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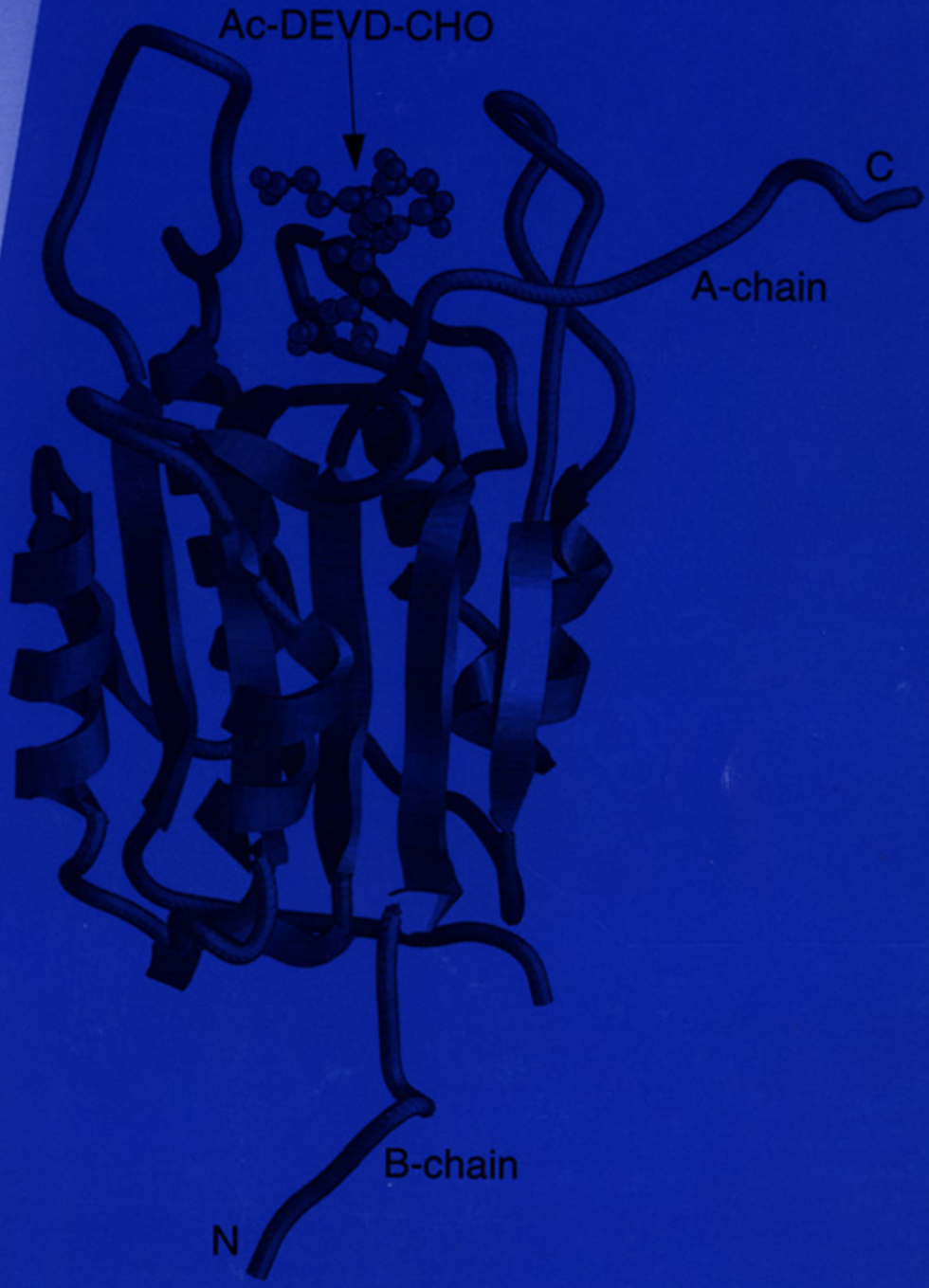

Caspase-8

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Prediction of the tertiary structure and substrate binding site of caspase-8

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Abstract The caspases represent a family of sulfhydryl proteases that play important regulatory roles in the cell. The tertiary structure of the protease domain of caspase-8, also called FLICE, has been predicted by a segment match modeling procedure. First, the atomic coordinates of the catalytic domain of caspase-3, also called CPP32, a member of the family that is closely related to caspase-8, were determined based upon the crystal structure of human caspase-1 (interleukin converting enzyme). Then, the caspase-3 structure was used as a template for modeling the protease domain of caspase-8. The resulting structure shows the expected level of similarity with the conformations of caspases-1 and -3 for which crystal structures have been determined. Moreover, the subsite contacts between caspase-8 and the covalently linked inhibitor, Ac-DEVD-aldehyde, are only slightly different from those seen in the caspase-3 enzyme/inhibitor complex. The model of caspase-8 can serve as a reference for subsite analysis relative to design of enzyme inhibitors that may find therapeutic application.

