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Stability of Separated ACE Inhibitory Peptides under Condition of Various pH, Temperature, Gastric Digestion (*In Vitro*)

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Introduction

Recently, the interest in the composition of food has been increased because potential anti-carcinogens and other therapeutic agents of food have been reported (Messina et al, 1994; Gibbs et al., 2004). Particular interest has been focused on bioactive peptides that are present in food proteins. These peptides are inactive within the sequence of the parent proteins but can be released by enzymatic proteolysis, for example, during gastrointestinal digestion or during food processing. Also, they are produced partly by the hydrolytic action of commercial enzymes (Gibbs et al., 2004). From the point of view of application of an antihypertensive material for functional food, a main consideration is not only the purification of an active peptide but also the stability of active peptide under various pH, temperature and gastric digestion. In this respect, separated peptides (Jang, 2004) were tested with various pH condition, temperature during 2 months storage to confirm if their activity can be changed and tested with commercial digest protease, pepsin, to identify whether these peptides stabilized or not under digestion with gastrointestinal enzymes.

Materials and Methods

1. Enzymes and Synthesis of Peptides

Pepsin (porcine stomach mucosa), trypsin (bovine pancreas), chymotrypsin (bovine pancreas), ACE from rabbit lung, hippuryl-histydylleucine (HHL) were obtained from Sigma, Co (USA). Acetonitrile and trifluoroacetic acid were purchased from Fisher, Co. and all the reagents used were analytical grade. Each peptide (325, 714, 1152, 1155, 1134) was synthesized in the solid phase in Peptron Co. (Dajeon, Korea).

2. Effect of pH and Temperature

Concentration of peptides adjusted to 0.1mg/ml and its range of the pH was adjusted to pH 6.0, 6.5, 7.0, 7.5, 8.0. Each pH level was adjusted with 0.5M HCl solution. The peptide solution was kept for 20min at 60, 70, 80, 90, 100℃ to make the study environment. It was kept at 4℃ for 2 months to see the change of its ACE inhibition activity.

3. *In Vitro* Digestion of Separated Peptides

Model peptides were dissolved in distilled water, adjusted to pH 2.0, and digested by pepsin at 37 ℃ for 5 hrs. The ratio of enzyme and substrate was 1:100 (w/w). The peptic digests were successively adjusted to pH 7.6 and pH 7.8, digested by trypsin and chymotrypsin (E/S = 1/100 (w/w)) for 5 h at 37 ℃. The reactions were inactivated at 100 ℃ for 5min.

4. Determination of Stability of Peptides for ACE Inhibitory Peptides

Stability of the inhibitory peptides for ACE was tested using HPLC equipped with an C-18 column (Waters, USA). All of the synthetic peptides used in this study were first found as ACE inhibitors.

Results and Discussion

1. Effect of pH Changes on ACE Inhibitory Activity of Peptides

ACE inhibition activity of peptides was measured after 2 months of storage at 4℃ under condition of pH 6.0, 6.5, 7.0, 7.5, 8.0(Table 1). After 2 months of chilled storage (4℃), there was no dramatic change and significance. This indicates that acidic, neutral, weak alkali conditions and storage did not affect ACE inhibitory activity of those peptides.

2. Effect of Temperature on ACE Inhibitory Activity of Peptides

Those synthesized peptides, 714, 325, 1155, 1152, 1134 had ACE inhibitory activity as 31.66, 34.77, 14.64, 25.09and 53.36%, respectively. These peptides were kept for 20min. at 60, 70,80,90, and 100℃ and measured any change of their activity. In all temperature, the activity of peptide 325 and 714 changed slightly, however, no significant difference was found(Table 2). Among peptide 1134, 1152, 1155, peptides from thermolysin + protease A hydrolysates, inhibition activity of peptide 1134 and 1152 was changed with the temperature. They showed similar inhibition activity from 70℃ to 100℃, however, the activity was significantly reduced at 60℃ of environment ($P<0.001$). Also, similar trend was found in peptide 1134 ($P<0.001$). This result confirmed by HPLC (Fig. 1). Chromatogram of peptide 1134, 1152, and 1155 at 60℃ was shown that different retention time compare to the rest of peptides. Even though peptide 1155 showed almost same inhibition activity,

Table 1. ACE inhibition activity of peptides on various pH condition after 2 months storage at 4°C (%)

pH \ Peptide*	714	325	1155	1152	1134
6.0	31.4±0.7	34.0±0.0	14.3±0.2	25.9±0.8	52.9±0.0
6.5	31.3±0.3	34.0±0.4	14.3±0.2	25.2±0.4	53.5±0.4
7.0	30.7±0.4	33.6±0.6	13.9±0.5	25.0±0.3	53.2±0.4
7.5	30.9±0.3	34.5±0.4	14.0±0.1	25.2±0.1	53.5±0.5
8.0	31.2±0.2	34.1±0.4	14.2±0.3	25.0±0.3	53.5±0.5

* peptide 714:DFHINQ, 325:GFHI, 1155:GLSDGEWQ, 1152:FHG, 1134:VLAQYK.

the retention time of the peptide at 60℃ was different. However, effect of temperature 60 ℃ on change of ACE inhibitory activity was not clear. This indicated that temperature can be changed the inhibition activity and profile of peptides.

