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—Original Article—

SPECIFIC INHIBITION OF HUMAN GROUP I PEPSINS BY TWO PEPSIN INHIBITORS

Kenji MURAKAMI, M.D., Fumio MISAKI, M.D., Zenji SASAKI, M.D.,
Taeko TANAKA, M.S. and Keiichi KAWAI, M.D. Prof.

Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto, Japan

Summary

Two pepsin-inhibitors, a peptide produced by *Streptomyces* EF-44-201 and a peptide produced by *Actinomycetes* have been found to inactivate completely the human group I pepsins, but to have little activity against the group II pepsins.

Key Words: pepsinogens, pepsin, isoenzymes of pepsinogens, isoenzymes of pepsins, pepsin inhibitor.

A peptide produced by Streptomyces EF-44-201 has been reported to inhibit peptic activity[1]. The inhibition appears to result from the interaction of the peptide with the substrate combining site of pepsin on an equimolar basis. However, there is no information about the possible specificity of the inhibitor against different molecular forms of pepsin. This study examines the specific activity of two inhibitory peptides against the human group I and group II pepsins[2].

Pepstatin, a peptide produced by *Actinomycetes*, is also known to have the same activity[3].

Materials and Method

The two pepsin inhibitors studied, a peptide produced by Streptomyces EF-44-201 (peptide A), and Pepstatin (peptide B), were dissolved in dilute hydrochloric acid, pH 2 (**Fig. 1**).

For the determination of antipeptic activity, graded amounts of each peptide were incubated with 1 ml human gastric juice, pH 2, for 30 minutes at 37 °C and the residual peptic activity was determined by a modified Anson-Mirsky's method[4].

To determine the inhibitory activity of each peptide against the different pepsins, human gastric juice was first incubated with each peptide in a final concentration of 100 μg/ml. The mixture was then subjected to electrophoretic analysis in agar gel at pH 5.7[5].

In additional experiments, an alkaline extract of human gastric mucosa was electrophoresed in agar gel at pH 8.3[6]. After electrophoresis for four hours at 400 V and 80 mA, the peptides in a concentration of 200 μg/ml at pH 2 were applied along the axis of electrophoretic migration. The plate was then incubated at 37 °C for five minutes to convert the pepsinogens to pepsins and to allow interaction between the inhibitor and the activated zymogens. The plate was then immersed in acid-hemoglobin for 15 minutes at 37 °C and was then processed in the routine manner. In control studies, the peptides were dissolved in water rather than hydrochloric acid before being applied to the agar gel. In addition, the gastric mucosal extract was incubated with each peptide at neutral

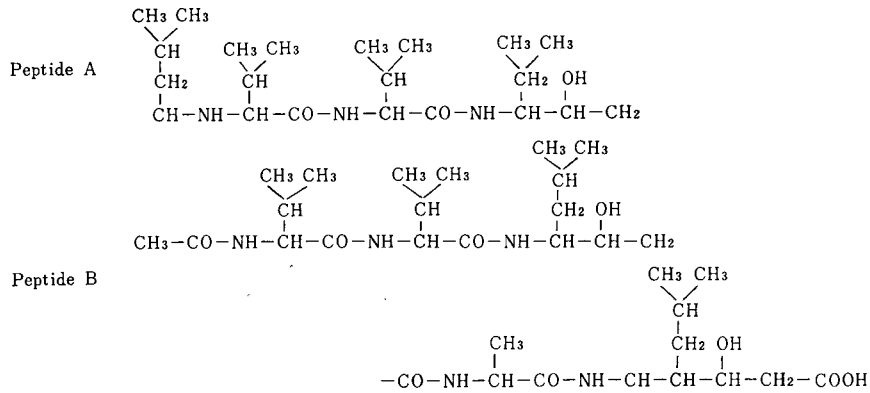

Fig. 1. Molecular structure of two pepsin inhibitors investigated.

pH for 30 minutes at 37°C before electrophoresis at pH 8.3.