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Key words: ICE family; CPP32; FLICE; Segment match modeling

1. Introduction

The caspase family of cysteine proteases occupies a central role in the induction of apoptosis in cells responding to a variety of insults. They have, therefore, attracted considerable attention as pharmaceutical targets for the treatment of neurodegenerative diseases, ischemic injury and cancer [1,2]. Pharmacological manipulation of the caspase proteases offers convincing evidence for their role in programmed cell death. Studies employing Crm-A, a cowpox virus protein that specifically inhibits certain caspases [3], provided the first pharmacologic evidence for cellular protection through inhibition of caspase-1, also called ICE (interleukin converting enzyme). Rat fibroblasts transfected with Crm-A are resistant to death induced by serum withdrawal or by overexpression of caspase-1 [4]. Microinjection of Crm-A into dorsal root ganglion neurons in vitro enhanced survival after withdrawal of nerve growth factor [5]. In addition, Crm-A provided protection from apoptosis induced by tumor necrosis factor and anti-fas stimulated cell death in several tumor cell lines [6–8]. Crm-A also replaces the protective effect of extracellular matrix proteins on cultures of primary breast epithelial cells [9]. Finally, peptide inhibitors of caspases prevent cell death in certain model systems, supporting the strategy of targeting these proteases with small molecule inhibitors [4,10,32].

To date, 10 caspase family members have been identified [11–22,33]. Phylogenetic analysis divides the existing family members into two subfamilies. One subfamily, typified by caspase-1, may play its role in cytokine processing. The second subfamily, represented by caspase-3 (also called CPP32), appears involved in the control of cell death. Biochemical studies are beginning to define the substrate and inhibitor specificities for individual members of the caspase family. Although there is some overlap in substrate specificities within the family, several notable distinctions allow biochemical dissection of protease activities in complex mixtures. In general, death effector proteases, typified by caspase-3, readily cleave DEVD-X sequences while the inflammatory cytokine processing caspases like caspase-1 recognize the sequence YVAD-X [23a,24].

Although numerous studies define a central role for caspases as effector molecules of the cell death pathway, the regulatory mechanism governing activation of the enzymes remains unclear. Muzio et al. [22] and Boldin et al. [23] recently described caspase-8 as a key regulator in the death cascade. Caspase-8, also called FLICE, displays a novel N-terminal domain with sequence homology to the death effector domain of FADD (fas associated protein with death domain) that allows association of caspase-8 with TNF/FAS family receptors. The association of caspase-8 with cell surface death receptors suggests this caspase as a proximal regulator of apoptosis and provides a clue for selection of the key death protease as a potential therapeutic target. Further evidence identifying caspase-8 as an interesting point for pharmacologic control of cell death comes from recent studies showing potent inhibition of caspase-8 by recombinant CrmA [25]. This inhibition may account for the ability of Crm-A to prevent apoptosis in a variety of cellular systems.

Recent crystallographic studies of caspase-1 and caspase-3 reveal similarity in their overall tertiary and quaternary structures. Notable differences within the enzyme active sites, however, impart the distinct macromolecular specificities and different biological functions of these two enzymes. These differences may provide the basis for design of specific caspase inhibitors. In view of the interest in regulating specific caspases in disease, we have predicted the structure of caspase-8 using the existing structural information of caspase-1 and caspase-3.

2. Materials and methods

Results of sequence alignment indicate that caspase-8 is closely related to caspase-3, but both are more distantly related to caspase-1. This is consistent with the fact that the caspase-8 and caspase-3 have similar biological function, i.e. both are involved in the control of cell death as mentioned above. Although the crystal structure of the protease domain of caspase-3 was determined recently [26], its atomic coordinates are not available to the public. In view of this, our strategy for predicting the three-dimensional structure of the pro-

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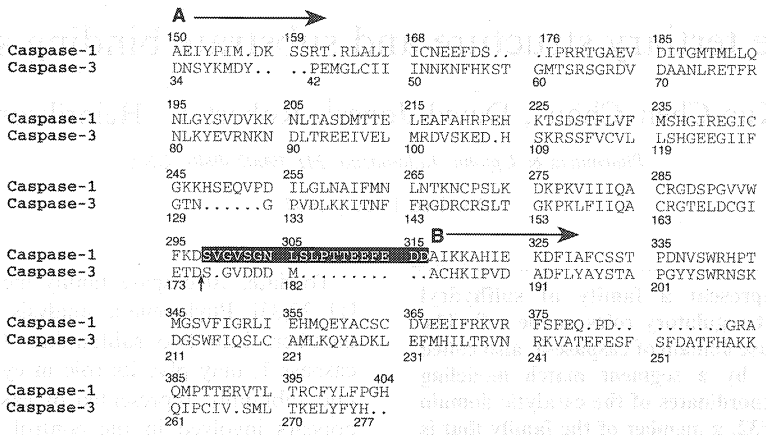

