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SYNTHESIS OF PEPTIDE FRAGMENTS RELATED TO EGLIN C AND EXAMINATION OF THEIR INHIBITORY EFFECT ON HUMAN LEUKOCYTE ELASTASE, CATHEPSIN G AND α-CHYMOTRYPSIN

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Summary: Various kinds of peptide fragments related to eglin c were prepared by the conventional solution method and their inhibitory effects on human leukocyte elastase, cathepsin G and α-chymotrypsin were examined. Peptide (31-40) inhibited cathepsin G ($Ki=2.3\times10^{-4}$ M), peptide (41-49) potently inhibited cathepsin G and α-chymotrypsin ($Ki=4.2\times10^{-5}$ M and 2.0×10^{-5} M, respectively), and peptide (60-63) inhibited leukocyte elastase ($Ki=1.6\times10^{-4}$ M), whereas, peptide (31-35) weakly inhibited both elastase and cathepsin G ($Ki=2.1\times10^{-3}$ M and 7.3×10^{-4} M, respectively).

Eglin c (1) isolated from the leech Hirudo medicinalis consists of 70 amino acid residues and inhibits chymotrypsin and subtilisin as well as the leukocyte elastase and cathepsin G, which have attracted our interest in recent year due to their possible involvement in connective tissue turnover and diseases such as inflammation, rheumatoid arthritis and emphysema (2). Therefore, eglin c is of potential medical interest as a therapeutic agent and N^{α}-acetyl eglin c has been prepared genetically (3), although its molecular weight is too large for practical therapeutic use.

This paper deals with the synthesis of various kinds of peptide fragments related to eglin c and the relationship between their structure and inhibitory effect on leukocyte elastase, cathepsin G and α-chymotrypsin.

Abbreviations: Suc, succinyl; pNA, p-nitroanilide; TFA, trifluoroacetic acid; AcOH, acetic acid.

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MATERIALS AND METHODS

Peptides shown in Fig. 1, were prepared by the conventional solution method. Full details of the synthesis and characterization of these peptides will be published elsewhere (Tsuboi, S. et al., in preparation).

Human leukocyte elastase (4) and cathepsin G (5) were prepared in our laboratory according to the procedure described previously. The cathepsin G preparation contains leukocyte elastase as an enzymatically active protein. α-Chymotrypsin was purchased from Miles Co. Ltd., Elkhart. Substrates, Suc-Ala-Tyr-Leu-Val-pNA (6) and Suc-Ile-Pro-Phe-pNA (7) were prepared in our laboratory.

Assay of leukocyte elastase, cathepsin G and α-chymotrypsin: Enzymatic activities of leukocyte elastase, cathepsin G and α-chymotrypsin were determined by the method described previously using Suc-Ala-Tyr-Leu-Val-pNA for leukocyte elastase and Suc-Ile-Pro-Phe-pNA for cathepsin G and α-chymotrypsin.

Effects of synthetic peptides on the enzymes: Synthetic peptide was dissolved in MeOH. Final concentration of MeOH for each peptide is indicated in Table I. Enzymatic activity was assayed in the presence and absence of the peptide examined.

RESULTS AND DISCUSSION

Peptide fragments synthesized [I-X] are shown in Fig. 1. The homogeneity of each peptide was ascertained by TLC, amino acid analysis of acid hydrolysate and elemental analysis. Inhibitory effects of the purified peptides [I-X] on leukocyte elastase, cathepsin G and α-chymotrypsin were examined. Of these, the peptides which exhibited significant effect on those enzymes are listed in Table I with Ki values. Peptide (31-35) [V-1] weakly inhibited both leukocyte elastase and cathepsin G ($Ki=2.1\times10^{-3}$ M and 7.3×10^{-4} M, respectively). Peptide (31-40)[V] inhibited cathepsin G ($Ki=2.3\times10^{-4}$ M) and peptide (41-49) [VI] inhibited cathepsin G and α-chymotrypsin, of which the enzymatic similarity was previously reported (8) ($Ki=4.0\times10^{-5}$ M and 2.0×10^{-5} M, respectively), although these values are 10^{5-6} times larger than the Ki values for the eglin c. Previously, Bode et al. (9) and McPhalen and James (10) reported that the nine residue of the binding loop (40-48) of eglin

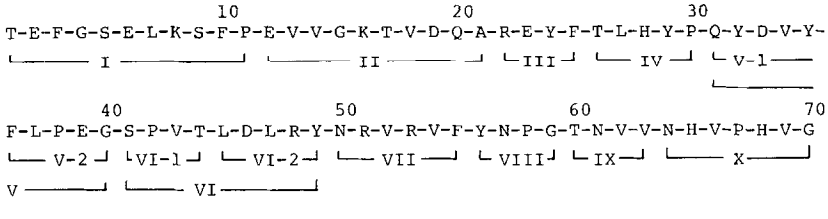

Figure 1. The structure of eglin c and peptide fragments [I-X].

