Notes

[Chem. Pharm. Bull. 36(8)3119—3124(1988)]

Synthesis of Tripeptide Amide Derivatives and Examination of Their Inhibitory Effect on Human Leukocyte Elastase (HLE)[1)]

YUKO TSUDA,[a] NAOKI TENO,[a] YOSHIO OKADA,*,[a]
YOKO NAGAMATSU[b] and UTAKO OKAMOTO[a]

Faculty of Pharmaceutical Sciences[a] and Faculty of Nutrition,[b] Kobe-Gakuin University, Nishi-ku, Kobe 673, Japan

(Received January 13, 1988)

Suc–Tyr–Leu–Val–*p*NA is a specific substrate for human leukocyte elastase (HLE) and Suc–Tyr–D-Leu–D-Val–*p*NA is a specific inhibitor of HLE. The *p*-nitroanilide moiety of the above peptides was replaced by *p*-acetylaniline, *p*-benzoylaniline and 4-benzylpiperidine to give Suc–Tyr–Leu–Val–ACA (**1**), Suc–Tyr–D-Leu–D-Val–ACA (**2**), Suc–Tyr–Leu–Val–BZA (**3**), Suc–Tyr–D-Leu–D-Val–BZA (**4**), Suc–Tyr–Leu–Val–BPP (**5**) and Suc–Tyr–D-Leu–D-Val–BPP (**6**), respectively. Tripeptide anilide derivatives (**1**—**4**) exhibited stronger inhibitory activity against HLE than the corresponding piperidine amide derivatives (**5** and **6**). It was found that L-D-D type peptides inhibited HLE competitively and more potently than the corresponding L-L-L type peptides. Suc–Tyr–D-Leu–D-Val–BZA (**4**) inhibited HLE with a K_i value of 60 μM, whereas Suc–Tyr–Leu–Val–BZA (**3**) inhibited HLE with a K_i value of 150 μM. Although these compounds inhibited HLE more strongly than human leukocyte cathepsin G, peptide (**3**) inhibited both HLE and cathepsin G with K_i values of 150 and 240 μM, respectively.

Keywords——human leukocyte elastase; human leukocyte cathepsin G; tripeptide amide derivative; *p*-nitroanilide; *p*-acetylanilide; *p*-benzoylanilide; 4-benzylpiperidine amide; stereoisomer; inhibitor; structure–activity relationship

Elastase and cathepsin G (chymotrypsin-like proteinase) from leukocytes participate in a fibrinolytic pathway.[2)] These proteinases have attracted our interest in recent years due to their possible involvement in connective tissue turnover[3)] and in diseases such as emphysema,[4)] inflammation[5)] and rheumatoid arthritis.[3)] Our studies have been directed to the synthesis of peptide substrates and inhibitors of human leukocyte elastase (HLE). It was reported that Suc–Tyr–Leu–Val–*p*NA was a specific substrate for HLE[6,7)] and the stereoisomer of the substrate, Suc–Tyr–D-Leu–D-Val–*p*NA was a specific and competitive inhibitor of HLE.[8)] Further, the *p*NA moiety of Suc–Tyr–D-Leu–D-Val–*p*NA played a very important role in increasing the affinity between Suc–Tyr–D-Leu–D-Val–*p*NA and some part of the active center of HLE, resulting in the manifestation of inhibitory activity.[9)] Thus, it seemed of interest to substitute some other aromatic compounds such as *p*-acetylaniline (ACA), *p*-benzoylaniline (BZA) and 4-benzylpiperidine (BPP) for the *p*-nitroanilide (*p*NA) moiety of the above peptides with the objective of obtaining more potent inhibitors of HLE.

This report deals with the synthesis of Suc–Tyr–Leu–Val–R (R = ACA, BZA and BPP) and Suc–Tyr–D-Leu–D-Val–R (R = ACA, BZA and BPP), and examination of their inhibitory activity against HLE.

