Inhibition of a mite protease (Df-protease) by synthetic inhibitors

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ABSTRACT

A protease (Df-protease) from house dust mites (*Dermatophagoides farinae*), which is closely related to mite-induced allergic diseases, catalyzes the activation of kallikrein-kinin system in plasma (3, 6, 12). Df-protease was competitively inhibited by guanidine and amidine derivatives with low-molecular weight. The kinetic study revealed that the K_i values of 1-[*p*-(*p*-amidinophenoxycarbonyl)benzoyl]-D-proline benzyl ester acetate and *N*-[*p*-(2-methoxycarbonyl-4-amidinophenoxycarbonyl)benzoyl]-*N*-phenylglycine ethyl ester acetate were 5×10^{-9} M and 6×10^{-9} M, respectively.

The allergic diseases derived from house dust are induced by two types of mite, *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp) (9, 13), in which cysteine and serine proteases are reported as allergens (2, 11). It was demonstrated that these proteases are closely associated with mite-related allergic diseases (2). Takahashi *et al.* (12) isolated a serine protease, Df-protease, from Df with a similar substrate specificity to that of blood coagulation factor XIIa. Df-protease catalyzed the activation of the kallikrein-kinin system. The protease gave rise to rhythmic contraction of rat uterine horns in factor XIIa-deficient human plasma (3) and enhancement of blood permeability in guinea pigs (5, 6). Df-protease liberated kinins from kininogens with low- and high-molecular weights (4) and a kinin induced allergic rhinitis, bronchial asthma and rhinovirus-caused cold (1, 10).

We previously found that Df-protease is inhibited by *p*-amionobenzamidine (an amidine derivative) or gabexate mesylate (a guanidine derivative) (6, 12). In the present paper, we further studied the inhibitory effect of a wide range of synthetic guanidine or amidine derivatives with low molecular weights, to explore effective therapeutic agents for mite-induced allergic diseases.

A synthetic substrate of blood coagulation factor XIIa, *t*-butyloxycarbonyl-L-glutaminyglycyl-

L-arginine 4-methylcoumaryl-7-amide(Boc-Gln-Gly-Arg-MCA), was purchased from the Peptide Institute (Osaka, Japan). Guanidine and amidine derivatives were kindly donated from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Df-protease was purified from culture extracts of mites (Df) by the method described previously (12). The protease was a serine-protease with molecular weight of 30,000 (12). Its enzymic activity was fluorometrically determined with the synthetic substrates, Boc-Gln-Gly-Arg-MCA in the presence or absence of guanidine or amidine derivatives according to the method described in a previous paper (6). The specific activity of the protease was 158 units/mg protein. Kinetic parameters, K_m and K_i values, were obtained from Lineweaver-Burk plots.

The kinetic study on Df-protease activity with a synthetic substrate of blood coagulation factor XIIa as substrate was carried out in the presence or absence of inhibitors. The K_m value of Df-protease was determined to be 8×10^{-6} M, which was obtained by reciprocal plot of $[v]$ and $[s]$ (line A in Fig. 1). Twenty compounds listed in Table 1 were subjected to the inhibition study, which are classified into two types, guanidine and amidine derivatives. Line B in Fig. 1 shows the reciprocal plot in the presence of 10 nM of *N*-[*p*-(2-methoxycarbonyl-4-amidinophenoxycarbonyl)benzoyl]-*N*-phenylglycine ethyl ester acetate (No. 6). This compound is

Fig. 1 Double-reciprocal plot of the initial rate of Df-protease activity toward synthetic substrate of XIIa in the presence and the absence of inhibitor. Line A (●), without inhibitor; line B (▲), in the presence of 10 nM of *N*-[*p*-(2-methoxycarbonyl-4-amidinophenoxycarbonyl)-benzoyl]-*N*-phenylglycine ethyl ester acetate. Df-protease concentration, 0.53 nM

a competitive inhibitor with K_i value of 6×10^{-9} M. Other compounds tested in the present study are all competitive inhibitors except one (No. 18). Table 1 shows chemical formula, molecular weight and K_i value of each inhibitor. K_i values of amidine derivatives are at the range between 10^{-8} M and 10^{-9} M, while those of guanidine derivatives are between 10^{-6} M and 10^{-8} M. Some of guanidinophenoxycarbonyl derivatives (Nos. 17, 19, 20) are not strong inhibitors ($K_i = 10^{-6}$ M– 10^{-7} M) and a guanidinophenoxycarbonyl derivative with a carboxyl group in benzene ring (No. 18) does not inhibit Df-protease at all. These results indicate that amidine derivatives are superior inhibitors than guanidine derivatives. Especially, 1-[*p*-(*p*-amidinophenoxycarbonyl)benzoyl]-D-proline benzyl ester acetate (No. 5) and *N*-[*p*-(2-methoxycarbonyl-4-amidinophenoxycarbonyl)benzoyl]-*N*-phenylglycine ethyl ester acetate (No. 6) were strong inhibitors with the K_i values of 5×10^{-9} M and 6×10^{-9} M, respectively. These inhibitors having rather small molecular weights may be used for treating mite-induced allergic diseases. In a pre-

