thornberry et al., 1994), an observation that is generally considered diagnostic for a diffusion-controlled encounter (Blacklow et al., 1988). However, it is also well below theoretical predictions of rates for diffusion-limited reactions (10^8–10^{10} M^{-1} s^{-1}) and is at the low end of the range of 10^6–10^8 M^{-1} s^{-1} typically observed for enzyme–substrate and protein–ligand interactions (Hammes & Schimmel, 1970; Fersht, 1985). An explanation that adequately accounts for both observations is that binding of a tetrapeptide inhibitor to ICE is accompanied by a viscosity-dependent conformational change, analogous to that proposed for binding of the transition state analogue inhibitor coformycin to adenosine deaminase (Kurz et al., 1987).

Inhibition by a cowpox serpin

The only macromolecular inhibitor described to date for ICE is the cowpox virus serpin, CrmA (Ray et al., 1992; Komiyama et al., 1994). This 31-kDa protein has been shown to play an important biological role in the host inflammatory response to infection (Ray et al., 1992) and has more recently been implicated in protection against cell death (Gagliardini et al., 1994). It is the only known member of the serpin superfamily to inhibit a cysteine, rather than a serine, protease, leading to the proposal that ICE has a substrate binding geometry similar to that of a serine protease (Komiyama et al., 1994). The rate constant for binding ($k_{on} = 1.7 \times 10^7$ M^{-1} s^{-1}) is among the fastest known for serpin–protease interactions, and the dissociation constant of the final complex is <4 pM (Komiyama et al., 1994). Unlike most enzyme–serpin interactions, the resulting complex is not stable to SDS-PAGE. Its selectivity for ICE is presumably determined by the putative P_1 Asp in the reactive site loop. CrmA may also be an excellent inhibitor of other members of this protease family, because it is virtually certain that they also require Asp in P_1 for catalysis (see below). This raises some uncertainty as to the target(s) of CrmA inhibition in vivo.

Composition and structure of active enzyme

Active ICE is composed of 2 subunits of molecular masses of 10,248 Da (p10) and 19,866 Da (p20). This was first demonstrated through purification of the enzyme to homogeneity from both human and murine monocytic cells by affinity chromatography using a peptide aldehyde immobilized on a Sepharose matrix (Thornberry et al., 1992; Molineaux et al., 1993). An alternately processed form of p20 with a molecular mass of 21,456 Da (p22) has also been isolated by affinity chromatography (Miller et al., 1993a). Several lines of evidence suggested that both subunits are required for catalytic activity (Cerretti et al., 1992; Thornberry et al., 1992; Miller et al., 1993a). This has been confirmed by crystal structures of enzyme bound to tetrapeptide inhibitors, which show p20 and p10 intimately associated, both contributing key residues to the active site (Walker et al., 1994; Wilson et al., 1994) (Fig. 4). The catalytic Cys^{285} and His^{237} reside in p20, whereas the P_1 aspartic acid binding pocket is formed by residues on both subunits. Not surprisingly, the side chains in P_2 and P_3 are solvent exposed, explaining why broad substitution is tolerated in the P_2 position in tetrapeptide substrates and inhibitors. The side chain of P_4 tyrosine, which is a critical determinant, binds in a hydrophobic channel formed by p10 residues. The oxyanion of the substrate tetrahedral intermediate is most likely stabilized by the backbone amide protons of Cys^{285} and Gly^{238}.

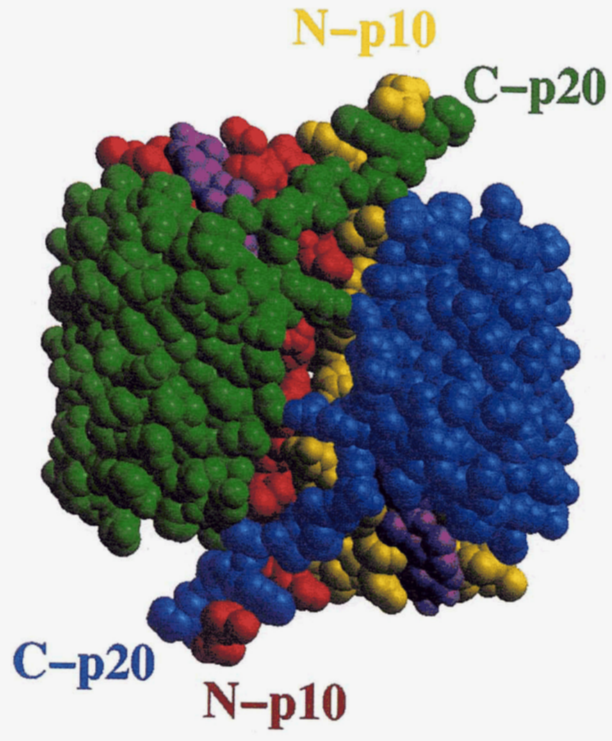


Fig. 4. CPK model of the $(p20)_2/(p10)_2$ tetramer. The p20 subunits (green and blue) and the p10 subunits (red and gold) form 2 catalytic domains (green/red) and (blue/gold). The tetrapeptide aldehyde inhibitor (**1**) is shown in purple. A model for proenzyme activation has been proposed in which the green p20 and gold p10 are derived from one proenzyme molecule, and the blue p20 and red p10 from another to form a tetramer of interdigitating subunits (Walker et al., 1994; Wilson et al., 1994). Reprinted with permission from *Nature* (Wilson et al., 1994).