As illustrated in Fig. 1, six kinds of peptide derivatives were prepared by the solution method. Boc–L- or D-Val–OH was coupled with ACA, BZA or BPP by the mixed anhydride method[10)] to give Boc–L or D-Val–R (R = ACA, BZA or BPP). Since the yield was very poor

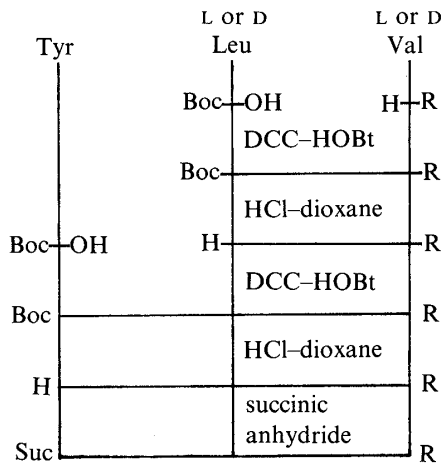

Fig. 1. Synthetic Scheme for Suc–Tyr–Leu–Val–R (**1**, **3** and **5**) and Suc–Tyr–D-Leu–D-Val–R (**2**, **4** and **6**)

1 and **2**, R = ACA; **3** and **4**, R = BZA; **5** and **6**, R = BPP.

TABLE I. Inhibition of HLE and Cathepsin G by the Peptides (**1**—**6**)

Compound	K_i value (mM)	
	HLE	Cathepsin G
Suc–L-Tyr–L-Leu–L-Val–*p*NA[a]	(K_m = 0.21 mM)	—[f]
Suc–L-Tyr–D-Leu–D-Val–*p*NA[b]	0.12	>2.0
Suc–L-Tyr–L-Leu–L-Val–ACA (**1**)[c]	0.54	>2.0
Suc–L-Tyr–D-Leu–D-Val–ACA (**2**)[c]	0.17	N.I.[g]
Suc–L-Tyr–L-Leu–L-Val–BZA (**3**)[d]	0.15	0.24
Suc–L-Tyr–D-Leu–D-Val–BZA (**4**)[e]	0.060	>2.0
Suc–L-Tyr–L-Leu–L-Val–Pipe[b,d]	>2.0	1.1
Suc–L-Tyr–D-Leu–D-Val–Pipe[b,d]	>2.0	>2.0
Suc–L-Tyr–L-Leu–L-Val–BPP (**5**)[d]	Enhance	0.80
Suc–L-Tyr–D-Leu–D-Val–BPP (**6**)[d]	0.90	>2.0

a) Data from Okada *et al.* (ref. 12). *b*) Data from Okada *et al.* (ref. 8). *c*) A buffer containing 2% CH_3OH was used. *d*) A buffer containing 5% CH_3OH was used. *e*) A buffer containing 5% CH_3OH and 5% DMSO was used. *f*) No hydrolysis. *g*) No inhibition.

in the case of Boc–D-Val–ACA, Z–D-Val–ACA was prepared by the phosphazo method[11] in a good yield. Suc–Tyr–Leu–Val–R (**1**, R = ACA; **3**, BZA; **5**, BPP), Suc–Tyr–D-Leu–D-Val–R (**2**, R = ACA; **4**, BZA; **6**, BPP) and their intermediates obtained here were each homogeneous upon thin-layer chromatography (TLC) on silica gel. Amino acid ratios in acid hydrolysates and the results of elemental analysis were in good agreement with the theoretically expected values.

Next, the inhibitory effect of the peptides (**1**—**6**) on HLE activity toward Suc–Ala–Tyr–Leu–Val–*p*NA[12] was examined and the results are summarized in comparison with their effect on human leukocyte cathepsin G in Table I. As can be seen in Table I, the peptides (**1**—**6**), which have a Val residue at the P_1 position, inhibited HLE but their inhibitory effect on cathepsin G which preferentially cleaves the phenylalanyl bond,[3] was negligible except for **3**. Regarding the inhibition of HLE, L-D-D type peptides (**2**, **4** and **6**) inhibited the enzyme competitively and more potently than the corresponding L-L-L type peptides (**1**, **3** and **5**). Peptide **4** inhibited HLE more potently than the corresponding *p*NA derivative (Suc–Tyr–D-Leu–D-Val–*p*NA), as we had expected from its inhibitory mechanism,[9] but the inhibitory activity of **6** was less potent than that of the corresponding *p*NA derivative, although a phenyl ring at the methyl group of the methylpiperidine moiety increased the inhibitory activity against HLE compared with that of the 4-methylpiperidine amide (Pipe)