Results

Peptides A and B exhibited significant antipeptic activity when incubated with human gastric juice (**Fig. 2**). The inhibitory effect of peptide A was greater than that of peptide B at all concentrations studied. Over 50% inhibition was achieved in a dose of 25 μg per ml of gastric juice. At a dose of 200 μg per ml of gastric juice, peptide A inhibited 92.2% and peptide B inhibited 78.9% of the available peptic activity.

Electrophoretic analysis of the peptide-gastric juice mixtures revealed complete inhibition of the most rapidly migrating zone of proteolytic activity, but only partial inhibition of the two proteolytic bands with slower electrophoretic mobility (**Fig. 3**). In addition, and additional band of proteolytic activity was seen close to the anodal side of the origin in the peptide-gastric juice mixtures.

When the peptides, at pH 2, were applied directly to the agar gel following electrophoretic separation of the pepsinogens, there was complete inhibition of the most rapidly migrating bands of proteolytic activity (group I pepsins[2]) and only partial inhibition of the slower migrating bands (group II pepsins) (**Fig. 4**). Peptide A appeared to be more potent than peptide B in inhibiting the group II pepsins. When the peptides were in-

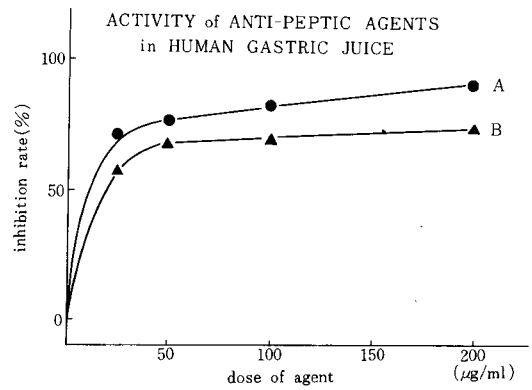

Fig. 2. Inhibition of peptic activity by peptides A and B. The dose of agent is per ml of gastric juice.

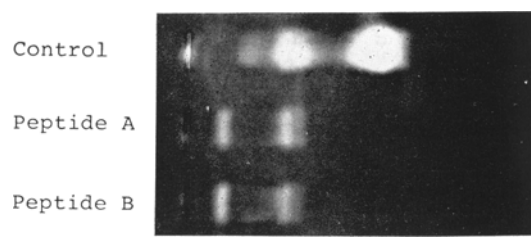

Fig. 3. Electrophoresis of a mixture o fpepsins and peptides A and B at pH 5.7. Peptides A and B inhibit only the most rapidly migrating fractions.

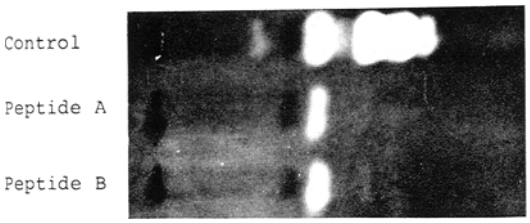

Fig. 4. Inhibition of potential peptic activity of pepsinogens 1 through 5 by peptides A and B. Electrophoresis was conducted at pH 8.3.

cubated with the gastric mucosal extract at alkaline pH before electrophoresis or were dissolved in water before being applied to the agar gel, no inhibitory effect was observed.

Discussion

The human pepsinogens have been shown to be separable into two distinct groups by a number of criteria and there is evidence that each group gives rise to a distinct group of pepsins[7,8]. The pepsinogens, which have been termed group I (PG I) and group II (PG II) contain, respectively, of five and two electrophoretically distinct fractions[2]. It has been shown the PG I and PG II differ in their mucosal distribution, cellular lacalization and antigenic relationships[2,9,10,11]. In addition, there is indirect evidence that the pepsins derived from PG I and PG II differ in their biochemical and biophysical characteristics. One of these differences is their specificity for certain peptide bonds.

Previous studies of peptides A and B have shown that their antipeptic activity is attributable to their ability to react at the substrate combining site on pepsin. The results of this study suggest that these peptides have a greater affinity for a combining site on the group I pepsins than on the group II pepsins. The observation that inhibition did not occur when the peptides were incubated with the pepsinogens suggests that the substrate combining site is not exposed when the molecule is in the zymogen form.

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Address requests for reprints to: Dr. Kenji Murakami, M.D., Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto, 602 Japan.