As shown in Figure 4, two p10/p20 heterodimers associate in the crystal to form a tetramer, and it has been proposed that this is the catalytically active form of the enzyme in solution (Walker et al., 1994; Wilson et al., 1994). Although the molecular weight of the enzyme in solution has yet to be rigorously determined, evidence for this hypothesis is compelling. First, the contacts between heterodimers in the crystal are extensive, covering an area of ~5,200 $Å^2$. Second, the residues that are located at the proposed tetramer interface are highly conserved. Finally, mutation of one of these residues eliminates ICE activity (Wilson et al., 1994).

The possibility that catalytically active enzyme is a tetramer has implications for inhibitor development. Prior to determination of the structure, it was found that dilution of the enzyme in solution causes loss of activity with a half-life of 2.7 h, which is fully restored upon reconcentration of the enzyme (Thornberry et al., 1992). This phenomenon is prevented by saturating levels of substrates or inhibitors and was initially thought to be due to dissociation of the p20/p10 heterodimer to catalytically inactive monomers. In view of the crystal structure, an alternative explanation is that this loss of activity is due to dissociation of the tetramer to form 2 catalytically inactive p20/p10

heterodimers. If this is true, then the interface between heterodimers offers a potential target for inhibitor design.

Proenzyme organization and activation

The mature p20/p10 form of ICE is derived from a 45-kDa proenzyme (p45) by proteolytic removal of an 11.5-kDa propeptide and a 2.0-kDa linker peptide between p20 and p10 (Fig. 5A) (Thornberry et al., 1992). The primary sequence also reveals that both p20 and p10 are flanked by Asp-X bonds, potential ICE cleavage sites. Given the enzyme's unusual substrate specificity, this strongly suggests that autocatalysis plays an important role in proenzyme activation. Definitive evidence for autoprocessing was obtained in both COS cells (Wilson et al., 1994) and baculovirus expression systems (Rolando et al., 1994; Wang et al., 1994b), where mutation of the catalytic Cys^{285} prevents processing of the proenzyme to p20/p10. The Asp-X bonds surrounding the mature subunits were confirmed to be the targets of autocatalytic cleavage by mutation of these sites (Rolando et al., 1994).

The arrangement of subunits in the crystal suggests a model for proenzyme activation via intermolecular autoproteolysis (Walker et al., 1994; Wilson et al., 1994). In a p20/p10 heterodimer, the C-terminal residue of the p20 is too far from the N-terminal p10 residue to be bridged by the 2.0-kDa linker peptide. Consequently, if the heterodimer is derived from a single proenzyme molecule, large conformational changes must accompany processing. An alternative hypothesis has been proposed in which 2 molecules of p45 associate and process to form a tetramer of interdigitating subunits. Each active site is formed from a p10 from one proenzyme molecule and a p20 from another, thus providing a rationale for the unique quaternary structure of the enzyme.

Role in processing and secretion of IL-1β

In contrast to the detailed understanding of the structure and catalytic mechanism of ICE described above, little is known of the cellular events leading to processing and secretion of mature IL-1β from activated monocytes. Among the prominent questions are the following.

First, how is activation of proenzyme regulated in monocytes? The vast majority (>99%) of ICE protein in both resting and stimulated monocytes is inactive cytosolic proenzyme, which is constitutively produced (Ayala et al., 1994). Active enzyme is present in such low abundance that it cannot be detected in intact cells by immunoprecipitation. What little active enzyme exists has been detected only by electron microscopy, which shows it predominantly associated with the plasma membrane (Singer et al., 1993). Taken together, these observations suggest that activation of proenzyme is tightly regulated and may be involved in controlling the rate of mature IL-1β release.