TABLE II. Kinetic Constants for Hydrolysis of the Peptides by HLE and Cathepsin G

Compound	Enzyme	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
Suc–L-Tyr–L-Leu–L-Val–*p*NA[a]	HLE	0.21	3.7	17600
	Cathepsin G			—[d]
Suc–L-Tyr–L-Leu–L-Val–ACA (**1**)[b]	HLE	0.40	2.7	6750
	Cathepsin G			—
Suc–L-Tyr–L-Leu–L-Val–BZA (**3**)[c]	HLE			N.C.[e]
	Cathepsin G			—

Kinetic constants were determined by measuring the ACA (330 nm) and BZA (370 nm) released from peptide 1 or 3, respectively. *a*) Data from Okada *et al.* (ref. 12). *b*) A buffer containing 2% CH_3OH was used. *c*) A buffer containing 5% CH_3OH was used. *d*) No hydrolysis. *e*) Not calculated. Only 0.6% of Val–BZA bond was cleaved in 5 min.

derivative.[8] For the increment of the affinity between an L-D-D type peptide and HLE, an anilide-type compound at the C-terminus of the L-D-D peptide seems to be more favorable than a piperidine amide-type compound. The peptide **3** is a unique inhibitor that can inhibit both HLE and cathepsin G, although the inhibitory activities were not so strong, presumably because the BZA moiety in **3** could bind with some part of the active site of cathepsin G.

It is possible that peptides **1** and **3** might be split by HLE to release *p*-acetylaniline and *p*-benzoylaniline, respectively. Thus, cleavage of the Val–R bond (R = ACA and BZA) of **1** and **3** by HLE was examined and the results are summarized in Table II. It was revealed that the Val–ACA bond was cleaved by HLE with a K_m value of 0.4 mM. However, only 0.6% of the Val–BZA bond of **3** was cleaved in 5 min. From the above results, it was found that the *p*-nitroanilide moiety and *p*-acetylanilide moiety exhibited similar behavior as a substrate or an inhibitor.

In order to obtain potent and selective inhibitors against HLE, an L-D-D type compound is more favorable than an L-L-L type peptide, because the former is stable to the enzyme and the replacement of the C-terminal moiety by various kinds of aromatic compounds may increase the potency of inhibitory activity.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates (110 °C, 18 h, 6 N HCl) were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co., Ltd.). On TLC (Kieselgel G, Merck), Rf^1, Rf^2 and Rf^3 values refer to the systems of $CHCl_3$, MeOH and water (89 : 10 : 1), $CHCl_3$, MeOH and AcOH (90 : 8 : 2) and $CHCl_3$, MeOH and water (8 : 3 : 1, lower phase), respectively.

General Procedure for Preparation of Boc–Val–R or Boc–D-Val–R (R = ACA, BZA and BPP)——Ethyl chloroformate (1.1 ml, 10 mmol) was added to a solution of Boc–Val–OH or Boc–D-Val–OH (2.8 g, 10 mmol) in tetrahydrofuran (THF) (30 ml) containing Et_3N (1.4 ml, 10 mmol) cooled with ice-salt. After 15 min, the above mixed anhydride was combined with H-R (10 mmol, R = ACA, BZA, or BPP) in THF (20 ml) at 0 °C. The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 5% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crystals, which were collected by filtration and purified by silica gel column chromatography, if necessary, as follows. The crude material in $CHCl_3$ (5 ml) was applied to a silica gel column (2.8 × 42 cm) equilibrated and eluted with $CHCl_3$. The solvent of the effluent (200—600 ml) was removed by evaporation and petroleum ether was added to the residue to give crystals. Yield, melting point, $[\alpha]_D$ value, *Rf* values and the elemental analysis data are summarized in Table III.

