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Review article

Angiotensin I-converting enzyme inhibitory peptides: Inhibition mode, bioavailability, and antihypertensive effects

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ABSTRACT

Bioactive peptides within the original food-derived proteins are inactive but can be activated by releasing them during food processing (by enzymatic hydrolysis or fermentation) or during gastrointestinal (GI) digestion. Among all the bioactive peptides, the antihypertensive peptides attract particular attention owing to the prevalence of high blood pressure, which plays an important role in cardiovascular diseases. These peptides have the ability to act as angiotensin I-converting enzyme (ACE) inhibitors. Previous studies have shown that the ACE inhibitory peptides functioned as competitive, noncompetitive, or uncompetitive inhibitors, and therefore, the structure–activity relationship of the peptides with various inhibition modes needs to be clarified. Besides, the ACE inhibitory activity of these peptides *in vitro* does not always suggest its antihypertensive effect *in vivo*, which is based on its fate to encounter GI enzymes and brush-border membrane peptidases, after oral administration. This paper reviews the current literature on ACE inhibitory peptides, focusing on the structure–activity relationship and inhibition mechanisms due to their inhibition modes. In addition, the *in vitro*–simulated GI digestion for assessing bioavailability and *in vivo* antihypertensive effects of the peptides are also summarized.

1. Introduction

Cardiovascular disease (CVD), a class of diseases that affects the heart and blood vessels, has been recognized as the biggest cause of death worldwide. High blood pressure, or hypertension, is a condition of sustained increase in blood pressure levels and is the primary risk factor for CVD. It is reported that >25% of the population worldwide (approximately 1 billion) had been affected by hypertension in 2000 and according to the findings of Kearney et al this figure is predicted to increase to 1.56 billion by 2025 [1].

The renin–angiotensin system is a hormone system that regulates blood pressure and fluid balance, and plays an important role in the pathophysiology of CVDs such as congestive heart failure and hypertension [2]. Plasma renin is responsible for the conversion of angiotensinogen released by

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the liver into angiotensin I, which subsequently undergoes proteolytic cleavage, in the presence of angiotensin I-converting enzyme (ACE), to form angiotensin II in the lungs. The hormone angiotensin II is a vasoconstrictor and its formation increases blood pressure. In addition, ACE also degrades bradykinin which has vasodilatation properties. Therefore, the use of ACE inhibitors is believed to lower hypertension and further prevent CVDs.

ACE inhibitors are originally synthesized from compounds found in pit viper venom, and synthetic ACE inhibitors such as captopril, enalapril, lisinopril, and ramipril are currently used in the treatment of hypertension in humans [3,4]. Although these synthetic inhibitors show a remarkable effect in treating hypertension, they also cause adverse side effects, such as cough, allergic reactions, taste disturbances, and skin rashes. Thus, the development of safe and natural ACE inhibitors is necessary for future treatment and prevention of hypertension.

Many studies have successfully produced and isolated ACE inhibitory peptides from various food proteins such as gelatin [5], milk [6], maize [7], sunflower [8], ovalbumin [9], and wheat germ [10]. These peptides have frequently been reported to act as competitive inhibitors of ACE [11–13]; however, in recent years, some noncompetitive and uncompetitive ACE inhibitory peptides have also been isolated [14–17]. Although some studies have demonstrated the relationship between ACE inhibitory activities and peptide structures, only few studies discussed about the activity and inhibition mode of these peptides. On the other hand, in order to reduce increased blood pressure levels after oral administration, the peptides possessing *in vitro* ACE inhibitory activity have to reach the target organ in the organism in an active form. However, because these peptides are degraded by gastrointestinal (GI) enzymes, there is an inconsistency between their *in vitro* ACE inhibitory activity and *in vivo* antihypertensive activity. Although some researchers have used *in vitro*-simulated GI digestion to evaluate the bioavailability and bioactivity of ACE inhibitory peptides [18–20], the correspondence between both *in vitro* and *in vivo* effects has not been investigated in many studies. In this review, the relationship between ACE inhibitory activity and inhibition mode is introduced first. Then, the discrepancy between *in vitro* and *in vivo* activity is examined in the view of bioavailability. Finally, some future perspectives of these peptides are also discussed.