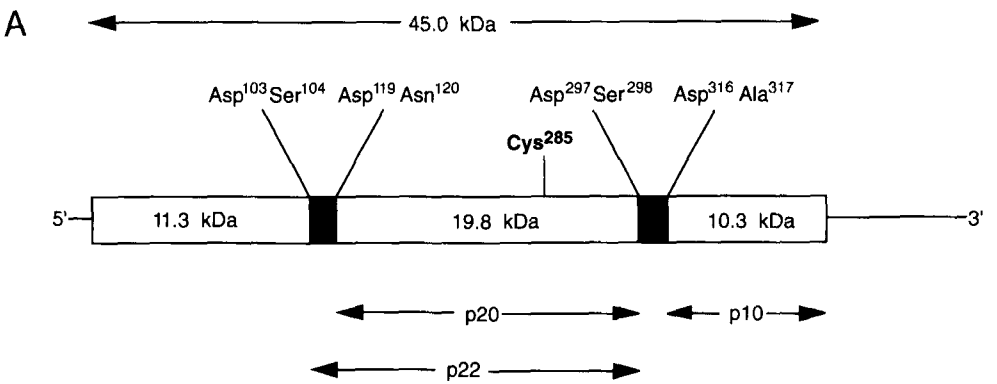
Second, how is mature IL-1β secreted from monocytes, and does ICE play a role in this process? It is clear from several lines of evidence that mature IL-1β is secreted through a nonclassical pathway that does not involve the Golgi/endoplasmic reticulum (ER) route (March et al., 1985; Singer et al., 1988; Rubartelli et al., 1990). Several alternative mechanisms for IL-1β release have been proposed (Rubartelli et al., 1990; Hogquist et al., 1991a, 1991b; Kuchler, 1993). One possibility is suggested by the notion that active ICE appears to be largely associated with the cell membrane. In this location it could function as part of the secretory machinery, perhaps as part of a transmembrane pore like that used for secretion of bacterial hemolysin A or yeast **a**-factor (Kuchler, 1993). This hypothesis is consistent with the observation that mature IL-1β is not found inside cells, indicating that processing and secretion are temporally linked (Limjuco et al., 1986; Hazuda et al., 1988).

Finally, why are potent, selective tetrapeptide inhibitors of the purified enzyme such poor inhibitors of mature IL-1β release from stimulated monocytes (Fig. 3)? Among the most reasonable explanations are proteolysis, poor cell penetration, and competition with proenzyme activation. There is also the unsettling possibility that the inhibitor profile of purified enzyme is not relevant to that of the active enzyme in monocytes, which may be membrane-associated. In this regard it is important to point out that the enzyme originally identified in, and purified from, the cytosol of monocytic cells (Kostura et al., 1989) was derived from the proenzyme following cell lysis (Ayala et al., 1994). Likewise, the enzyme used for structural studies was derived from recombinant proenzyme (Wilson et al., 1994) or refolded from p20 and p10 (Walker et al., 1994). The crystal structure of this enzyme does not reveal any membrane binding domains. Thus, if active enzyme is indeed membrane bound, it may be posttranslationally modified, or associated with membrane proteins, or both. The resolution of these issues clearly has important implications for future inhibitor design.

Evidence for a role in programmed cell death and apoptosis

When ICE was first purified, cloned, and sequenced in 1992, it was found to be unrelated to any known protease (Cerretti et al., 1992; Thornberry et al., 1992). The crystal structure revealed that the topology of the enzyme is also novel with respect to known protein structures (Walker et al., 1994; Wilson et al., 1994). Recently it was shown to have sequence similarity to CED-3, the product of a gene required for programmed cell death in *C. elegans* (Yuan et al., 1993). It is now clear that ICE is the first member of a new family of cysteine proteases that includes CED-3 and NEDD-2, the product of a murine gene also implicated in cell death (Kumar et al., 1994). A sequence alignment of these proteins with human ICE, shown in Figure 5B, shows that all of the residues implicated in catalysis and binding by the crystal structure, including the 4 amino acids important for recognition of the P_1 aspartic acid, are conserved. These residues are also conserved in the human *nedd2* gene, which has recently been isolated and characterized (Wang et al., 1994a). Consequently, it is virtually certain that *ced-3* and *nedd2* encode cysteine proteases resembling ICE that cleave after an aspartic acid in substrates that are, as yet, unidentified. The finding that CED-3 has mammalian homologs, together with the discovery that a gene required for protection against cell death in *C. elegans* (*ced-9*) has a mammalian homolog with similar function (*bcl-2*) (Hengartner et al., 1992; Vaux et al., 1992; Hengartner & Horvitz, 1994), strongly suggests that the mechanism of cell death in the worm is conserved in vertebrates.

What is the evidence that ICE is the mammalian counterpart of CED-3? Yuan and her colleagues have found that overexpression of the *ice* gene or the *ced-3* gene in fibroblasts induces apoptosis (Miura et al., 1993). Cell death was not observed upon



B

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Human ICE          ------------------------MADKVLKEKRKLFIRSMGEG-----TINGLLDELLQTRVLNKEEMEKVKRENATVMDKTRALIDSVIPKGAQAC   69
C. elegans Ced-3   ----------------------------MMRQDRRSLLERNINMFSSHLKVDEILEVLIAKQVLNSDNGDMIN-SCGTVREKRREIVKAVQRRGDVAF   69
Murine Nedd-2      MAAPSGRSQSSLHRKGLMAADRRSRILAVCGMHPDHQETLKKNRVVLAKQLLLSELLEHLLEKDIITLEMRELIQ-AKGGSFSQNVE-LNLLPKRGPQAF   96