Z–D-Val–ACA——PCl_3 (0.9 ml, 10 mmol) was added to a solution of 4-acetylaniline (2.7 g, 20 mmol) in dry pyridine (50 ml) cooled with ice-salt. The reaction mixture was stirred at the same temperature for 15 min and then at room temperature for 30 min. Z–D-Val–OH (5.0 g, 20 mmol) was added to the above solution and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. Ether was added to the residue to give crystals, which were collected by filtration. Yield, melting point, $[\alpha]_D$ value, *Rf*

TABLE III. Yields, Melting Points, $[\alpha]_D$ Values, Elemental Analyses and *Rf* Values of Boc–L-Val–R and Boc (or Z)–D-Val–R (R = ACA, BZA and BPP)

Compound	Yield (%)	mp (°C)	$[\alpha]_D^{25}$	Formula	Elemental analysis Calcd (Found) C H N	TLC *Rf*¹	TLC *Rf*²
Boc–L-Val–ACA	36	155—158	+15.9° (*c*=0.8, DMF)	$C_{18}H_{26}N_2O_4$	64.6 7.84 8.38 (64.2 7.95 8.72)	0.70	0.76
Z–D-Val–ACA	95	200—202	+16.8° (*c*=1.0, MeOH)	$C_{21}H_{24}N_2O_4$	68.5 6.57 7.60 (68.4 6.55 7.63)	0.73	0.76
Boc–L-Val–BZA	45	138—142	−19.4° (*c*=0.9, MeOH)	$C_{23}H_{28}N_2O_4$	69.7 7.12 7.07 (69.5 7.06 7.03)		0.72
Boc–D-Val–BZA	71	138—145	+19.2° (*c*=0.9, MeOH)	$C_{23}H_{28}N_2O_4$	69.7 7.12 7.07 (69.5 7.06 7.03)		0.72
Boc–L-Val–BPP	40	96—97	+17.5° (*c*=1.0, DMF)	$C_{22}H_{34}N_2O_3$	70.6 9.15 7.48 (70.6 9.23 7.53)	0.77	0.68
Boc–D-Val–BPP	40	92—95	−16.2° (*c*=1.0, DMF)	$C_{22}H_{34}N_2O_3$	70.6 9.15 7.48 (70.5 9.26 7.40)	0.77	0.68

TABLE IV. Yields, Melting Points, $[\alpha]_D$ Values, Elemental Analyses and *Rf* Values of Boc–L-Leu–L-Val–R and Boc–D-Leu–D-Val–R (R = ACA, BZA and BPP)

Compound	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found) C H N	TLC *Rf*¹	TLC *Rf*²
Boc–L-Leu–L-Val–ACA	66	98—101	−58.0° (*c*=1.0)	$C_{24}H_{37}N_3O_5$	64.4 8.33 9.39 (64.3 8.38 9.40)	0.58	0.64
Boc–D-Leu–D-Val–ACA	81	164—168	+56.7° (*c*=1.1)	$C_{24}H_{37}N_3O_5$	64.4 8.33 9.39 (64.5 8.55 9.38)	0.58	0.64
Boc–L-Leu–L-Val–BZA	44	98—101	−48.5° (*c*=0.8)	$C_{29}H_{39}N_3O_5$	68.3 7.71 8.25 (68.1 7.90 8.16)		0.63
Boc–D-Leu–D-Val–BZA	68	99—105	+55.0° (*c*=0.8)	$C_{29}H_{39}N_3O_5$	68.3 7.71 8.25 (68.5 7.98 8.11)	0.64	
Boc–L-Leu–L-Val–BPP	100	Oil				0.63	0.71
Boc–D-Leu–D-Val–BPP	94	Oil				0.63	0.71

values and the elemental analysis data are summarized in Table III.

