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A combinatorial approach for determining protease specificities: application to interleukin-1β converting enzyme (ICE)

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Abstract:

Background: Interleukin-1β converting enzyme (ICE/caspase-1) is the protease responsible for interleukin-1β (IL-1β) production in monocytes. It was the first member of a new cysteine protease family to be identified. Members of this family have functions in both inflammation and apoptosis.
Results: A novel method for identifying protease specificity, employing a positional-scanning substrate library, was used to determine the amino-acid preferences of ICE. Using this method, the complete specificity of a protease can be mapped in the time required to perform one assay. The results indicate that the optimal tetrapeptide recognition sequence for ICE is WEHD, not YVAD, as previously believed, and this led to the synthesis of an unusually potent aldehyde inhibitor, Ac-WEHD-CHO (Ki=56 pM). The structural basis for this potent inhibition was determined by X-ray crystallography.
Conclusions: The results presented in this study establish a positional-scanning library as a powerful tool for rapidly and accurately assessing protease specificity. The preferred sequence for ICE (WEHD) differs significantly from that found in human pro-interleukin-1β (YVHD), which suggests that this protease may have additional endogenous substrates, consistent with evidence linking it to apoptosis and IL-1a production.

Introduction

A detailed knowledge of the specificity of a particular protease!can provide clues to its biological function(s), and!is invaluable for designing appropriate peptide-based!substrates and potent, selective inhibitors. Traditionally,!most investigations of protease specificity have required!the synthesis of large numbers of peptides and/or peptidebased!inhibitors, which were analyzed individually for!cleavage or inhibition. More recently, several investigators!have developed more efficient techniques for determining!protease specificity, including substrate phage display, methods for rapid analysis of peptide mixtures, and use of peptide nucleophiles.!Positional-scanning synthetic combinatorial libraries (PSSCL) have recently emerged as a powerful method!for rapidly identifying high-affinity peptide sequences!from a mixture of compounds. Peptide-based PS-SCLs!are generally composed of several sub-libraries in which!one position is defined with an amino acid, and the!remaining positions contain a

mixture of amino acids!present in approximately equimolar concentrations.!Analysis of the library will identify the preferred amino!acids for each position. Such libraries have been successfully!employed for the identification of potent receptor!ligands, enzyme inhibitors , and specific!antigens , but have not yet been used to determine!protease specificity.!Here we describe a novel PS-SCL designed to investigate!the specificity of interleukin-1β converting enzyme!(ICE/caspase-1, EC 3.4.22.36), the cysteine protease!responsible for the production of IL-1β in monocytes. Since its discovery in 1989, this enzyme!has been the subject of intense interest as a potential therapeutic!target for the treatment of inflammatory diseases.!The finding that ICE-deficient mice are resistant to!lipopolysaccharide-induced endotoxic shock supports!the hypothesis that this enzyme is a key mediator of!inflammation. Surprisingly, these mice are defective!not only in IL-1β; their ability to make mature IL-1a!is also impaired, suggesting that this enzyme has multiple!biological functions.!ICE is the first identified member of a new cysteine!protease family that includes CED-3, the product of!a gene required for programmed cell death in the nematode!*Caenorhabditis elegans*. The ICE/CED-3 or!caspase family currently includes ten human proteases.!

(e)

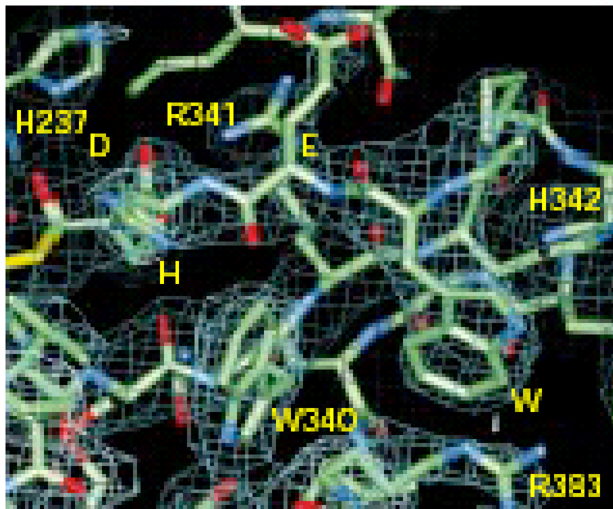

Figure 3:
(e) Electron density in the!vicinity of the bound Ac-WEHD-CHO inhibitors. The model is!displayed together with electron density from the final map. Residues!in the inhibitor are identified with the one-letter code, and residues in!the protein by one-letter code and amino-acid sequences number.!