Human ICE          QICITYICE-EDSYLAGTLG----------------------------LSADQTSGNYLNMQDSQGVLSS------------------------------   110
C. elegans Ced-3   DAFYDALRSTGHEGLAEVLEPLARSVDSNAVEFECPMSPASHRRSRALSPAGYTSPTRVHRDSVSSVSSFTS-YQDIYSRARSRSR-SRALHSSDRHNYS   167
Murine Nedd-2      DAFCEALRETRQGHLEDLLLTTLSDIQHVLPPLSCDYDT-----SLPFSVCESCPPHKQLRLSTDAT-------------------------------   160

Human ICE          ----------------------------FPAPQAVQDNPAMPTSSGSEGNVKLCSLEEAQRIWKQKSAEIYPIMDKSSRTRLALIICNEEF---DSIPRRT   180
C. elegans Ced-3   SPPVNAFPSQPSSANSSFYGCSSLGYSSSRNRSFSKASGPTQYIFHEEDMNFVDAPTISRVFDEKTM----YRNFSSPRGMCLIINNEHF---EQMPTRN   260
Murine Nedd-2      ------------------------------EHSLDNGDGPPCLLVKPCTPEFYQA-------HYQLA----YRLQSQPRGLALVLSNVHFTGEKDLEFRS   219

Human ICE          GAEVDITGMTMLLQNLGYSVDVKKNLTASDMTTELEAFAHRPEHKTSDSTFLVFMSHGIREGICGKKHSEQVPDILQLNAIFNMLNTKNCPSLKDKPKVI   280
C. elegans Ced-3   GTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIRDFAKHESH--GDSAILVILSHGEENVIIGVD-----DIPISTHEIYDLLNAANAPRLANKPKIV   353
Murine Nedd-2      GGDVDHTTLVTLFKLLGYNVHVLHDQTAQEMQEKLQNFAQLPAHRVTDSCVVALLSHGVEGGIYGVD-----GKLLQLQEVFRLFDNANCPSLQNKPKMF   314

                                  *
Human ICE          IIQACRGDSPGVVW-FKDSV------------GVSGNLSLPTTEEFEDDAI-KKAHIEKDFIAFCSSTPDNVSWRHPTMGSVFIGRLIEHMQEYACSCD   365
C. elegans Ced-3   FVQACRGERRDNGFPVLDSVDGVPAFLRRGWDNRDGPLFNFLGCVRPQVQQVWRKKPSQADILIAYATTAQYVSWRNSARGSWFIQAVCEVFSTHAKDMD   453
Murine Nedd-2      FIQACRGDETDRG---VDQQDGKNHTQSPGCEESDAGKEELM---------KMRLPTRSDMICGYACLKGNAAMRNTKRGSWYIEALTQVFSERACDMH   401

Human ICE          VEEIFRKVR--------FSFEQPDGRAQMPTTERVTLTRCFYLFPGH-------   404
C. elegans Ced-3   VVELLTEVNK--KVACGFQTSQGSNILKQMPEMTSRLLKKFYFWPE--ARNSAV   503
Murine Nedd-2      VADMLVKVNALIKEREGYAPGTEFHRCKEMSEYCSTLCQQLYLFPGYPPT----   451
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p20

p10

Fig. 5. Proenzyme organization (**A**) and sequence comparison (**B**) of human ICE, *C. elegans* CED-3, and murine Nedd-2. The sequence alignment shows that all of the residues implicated in catalysis (Cys^{285} and His^{237}), binding of the aspartic acid (Arg^{179}, Gln^{283}, Arg^{341}, Ser^{347}), and stabilization of the oxyanion of the substrate tetrahedral intermediate (Gly^{238}, Cys^{285}) are conserved (black). The active site Cys^{285} is also denoted with an asterisk. Other identical residues are indicated in bold. The p20 and p10 subunits of ICE are outlined in black. It is clear from the sequence alignment that the *ced-3* and *nedd-2* genes encode cysteine proteases that cleave after an aspartic acid in their unknown substrates.

overexpression of mutants lacking the active site cysteine, indicating that this effect depends on the proteolytic activity of these proteins. The most provocative evidence is provided by the finding that expression of the cowpox serpin CrmA prevents apoptosis in chick dorsal root ganglion neurons deprived of nerve growth factor (NGF) (Gagliardini et al., 1994), although expression of ICE protein in these cells was not documented.

