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INHIBITORY EFFECTS OF ω-GUANIDINO ACID ESTERS ON TRYPSIN, PLASMIN, PLASMA KALLIKREIN AND THROMBIN

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SUMMARY

The inhibitory effects of various esters of ω-guanidino acids on trypsin (EC 3.4.4.4), plasmin (EC 3.4.4.14), plasma kallikrein (EC 3.4.4.21) and thrombin (EC 3.4.4.13) were examined. Among the various inhibitors tested, phenyl and *p*-carbethoxyphenyl ε-guanidinocaproate were the most effective inhibitors of these four enzymes.

Previously[1], we reported the competitive inhibitory effects of saturated aliphatic esters of various ω-guanidino acids on trypsin (EC 3.4.4.4), plasmin (EC 3.4.4.14) and kallikrein (EC 3.4.4.21), and their protective effects on the inactivation of trypsin by diisopropylphosphofluoridate. Moreover, we[2] reported that various esters of ε-amino caproic and *trans*-4-aminomethylcyclohexanecarboxylic acid extensively inhibited trypsin, plasmin, plasma kallikrein and thrombin (EC 3.4.4.13). Among the various esters examined, aromatic esters, such as phenyl and 4′-(2″-carboxy)ethylphenyl esters, were more inhibitory than saturated aliphatic esters, such as the hexyl ester. Therefore, it seems likely that aromatic esters of ω-guanidino acid should inhibit trypsin, plasmin, kallikrein and thrombin more than saturated aliphatic esters. Mares-Guia and Shaw[3] reported the irreversible inhibitory effect of ethyl *p*-guanidinobenzoate on trypsin, and Chase and Shaw[4] reported that of *p*-nitrophenyl-*p*′-guanidinobenzoate. The latter compound has been used in titration of the active site of trypsin[4], plasmin and thrombin[5].

This report describes the strong, reversible inhibitory effects of various ω-guanidino acid esters on trypsin, plasmin, plasma kallikrein and thrombin.

The various enzymes used, such as bovine trypsin, human plasmin, human plasma kallikrein and thrombin, and the various substrates, such as methyl N^{α}-tosyl-

Abbreviations: TAME, N^{α}-tosyl-L-argininate; BAEE, ethyl N^{α}-benzoyl-L-argininate; BAPA, N^{α}-benzoyl-DL-arginine *p*-nitroanilide; ε-GCA-Hex, hexyl ε-guanidinocaproate; δ-GVA-Hex, hexyl δ-guanidinovalerate; γ-GBA-Hex, hexyl γ-guanidinobutyrate; ε-GCA-Bzl, benzyl ε-guanidinocaproate bicarbonate; ε-GCA-Phe, phenyl ε-guanidinocaproate *p*-toluenesulfonate; ε-GCA-CEP, *p*-carbethoxyphenyl ε-guanidinocaproate monophosphate; AMCHA-Phe, phenyl *trans*-4-aminomethylcyclohexane carboxylate.

L-argininate (TAME), ethyl N^{α}-benzoyl-L-argininate (BAEE) and N^{α}-benzoyl-DL-arginine *p*-nitroanilide (BAPA) were described previously[2]. Enzyme activities were also measured as described previously[2]. Hexyl ε-guanidinocaproate (ε-GCA-Hex), δ-guanidinovalerate (δ-GVA-Hex) and γ-guanidinobutyrate (γ-GBA-Hex) were prepared as described previously[1]. Benzyl ε-guanidinocaproate bicarbonate (ε-GCA-Bzl), phenyl ε-guanidinocaproate *p*-toluenesulfonate (ε-GCA-Phe) and *p*-carbethoxyphenyl ε-guanidinocaproate monophosphate (ε-GCA-CEP) were prepared in the Research Laboratories of Ono Pharmaceutical Company, Osaka.

The effects of various esters of ε-guanidinocaproic acid on the caseinolytic activities of trypsin and plasmin were examined, and the concentrations required for 50% inhibition are shown in Table I. The concentrations of ε-GCA-Bzl and ε-GCA-Phe causing 50% inhibition in fibrinogenolysis by plasmin were 0.72 mM and 0.1 mM, respectively. Table II shows the K_i values of various inhibitors in hydrolysis

TABLE I

CONCENTRATIONS OF INHIBITORS FOR 50% INHIBITION OF CASEINOLYSIS

Incubations were carried out in 0.1 M borate buffer, pH 7.4, at 37°. The experimental procedure is described in the text.

