JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Inhibition Strength of Short Peptides Derived from an ACE Inhibitory Peptide

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ABSTRACT: A series of peptides, derived from an ACE inhibitory peptide (VTVNPYKWLP) found in our previous work, were synthesized. Their half maximal inhibition concentrations (IC_{50}) for ACE inhibition have been determined. The effect of amino acid sequence on ACE inhibition was discussed on the basis of IC_{50} of the synthetic peptides, and the following characteristics of the ACE inhibitory peptide have been clarified. First, the active portion of this peptide for ACE inhibition is KW. Second, the amino acid sequences near this dipeptide (KW) have a strong effect on the inhibitory activity. Especially, the proline residue in the C-terminal end strongly enhanced ACE inhibition. It should be noted that the IC_{50} value of KWLP (5.5 μM) is the same as the ACE inhibitory peptide (VTVNPYKWLP) and that the IC_{50} value of KW is 7.8 μM. The stability and absorption efficiency in vivo would be significantly improved by shortening the peptide length from 10 amino acids to four amino acids or two amino acids.

KEYWORDS: Angiotensin, ACE, inhibition, peptide, hypertensive

INTRODUCTION

The so-called renin−angiotensin system is one of the physiologically important mechanisms regulating blood pressure.[1,2] Angiotensin-converting enzyme (ACE), a key enzyme in this system, produces a potent vasoconstrictive peptide, angiotensin II, from angiotensin I. This enzyme is a dipeptidyl carboxy peptidase, and an antihypertensive effect is expected by the inhibition of this enzyme. Although synthetic ACE inhibitors prescribed to the hypertensive, captopril, for example, show very strong activity, they are known to have various side effects such as coughing, skin rashes, and angioedema.[3,4] In the food industry, therefore, many researchers have tried to isolate the ACE inhibitors from natural resources for decades. Various bioactive peptides derived from a wide range of foods were reported as ACE inhibitors in the literature. The ACE inhibitory peptides, for example, were isolated from collagenase hydrolysate of gelatin,[5] trypsin hydrolysate of casein,[6] thermal hydrolysate of tuna,[7] corn,[8] soy beans,[9] sardine muscle,[10] and dried bonito.[11] Inhibitory effects of the various food-derived ACE inhibitory peptides were demonstrated also in vivo using spontaneously hypertensive rats (SHR).[12,13] To keep the blood pressure to a healthy level, the intake of foods containing such ACE inhibitory peptides is considered to be important and effective.[14]

The kinetic studies reported that competitive inhibition, concompetitive inhibition, or mixed type inhibition was observed depending on the ACE inhibitory peptides,[15,16] and any common sequence for ACE inhibition among the ACE inhibitory peptides has not been reported. For better understanding of the blood pressure regulation by the renin−angiotensin system and also for developing better nutraceuticals for hypertension, the effects of amino acid sequences of the ACE inhibitory peptides on their inhibitory strength should be clarified.

We isolated four ACE inhibitory peptides from pepsin hydrolysate of boneless chicken leg meat.[17] One of the identified peptides, VTVNPYKWLP, was a part of myosin heavy chain (AA 125−135 of myosin), and its IC_{50} was determined as 5.5 μM. The IC_{50} of this peptide was of the same order of those of the lactotripeptides, VPP and IPP,[18] which are antihypertensive peptides contained in a nutraceutical commercialized by Calpis Co., Ltd. (Japan). To clarify the effect of amino acid sequence on the inhibition strength of the ACE inhibitory peptide VTVNPYKWLP, a series of the peptides were synthesized, and their IC_{50} values were determined in this work. The effect of amino acid sequence on the intensity of ACE inhibition has been discussed on the basis of the determined IC_{50} values.

MATERIALS AND METHODS

Materials. Hippuryl-L-histdyl-L-leucine (HHL) and a hydrophobic column for high-performance liquid chromatography (HPLC), Cosmosil 5C_{18}-MS-II (4.6 mm × 150 mm), were purchased from Nacalai Tesque, Inc. (Japan). ACE was purchased from Sigma-Aldrich (Japan). Synthetic peptides were purchased from Hokkaido System Science Co., Ltd. (Japan). All other reagents purchased from Wako Pure Chemicals Industries, Ltd. (Japan) were of reagent grade.