Inhibition of ICE by Ac-WEHD-CHO

Upon discovering that the preferred substrate for!ICE is Ac-WEHD-AMC, the corresponding tetrapeptide!aldehyde was synthesized and tested, in anticipation!that it would be a more potent peptide-based inhibitor!than those previously described for this enzyme. Peptide!aldehydes are effective, competitive, reversible inhibitors!of cysteine

proteases, forming a thiohemiacetal with the!active site cysteine. Inhibition of!ICE by Ac-WEHD-CHO is characterized by a relatively!slow association rate constant, 2.6´10^{5} $M^{-1}s^{-1}$, and a dissociation!rate constant of 1.5´10^{-5} s^{-1}, corresponding to an!overall dissociation constant (K_i=k_{off}/k_{on}) of 56pM,!making it among the most potent reversible inhibitors!described for any caspase. This represents an improvement!in binding affinity of 14-fold over Ac-YVAD-CHO,!the compound previously thought to be the best peptidebased,!reversible inhibitor of ICE. Ac-WEHD-CHO is a!more potent inhibitor than Ac-YVAD-CHO because there!is an increase in the lifetime of the enzyme–inhibitor!complex ($t_{1/2\ YVAD}$=0.66 h, $t_{1/2\ WEHD}$=12.8 h) (Table 2).!

Three-dimensional structure of ICE|Ac-WEHD-CHO!

To understand the structural basis of the improved affinity!of Ac-WEHD-CHO, the three-dimensional structure!of the ICE|Ac-WEHD-CHO complex was determined by!X-ray crystallography, and is compared to that of ICE|Ac-!YVAD-CHO in Figure 3. The overall structures of!the two complexes are, in many ways, quite similar. The!same three mainchain hydrogen bonds are formed!(P_1N–Ser_{339} O, P_3N–Arg_{341} O and P_3O–Arg_{341} N) and comparable!interactions are involved in the stabilization of the!P_1 Asp. In addition, in both structures the chiral center of!the hemithioacetal is predominantly in the nontransition!state configuration, allowing the hydroxyl group to make a!polar interaction with the sidechain of the catalytic His_{237}.!In contrast to these similarities, and despite the relatively!low resolution (2.7 Å) of these studies, there are clear differences!in the protein–inhibitor interactions in the S_2, S_3!and S_4 subsites that appear to account for the increased!potency of Ac-WEHD-CHO. First, in both structures the!aromatic sidechain contacts the imidazole ring of His_{342} in!S_4. In the ICE|Ac-WEHD-CHO crystal, however, the!sidechain of Arg_{383} is rotated away from the conformation!seen in the YVAD complex and into a position that would!allow a favorable interaction with the partial negative!center of the P_4 Trp (Fig. 3a,b). Second, in S_3, increased!binding energy apparently comes from a salt link between!the carboxylate of the P_3 Glu and the N_{h2} of Arg_{341} (Fig.!3c,d). Finally, additional binding energy in S_2 appears to!arise from a relationship between the inhibitor's histidyl!sidechain and Trp_{340} (Fig. 3d). The N_{e2} atom of the imidazole!is appropriately positioned to make a favorable interaction!with the partial charges on both the C_{h2} and C_{z3}!atoms of the indole sidechain.!

Table 2

Inhibition of ICE by peptide aldehydes.

	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_i (nM)
Ac-YVAD-CHO	3.8×10^5	2.9×10^{-4}	760
Ac-WEHD-CHO	2.6×10^5	1.5×10^{-5}	56
Selectivity			14-fold