These results provide compelling evidence for a role for ICE in programmed cell death, but they are inconclusive. It is possible that overexpression of any protease in fibroblasts will cause nonspecific induction of cell death. Regarding the inhibition of apoptosis by CrmA in neurons, cell death could be due to an ICE homolog that is also inhibited by this serpin, analogous to the broad inhibition of serine proteases by α-1-proteinase inhibitor (Beatty et al., 1980). One candidate is NEDD-2, the homolog that is expressed in the brain during development and is down-regulated in adult brain, consistent with a function for this protein in neuronal cell death (Kumar et al., 1994). Another is prICE, a recently discovered proteolytic activity in cytosolic extracts of apoptotic chick hepatoma cells (Lazebnik et al., 1994). This enzyme, which appears to be a cysteine protease with a requirement for Asp in P_1, cleaves the nuclear enzyme polyADP ribose polymerase (PARP) at a highly conserved sequence that is identical to the upstream cleavage site for ICE in pro-IL-1β. Inactivation of PARP by cleavage at this site is known to be a hallmark of apoptosis in a variety of cell types (Kaufmann et al., 1993). Despite the similarities between prICE and ICE, they clearly have different macromolecular substrate specificities: prICE does not cleave mammalian pro-IL-1β, and ICE does not cleave PARP, implying that the enzymes have distinct functions in vivo. These important studies have provided the first insights into mechanisms by which members of this cysteine protease family may participate in apoptosis.

Taken together, these results provide clear evidence that homologs of CED-3 function in cell death in higher organisms, but a role for ICE itself has yet to be proven. Clearly, potent, selective inhibitors would be useful in delineating the biological roles of this enzyme and its relatives. In this regard, it is important to note that it is not yet known if the potent tetrapeptide inhibitors of ICE described in this review are good inhibitors of other family members. Two lines of evidence suggest they will not be. First, although there is excellent conservation of the active site residues involved in catalysis and binding of the P_1 aspartic acid, the homology does not appear to extend to the S_4 subsite, a critical determinant for ICE (Wilson et al., 1994). Consistent with this view, the sequence of the PARP cleavage site suggests that prICE prefers an acidic residue in this position, in contrast to the hydrophobic residues favored by ICE. Second, although the tetrapeptide halomethylketone Ac-Tyr-Val-Ala-Asp-CO-CH_2-Cl appears to be an inhibitor of prICE (Lazebnik et al., 1994), it is 1,000-fold less potent than we would expect for inhibition of ICE, by analogy to the rates of inactivation observed with the corresponding tetrapeptide acyloxymethylketones ($k_{inact}/K_i = 10^6\ M^{-1}\ s^{-1}$), which contain less reactive leaving groups. Thus, present indications are that tetrapeptide inhibitors of ICE probably will not be potent probes against other members of this family; conversely, with a knowledge of individual sequence specificities it should be possible to design potent, selective inhibitors for each enzyme built on the same fundamental motifs that have been successful with ICE. Finally, viewed as probes of biological function, it should be kept in mind that none of the present ICE inhibitors is potent against whole cells, for reasons that remain to be established.

Challenges for future research

The structure and catalytic mechanism of ICE are reasonably well understood, and efforts to find potent, selective, nonpeptide inhibitors with suitable pharmacologic properties for studies in vivo will inevitably succeed. Such inhibitors may prove crucial to addressing the serious questions that remain regarding the biological role(s) of ICE and its relatives in inflammation and cell death.

First, will inhibition of IL-1β alone be effective in treating chronic and/or acute inflammation, or is IL-1α also an important player? Recent studies with neutralizing antibodies to IL-1β suggest a major role for this cytokine in animal models of arthritis (Geiger et al., 1993). More studies of this type will be necessary to establish the relative importance of IL-1α and IL-1β in other models of inflammation. Second, is IL-1β a suitable target for chronic therapy in diseases such as rheumatoid arthritis? IL-1 has long been thought to be a key mediator of the immune response. Cowpox virus encodes 2 proteins, a potent ICE inhibitor (CrmA) (Ray et al., 1992) and an IL-1β binding protein (Alcami & Smith, 1992; Spriggs et al., 1992), that facilitate infection through inhibition of the host inflammatory response. Although these findings support the view that ICE and IL-1β are important in inflammation, they also raise serious questions as to the safe administration of ICE inhibitors.

Finally, is ICE itself involved in cell death? The results so far are not definitive; however, they do clearly establish that at least some family members are key participants. These findings have intensified interest in understanding the role of these proteases in normal mechanisms of cell death, based on the possibility that abnormally controlled cell death may be the underlying cause of neurodegenerative diseases such as Alzheimer's or Parkinson's (Barinaga, 1993). There is no good evidence for this at the moment, and clearly more research is required to assess the therapeutic potential of inhibitors of ICE and its homologs for treatment of these debilitating diseases. Specificity of inhibition will also be a major issue in this area, because programmed cell death is generally considered an essential component of development, tissue homeostasis, and aging (Ellis et al., 1991).

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References

Alcami A, Smith GL. 1992. A soluble receptor for interleukin-1β encoded by vaccinia virus: A novel mechanism of virus modulation of the host response to infection. *Cell 71*:153–167.

Angelastro MR, Mehdi S, Burkhart JP, Peet NP, Bey P. 1990. α-Diketone and α-keto ester derivatives of N-protected amino acids and peptides as novel inhibitors of cysteine and serine proteinases. *J Med Chem 33*:11–13.

Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc Natl Acad Sci USA 81*:7907–7911.

Ayala JM, Yamin TT, Egger LA, Chin J, Kostura MJ, Miller DK. 1994. IL-1β-converting enzyme is present in monocytic cells as an inactive 45-kDa precursor. *J Immunol 153*:2592–2599.