Table I. Ki Values of Eglin c Fragments[a)]

	Ki (M)		
	LE[b)]	Cathepsin G[c)]	α-Chymotrypsin[c)]
H-(22-25)-OMe.2AcOH(III)	-[d)]	8.0×10^{-4}(11)[e)]	7.0×10^{-5}(7)
H-(31-35)-OMe.TFA(V-1)	2.1×10^{-3}(6)	7.3×10^{-4}(10)	-
H-(31-40)-OMe.TFA(V)	-	2.3×10^{-4}(7)	-
H-(41-49)-OMe.2AcOH(VI)	-	4.0×10^{-5}(8)	2.0×10^{-5}(4)
H-(60-63)-)Me.HCl(IX)	1.6×10^{-4}(4)	-	-
eglin c[f)]	2.0×10^{-10}	2.8×10^{-10}	

a) The reaction mixture contained 0.5mM of inhibitor. The reaction was carried out in Tris-HCl buffer(0.1M,pH8.0) containing 0.2M of NaCl for leukocyte elastase and cathepsin G. b) Substrate for LE(leukocyte elastase) was Suc-Ala-Tyr-Leu-Val-pNA(0.5mM). c) Substrate for cathepsin G and α-chymotrypsin was Suc-Ile-Pro-Phe-pNA(0.5mM). d) Not detectable. e) The final concentration of MeOH(%) is indicated in parenthesis. f) U.Seemüller et al., Hoppe-Seyler's Z. Pysiol. Chem., 358, 1105(1977).

c are involved in direct contact with subtilisin as a results of the determination of the crystal structure of the complex formed between eglin c and subtilisin Carlsberg by X ray analysis. In eglin c molecule, Thr^{41}, Asp^{46} and Arg^{48} form electrostatic and hydrogen bonds with Arg^{55}, Arg^{51} and Gly^{70}, respectively, to stabilize the reactive site. However, the peptide (41-49) [VI] does not have the electrostatic and hydrogen bonds needed to maintain the comfortable and rigid conformation for interaction with enzyme. This might be a possible reason why Ki values of peptide (41-49) [VI] are 10^{5-6} times larger than those of eglin c. The fact that peptide (41-44) [VI-1] and peptide (45-49) [VI-2] which contains active site, Leu^{45}-Asp^{46}, did not exhibit any detectable inhibitory effect on these enzymes, suggests that some peptide length is required for manifestation of inhibitory activity. It was also revealed that eglin c present in the complex with subtilisin (9) was shortened N-terminally by 7 amino acid residues. This observation indicates that eglin c derivatives shortened from the N-terminus by up to 7 amino acid residues should be active as an inhibitor. In fact, the eglin c derivatives consisting of positions 5-70 and 7-70 which were prepared enzymatically using cathepsin C, did not influence the equilibrium dissociation constant for the interaction of eglin c with chymotrypsin (11). Peptide fragment (1-11) [I], however, weakly inhibited leukocyte elastase, cathepsin G and α-chymotrypsin. Peptide (60-63) [IX] inhibited leukocyte elastase with Ki value of 1.6×10^{-4} M but not cathepsin G and α-chymotrypsin, indicating that the intera-

cting site of eglin c with leukocyte elastase and cathepsin G and α-chymotrypsin might be different. The fact that peptide (22-25) [III] enhanced leukocyte elastase activity suggests that this peptide binds with some part of the enzyme to change the conformation to an active form.

Further studies on the relationship between the structure of eglin c and its inhibitory activity against leukocyte elastase, cathepsin G and α-chymotrypsin are under way in our laboratory with the aim of obtaining potent and selective inhibitors with smaller molecular weight for practical therapeutic use.

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