2. Inhibition mode of ACE inhibitory peptides

Proteins are well-known precursors of a range of biologically active peptides. The biologically active (also called “bioactive”) peptides are derived from food proteins that have a physiological effect in the body in addition to their nutritional value. The fact that proteins are precursors of biologically active molecules is particularly attractive for the development of functional foods, because the bioactive peptides are commonly-used food ingredients and are of natural origin. As compared with chemosynthetic drugs, food protein-derived peptides can be used as potent pharmaceuticals as alternatives to synthetic drugs because of the increasing interest for safe and economical use of drugs. The bioactive peptides are activated when released from proteins by enzymatic or acidic hydrolysis, and their biological activity is determined by their native amino acid composition and sequence [21].

Since the isolation of the first ACE inhibitory peptide from snake venom [22], many other ACE inhibitory peptides have been discovered in the enzymatic hydrolysates of various food proteins, including animal-, plant-, and microorganism-derived peptides. Table 1 [12,16,17,19,23–27,30,31,33–36] shows a summary of ACE inhibitory peptides from different food proteins categorized by their inhibition mode.

2.1. *Competitive inhibitor*

The inhibition mode of ACE inhibitory peptides is evaluated using Lineweaver–Burk plots. The competitive inhibitors can bind to the active site to block it or to the inhibitor-binding site that is remote from the active site so as to alter the enzyme conformation such that the substrate no longer binds to the active site (Fig. 1) [23]. For example, NLP [24], NG [24], YN [25], LGFPTTKTYFPHF [26], VVYPWT [26], LNVPGEIVE [27], NIPPLTQTPV [27], DKIHPF [27], LF [28], WA [28], WM [28], were reported to be competitive inhibitors. A previous study has reported that the active sites of two domains of somatic ACE are structurally and functionally homologous to a dipeptidyl carboxypeptidase, and that the zinc-coordination geometry is critical for their hydrolytic action [3]. However, the two catalytic sites are differentially activated by chloride ions and the physiological substrate angiotensin I is preferentially bound to the C-domain catalytic site. The substrate also makes a contribution to the chloride-mediated activation of the active site. Therefore, these differences indicate that despite the higher level of primary sequence homology, structural and functional differences do exist between two active sites of C and N domains. Three subsites, S1 (antepenultimate), S1′ (penultimate), and S2′ (ultimate), with unshared distinct characteristics for the binding of C-terminal amino acids of substrates or inhibitor are located on two homologous active sites. For the inhibitor–enzyme binding and interaction, three main subsites on the active site of the enzyme with different amino acid sequence should be bound with the substrate. Binding of inhibitor or the natural substrate to the enzyme takes place predominantly via the C-terminal tripeptide residues. The peptides with high ACE inhibitory activity have Trp, Phe, Tyr, or Pro at their C terminus and the branched aliphatic amino acids at the N terminus, and ACE is known to have little affinity toward inhibitors with C-terminal dicarboxylic amino acid, such as Glu [29]. More specifically, the presence of aromatic amino acids, Pro, Ala, Val, and Leu are most favorable for the antepenultimate position (S1), while Ile is most favorable for the penultimate position (S1′). Pro and Leu in the substrate sequence are most favorable for the ultimate position (S2′) with regard to the affinity exerted on the enzyme [30,31]. However, there are several peptides such as NNTGHNFENTGEAM [32] and WM [28] that do not fit into the model.

2.2. *Noncompetitive inhibitor*

The noncompetitive inhibition system shows that both the inhibitor and the substrate can be bound to the enzyme at any given point of time. When both the substrate and the inhibitor

Table 1 – Protein-derived ACE inhibitory peptides categorized by inhibition modes.

