Communications to the Editor

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SIGNIFICANT EFFECTS OF SYNTHETIC GLN-VAL-VAL-ALA-GLY AND ITS DERIVATIVES, COMMON SEQUENCES OF THIOL PROTEINASE INHIBITORS, ON THIOL PROTEINASE[1)]

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The Gln-Val-Val-Ala-Gly sequence occurs frequently in several thiol proteinase inhibitors, suggesting that this conserved sequence may be one of the reactive sites in the inhibitors. In order to study the effects of the sequence on thiol proteinases, we synthesized H-Gln-Val-Val-Ala-Gly-OH (VI) and its related peptide derivatives by the conventional solution method and examined their effects on the enzymatic activity of papain. We found that Z-Gln-Val-Val-Ala-Gly-OMe (IV) not only inhibits papain but also protects it from T-kininogen, which is one of thiol proteinase inhibitors. Apparently this peptide (IV) binds with some part of papain in competition with T-kininogen.

KEYWORDS——thiol proteinase inhibitor; conserved sequence; Gln-Val-Val-Ala-Gly; chemical synthesis; inhibitory activity; papain; structure activity relationship

Thiol proteinase inhibitors with high (60,000-70,000) and low (10,000-14,000) molecular weights have been isolated from plasma and various tissues and studies extensively. Their physiological function appears to involve the regulation of thiol proteinases. Recently, the amino acid sequence of α_2-thiol proteinase inhibitor (MW = 70,000) isolated from human plasma[2)] was determined by analyzing the base sequence of cDNA and its identity with low molecular weight kininogen was reproted.[3)] Kininogens from various origins were found to inhibit thiol proteinase in addition to being a source for vasoactive bradykinin.[3,4)] The sequence Gln-Val-Val-Ala-Gly is one of the highly conserved amino acid sequences in the inhibitors and kininogens such as α_2-thiol proteinase inhibitor (α_2-TPI),[3)] human cystatin,[5)] human stefin,[6)] chicken cystatin,[7)] rat liver thiol proteinase inhibitor,[8)] rat epidermal thiol proteinase inhibitor,[9)] bovine low molecular weight kininogen,[10)] rat major acute phase α_1-protein,[11)] T-kininogen[12)] and others. The clear conservation of this sequence indicates that this may be one of the reactive sites for these proteinase inhibitors.

In order to elucidate the reactive site of thiol proteinase inhibitors, the synthesis of the conserved sequence and its derivatives has been undertaken in our laboratory. This communication deals with the synthesis of Z-Gln-Val-Val-Ala-Gly-OMe (IV) and related peptides and their effects on the enzymatic activity of thiol proteinase (papain) and the inhibition of papain by T-kininogen. The peptides were synthesized by the stepwise condensation of Z-amino acids starting with H-Gly-OMe obtained either by the DCC, mixed anhydride or active ester method to afford Z-Val-Val-Ala-

Gly-OMe (III) [mp 182-185°C, $[\alpha]_D^{28}$ -3.4° (c=1.0, DMF), Anal. Calcd for $C_{24}H_{36}N_4O_7 \cdot H_2O$: C, 56.5; H, 7.45; N, 11.0. Found: C, 56.5; H, 7.77; N, 11.1.], Z-Gln-Val-Val-Ala-Gly-OMe (IV) [mp 252-256°C, $[\alpha]_D^{28}$ -24.9° (c=0.8, DMSO), Anal. Calcd for $C_{29}H_{44}N_6O_9 \cdot H_2O$: C, 54.6; H, 7.21; N, 13.2. Found: C, 54.8; H, 7.34; N, 13.0.], Z-Gln-Val-Val-Ala-Gly-OH (V) [mp 270°C (dec.), $[\alpha]_D^{28}$ -26.2° (c=0.2, DMSO), Anal. Calcd for $C_{28}H_{42}N_6O_9 \cdot 2H_2O$: C, 52.4; H, 7.16; N, 13.1. Found: C, 52.7; H, 7.03; N, 13.4.] and H-Gln-Val-Val-Ala-Gly-OH (VI) [amorphous powder, $[\alpha]_D^{28}$ +181.6° (c=0.1, DMSO), Anal. Calcd for $C_{20}H_{36}N_6O_7 \cdot 3H_2O$: C, 45.7; H, 7.98; N, 16.0. Found: C, 45.7; H, 7.86; N, 15.7.].