Fig. 1. The amino acid sequence alignment between caspase-1 (ICE) and caspase-3 (CPP32). The alignment was conducted by matching conformationally equivalent residues in the two protein chains [26]. The sequence positions for some amino acids are respectively marked by a number closest to them. From these numbers, the sequence locations for all the other amino acids can be easily identified. The caspases are proteolytically processed in a region just C-terminal to the active site cysteine residue (285/163). In the case of caspase-1 two cleavages occur to remove a 19 residue segment shown in a black box. For caspase-3, only a single hydrolysis takes place as indicated by the arrow.

tease domain of caspase-8 was composed of the following two procedures. First, we determined the atomic coordinates of the protease domain of caspase-3 based on the crystal structure of human caspase-1 [27]. During the modeling process [28], the amino acid sequence alignment of the two proteins was based on the report by Rotonda et al. [26]. This reduced considerably the deviation of the

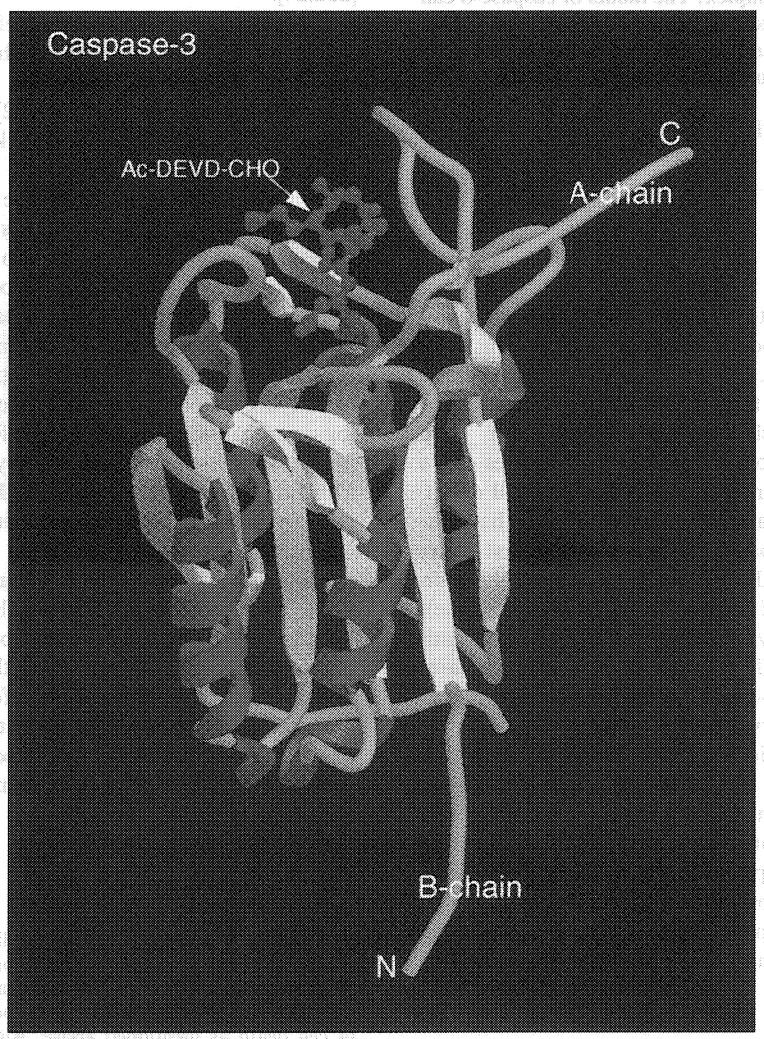

Fig. 2. Schematic representation of caspase-3:Ac-DEVD-CHO. The ribbon drawing was used for the enzyme, where β-strands are in yellow and α-helices in blue. The bound inhibitor Ac-DEVD-CHO is located at the top center of the figure that is represented by a ball-and-stick drawing with purple.

modeled structure of caspase-3 from its crystal structure because the alignment was based on the conformational alignment of the three-dimensional structures of caspase-1 and caspase-3. Second, the atomic coordinates thus obtained for the protease domain of caspase-3 were used as a template to predict the protease domain of caspase-8 [28].

3. Results

3.1. From caspase-1 to caspase-3

The amino acid sequence alignment between caspase-1 and caspase-3 by Rotonda et al. [26] is shown in Fig. 1, where the numbers above the blocks indicate the sequence positions of amino acids in caspase-1, while the numbers under the blocks indicate those in caspase-3. Because of autoprocessing, each of the two protein molecules consists of an A chain of about 17–20 kDa, and a B chain of about 10–12 kDa. In the case of caspase-1, a 19 amino acid segment (from Ser-298 to Asp-316) is removed during this process and this segment is marked with a black box with white codes in Fig. 1. For caspase-3, however, only a single cleavage occurs between Asp-175 and Ser-176 (see the arrow in Fig. 1) with no loss of a peptide connecting segment [32,34].