Barinaga M. 1993. Death gives birth to the nervous system. But how? *Science 259*:762–763.

Beatty K, Bieth J, Travis J. 1980. Kinetics of association of serine proteinases with native and oxidized α-1-proteinase inhibitor and α-1-antichymotrypsin. *J Biol Chem 255*:3931–3934.

Black RA, Kronheim SR, Sleath PR. 1989. Activation of interleukin-1β by a co-induced protease. *FEBS Lett 247*(2):386–390.

Blacklow SC, Raines RT, Lim WA, Zamore PD, Knowles JR. 1988. Triosephosphate isomerase catalysis is diffusion controlled. *Biochemistry 27*: 1158–1167.

Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, March CJ, Kronheim SR, Druck T, Cannizzaro LA, Huebner K, Black RA. 1992. Molecular cloning of the interleukin-1β converting enzyme. *Science 256*:97–100.

Chapman KT. 1992. Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. *Bioorg Med Chem Lett 2*:613–618.

Dinarello CA. 1991. Interleukin-1 and interleukin-1 antagonism. *Blood 77*: 1627–1652.

Dinarello CA, Thompson RC. 1991. Blocking IL-1: Interleukin-1 receptor antagonist in vivo and in vitro. *Immunol Today 12*:404–410.

Dolle RE, Hoyer D, Prasad CVC, Schmidt SJ, Helaszek CT, Miller RE, Ator MA. 1994. P_1 aspartate-based peptide α-((2,6-dichlorobenzoyl)oxy)-methyl ketones as potent time-dependent inhibitors of interleukin-1β converting enzyme. *J Med Chem 37*:563–564.

Ellis RE, Yuan J, Horvitz HR. 1991. Mechanisms and functions of cell death. *Annu Rev Cell Biol 7*:663–698.

Fersht A. 1985. *Enzyme structure and mechanism, 2nd ed.* New York: W.H. Freeman and Co. pp 150–152.

Frankfater A, Kuppy T. 1981. Mechanism of association of *N*-acetyl-L-phenylalanylglycinal to papain. *Biochemistry 20*:5517–5524.

Gagliardini V, Fernandez PA, Lee RKK, Drexler HCA, Rotello RJ, Fishman MC, Yuan J. 1994. Prevention of vertebrate neuronal death by the *crmA* gene. *Science 263*:826–828.

Geiger T, Towbin H, Cosenti-Vargas A, Zingel O, Arnold J, Rordorf C, Glatt M, Vosbeck K. 1993. Neutralization of interleukin-1β activity in vivo with a monoclonal antibody alleviates collagen-induced arthritis in DBA/1 mice and prevents the associated acute-phase response. *Clin Exp Rheumatol 11*:515–522.

Hammes GG, Schimmel PR. 1970. Rapid reactions and transient states. *The Enzymes 2*:67–114.

Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC. 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature 343*:336–340.

Hazuda DJ, Lee JC, Young PR. 1988. The kinetics of interleukin 1 secretion from activated monocytes. *J Biol Chem 263*(17):8473–8479.

Hengartner MO, Ellis RE, Horvitz HR. 1992. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature 356*:494–499.

Hengartner MO, Horvitz HR. 1994. *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell 76*:665–676.

Hogquist KA, Nett MA, Unanue ER, Chaplin DD. 1991a. Interleukin 1 is processed and released during apoptosis. *Proc Natl Acad Sci USA 88*: 8485–8489.

Hogquist KA, Unanue ER, Chaplin DD. 1991b. Release of IL-1 from mononuclear phagocytes. *J Immunol 147*:2181–2186.

Howard AD, Kostura MJ, Thornberry N, Ding GJF, Limjuco G, Weidner J, Salley JP, Hogquist KA, Chaplin DD, Mumford RA, Schmidt JA, Tocci MJ. 1991. IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31 kDa IL-1α. *J Immunol 147*:2964–2969.

Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. 1993. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: An early marker of chemotherapy-induced apoptosis. *Cancer Res 53*:3976–3985.

Komiyama T, Ray CA, Pickup DJ, Howard AD, Thornberry NA, Peterson EP, Salvesen G. 1994. Inhibition of interleukin-1β converting enzyme by the cowpox virus serpin crmA. *J Biol Chem 269*(30):19331–19337.

Kostura MJ, Tocci MJ, Limjuco G, Chin J, Cameron P, Hillman AG, Chartrain NA, Schmidt JA. 1989. Identification of a monocyte specific pre-interleukin 1β convertase activity. *Proc Natl Acad Sci USA 86*:5227–5231.

Krantz A, Copp LJ, Coles PJ, Smith RA, Heard SB. 1991. Peptidyl (acyloxy)-methyl ketones and the quiescent affinity label concept: The departing group as a variable structural element in the design of inactivators of cysteine proteinases. *Biochemistry 30*:4678–4687.