The papain activity was assayed by the method of Barrett[13] using N^{α}-Bz-DL-Arg-2-naphthylamide as the substrate: Papain (0.17 μg; Type III, Sigma) was preincubated with cysteine (2.7 mM), Na_2EDTA (1.3 mM) and 0.1 M Na,K-phosphate (pH 6.0) in a total volume of 500 μl at 37°C for 5 min. Reaction was initiated by adding the substrate (final concentration, 4.6 mM) in DMSO (25 μl). Liberated 2-naphthylamine was assayed by the Barrett method.[13] The inhibitory activity of the peptides was determined by adding a peptide dissolved in DMSO (30 μl) to the preincubation mixture. Because of the limited solubility of the peptides, 0.18 mM of peptide was used as the final concentration in the reaction mixture. The results are summarized in Table I. As can be

Table I. Effects of Gln-Val-Val-Ala-Gly and Its Derivatives, Common Sequence of Thiol Proteinase Inhibitors, on the Enzymatic Activity of Papain and the Inhibitory Activity of T-Kininogen toward Papain

Peptide	Inhibition of papain (%)[a]	Protection of papain from T-kininogen-induced inhibition[b]
Z-Ala-Gly-OMe (I)	5.4	1.0
Z-Val-Ala-Gly-OMe (II)	21.9	1.0
Z-Val-Val-Ala-Gly-OMe (III)	21.9	1.0
Z-Gln-Val-Val-Ala-Gly-OMe (IV)	24.3	5.8
Z-Gln-Val-Val-Ala-Gly-OH (V)	11.1	1.0
H-Gln-Val-Val-Ala-Gly-OH (VI)	6.8	1.0

a) The inhibitory effects of the peptides were determined by adding the peptides (0.18 mM) to the assay medium. The values represent the means of 4 experiments.

b) The protective effect of the peptide on the T-kininogen-induced inhibition of papain was calculated as follows:

$$\text{Factor} = \frac{ID_{50} \text{ of T-kininogen in the presence of peptide}}{ID_{50} \text{ of T-kininogen in the absence of peptide}}$$ (see Fig. 1)

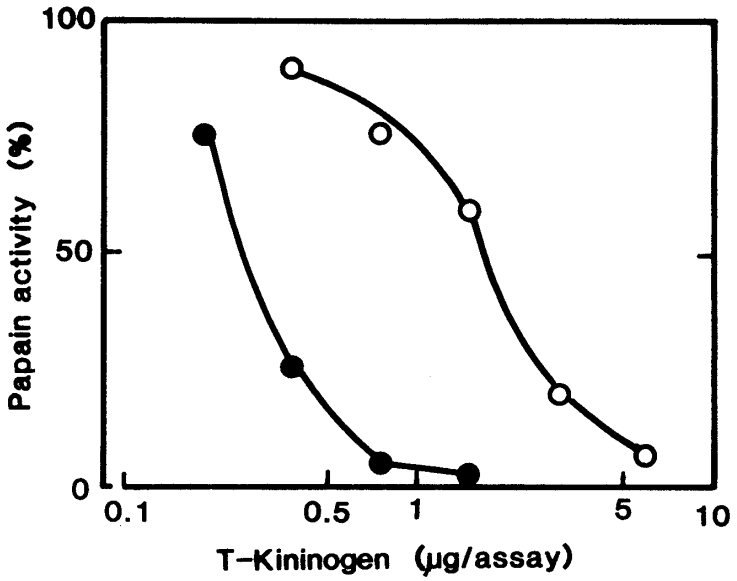
seen in the table, the peptides (II, III and IV) exhibited significant inhibitory activity against papain in a dose-response manner (data not shown). Z-Gln-Val-Val-Ala-Gly-OH (V) and H-Gln-Val-Val-Ala-Gly-OH (VI) showed less inhibitory effect on papain than that of II-IV. Although the inhibitory effect is not so strong, it is interesting that such a small peptide inhibits thiol proteinase. This supports the hypothesis that the sequence, Gln-Val-Val-Ala-Gly, may be a reactive site of thiol proteinase inhibitors.