Table 1 Inhibition Constant (K_i) of Various Compounds That Inhibit Df-Protease

Number	MW	K_i (M)	Chemical formula	
Amidine derivatives				
1 (z)-5-[<i>p</i> -(<i>p</i> -Amidinophenoxycarbonyl)-benzylidene]-rhodanine-3-acetic acid ethyl ester acetate	529	5×10^{-8}		a
2 <i>p</i> -Amidinophenyl <i>p</i> -[(2-isopropyl-1-imidazolyl)methyl]benzoate dihydrochloride	435	1×10^{-8}		b
3 <i>N</i> ^α -[<i>p</i> -(<i>p</i> -Amidinophenoxycarbonyl)-benzoyl]-DL-tryptophan ethyl ester acetate	558	2×10^{-8}		a
4 1-[<i>p</i> -(<i>p</i> -Amidinophenoxycarbonyl)-benzoyl]-L-proline ethyl ester acetate	469	3×10^{-8}		a
5 1-[<i>p</i> -(<i>p</i> -Amidinophenoxycarbonyl)-benzoyl]-D-proline benzyl ester acetate	532	5×10^{-9}		a
6 <i>N</i> -[<i>p</i> -(2-Methoxycarbonyl-4-amidinophenoxycarbonyl)benzoyl]- <i>N</i> -phenylglycine ethyl ester acetate	563	6×10^{-9}		a
7 <i>N</i> ^α -[<i>p</i> -(<i>p</i> -Amidinophenoxycarbonyl)-α-methylcinnamoyl]-L-tryptophan ethyl ester mesylate	635	2×10^{-8}		d

continued

Table 1 *Continued*

	Number	MW	Ki (M)	Chemical formula	
Guanidine derivatives					
8	(<i>N,N</i> -Dimethylcarbamoyl)methyl 2-(<i>p</i> -guanidinobenzoyloxy)-1-naphthoate hydrochloride	470	2×10^{-8}		b
9	Ethyl {2-[<i>p</i> -(<i>p</i> -guanidinobenzoyloxy)phenyl]- <i>N</i> -phenylacetamide}acetate acetate	534	4×10^{-8}		a
10	Ethyl 1-[<i>p</i> -(<i>p</i> -guanidinobenzoyloxy)-phenylacetyl]-indoline-2-carboxylate acetate	547	2×10^{-8}		a
11	Ethyl 2-[<i>p</i> -(<i>p</i> -guanidinobenzoyloxy)-benzoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylate acetate	547	3×10^{-8}		a
12	Ethyl 1-[<i>p</i> -(<i>p</i> -guanidinobenzoyloxy)-benzoyl]-indoline-2-carboxylate acetate	533	2×10^{-8}		a
13	<i>N</i> -Benzyl- <i>N</i> -[<i>p</i> -(<i>p</i> -guanidinobenzoyloxy)phenylsulfonyl]glycine ethyl ester acetate	570	1×10^{-8}		a
14	3-Chloro-5-hydroxyphenyl <i>p</i> -guanidinobenzoate hydrobromide	374	7×10^{-8}		c
15	3-Chloro-4-pivaroyloxyphenyl <i>p</i> -guanidinobenzoate acetate	449	3×10^{-8}		a
16	4-Sulfamoylphenyl <i>p</i> -guanidinobenzoate mesylate	430	4×10^{-8}		d
17	<i>N</i> -[<i>p</i> -(4-Guanidino-2-methoxycarbonylbenzoyl)benzoyl]- <i>N</i> -phenylglycine ethyl ester hydrochloride	555	2×10^{-6}		b
18	2-[<i>p</i> -(<i>N</i> -Ethoxycarbonylmethyl- <i>N</i> -phenylcarbamoyl)-benzoyloxy]-5-guanidinobenzoic acid hydrochloride	541	Not inhibited		b
19	Ethyl 1-[<i>p</i> -(<i>p</i> -guanidinophenoxycarbonyl)benzoyl]indoline-2- <i>RS</i> -carboxylate acetate	532	4×10^{-7}		a
20	1-[<i>p</i> -(<i>p</i> -Guanidinophenoxycarbonyl)-benzoyl]-L-proline ethyl ester acetate	485	2×10^{-6}		a

a, b, c and d: $\cdot\text{CH}_3\text{COOH}$, $\cdot\text{HCl}$, $\cdot\text{HBr}$ and $\cdot\text{CH}_3\text{SO}_3\text{H}$, respectively. All compounds, except 18, are competitive inhibitors.

vious study, we demonstrated that aprotinin ($K_i=4 \times 10^{-10}$ M) and soybean trypsin inhibitor ($K_i=3 \times 10^{-9}$ M) have strong inhibitory action (6). However, these are proteins with the molecular weight of 6,511 and 22,000, respectively. It is interesting to note that the Df-protease was not inhibited by antithrombin, α_2 -macroglobulin and Cl-esterase inhibitor. Quite recently, we found that Df-protease activates not only blood coagulation system (7) but also fibrinolytic system (8).

It can be concluded that Df-protease from mites is closely associated with not only allergic diseases but also blood coagulation system and fibrinolysis in blood. Therefore, the finding of inhibitors of Df-protease may serve to explore the therapy for these allergic diseases.

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