Source	Preparation	Peptide	IC_{50} (μM)	Reference
1. *Competitive inhibitors*				
Soy	Alcalase	NLP	4.8	[23]
		NG	12.3	
Hard clam meat	Protamex	YN	51	[24]
Porcine hemoglobin	Pepsin	LGFPTTKTYFPHF	4.92	[25]
		VVYPWT	6.02	
Milk	Fermentation	LNVPGEIVE	300.1	[26]
		NIPPLTQTPV	173.3	
		DKIHPF	256.8	
Mushroom	Hot water extraction	VIEKYP	129.7	[30]
Chum salmon muscle	Thermolysin	LF	383.2	[27]
		WA	277.3	
		WM	98.6	
Marine rotifer	Alcalase	NNTGHNFENTGEAM	9.64	[31]
2. *Noncompetitive inhibitors*				
Tofuyo (fermented soybean)	Fermentation	IFL	44.8	[19]
		WL	29.9	
Hen ovotransferrin	Chymotrypsin	KVREGTTY	102.8	[33]
	ACE	KVREGT	9.1	
Oyster	Pepsin	VVYPWTQRF	66	[34]
Oyster	Trypsin	DLTDY	143	[35]
Algae protein waste	Pepsin	VECYGPNRPQF	29.6	[36]
Yellowfin sole frame	α-Chymotrypsin	MIFPGAGGPEL	26.4	[37]
Tuna frame	Pepsin	GNLGKTTTVSNWSPPKWKNTP	11.28	[12]
Chum salmon muscle	Thermolysin	FL	13.6	[27]
		AW	6.4	
3. *Uncompetitive inhibitor*				
Wakame	Protease S	IW	1.5	[16]
		FY	42.3	
		AW	18.8	
Human plasma	Trypsin	YLYEIARR	86	[17]

ACE = angiotensin I-converting enzyme; IC_{50} = median inhibitory concentration.

are bound, the enzyme–substrate–inhibitor complex cannot form a product but can only be converted back into the enzyme–substrate complex or the enzyme–inhibitor complex [33]. Some food protein–derived peptides were reported to be noncompetitive inhibitors of ACE. These include IFL [19], WL [19], KVREGTTY [34], KVREGT [34], VVYPWTQRF [20], DLTDY [35], VECYGPNRPQF [36], and MIFPGAGGPEL [37].

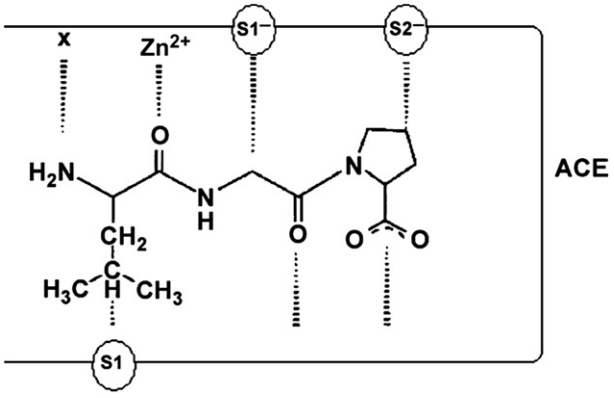

Fig. 1 – Active site of angiotensin I-converting enzyme (ACE) showing the interactions between ACE inhibitory peptides and ACE [23].

The inhibition site of these peptides is not specified because of the various structures of the peptides derived from different parent proteins, and the noncompetitive inhibition mechanism of ACE inhibitory peptides is not clear yet. In order to understand the inhibition mechanism, TPTQQS, a hexapeptide that acts as a noncompetitive inhibitor of ACE to prevent the formation of the reaction product His-Ala, was used to investigate the interactions between ACE, TPTQQS, and the nonphysiological substrate, hippuryl-His-Leu [38]. The results obtained showed that when ACE is in the unbound form, the zinc ion and HEXXH (the key amino acid residues of ACE active site) motif compose the complete active site of ACE, and hippuryl-histidyl-leucine can enter the active site and be converted into the reaction product. After TPTQQS enters ACE, the Thr1, Thr3, and Gln4 residues of TPTQQS allow the peptide to interact with the lid structure of testos ACE (tACE), and the C-terminal Ser6 pushes the zinc ion away from the active site through the coordination bonds between the Ser and the zinc ion, resulting in noncompetitive inhibition of ACE by TPTQQS (Fig. 2) [38]. Although the noncompetitive inhibition model of ACE using TPTQQS has been established, the other inhibitory peptides that function as noncompetitive inhibitors may not fit into this model because of their various peptide lengths and composition. Further investigations are being carried out to identify the relationship between the inhibition mechanism