Next, the protection of papain from T-kininogen-induced inhibition by the synthetic peptides was examined as follows: T-Kininogen isolated from rat plasma was used as a natural thiol proteinase inhibitor. The mixture of papain and various amount of T-kininogen was incubated with peptide (0.18 mM) at 37°C for 5 min, and the remaining

activity of papain was assayed as described above. The amount of T-kininogen required to inhibit papain by 50% (ID_{50}) was estimated from dose-inhibition curve in the absence or presence of peptide (Fig. 1). The protective effect was expressed as the

Fig. 1. Dose-Inhibition Curve of T-Kininogen against Papain in the Absence and Presence of Z-Gln-Val-Val-Ala-Gly-OMe (IV)

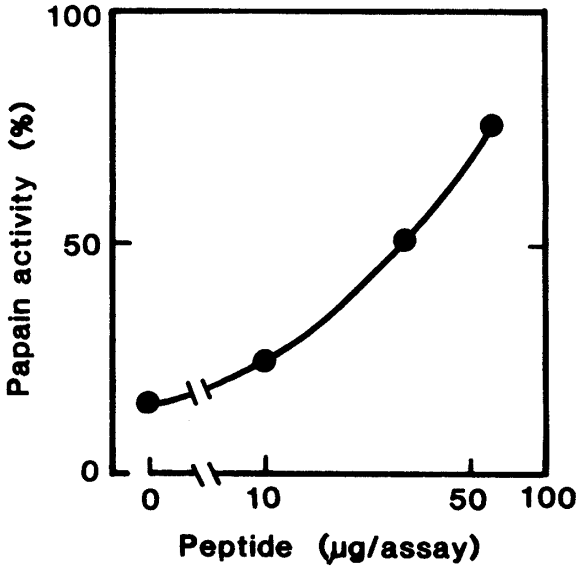
Various amounts of T-kininogen were preincubated with papain (0.17 μg) with (o) or without (●) 60 μg of Z-Gln-Val-Val-Ala-Gly-OMe (IV) at 37°C for 5 min. The residual activity of papain was then assayed, and expressed as % of control activity (without T-kininogen and peptide). Each point represents the mean of 3 experiments.



relative value of the ID_{50} of T-kininogen compared with that in the absence of peptide as summarized in Table I. The results show that Z-Gln-Val-Val-Ala-Gly-OMe (IV) significantly and dose dependently protected papain from T-kininogen-induced inhibition (Fig. 2).

Fig. 2. Dose-Dependent Protection of Papain from T-Kininogen-induced Inhibition by Z-Gln-Val-Val-Ala-Gly-OMe (IV)

Papain (0.17 μg) was preincubated with T-kininogen (0.6 μg) in the presence of 0, 10, 30 or 60 μg of Z-Gln-Val-Val-Ala-Gly-OMe (IV) at 37°C for 5 min. The residual activity was expressed as described in Fig. 1. Each point represents the mean of 3 experiments.



The inhibition by the small peptides may be a result of their binding with some part of the papain. The protection of papain from T-kininogen-induced inhibition by the peptide (IV) may be due to binding of the peptide with some part of papain in competition with T-kininogen. Further studies are needed to determine the inhibitory mechanism of these peptides against papain.

The information obtained here may help us to design small peptide inhibitors against thiol proteinases instead of natural thiol proteinase inhibitors, whose molecular weights are more than 10,000. Such small peptide inhibitors may provide us a new methodology to study thiol proteinases.

REFERENCES AND NOTES

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