ACE Activity Measurement. The activity of ACE was measured as described in the previous paper.[19] Briefly, 25 μL of phosphate buffer (50 mM KH_2PO_4, pH 8.3), 10 μL of peptide solution, and 10 μL of ACE solution (0.2 units/mL, 50 mM KH_2PO_4, pH 8.3) were mixed and preincubated for 10 min at 37 °C. After 25 μL of HHL solution (8.3 mM HHL, 133 mM KH_2PO_4, and 500 mM NaCl) was added, the reaction mixture was further incubated for 30 min at 37 °C. The ACE reaction was terminated by adding 70 μL of 1 M HCl. After the reaction mixture was filtered with Millex-LG (Millipore Corp.), 10 μL of the reaction mixture was injected to a HPLC system (Shimadzu LC-10, Japan) equipped with a hydrophobic column (Cosmosil 5C18-MS-II, 4.6 mm × 150 mm). An isocratic mobile phase was 80% Milli-Q water containing 0.1% (v/v) trifluoroacetic acid and 20% acetonitrile containing

Received: March 25, 2011
Accepted: September 19, 2011
Revised: September 19, 2011
Published: September 19, 2011

dx.doi.org/10.1021/jf202902r | J. Agric. Food Chem. 2011, 59, 11234–11237

Table 1. IC_{50} Values of the ACE Inhibitory Peptide Determined in This Work

peptides	IC_{50} (μM)
VTVNPYKWLP	5.5
PYKWLP	5.5
KW	7.8
PYK	2400
YKW	13.3
KWL	43.6
WLP	400
YKWL	38.7
PYKW	17.2
YKWLP	17.2
KWLP	5.0

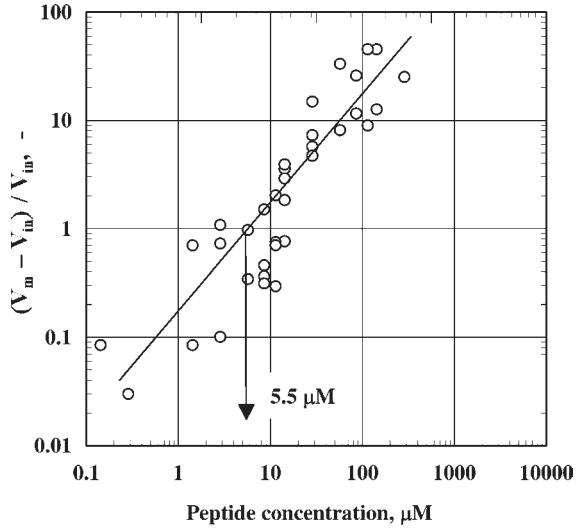

Figure 1. Determination of IC_{50} for peptide PYKWLP.

0.1% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL/min. Peak heights of hippuric acid (HA) generated by ACE reaction and the unreacted HHL were monitored at 228 nm with a detector (Shimadzu SPD-20A). The retention times of HA and HHL were 4.5 and 9.7 min, respectively.

Determination of Half Maximal Concentration IC_{50}. The half maximal concentration IC_{50} was used as a measure of the effectiveness of the peptides in inhibiting ACE. The ACE reaction rates were measured with the different peptide concentrations, and $[(V_m - V_{in})/V_{in}]$ was plotted against the peptide concentration on a logarithmic scale, where V_m is the reaction rate without the inhibitor and V_{in} is that with the inhibitor. This plot should give a straight line with the slope of 1.0 irrespective of inhibition mechanism. After a straight line was drawn with the least-squares method, the peptide concentration that gave $[(V_m - V_{in})/V_{in}] = 1$ was determined as IC_{50}.

RESULTS AND DISCUSSION

The half maximal inhibition concentrations, IC_{50} values, of the synthesized peptides were determined from the logarithmic plots of $[(V_m - V_{in})/V_{in}]$ versus the peptide concentration. For example, the IC_{50} of PYKWLP was determined as 5.5 μM from the logarithmic plot shown in Figure 1. The IC_{50} values for the various peptides determined in this work are summarized in Table 1. It should be noted that the IC_{50} of PYKWLP was the same as that of VTVNPYKWLP (5.5 μM) reported in the previous work.[17] This result strongly suggests that the inhibitory action arises from PYKWLP. Then, IC_{50} values of four tripeptides derived from this region, PYK, YKW, KWL, and WLP, were compared. While the IC_{50} of YKW (13.3 μM) was close to that of PYKWLP (5.5 μM), IC_{50} values of PYK (2400 μM) and WLP (400 μM) were surprisingly large. It should be also noted that the IC_{50} of KW was 7.8 μM. These results clearly show that the dipeptide structure, KW, is crucially important to ACE inhibition. Because ACE is a zinc-containing exopeptidase and is inhibited by EDTA and other metal-chelating agents,[20,21] the imidazole group of tryptophan residue should bind to the zinc molecule of ACE. The strength of the binding is likely to be affected by the amino acid sequence near the tryptophan residue, and thus, the IC_{50} value of the peptides containing KW significantly varies. The IC_{50} of KWL (43.6 μM) was about three times larger than that of YKW (13.3 μM), suggesting that leucine in KWL weakened the binding of the peptide to ACE. This idea was supported by the fact that IC_{50} of YKWL (38.7 μM) was three times larger than that of YKW (13.3 μM). IC_{50} values of tetrapeptides PYKW and KWLP were 17.2 and 5.5 μM, respectively. The IC_{50} value of PYKW (17.2 μM) was almost same as that of YKW (13.3 μM), suggesting that proline in this position