Based on the sequence alignment of Fig. 1 and the X-ray structure of human caspase-1 [27], the corresponding three-dimensional structure for caspase-3 was derived by means of the segment match modeling method [28]. The structure thus obtained is given in Fig. 2, where both the tertiary structures of the A chain and B chain, as well as their relative position, are uniquely defined. Furthermore, based on the coordinates of the T chain, a tetrapeptide (Ac-YVAD-CHO) inhibitor in complex with caspase-1 [27], the binding site of the peptide-aldehyde inhibitor (Ac-DEVD-CHO) to caspase-3 is also given. This procedure has been shown to be highly accurate for eight test proteins ranging in size from 46 to 323 residues, where the all-atom root-mean-square deviation of the modeled structures is between 0.93 Å and 1.73 Å [28].

3.2. From caspase-3 to caspase-8

The atomic coordinates obtained above for caspase-3 served as a template to predict the structure of caspase-8. First, the sequences of caspase-3 and caspase-8 were aligned using the BESTFIT program in the GCG package [29]. The aligned result is given in Fig. 3, where the numbers above the blocks indicate the sequence positions of amino acids in caspase-3, while the numbers under the blocks indicate those in caspase-8. As in the case for caspase-1, processing of caspase-8 leads to loss of a short peptide segment connecting the amino domain (~20 kDa) and carboxyl domain (~10 kDa) domain; this decapeptide segment (from Ser-375 to Asp-384) is shown in a black box of Fig. 3. Thus, based on the sequence alignment of Fig. 3 and the atomic coordinates of caspase-3 obtained above, the corresponding three-dimensional structure for caspase-8 was derived, once more, by means of the segment match modeling method [28]. The structure thus obtained is given in Fig. 4, where both the tertiary structures of the A chain and B chain, as well as their relative position, are uniquely defined. Furthermore, based on the coordinates of caspase-3:Ac-DEVD-CHO complex, the binding site of the peptide-aldehyde inhibitor (Ac-DEVD-CHO) to caspase-8 is also given.

4. Discussion

It has been recognized for decades that the catalytic apparatuses of digestive proteinases can be conscripted among families of related enzymes which engage in highly selective regulatory processes. The recognition of the caspase family of enzymes, following upon the identification of their progenitor, ICE or caspase-1, is just another variation on that theme. These are sulfhydryl proteinases, reminiscent of papain and other digestive enzymes, but they share a unique aspect of processing and of tertiary structural organization in which monomeric units of a homodimer are cleaved in a region just C-terminal to the active site thiol, so that the dimmer consists of four polypeptide chains [26,27]. The discovery of caspase-1, typifying one subfamily of these enzymes, had an immediate impact on our understanding of the activation mechanism of IL-1, an important pro-inflammatory cytokine. Now, more recently, the identification of another subfamily of the caspases, including caspases-3 and -8 as players in a cascade of events leading to programmed cell death, extends the repertoire of this important class of sulfhydryl proteinases.

Because it appears that caspase-8 may play a role in the progression of events leading to apoptosis that is most proximal to the original signal, we were interested in knowing more about its catalytic site and mode of substrate binding.

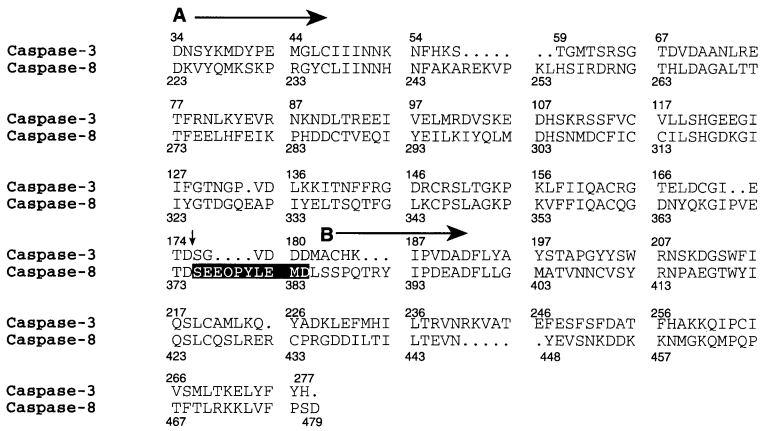

Fig. 3. The amino acid sequence alignment between caspase-3 and caspase-8 obtained by running the BESTFIT program in the GCG package [29]. The black box indicates the decapeptide segment removed from caspase-8 during processing. See the legend to Fig. 1 for further explanation.