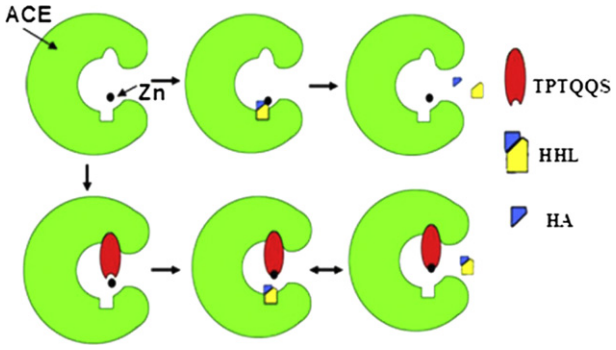

Fig. 2 – Model of the inhibition of angiotensin I-converting enzyme (ACE) by TPTQQS. The model shows that TPTQQS moves the zinc ion away from the active site to inhibit ACE [38]. HHL = hippuryl-histidyl-leucine.

and the structure of these peptides. In any case, this was the first study to report the noncompetitive inhibition mechanism of ACE inhibitory peptides, and this study has also provided fresh thoughts for designing drugs or functional foods against enzyme targets.

2.3. *Uncompetitive inhibitor*

In the uncompetitive inhibition system, the inhibitor can bind only to substrate–enzyme complex and decrease the maximum enzyme activity, so that it takes longer for the substrate or product to leave the active site. Peptides such as IW [16], FY [16], AW [16], and YLYEIARR [17] were reported to act as the uncompetitive inhibitors of ACE. However, the inhibition mechanism of this mode is not clear yet.

The fact that ACE is a dipeptidase makes it plausible for further hydrolysis of ACE inhibitory peptides and affects their antihypertensive activity *in vivo*. Depending on the outcome of GI digestion and other enzyme action *in vivo*, ACE inhibitory peptides can be classified as either true inhibitor (i.e., ACE inhibitory activity remains unchanged), prodrug (i.e., increased ACE inhibitory activity), or substrate (i.e., decreased ACE inhibitory activity) types of ACE inhibitors [39]. However, the active sites or these peptides which act as competitive, noncompetitive, or uncompetitive inhibitors are not specified, and the exact inhibition mechanism of ACE inhibitory peptides remains unclear. Further studies are necessary to figure out the correlation between the inhibition mode and the structure of the peptides.

3. Bioavailability

In order to administer the ACE inhibitory peptides orally in hypertensive patients, these peptides have to pass through the digestive tract to be absorbed through the intestinal epithelium. Digestion of proteins and peptides starts in the stomach by the action of pepsin at acidic pH, and then the polypeptides are further truncated by the pancreatic proteases, trypsin, α-chymotrypsin, elastase, and carboxypeptidases A and B at more alkaline pH. The *in vitro* bioavailability of the bioactive peptides is usually tested by sequential hydrolysis of pepsin and mimicking the actions of pancreatic enzymes so as to simulate the conditions of the GI. The inhibitory activity of some ACE inhibitory peptides was reported to decrease during simulated GI digestion. After sequential treatment with pepsin, chymotrypsin, and trypsin, the median inhibitory concentration (IC_{50}) values of IFL and WL (both isolated from tofuyo extract) against ACE varied from 44.8 to 117.9 μM and from 29.9 to 103.1 μM, respectively [19]. The ACE inhibitory activity of TQVY isolated from rice protein also showed a slight decrease after simulated GI digestion [40]. Some studies also demonstrated that several peptides are resistant to the digestive proteases, such as VIEKYP (from mushroom) [31], LNVPGEIVE, NIPPLTQTPV, DKIHPF (from fermented milk) [27], WVPSV, YTVF, VVYPW (from porcine hemoglobin) [41], and IPP, VPP (from β-casein) [42]. Furthermore, DLTDY was hydrolyzed by the simulated GI digestion to a shorter active form, DY, and the IC_{50} value against ACE decreased from 143 μM for DLTDY to 28 μM for DY [35]. In one of the studies, egg-derived ACE inhibitory peptides YAEERYPIL and RADHPFL were hydrolyzed to other active forms after simulated GI digestion [43]. There was also a study in which the peptide KVLPVPQ derived from β-casein showed low ACE inhibitory activity (IC_{50} = 1000 μM); however, the shorter peptide KVLPVP obtained after pancreatic digestion showed greater ACE inhibitory activity at the IC_{50} value of 5 μM [44]. In the same study, YKVPQL with strong ACE inhibitory activity (IC_{50} = 22 μM) failed to act as a potent ACE inhibitor with IC_{50} > 1000 μM after pancreatic digestion.