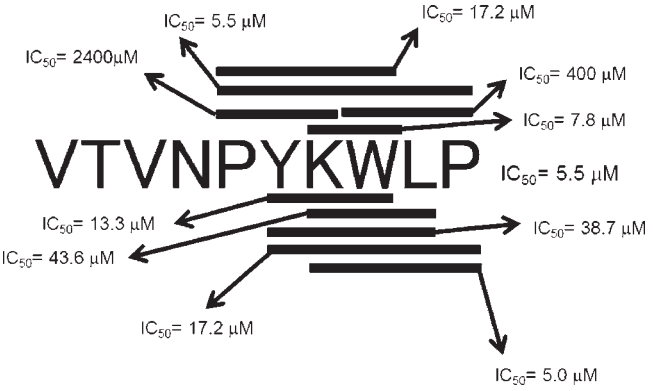

Figure 2. Effect of amino acid sequence on IC_{50}.

Table 2. ACE Inhibitory Dipeptides and Tripeptides Containing Tryptophan or Lysine

peptides	IC_{50} (μM)	ref
AW	10	20
DW	13	23
GW	30	20
IW	2.0	20
RW	16	20
VW	1.6	20
KF	116	24
AKK	3.1	10
GKP	352	25
HIK	>100	26
IKP	1.7	11
IKW	0.21	27
LEK	800	28
LKP	0.32	22
TKY	2.3	24

Table 3. ACE Inhibitory Peptides Show IC_{50} Less Than 1.0 μM

peptides	IC_{50} (μM)	ref
IVY	0.48	29
IKW	0.21	27
LIY	0.82	30
LKP	0.32	26
LRP	0.27	8
VFPS	0.46	29
DYVGN	0.72	29
TYLGS	0.86	29
GGVIPN	0.74	29
PTHIKWGD	0.9	24

did not enhance the binding of the peptides to ACE. However, the IC_{50} of YKWLP (17.2 μM) was less than half of that of YKWL (38.7 μM). Because the IC_{50} of YKWLP (17.2 μM) was close to the IC_{50} of YKW (13.3 μM), the addition of the proline residue to the C-terminal end most likely strengthens the peptide binding to ACE. Three substies were proposed for peptide binding in the hypothetical model that triggered the development of captopril.[20,21] The active site of this model consists of subsite 1, active center (Zn^{2+}), subsite 1′, and subsite 2′. The proline residues of a venom peptide analogue, FAP, were considered to bind strongly to the subsite 2′ positioned to the C-terminal side. Our results revealed that proline residue in the C-terminal side of KW strengthened the binding of the peptide to ACE. The significant effect of proline in this position was also demonstrated by the fact that IC_{50} of KWL (43.6 μM) has decreased to that of KWLP (5.5 μM) by adding proline to the C-terminal end of KWL. Figure 2 summarizes the above-mentioned effects of amino acid sequences on IC_{50} values.

A range of dipeptides and tripeptides containing tryptophan or lysine have been reported as the ACE inhibitory peptides in the literature (Table 2). IC_{50} values of those peptides significantly vary depending on the amino acid sequences, suggesting that a small change in peptide structures significantly affects the binding properties of those peptides to ACE. In Table 3, the ACE inhibitory peptides with IC_{50} values less than 1.0 μM are summarized. Although any common sequence has not found in the list, it should be noted that the sequence KW, the importance of which is suggested in our current work, is found in IKW and PTHIKWGD. Because our systematic work has demonstrated that even a single substitution or addition of amino acid residue in the ACE inhibitory peptides strongly affect IC_{50} values, the IC_{50} values of the peptides listed in Table 3 can be improved by changing amino acid sequences of the peptides.

Thus, a series of experiments in this work have clearly shown that the amino acid sequence of the inhibitory peptide has significant effects on its activity and revealed the following important characteristics of the ACE inhibitory peptide. First, the active portion of this peptide (VTVNPYKWLP) for ACE inhibition is KW. Second, the amino acid sequences in the vicinity of this dipeptide (KW) have a strong effect on the inhibitory activity. These results suggest that the activity of ACE inhibitory peptides can be controlled by choosing proper amino acid sequences. It should be noted that the IC_{50} value of tetrapepide KWLP (5.5 μM) is the same as the peptide VTVNPYKWLP found in our previous work[17] and that of KW is 7.8 μM. Stability and absorption efficiency in vivo would be significantly improved by shortening the peptide length from 10 amino acids to four amino acids or two amino acids. Inhibitory activities of KWLP (IC_{50} = 5.5 μM) and KW (IC_{50} = 7.8 μM) are considered to be sufficiently strong for practical uses, because VPP and IPP, commercialized antihypertensive peptides from Calpis Co., Ltd. (Japan), have IC_{50} values 9 and 5 μM, respectively.[18]

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ACKNOWLEDGMENT

We appreciate Yuki Kimoto, Kaori Moriyama, and Naoko Sawada for their technical support.

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