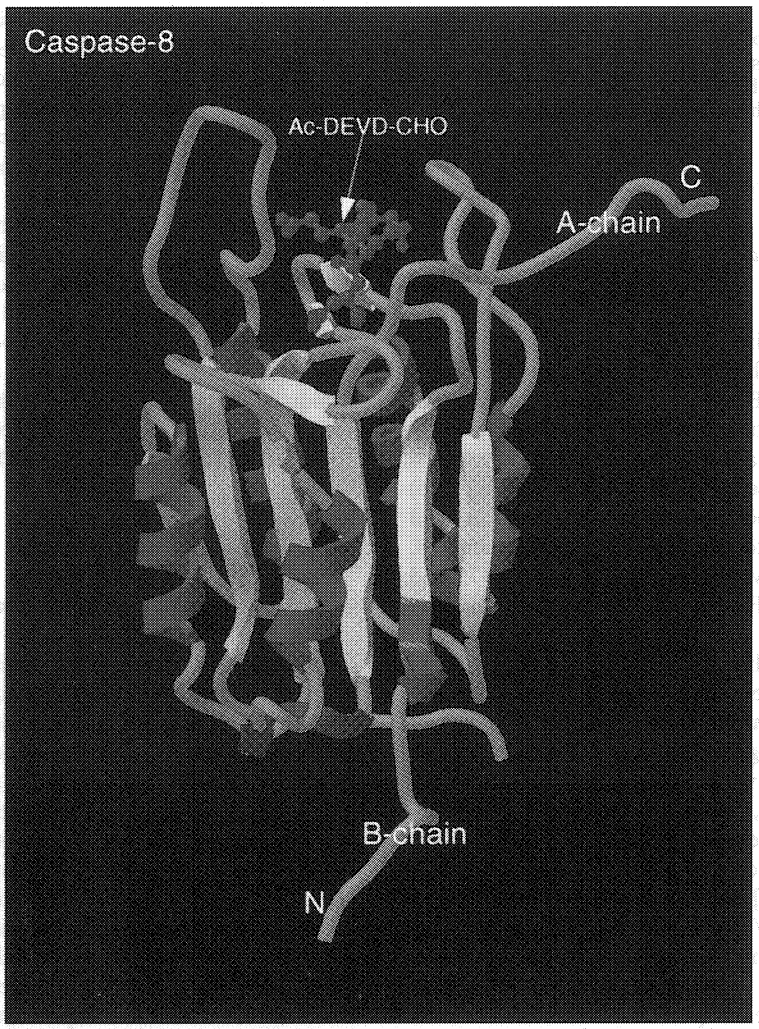

Fig. 4. Schematic representation of caspase-8:Ac-DEVD-CHO. See the legend to Fig. 2 for further explanation.

Two structural papers, one on caspase-1 [27] and one on caspase-3 [26], were available for the purposes of modeling of caspase-8. Since caspase-8 is more closely related to caspase-3, both in terms of the degree of sequence identity and substrate preference, it was desirable to base the modeling on the caspase-3:Ac-DEVD-CHO complex. Since X-ray coordinates were available only for caspase-1, we adopted a strategy that involved first modeling of the caspase-3:Ac-DEVD-CHO structure from the caspase-1:Ac-YVAD-CHO complex, and then using the resulting caspase-3:Ac-DEVD-CHO model to predict that for caspase-8:Ac-DEVD-CHO. We were greatly aided in this work by the availability of a sequence alignment for caspase-1 and caspase-3 [26] that was based upon conformational equivalent residues in each protein. This allowed us, by inference, to make similar structural assignments for residues in caspase-8, since this enzyme shows a high degree of identity to caspase-3.

The predicted structure of caspase-8 shown in the schematic model of Fig. 4 and the stereo depiction in Fig. 5 shows two views of the molecule with different orientations of the inhibitor in the binding pocket. As might be expected, the secondary and super-secondary structural arrays are conserved in caspases-1 and -3. Thus, the arrays of β-strand/sheet surrounded by surface α-helices are shared, by and large, by the two caspases. As is the case with other ICE protease family members, the C-terminus of the A chain and the N-terminus of the B chain lie at opposite ends of the molecule (Fig. 4), and these segments appear to play a role in the formation of protease homodimers. Residues shown in green in Fig. 5 are highlighted to indicate their involvement in subsite interactions with the bound inhibitor, Ac-DEVD-CHO (yellow). For the most part, these residues are conserved between caspase-3 [26] and caspase-8.