3. Effect of Gastric Enzymes on the Purified ACE Inhibitory Peptides

When the ACE inhibitors were consecutively digested with pepsin, trypsin, and α -chymotrypsin, the ACE inhibitory activities increased only slightly (Table 3). The ACE inhibitory activity of peptide 325 was slightly decreased, however, its of the rest peptides had not changed. These results indicated that if the ACE inhibitor peptide were orally administered, it would remain stable in the stomach.

SUMMARY

Table 2. ACE inhibition activity of peptides on various temperature condition after 2 months storage at 4°C (%)

℃ \ Peptide*	714	325	1155	1152***	1134***
60	31.5±1.1	34.5±0.5	15.5±1.2	18.2±0.4^{b}	37.1±0.4^{b}
70	32.4±0.8	34.2±0.8	14.2±0.5	24.6±1.3^{a}	53.4±0.6^{a}
80	31.2±1.2	35.2±1.5	14.8±0.6	25.4±1.4^{a}	53.7±1.5^{a}
90	31.2±0.6	34.8±1.7	14.6±0.4	24.6±0.6^{a}	53.9±0.6^{a}
100	30.9±0.4	34.5±1.0	15.7±1.7	24.8±0.8^{a}	53.5±0.4^{a}

***; $p<0.001$.

* peptide 714:DFHINQ, 325:GFHI, 1155:GLSDGEWQ, 1152:FHG, 1134:VLAQYK.

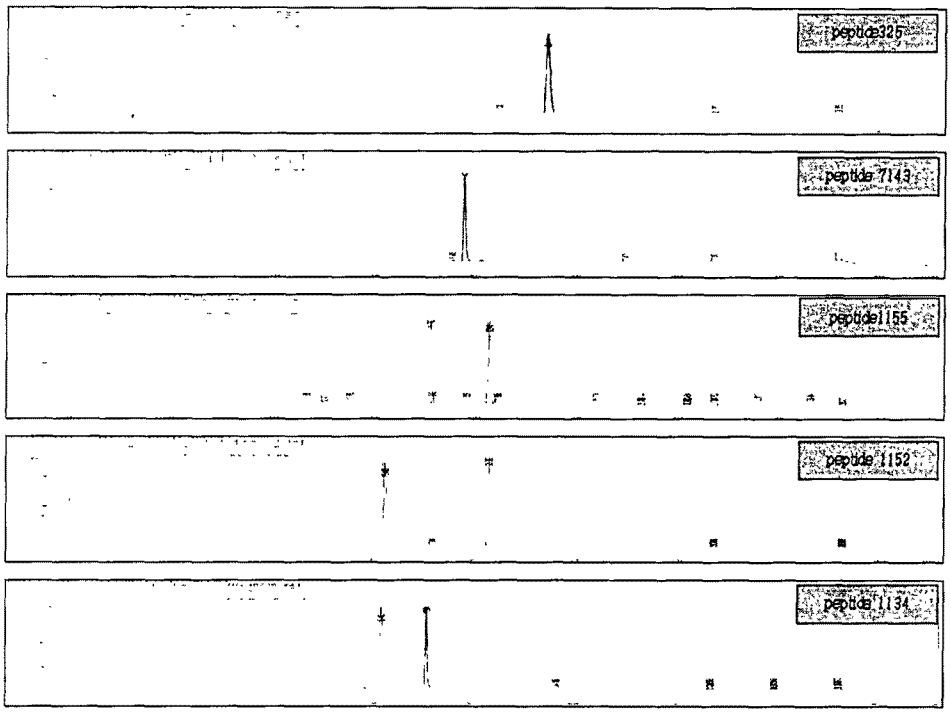

Fig. 1. Chromatogram of peptides 325, 7143, 1155, 1152, 1134 after heating treatment at 60, 70, 80, 90, 100°C.

* peptide 714:DFHINQ, 325:GFHI, 1155:GLSDGEWQ, 1152:FHG, 1134:VLAQYK.

Table 3. Effect of gastric enzymes on ACE inhibition activity of separated peptides

Peptide* / Enzyme	714	325	1155	1152	1134
Pre-enzyme	31.66	34.77	14.64	25.09	53.36
Pepsin	31.6±1.2	35.2±0.4	14.8±0.2	25.6±0.6	31.3±0.5
Trypsin	31.4±0.5	35.0±0.5	14.6±0.1	25.8±0.3	31.3±1.1
Chymotrypsin	31.5±0.3	34.4±0.5	14.5±0.3	25.8±0.4	31.1±0.3

* peptide 714:DFHINQ, 325:GFHI, 1155:GLSDGEWQ, 1152:FHG, 1134:VLAQYK.

ACE inhibition activity of peptides was measured after 2 months of storage at 4℃ under condition of pH 6.0, 6.5, 7.0, 7.5, 8.0. and the ACE inhibitory activity were changed only slightly. After 2 months of chilled storage (4℃), no dramatic change and significance was found. This indicates that acidic, neutral, weak alkali conditions did not affect ACE

inhibitory activity of those peptides. Among peptide 1134, 1152, and 1155, peptides from thermolysin + protease A hydrolysates, inhibition activity of peptide 1134 and 1152 was decreased significantly at 60℃, however, they showed stable inhibition activity from 70℃ to 100℃ ($P<0.001$). Also, chromatogram of peptide 1134, 1152, and 1155 was shown that retention time of peptide of 60℃ was not correspond to the retention time of the rest of peptides. This indicated that temperature may change the inhibitory activity and profile of peptides.

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