SYNTHESIS OF A HEPTACONTAPEPTIDE CORRESPONDING TO THE ENTIRE AMINO ACID SEQUENCE OF EGLIN C[1)]

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A heptacontapeptide corresponding to the entire amino acid sequence of eglin c was synthesized by the conventional solution method using a minimal protecting method. The synthetic eglin c exhibited a symmetrical single peak on HPLC at the same retention time as an authentic eglin c, and had the same inhibitory activity against human leukocyte elastase, cathepsin G and α-chymotrypsin (Ki = 6.0×10^{-9} M, 5.5×10^{-9} M and 2.5×10^{-9} M, respectively) as N^{α}-acetyl-eglin c synthesized genetically (Ki = 5.1×10^{-9} M, 1.5×10^{-8} M and 2.2×10^{-9} M, respectively).

KEYWORDS heptacontapeptide; eglin c; total synthesis; inhibitory activity; human leukocyte elastase; cathepsin G; α-chymotrypsin

Eglin c, isolated from the leech *Hirudo medicinalis*,[2)] consists of 70 amino acid residues and effectively inhibits chymotrypsin and subtilisin as well as leukocyte elastase and cathepsin G.[3)] The latter two enzymes became the focus of increasing attention due to their possible involvement in connective tissue turnover and diseases such as emphysema, rheumatoid arthritis and inflammation.[4,5)] Therefore, eglin c is a candidate agent for emphysema and inflammation therapy. Rink *et al.* prepared N^{α}-acetyleglin c genetically,[6)] but its molecular weight is too large for practical therapy.

Our studies were directed to the synthesis of eglin c and its related peptides with the objectives of studying the relationship between the structure and the inhibitory activities and of obtaining potent inhibitors against leukocyte elastase and cathepsin G with a small molecular size for practical therapeutic use for emphysema or inflammation. This paper deals with the total synthesis of eglin c, examination of its inhibitory activity, and studies of its structure-activity relationship.

Starting with the C-terminal heptapeptide (1), the sixteen peptide fragments in the Scheme (Fig. 1) were coupled successively by the azide procedure[7)] in order to minimize racemization and to avoid as much as possible the need for protecting the side chain functional groups of the amino acid residues during the synthesis (Fig. 1). The α-amino functions of the amino acids were protected by the Boc group. The Bzl protecting group of β- or γ-carboxy function of Asp or Glu was removed by catalytic hydrogenation over palladium prior to the synthesis of the corresponding hydrazide (5, 7, 8, 10, 11, 13, 15 and 16). The carboxy-group of the C-terminal Gly residue was protected as its Bzl ester. To protect the guanidino group of Arg, the ε-amino group of Lys, and the imidazole nitrogen of His, we used respectively mesitylenesulphonyl (Mts), Z and benzyloxymethyl (Bom), removable by treatment with HF at 0°C for 60 min[8)] or trimethylsilyl bromide at 0°C for 3 h.[9)] To introduce the bulky amino acid (Val) in synthesizing the peptide fragments (1-16), we used the newly developed 6-chloro-2-pyridyl ester.[10)] To introduce the Arg residue, the diphenylphosphoryl-azide (DPPA) method[11,12)] was used to avoid lactam formation.

After azide coupling, the protected peptide products were isolated and purified by reprecipitation from DMF and MeOH and by column chromatography on Sephadex LH 20 or 60 using DMF as an eluant (for peptides 21 and 29) to afford purified peptide intermediates (17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30) and finally to make the protected heptacontapeptide (31) [yield 104 mg, 81.2%, mp 229-239°C, $[\alpha]_D$ -42.7° (c=0.1, DMSO), *Anal.* Calcd for $C_{453}H_{632}N_{96}O_{117}S_4 \cdot 9H_2O$: C, 56.8; H, 6.85; N, 14.0. Found: C, 56.5; H, 6.65; N, 13.8, amino acid analysis of acid hydrolysate (6N HCl, 110°C, 72h), Asp (7) 6.99; Thr (5) 3.95; Ser (3) 2.76; Glu (7) 7.13; Gly (5) 4.71; Ala (1) 1.12; Val (11) 10.00; Leu (5) 4.92; Tyr (6) 6.09; Phe (5) 5.27; Lys (2) 2.02;