Of special interest is the similarity in subsites S_1 through S_4 seen in caspase-3 [26] and predicted in caspase-8. A comparison of subsite/inhibitor interactions seen in caspase-3 and predicted in caspase-8 is given in Table 1, where the corresponding subsites in the inhibitor are expressed by R_1–R_4 [30,31]. First, as we can see, like the binding of caspase-3 with Ac-DEVD-CHO [26], a covalent bond linking the aldehyde of the inhibitor to the thiol of Cys-360 can also be formed for the caspase-8:Ac-DEVD-CHO complex. Next, the protein subsites for the inhibitor R_1-R_4 sites were examined, maintaining the general binding features of Ac-YVAD-CHO and Ac-DEVD-CHO seen in caspase-1 [27] and caspase-3 [26], respectively. As might be expected, the interactions between the inhibitor and S_1 were completely in accord with what was seen in caspase-3 [26]. The Asp residue at R_1 of the

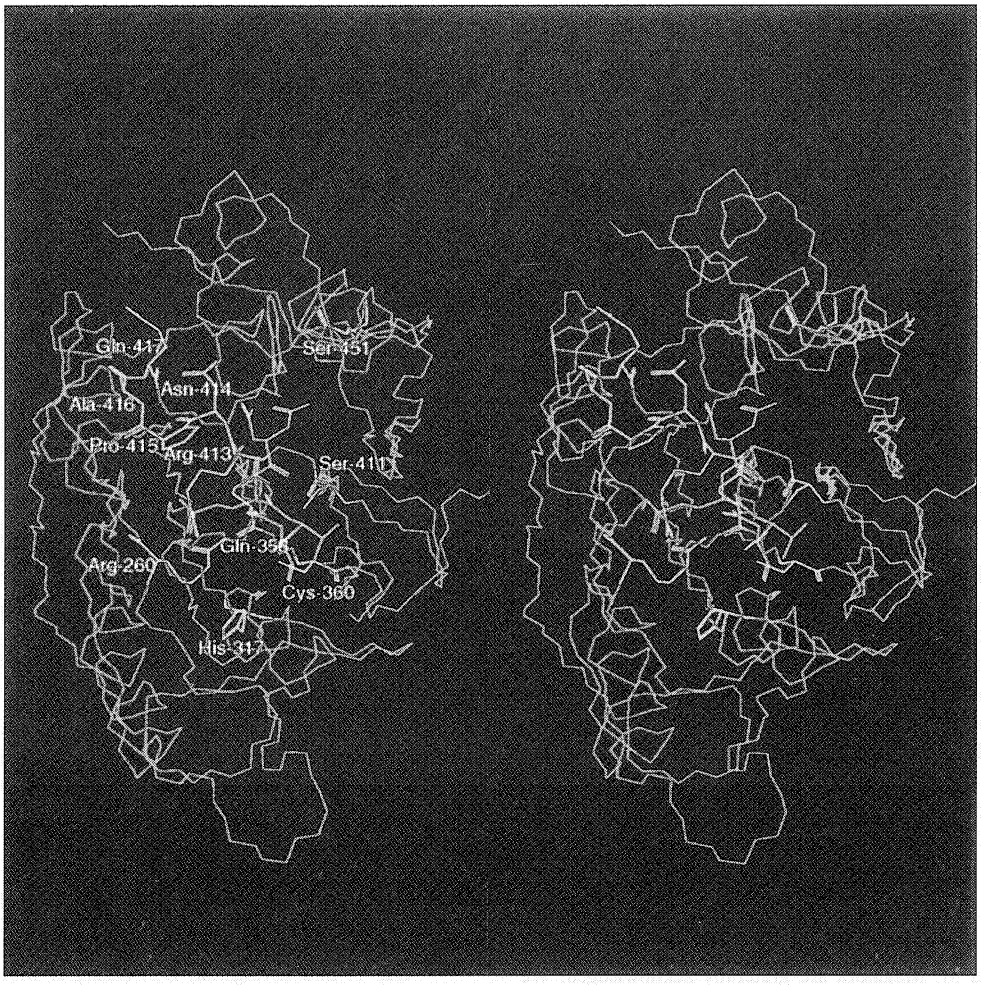

Fig. 5. Stereo drawing to show the binding interaction of caspase-8 with the inhibitor Ac-DEVD-CHO (in yellow). For clarity, only the backbone of the enzyme (red) is shown except those residues that directly interact with the inhibitor as described in Table 1. For these residues functioning as a binding pocket, the side chains (green) are also included.