General Procedure for Preparation of Boc–Leu–Val–R or Boc–D-Leu–D-Val–R (R = ACA, BZA and BPP)——Boc–Leu–OH or Boc–D-Leu–OH (0.39 g, 4.0 mmol), H–Val–R·HCl or H–D-Val–R·HCl (4.0 mmol) [prepared from 4.3 mmol of the corresponding Boc–Val–R and 3.0 ml (21.4 mmol) of 7.3 N HCl–dioxane, in the case of Z–D-Val–ACA, the Z group was removed by 25% HBr–AcOH] and HOBt (0.54 g, 4.3 mmol) were dissolved in DMF (50 ml) containing Et_3N (0.56 ml, 4.0 mmol) and the solution was cooled with ice-salt. DCC (1.0 g, 4.8 mmol) was added and the reaction mixture was stirred at 4 °C overnight. After removal of the dicyclohexylurea and the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 5% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and purified by silica gel column chromatography, if necessary, as follows. The crude material in $CHCl_3$ (6 ml) was applied to a silica gel column (2.0 × 46 cm) equilibrated and eluted with $CHCl_3$. After removal of the solvent of the effluent (250—450 ml), petroleum ether was added to the residue to give crystals. Yield, melting points, $[\alpha]_D$ value, *Rf* values and the elemental analysis data are summarised in Table IV.

General Procedure for Preparation of Boc–Tyr–Leu–Val–R or Boc–Tyr–D-Leu–D-Val–R (R = ACA, BZA and BPP)——Boc–Tyr–OH (2.0 mmol), H–Leu–Val–R·HCl or H–D-Leu–D-Val–R·HCl [prepared from 2.0 mmol of the corresponding Boc–Leu–Val–R and 1.3 ml (9.8 mmol) of 7.3 N HCl–dioxane] and HOBt (0.26 g, 2.0 mmol) were dissolved in DMF (15 ml) containing Et_3N (0.28 ml, 2.0 mmol) and the solution was cooled with ice-salt. DCC (0.48 g, 2.4 mmol) was added, and the reaction mixture was stirred at 4 °C overnight. After removal of the dicyclohexylurea

TABLE V. Yields, Melting Points, $[\alpha]_D$ Values, Elemental Analyses and *Rf* Values of Boc–L-Tyr–L-Leu–L-Val–R and Boc–L-Tyr–D-Leu–D-Val–R (R = ACA, BZA and BPP)

Compound	Yield (%)	mp (°C)	$[\alpha]_D^{25}$	Formula	Elemental analysis Calcd (Found) C	H	N	TLC Rf^1	Rf^2
Boc–L-Tyr–L-Leu–L-Val–ACA	68	135—142	+5.1° (*c* = 0.9, DMF)	$C_{33}H_{46}N_4O_7$	64.9 (64.6)	7.54 (7.74)	9.17 (9.04)	0.64	0.58
Boc–L-Tyr–D-Leu–D-Val–ACA	58	224—227	+7.5° (*c* = 0.9, DMF)	$C_{33}H_{46}N_4O_7 \cdot 1/2\,H_2O$	64.0 (64.2)	7.64 (7.81)	9.04 (9.32)	0.62	0.60
Boc–L-Tyr–L-Leu–L-Val–BZA	92	124—129	+6.2° (*c* = 1.0, DMF)	$C_{38}H_{48}N_4O_7 \cdot 3/2\,H_2O$	65.2 (65.5)	7.34 (7.15)	8.00 (8.28)	0.47	0.67
Boc–L-Tyr–D-Leu–D-Val–BZA	36	235—238	+6.8° (*c* = 0.3, DMF)	$C_{38}H_{48}N_4O_7 \cdot H_2O$	66.1 (65.8)	7.30 (6.90)	8.11 (8.27)	0.45	0.67
Boc–L-Tyr–L-Leu–L-Val–BPP	28	Amorphous	+1.4° (*c* = 1.0, MeOH)	$C_{37}H_{54}N_4O_6$	68.3 (68.4)	8.36 (8.61)	8.61 (8.54)	0.31	0.58
Boc–L-Tyr–D-Leu–D-Val–BPP	21	122—126	−4.5° (*c* = 1.0, DMF)	$C_{37}H_{54}N_4O_6$	68.3 (68.3)	8.36 (8.63)	8.61 (8.74)	0.24	0.50