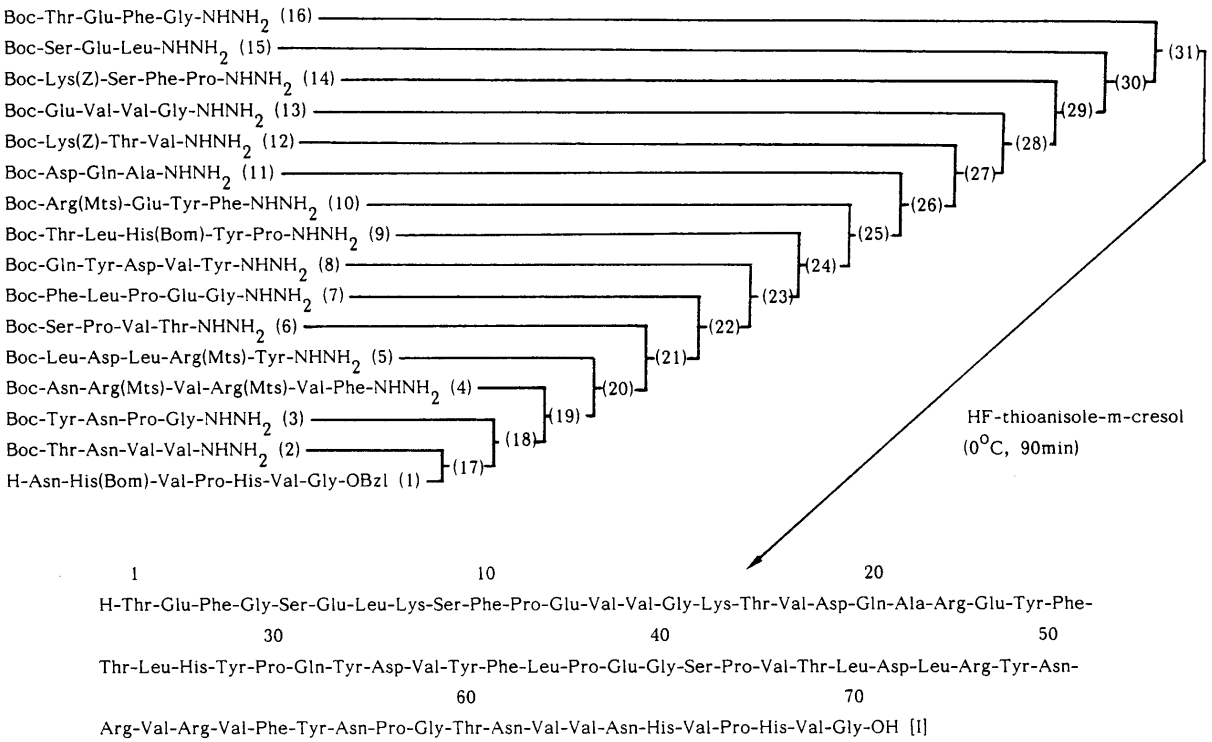

Fig. 1. Synthetic Scheme for a Heptacontapeptide [I]