Table 1
A comparison of Ac-DEVD-CHO binding sites in caspase-3 and caspase-8

Subsite	Bonding	Seen in caspase-3[a]	Predicted in caspase-8
S_1	Covalent thiohemiacetal[b]	Cys-163 (285)	Cys-360
	H-bond from Im[c] to C=O of aldehyde	His-121 (237)	His-317
	H-bond from backbone C=O to HN of R_1	Ser-205 (339)	Ser-411
	H-bond from γ-NH to β-COOH at R_1	Gln-160 (283)	Gln-358
	H-bond from Gu[d] to β-COOH at R_1	Arg-64 (179)	Arg-260
	H-bond from Gu[d] to β-COOH at R_1	Arg-207 (341)	Arg-413
S_2	H-bond from Gu[d] to C=O of R_2	Arg-207 (341)	Arg-413
S_3	H-bond from Gu[d] to γ-COOH of R_3	Arg-207 (341)	Arg-413
	H-bond from β-OH to γ-COOH at R_3	Ser-209 (343)	not possible
S_4	H-bond from NH to β-COOH of R_4	Phe-250 (381b)	Ser-451
	H-bond from β-NH_2 to β-COOH of R_4	Asn-208 (342)	Asn-414
	H-bond network to Asn β C=O	Residues 209–211 (343–345)	Residues 415–417
	H-bond from β-OH to acetyl C=O	Ser-209 (343)	not possible
	H-bond from NH to acetyl C=O	Ser-209 (343)	not possible
	H-bond from NH to N_{342} in caspase-3	Ser-209 (343)	not possible

[a]Residue numbers are as defined in Fig. 3 with respect to the alignment with caspase-8. Numbers in parentheses are those used by Rotonda et al. [26] that are defined in the sequence frame of caspase-1.
[b]Bond linking aldehyde of inhibitor to thiol of Cys.
[c]Im = imidazole.
[d]Gu = guanadino group.

inhibitor is required for enzyme specificity, and its binding involves amino acids in the enzyme which are entirely conserved. Thus, the β-COOH group of Asp-1 of the tetrapeptide inhibitor is nicely coordinated with Arg-260, Gln-358, and Arg-413, and the inhibitor's main-chain CO and NH moieties are hydrogen-bonded to the enzyme's His-317 and Ser-411, respectively.

Subsite S_2, also involving an interaction between the NH of the exposed Val-2 peptide backbone of the inhibitor and Arg-413 of the enzyme, appears to be identical to what was documented for caspase-3 [26]. Arg-413 is also part of subsite S_3, as it is involved in an intricate network of H-bonds including the γ-COOH of Glu-3. As was the case for caspases-1 and -3, differences begin to emerge in the S_4 subsite. In the case of the aforementioned comparison, dramatic differences might have been expected since R_4 in caspase-1 is a tyrosine residue, and in caspase-3 R_4 is an aspartic acid. We know that caspases-3 and -8 differ subtly in specificity, so one might expect to see small differences in substrate binding to these enzymes. The most apparent difference we see in the predicted caspase-8:substrate complex is a proline in S_4 replacing a serine in caspase-3. The serine NH and OH groups appear to play a significant role in enzyme/inhibitor interactions that will not be possible for the amino acid replacement in caspase-8. This may serve to explain, at least in part, why the DEVD-based motif is preferred by caspase-3 over caspase-8.

The model for caspase-8 predicted in this study will serve as a reference for thinking about design of inhibitors while we await publication of the X-ray coordinates of caspase-3. This new information should allow a significant refinement in our model.