TABLE VI. Yields, Melting Points, $[\alpha]_D$ Values, Elemental Analyses and *Rf* Values of Suc–L-Tyr–L-Leu–L-Val–R and Suc–L-Tyr–D-Leu–D-Val–R (R = ACA, BZA and BPP)

Compound	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (MeOH)	Formula	Elemental analysis Calcd (Found) C	H	N	TLC Rf^2	Rf^3
1	68	128—135	−41.8° (*c* = 1.0)	$C_{32}H_{42}N_4O_8 \cdot 1/2\,H_2O$	62.0 (61.7)	6.99 (6.97)	9.04 (8.86)	0.26	0.42
2	59	219—224	+99.2° (*c* = 0.84)	$C_{32}H_{42}N_4O_8 \cdot 1/2\,H_2O$	62.0 (61.8)	6.99 (7.08)	9.04 (9.24)	0.26	0.27
3	65	123—133	−32.6° (*c* = 0.9)	$C_{37}H_{44}N_4O_8 \cdot H_2O$	64.3 (64.1)	6.71 (6.65)	8.11 (8.05)	0.31	0.42
4	43	213—217	+110.8° (*c* = 0.7)	$C_{37}H_{44}N_4O_8 \cdot H_2O$	64.3 (64.7)	6.71 (6.54)	8.11 (8.17)	0.31	0.45
5	62	125—130	−19.6° (*c* = 0.9)	$C_{36}H_{50}N_4O_7 \cdot 1/2\,H_2O$	65.5 (65.6)	7.79 (7.78)	8.49 (8.53)	0.44	0.36
6	57	112—117	+30.2° (*c* = 1.0)	$C_{36}H_{50}N_4O_7 \cdot 1/2\,H_2O$	65.5 (65.3)	7.79 (7.79)	8.49 (8.42)	0.44	0.70

and the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 5% citric acid and water, dried over Na_2SO_4 and evaporated down. The residue was crystallized from AcOEt and petroleum ether. If necessary, crude materials were purified by gel-filtration on Sephadex LH-20 or by silica gel column chromatography. Yield, melting point, $[\alpha]_D$ value, *Rf* values and the elemental analysis data are summarized in Table V.

General Procedure for Preparation of Suc–Tyr–Leu–Val–R (1, R = ACA; 3, R = BZA; 5, R = BPP) and Suc–Tyr–D-Leu–D-Val–R (2, R = ACA; 4, R = BZA; 6, R = BPP)——H–Tyr–Leu–Val–R·HCl or H–Tyr–D-Leu–D-Val–R·HCl [prepared from 0.74 mmol of the corresponding Boc–Tyr–Leu–Val–R and 0.51 ml (3.7 mmol) of 7.3 N HCl–dioxane] was dissolved in pyridine (3.0 ml) containing Et_3N (0.14 ml, 0.74 mmol). Succinic anhydride (0.18 g, 1.9 mmol) was added to the above solution in three equal portions over a period of 30 min. During the reaction, the pH of the solution was maintained at 8—9 by adding Et_3N. The reaction mixture was stirred at room temperature overnight. After removal of the solvent, AcOEt and 10% AcOH were added to the residue. The AcOEt layer was washed with water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were dissolved in MeOH (30 ml) containing 1 N NaOH (1.1 ml). After 90 min at room temperature, the solution was neutralized with AcOH and the solvent was removed by evaporation. The residue was extracted with AcOEt and the extract was washed with 10% AcOH and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration. Yield, melting point, $[\alpha]_D$ value, the elemental analysis data

TABLE VII. Results of Amino Acid Analyses of Suc–L-Tyr–L-Leu–L-Val–R and Suc–L-Tyr–D-Leu–D-Val–R (R = ACA, BZA and BPP)