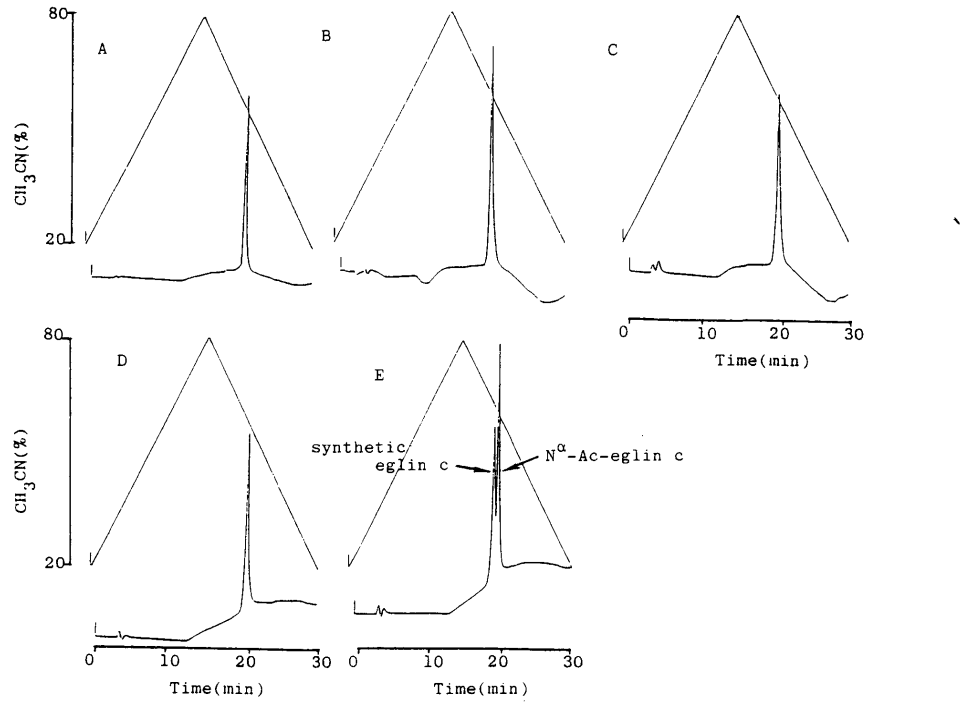

Fig. 2. Analytical HPLC of the Synthetic Heptacontapeptide [I]

A: synthetic eglin c [I]; B: authentic eglin c[14]; C: synthetic eglin c [I] + authentic eglin c; D: N^{α}-acetyleglin c[6]; E: synthetic eglin c [I] + N^{α}-acetyleglin c.
Column: YMC-Pack R-ODS-5 (4.6x250 mm); Solvent: a=H_2O (0.05% TFA), b=CH_3CN (0.05% TFA), gradient (a/b 80/20 - 15 min ► 20/80 - 15 min ► 80/20); Flow rate: 1.0 ml/min; Absorbanc: 210 nm.

His (3) 2.58; Arg (4) 4.15; Pro (6) 5.65 (average recovery 83.3%)]. The homogeneity of other peptide intermediates was also ascertained by TLC, elemental analysis and amino acid analysis.

The protected heptacontapeptide thus obtained was treated with HF in the presence of *m*-cresol and thioanisole.[13] This treatment was repeated two times to ensure complete deprotection. The crude product was purified by gel-filtration on Sephadex G-50 two times using 3% acetic acid as an eluant and then by preparative reversed phase HPLC.

The purified heptacontapeptide [I] exhibited a single peak (Fig. 2) at the same retention time (19.6 min) as that of eglin c^{14} derived from N^{α}-acetyleglin c,[6] yield 10.9 mg (32.1%), $[\alpha]_D$ -62.5° (c=0.10, 5% AcOH), amino acid analysis: Asp(7) 6.91; Thr(5) 4.83; Ser(3) 2.93; Glu(7) 6.90; Gly(5) 5.00; Ala(1) 0.90; Val(11) 10.11; Leu(5) 5.23; Tyr(6) 5.67; Phe(5) 5.01; Lys(2) 1.91; His(3) 2.80; Arg(4) 4.18; Pro(6) 5.96 (average recovery 75.1%). This synthetic heptacontapeptide [I] exhibited the same potent inhibitory activities as those of N^{α}-acetyleglin c against human leukocyte elastase, porcine pancreatic elastase, cathepsin G and α-chymotrypsin (Ki of the synthetic eglin c for the above enzymes: 6.0×10^{-9} M, 2.9×10^{-8} M, 5.5×10^{-9} M and 2.5×10^{-9} M, respectively and Ki of N^{α}-acetyleglin c: 5.1×10^{-9} M, 2.5×10^{-8} M, 1.5×10^{-8} M and 2.2×10^{-9} M, respectively).

Synthetic intermediates (17-30) were also treated with HF to give the corresponding peptides, which were used to study the relationship between the structure and the inhibitory activity of eglin c. Eglin c (60-70) inhibited leukocyte elastase (Ki=1.7×10^{-3} M) but not cathepsin G and α-chymotrypsin. Although eglin c (50-70) and eglin c (45-70) inhibited leukocyte elastase and α-chymotrypsin but not cathepsin G, eglin c (41-70) inhibited the above three enzymes. It is of interest that the sequence 41-44 of eglin c is very important for inhibiting cathepsin G. Eglin c (31-70) and eglin c (22-70) inhibited leukocyte elastase, cathepsin G and α-chymotrypsin with Ki values similar to those of eglin c (41-70) (Ki=1.2×10^{-4} M, 2.1×10^{-4} M and 7.0×10^{-6} M, respectively). Interestingly, eglin c (8-70) was slightly more inhibitory than synthetic eglin c against the above three enzymes.

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RFERENCES AND NOTES

1) Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, 5, 3485 (1966); *idem*, *ibid.*, 6, 362 (1967) and *idem*, *ibid.*, 11, 1726 (1972).
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