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References

[1] Nicholson, D.W. (1996) Nature Biotech. 14, 297–301.
[2] Thornberry, N.A., Miller, D.K. and Nicholson, D.W. (1995) Perspect. Drug Discov. Design 2, 389–399.
[3] Ray, C.A., Black, R.A., Kronheim, S.R., Greenstreet, T.A., Sleath, P.R., Salvesen, G.S. and Pickup, D.J. (1992) Cell 69, 597–604.
[4] Miura, M., Zhu, H., Rotello, R., Hartwieg, E.A. and Yuan, J. (1993) Cell 75, 653–660.
[5] Gagliardini, V., Fernandez, P.A., Lee, R.K.K., Drexler, H.C.A., Rotello, R.J., Fishman, M.C. and Yuan, J. (1994) Science 263, 826–828.
[6] Tewari, M. and Dixit, V.M. (1995) J. Biol. Chem. 270, 3255–3260.
[7] Enari, M., Hug, H. and Nagata, S. (1995) Nature 375, 78–81.
[8] Miura, M., Friedlander, R.M. and Yaun, J. (1995) Proc. Natl. Acad. Sci. USA 92, 8318–8322.
[9] Boudreau, N., Sympson, C.J., Werb, Z. and Bissell, M.J. (1995) Science 267, 891–893.
[10] Milligan, C.E., Prevette, D., Yaginuma, H., Homma, S., Cardwell, C., Fritz, L.C., Tomaselli, K.J., Oppenheim, R.W. and Schwartz, L.M. (1995) Neuron 15, 385–393.
[11] Kumar, S., Kinoshita, M., Noda, M., Copeland, N.G. and Jenkins, N.A. (1994) Genes Dev. 8, 1613–1626.
[12] Wang, L., Miura, M., Bergeron, L., Zhu, H. and Yuan, J. (1994) Cell 78, 739–750.
[13] Fernandes-Alnermi, T., Litwack, G. and Alnermi, E.S. (1994) J. Biol. Chem. 269, 30761–30764.
[14] Fernandes-Alnemri, T., Litwack, G. and Alnemri, E.S. (1995) Cancer Res. 55, 2737–2742.
[15] Fernandes-Alnemri, T., Takahashi, A., Armstrong, R., Krebs, J., Fritz, L., Tomaselli, K.J., Wang, L., Yu, Z., Croce, C.M., Salveson, G., Earnshw, W.C., Litwack, G. and Alnemri, E.S. (1995) Cancer Res. 55, 6045–6052.
[16] Faucheu, C., Diu, A., Chan, A.W.E., Blanchet, A.M., Miossec, C., Herve, F., Collard-Dutilleul, V., Gu, Y., Aldape, R.A., Lippke, J.A., Rocher, C., Su, M.S.-S., Livingston, D.J., Hercend, T. and Lalanne, J.L. (1995) EMBO J. 14, 1914–1922.
[17] Kamens, J., Paskind, M., Hugunin, M., Talanian, R.V., Allen, H., Danach, D., Bump, N., Hackett, M., Johnston, C.G., Li, P., Mankovich, J.A., Terranova, M. and Ghayur, T. (1995) J. Biol. Chem. 270, 15250–15256.
[18] Munday, N.A., Vaillancourt, J.P., Ali, A., Casano, F.J., Miller, D.K., Molineaux, S.M., Yamin, T.T., Yu, V.L. and Nicholson, D.W. (1995) J. Biol. Chem. 270, 15870–15876.
[19] Lippke, J.A., Gu, Y., Samecki, C., Caron, P.R. and Su, M.S.-S. (1996) J. Biol. Chem. 271, 1825–1828.
[20] Duan, H., Chinnaiyan, A.M., Hudson, P.L., Wing, J.P., He, W.W. and Dixit, V.M. (1996) J. Biol. Chem. 271, 1621–1625.
[21] Duan, H., Orth, K., Chinnaiyan, A., Poirier, G., Froelich, C.J., He, W.W. and Dixit, V.M. (1996) J. Biol. Chem. 271, 16720–16724.
[22] Muzio, M., Chinnaiyan, A.M., Kischkel, F.C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J.D., Zhang, M., Gentz, R., Mann, M., Krammer, P.H., Peter, M.E. and Dixit, V.M. (1996) Cell 85, 817–827.
[23] Boldin, M.P., Goncharov, T.M., Goltsev, Y.V. and Wallach, D. (1996) Cell 85, 803–815.
[23a] Talanian, R.V., Quinlan, C., Trautz, S., Hackett, M.C., Mankovich, J.A., Banach, D., Ghayur, T., Brady, K.D. and Wong, W.W. (1997) J. Biol. Chem. 272, 9677–9682.
[24] Argolin, N., Raybuck, S.A., Wilson, K.P., Chen, W., Fox, T., Gu, Y. and Livingston, D.J. (1997) J. Biol. Chem. 272, 7223–7228.
[25] Zhou, Q., Snipas, S., Orth, K., Muzio, M., Dixit, V.M. and Salvesen, G.S. (1997) J. Biol. Chem. 272, 7797–7800.
[26] Rotonda, J., Nicholson, D.W., Fazil, K.M., Gallant, M., Gareau, Y., Labelle, M., Peterson, E.P., Rasper, D.M., Ruel, R., Vaillancourt, J.P., Thornberry, N.A. and Becker, J.W. (1996) Nature Struct. Biol. 3, 619–625.
[27] Wilson, K.P., Black, J.F., Thomson, J.T., Kim, E.K., Griffith, J.P., Navia, M.N., Murcko, M.A., Chambers, S.P., Aldape, R.A., Raybuck, S.A. and Livingston, D.L. (1994) Nature 370, 270–274.
[28] Levitt, M. (1992) J. Mol. Biol. 226, 507–533.
[29] Devereux, J. (1994) The Wisconsin Sequence Analysis Package, Version 8.0, Genetic Computer Group, Madison, WI.
[30] Chou, K.C., Tomasselli, A.G., Reardon, I.M. and Heinrikson, R.L. (1996) Proteins Struct. Funct. Genet. 24, 51–72.
[31] Chou, K.C. (1996) Anal. Biochem. 233, 1–14.
[32] Nicholson, D.W., Ali, A., Thornberry, N.A., Vaillancourt, J.P., Ding, C.K., Gallant, M., Gareau, Y., Griffin, P.R., Labelle, M., Lazebnik, Y.A., Munday, N.A., Raju, S.M., Smulson, M.E., Yamin, T.T., Yu, V.L. and Miller, D.K. (1995) Nature 376, 37–43.
[33] Tewari, M., Quan, L.T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D.R., Poirier, G.G., Salvesen, G.S. and Dixit, V.M. (1995) Cell 81, 801–809.
[34] Han, Z., Hendrickson, E.A., Bremner, T.A. and Wyche, J.H. (1997) J. Biol. Chem. 272, 13432–13436.