Compound	Amino acid ratios of acid hydrolysate (110 °C, 18 h)			Average recovery (%)
	Tyr	Leu	Val	
1	0.87	0.91	1.00	74
2	1.01	1.03	1.00	80
3	0.75	1.10	1.00	70
4	0.91	0.96	1.00	86
5	1.10	1.20	1.00	81
6	0.98	0.89	1.00	66

and *Rf* values are summarized in Table VI and the amino acid ratios in an acid hydrolysate are summarized in Table VII.

Kinetic Measurement——HLE[2] and cathepsin G[13] were isolated in our laboratory according to the procedures described previously. This cathepsin G preparation contains leukocyte elastase as an enzymatically active protein. The amidolytic activities of HLE and cathepsin G were assayed by measuring the *p*-nitroaniline (410 nm) released from the specific substrates, Suc–Ala–Tyr–Leu–Val–*p*NA[12] and Suc–Val–Pro–Phe–*p*NA,[3] respectively. The enzyme reaction was carried out at 37 °C in Tris–HCl buffer (0.1 M, pH 8.0 for HLE and 0.1 M, pH 7.5 for cathepsin G). The kinetic constants were estimated from the initial rate of amidolysis of substrates by the enzymes according to Lineweaver–Burk plots.[14] The K_i values were determined in the same manner after adding the peptide to be examined (synthetic peptide was dissolved in DMSO-containing buffer).

References and Notes

1) The customary L indication for amino acid residues is omitted; only D isomers are indicated. Standard abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 3485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Other abbreviations used: Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Suc, succinyl; *p*NA, *p*-nitroanilide; BZA, *p*-benzoylanilide; BPP, 4-benzylpiperidine amide; Pipe, 4-methylpiperidine amide; ACA, *p*-acetylanilide; DCC, *N,N'*-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; AcOEt, ethyl acetate; DMF, dimethylformamide; AcOH, acetic acid; DMSO, dimethylsulfoxide.
2) Y. Nagamatsu, U. Okamoto, Y. Tsuda and Y. Okada, *Throm. Haemostas.*, **51**, 243 (1984).
3) T. Tanaka, Y. Minematsu, C. F. Reilly, J. Travis and J. C. Powers, *Biochemistry*, **24**, 2040 (1985).
4) P. M. Senior, H. Tegner, C. Kuhn, K. Ohlsson, B. C. Starcher and J. A. Pierce, *Am. Rev. Respir. Dis.*, **116**, 469 (1977).
5) A. Janoff, *Ann. Rev. Med.*, **23**, 177 (1972).
6) U. Okamoto, Y. Nagamatsu, Y. Tsuda and Y. Okada, *Biochem. Biophys. Res. Commun.*, **97**, 28 (1980).
7) Y. Okada, Y. Tsuda, Y. Nagamatsu and U. Okamoto, *Int. J. Peptide Protein Res.*, **17**, 560 (1981).
8) Y. Okada, Y. Tsuda, Y. Nagamatsu and U. Okamoto, *Int. J. Peptide Protein Res.*, **24**, 347 (1984).
9) Y. Okada, Y. Tsuda, N. Teno, Y. Nagamatsu, U. Okamoto and N. Nishi, *Chem. Pharm. Bull.*, **33**, 5301 (1985).
10) G. W. Anderson, F. M. Callahan and J. E. Zimmerman, *J. Am. Chem. Soc.*, **88**, 1338 (1966).
11) S. Goldschmidt and G. Roscult, *Chem. Ber.*, **93**, 2387 (1960).
12) Y. Okada, Y. Tsuda, A. Hirata, Y. Nagamatsu and U. Okamoto, *Chem. Pharm. Bull.*, **30**, 4060 (1982).
13) Y. Nagamatsu, Y. Nakaya, U. Okamoto, Y. Tsuda and Y. Okada, *Blood & Vessel*, **18**, 257 (1987).
14) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).