Kuchler K. 1993. Unusual routes of protein secretion: The easy way out. *Trends Cell Biol 3*:421–426.

Kumar S, Kinoshita M, Noda M, Copeland NG, Jenkins NA. 1994. Induction of apoptosis by the mouse *Nedd2* gene, which encodes a protein similar to the product of *Caenorhabditis elegans* cell death gene *ced-3* and the mammalian IL-1β-converting enzyme. *Genes & Dev 8*:1613–1626.

Kurz LC, Weitkamp E, Frieden C. 1987. Adenosine deaminase: Viscosity studies and the mechanism of binding of substrate and of ground- and transition-state analogue inhibitors. *Biochemistry 26*:3027–3032.

Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, Earnshaw WC. 1994. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature 371*:346–347.

Limjuco G, Galuska S, Chin J, Cameron P, Boger J, Schmidt JA. 1986. Antibodies of predetermined specificity to the major charged species of human interleukin 1. *Proc Natl Acad Sci USA 83*:3972–3976.

Mackenzie NE, Grant SK, Scott AI, Malthouse JPG. 1986. ^{13}C NMR study of the stereospecificity of the thiohemiacetals formed on inhibition of papain by specific enantiomeric aldehydes. *Biochemistry 25*:2293–2298.

March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G, Price V, Gillis S, Henney CS, Kronheim SR, Grabstein K, Conlon PJ, Hopp TP, Cosman D. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature 315*:641–647.

Ménard R, Carrière J, Laflamme P, Plouffe C, Khouri HE, Vernet T, Tessier DC, Thomas DY, Storer AC. 1991. Contribution of the glutamine 19 side chain to transition-state stabilization in the oxyanion hole of papain. *Biochemistry 30*:8924–8928.

Miller DK, Ayala JM, Egger LA, Raju SM, Yamin TT, Ding GJF, Gaffney EP, Howard AD, Palyha OC, Rolando AM, Salley JP, Thornberry NA, Weidner JR, Williams JH, Chapman KT, Jackson J, Kostura MJ, Limjuco G, Molineaux SM, Mumford RA, Calaycay JR. 1993a. Purification and characterization of active human interleukin-1β-converting enzyme from THP.1 monocytic cells. *J Biol Chem 268*(24):18062–18069.

Miller DK, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Molineaux SM, Thornberry NA. 1993b. The IL-1β converting enzyme as a therapeutic target. *Ann NY Acad Sci 696*:133–148.

Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J. 1993. Induction of apoptosis in fibroblasts by Il-1β converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell 75*:653–660.

Mjalli AMM, Chapman KT, MacCoss M. 1993a. Synthesis of a peptidyl 2,2-difluoro-4-phenylbutyl ketone and its evaluation as an inhibitor of interleukin-1β converting enzyme. *Bioorg Med Chem Lett 3*(12):2693–2698.

Mjalli AMM, Chapman KT, MacCoss M, Thornberry NA. 1993b. Phenylalkyl ketones as potent reversible inhibitors of interleukin-1β converting enzyme. *Bioorg Med Chem Lett 3*(12):2689–2692.

Molineaux SM, Casano FJ, Rolando AM, Peterson EP, Limjuco G, Chin J, Griffin PR, Calaycay JR, Ding GJF, Yamin TT, Palyha OC, Luell S, Fletcher D, Miller DK, Howard AD, Thornberry NA, Kostura MJ. 1993. Interleukin 1β (IL-1β) processing in murine macrophages requires a structurally conserved homologue of human IL-1β converting enzyme. *Proc Natl Acad Sci USA 90*:1809–1813.

Mosley B, Urdal DL, Prickett KS, Larsen A, Cosman D, Conlon PJ, Gillis S, Dower SK. 1987. The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. *J Biol Chem 262*:2941–2944.

Odake S, Kam CM, Narasimhan L, Poe M, Blake JT, Krahenbuhl O, Tschopp J, Powers JC. 1991. Human and murine cytotoxic T lymphocyte serine proteases: Subsite mapping with peptide thioester substrates and inhibition of enzyme activity and cytolysis by isocoumarins. *Biochemistry 30*:2217–2227.

Pennington MW, Thornberry NA. 1994. Synthesis of a fluorogenic interleukin-1β converting-enzyme substrate based on resonance energy-transfer. *Pept Res 7*(2):72–76.

Poe M, Blake JT, Boulton DA, Gammon M, Sigal NH, Wu JK, Zweerink HJ. 1991. Human cytotoxic lymphocyte granzyme B. *J Biol Chem 266*: 98–103.

Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS, Pickup DJ. 1992. Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1β converting enzyme. *Cell 69*: 597–604.

Reiter LA. 1994. Peptidic *p*-nitroanilide substrates of interleukin-1β-converting enzyme. *Int J Pept Protein Res 43*:87–96.

Rich DH. 1986. Inhibitors of cysteine proteases. In: Barrett AJ, Salvesen G, eds. *Proteinase inhibitors*. Amsterdam: Elsevier. pp 153–178.