Inhibitor	*Inhibitor concn. for 50% inhibition (mM)*	
	Trypsin	*Plasmin*
ε-GCA-Hex	>4	4.5
ε-GCA-Bzl	0.48	0.25
ε-GCA-Phe	0.013	0.035
ε-GCA-CEP	0.0054	0.066

TABLE II

K_i VALUES FOR TRYPSIN, PLASMIN, PLASMA KALLIKREIN AND THROMBIN

K_i values were estimated in 0.05 M Tris–HCl, pH 8.0, at 20°. With trypsin, the buffer contained 0.01 M $CaCl_2$. BAEE was used as substrate.

Inhibitor	K_i *(M)*			
	Trypsin	*Plasmin*	*Kallikrein*	*Thrombin*
δ-GVA-Hex	$2.1 \cdot 10^{-5}$	—	—	—
γ-GBA-Hex	$1.7 \cdot 10^{-4}$	—	—	—
ε-GCA-Bzl	$9.4 \cdot 10^{-6}$		$3.2 \cdot 10^{-5}$	

of BAEE by trypsin, plasmin, plasma kallikrein and thrombin. Table III shows the concentrations required for 50% inhibition of hydrolysis of TAME by these four enzymes. The concentrations causing 50% inhibition and the K_i values in hydrolysis of BAPA by trypsin are shown in Table IV.

Previously, we reported that aromatic esters of ω-amino acid were more inhibitory than saturated aliphatic esters. This was confirmed in the present report,

TABLE III

CONCENTRATIONS OF INHIBITORS FOR 50% INHIBITION OF ESTEROLYSIS

Incubations were carried out in 0.15 M KCl containing 10 mM $CaCl_2$, at 25°. TAME was used as substrate (10 mM).

Inhibitor	*Inhibitor concn. for 50% inhibition (mM)*			
	Trypsin	*Plasmin*	*Kallikrein*	*Thrombin*
ε-GCA-Hex	>8	>8	3	>10
ε-GCA-Bzl	>1	>2	0.19	>10
ε-GCA-Phe	0.18	0.35	0.0075	0.075
ε-GCA-CEP	0.0086	0.060	0.0074	0.064

TABLE IV

I_{50} AND K_i VALUES FOR TRYPTIC HYDROLYSIS OF BAPA

Incubations were carried out in 0.05 M Tris–HCl buffer containing 0.02 M $CaCl_2$, pH 8.2, at 25°. For details see text. In experiments to determine I_{50} values, the final substrate concentrations were 1 mM.

Inhibitor	I_{50} *(M)*	K_i *(M)*
γ-GBA-Hex	$1.5 \cdot 10^{-4}$	$2.9 \cdot 10^{-5}$
δ-GVA-Hex	$5.0 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
ε-GCA-Hex	$9.0 \cdot 10^{-5}$	$2.0 \cdot 10^{-5}$
ε-GCA-Phe	$2.2 \cdot 10^{-6}$	$8.0 \cdot 10^{-7}$

as shown in Tables I to IV. The phenyl ester was one of the strongest inhibitors of trypsin, plasmin, kallikrein and thrombin, and the K_i value for hydrolysis of BAPA by trypsin was $8.0 \cdot 10^{-7}$ M (Table IV). This value is the same as that of phenyl *trans*-4-aminomethylcyclohexane carboxylate (AMCHA-Phe) reported previously[2]. However, Lineweaver–Burk plots of the inhibition of hydrolysis of BAME by trypsin, plasmin, kallikrein and thrombin by this compound were not linear so that the K_i values could not be obtained. Similarly, the K_i values of ε-GCA-Bzl for plasmin and thrombin could not be obtained (Table II).

AMCHA-Phe strongly inhibited trypsin and plasmin, but had little affect on kallikrein and thrombin: the concentrations causing 50% inhibition of TAME hydrolysis by trypsin, plasmin, kallikrein and thrombin were 0.06 mM, 0.01 mM, 0.13 mM and 0.34 mM, respectively[2]. On the other hand, as shown in Table III, the concentrations for 50% inhibition of ε-GCA-Phe were 0.18 mM, 0.35 mM, 0.0075 mM and 0.075 mM, respectively. These results show that the inhibitory effects of this compound on kallikrein and thrombin were greater than those of AMCHA-Phe, while its effects on trypsin and plasmin were less. On the other hand, the carbethoxyphenyl ester strongly inhibited all these four enzymes, as shown in Tables I and III.

Various compounds described above and in our previous report[2] were reversible inhibitors on trypsin, plasmin, plasma kallikrein and thrombin. For example, they were easily removed from the inhibited enzyme by dialysis. The inhibition mechanism and behavior of these inhibitors on trypsin will be described elsewhere.

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