In general, Pro- and hydroxyproline-containing peptides are resistant to degradation by digestive proteases, such that the tripeptides with the C-terminal Pro–Pro are reported to be resistant to Pro-specific peptidases [45,46]. This might be the reason that some ACE inhibitory peptides derived from casein and gelatin have been shown to exert *in vivo* antihypertensive effect, as the Pro content in these two proteins is high.

4. Antihypertensive effect

The inhibitory activity of the peptides against ACE does not always correlate with their *in vivo* antihypertensive effects. There are two ways in which the ACE inhibitory peptides exert an antihypertensive effect *in vivo* after oral administration. First, the peptides retain their intact structure and second, they are hydrolyzed into active products, after the action of digestive enzymes, absorbed in the intestine and finally reach their target sites. For this purpose, *in vitro*–simulated GI digestion is a simple and cheap experimental method that is usually used for mimicking the *in vivo* effect. However, the relationship between the *in vitro* ACE inhibitory activity of the peptides via simulated GI digestion and *in vivo* antihypertensive activity is not clear.

An ACE-inhibitory peptide KVLPVPQ derived from casein was degraded to KVLPVP after the simulated GI digestion, and they showed the similar ACE inhibitory activity. After oral administration at a dose of 2 mg/kg, the two peptides KVLPVP and KVLPVPQ got a systolic blood pressure (SBP) reduction of 32.2 and 31.5 mmHg, respectively [44]. In the same study, YLVPQL possessing a strong ACE inhibitory activity was hydrolyzed by simulated GI digestion to YLVP with low ACE

inhibitory activity, and the YLVP showed no significant antihypertensive effect for SHR. Another ACE inhibitory peptide DLTDY with an IC_{50} value of 143 μM was degraded by simulated GI digestion to a dipeptide DL with an IC_{50} value of 28 μM. After both of these ACE inhibitory peptides were orally administrated to SHRs (at dosages of 8 and 10 mg/kg, respectively), their SBPs significantly decreased by about 15 and 20 mmHg at 3–6 and 3 hours [35], respectively. In another study, the ACE inhibitory activity of the peptide TQVY that had an initial IC_{50} value of 18.2 μM slightly decreased after simulated GI digestion. The peptide also showed the antihypertensive activity (by decreasing SBP to 40 mmHg) in SHR when a dosage of 30 mg/kg was administered 6 hours after the oral administration [40].

However, some studies showed inconsistent results. In one of the studies, two peptides YPI and RADHP obtained from the ACE inhibitory peptides YAEERYPIL and RADHPFL, respectively, by simulated GI digestion were not as active as ACE inhibitors ($IC_{50} > 1000$ and $= 153$ μg/mL), but showed a great antihypertensive effect on SHR at a dosage of 2 mg/kg 2–4 hours after oral administration (SBP: 31.6 and 34.0 mmHg, respectively) [43]. In another study, three ACE inhibitory peptides WVPSV, YTVF, and VVYPW had initial IC_{50} values of 0.368, 0.226, and 0.254 mg/mL, respectively [41]. After the simulated GI digestion, the ACE inhibitory activity of WVPSV and YTVF slightly increased, while that of VVYPW remained almost unchanged. Three hours after the oral administration of the former peptides, the SBPs of SHRs decreased by 22.5 and 18.5 mmHg, respectively, whereas after administration of the latter peptide it decreased only by 9.6 mmHg. The possible explanation is that these peptides need further activation by intestinal brush-border or plasma peptidases [43].