Rolando AM, Palyha OC, Ding GJF, Howard AD, Molineaux SM. 1994. Intermolecular, autocatalytic processing of the proenzyme of Il-1β converting enzyme (ICE) in baculovirus-infected cells. *J Cell Biochem 18D*:147.

Rubartelli A, Cozzolino F, Talio M, Sitia R. 1990. A novel secretory pathway for interleukin-1β, a protein lacking a signal sequence. *EMBO J 9*(5):1503–1510.

Schröder E, Phillips C, Garman E, Harlos K, Crawford C. 1993. X-ray crys-

tallographic structure of a papain-leupeptin complex. *FEBS Lett 315*: 38-42.

Shaw E. 1990. Cysteinyl proteinases and their selective inactivation. *Adv Enzymol 63*:271-347.

Singer II, Scott S, Chin J, Kostura MJ, Miller DK, Chapman K, Bayne EK. 1993. Interleukin-1β converting enzyme is localized on the external cell surface membranes and in the cytoplasmic ground substance of activated human monocytes by immuno-electronmicroscopy. *Lymphokine Cytokine Res 12*:340.

Singer II, Scott S, Hall GL, Limjuco G, Chin J, Schmidt JA. 1988. Interleukin 1β is localized in the cytoplasmic ground substance but is largely absent from the golgi apparatus and plasma membranes of stimulated human monocytes. *J Exp Med 167*:389-407.

Sleath PR, Hendrickson RC, Kronheim SR, March CJ, Black RA. 1990. Substrate specificity of the protease that processes human interleukin-1β. *J Biol Chem 265*(24):14526-14528.

Smith RA, Copp LJ, Coles PJ, Pauls HW, Robinson VJ, Spencer RW, Heard SB, Krantz A. 1988a. New inhibitors of cysteine proteinases. Peptidyl acyloxymethyl ketones and the quiescent nucleofuge strategy. *J Am Chem Soc 110*:4429-4431.

Smith RA, Copp LJ, Donnelly SL, Spencer RW, Krantz A. 1988b. Inhibition of cathepsin B by peptidyl aldehydes and ketones: Slow-binding behavior of a trifluoromethyl ketone. *Biochemistry 27*:6568-6573.

Spriggs MK, Hruby DE, Maliszewski CR, Pickup DJ, Sims JE, Buller RML, VanSlyke J. 1992. Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. *Cell 71*:145-152.

Thornberry NA. 1994. Interleukin-1β converting enzyme. *Methods Enzymol 244*:615-631.

Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, Elliston KO, Ayala JM, Casano FJ, Chin J, Ding GJF, Egger LA, Gaffney EP, Limjuco G, Palyha OC, Raju SM, Rolando AM, Salley JP, Yamin TT, Lee TD, Shively JE, MacCoss M, Mumford RA, Schmidt JA, Tocci MJ. 1992. A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. *Nature 356*:768-774.

Thornberry NA, Peterson EP, Zhao JJ, Howard AD, Griffin PR, Chapman KT. 1994. Inactivation of interleukin-1β converting enzyme by peptide (acyloxy)methyl ketones. *Biochemistry 33*:3934-3940.

Vaux DL, Weissman IL, Kim SK. 1992. Prevention of programmed cell death in *Caenorhabditis elegans* by human bcl-2. *Science 258*:1955-1957.

Walker NPC, Talanian RV, Brady KD, Dang LC, Bump NJ, Ferenz CR, Franklin S, Ghayur T, Hackett MC, Hammill LD, Herzog L, Hugunin M, Houy W, Mankovich JA, McGuiness L, Orlewicz E, Paskind M, Pratt CA, Reis P, Summani A, Terranova M, Welch JP, Xiong L, Möller A, Tracey DE, Kamen R, Wong WW. 1994. Crystal structure of the cysteine protease interleukin-1β-converting enzyme: A $(p20/p10)_2$ homodimer. *Cell 78*:343-352.

Wang L, Miura M, Bergeron L, Zhu H, Yuan J. 1994a. *Ich-1*, an *Ice/ced-3*-related gene, encodes both positive and negative regulators of programmed cell death. *Cell 78*:739-750.

Wang XM, Helaszek CT, Winter LA, Lirette RP, Dixon DC, Ciccarelli RB, Kelley MM, Malinowski JJ, Simmons SJ, Huston EE, Koehn JA, Kratz D, Bruckner RC, Graybill T, Ator MA, Lehr RV, Stevis PE. 1994b. Production of active human interleukin-1β-converting enzyme in a baculovirus expression system. *Gene 145*:273-277.

Westerik JO, Wolfenden R. 1972. Aldehydes as inhibitors of papain. *J Biol Chem 247*(24):8195-8197.

Wilson KP, Black JF, Thomson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA, Livingston DJ. 1994. Structure and mechanism of interleukin-1β converting enzyme. *Nature 370*:270-275.

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. 1993. The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell 75*:641-652.