In fact, it is difficult to establish a model that directly correlates with *in vitro* ACE inhibitory activity and *in vivo* antihypertensive activity of peptides. The first reason is the bioavailability after oral administration, and the other is that some other antihypertensive mechanisms other than ACE inhibition may be of interest. Although the ACE inhibitory activity of the peptides through *in vitro*–simulated GI digestion may not always ensure their *in vivo* antihypertensive activity, simulated GI digestion is still a good method for preliminary test that is carried out to understanding the possible changes of the peptide structure and screening the potent antihypertensive peptides. Some studies have demonstrated the existence of vasorelaxant peptides that exert their effects through the stimulation of opioid receptors [47]. Moreover, these peptides exhibited a direct or indirect action on vascular smooth muscles [48,49]. In addition, strong evidence indicates that oxidative stress and associated oxidative damage are mediators in cardiovascular pathologies, and thus, antioxidant activity can also be responsible for antihypertensive effects [50].

5. Conclusions and future perspectives

Much work has been done with food protein–derived ACE inhibitory peptides and evidence of their *in vivo* antihypertensive effect has been built in animal and clinical studies. However, the ACE inhibition mechanisms of the peptides with different inhibition modes are still vague due to the various lengths and sequences of these peptides derived from different parent proteins. It is difficult to establish a model and a rule to describe the structure–activity relationship. "BIOPEP" database (http://www.uwm.edu.pl/biochemia/index.php/en/biopep) is responsible for collecting all the information about bioactive peptides from academic literature, and currently the database lists the sequences of 556 ACE inhibitory peptides. Further studies on the establishment of the model of structure–activity relationship are necessary, which can be achieved by using computing methods.

The most challenging task in the antihypertensive peptide researches is the establishment of a detection model for the identification of possible mechanisms by which they can exert *in vivo* antihypertensive activity. Although the simulated GI digestion is a kind of model mimicking the actions of human GI enzymes, the antihypertensive effect of the peptides released from the ACE inhibitory peptides through simulated GI digestion is not guaranteed to be similar to that observed in the GI tract *in vivo*. Therefore, the possible strategies for increasing the resistance to digestive enzymes and cellular permeability of antihypertensive peptides should be also investigated.

REFERENCES

[1] Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005;365:217–23.
[2] Brunner HR, Laragh JH, Baer L, Newton MA, Goodwin FT, Krakoff LR, et al. Essential hypertension: renin and aldosterone, heart attack and stroke. N Engl J Med 1972;286:441–9.
[3] Ondetti MA, Rubin B, Cushman DW. Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. Science 1977;4288:441–4.
[4] Patchett AA, Harris E, Tristram EW, Wyvratt MJ, Wu MT, Taub D, et al. A new class of angiotensin-converting enzyme inhibitors. Nature 1980;298:280–3.
[5] Oshima G, Shimabukuro H, Nagasawa K. Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. Biochim Biophys Acta 1979;566:128–37.
[6] Mullally MM, Meisel H, FitzGerald RJ. Identification of a novel angiotensin-I-converting enzyme inhibitory peptide corresponding to a tryptic fragment of bovine beta-lactoglobulin. FEBS Lett 1997;402:99–101.
[7] Yano S, Suzuki K, Funatsu G. Isolation from alpha-zein of thermolysin peptides with angiotensin I-converting enzyme inhibitory activity. Biosci Biotechnol Biochem 1996;60:661–3.
[8] Megías C, del Mar Yust M, Pedroche J, Lquari H, Girón-Calle J, Alaiz M, et al. Purification of an ACE inhibitory peptide after hydrolysis of sunflower (*Helianthus annuus* L.) protein isolates. J Agric Food Chem 2004;52:1928–32.
[9] Yoshikawa M, Fujita H, Matoba N, Takenaka Y, Yamamoto T, Yamauchi R, et al. Bioactive peptides derived from food proteins preventing lifestyle-related diseases. Biofactors 2000;12:143–6.
[10] Matsui T, Li CH, Osajima Y. Preparation and characterization of novel bioactive peptides responsible for angiotensin I-converting enzyme inhibition from wheat germ. J Pept Sci 1999;5:289–97.

[11] Je JY, Park JY, Jung WK, Park PJ, Kim SK. Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. Food Chem 2005; 90:809–14.
[12] Lee SH, Qian ZJ, Kim SK. A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 2010;118:96–102.
[13] Zhao Y, Li B, Dong S, Liu Z, Zhao X, Wang J, et al. A novel ACE inhibitory peptide isolated from *Acaudina molpadioidea* hydrolysate. Peptides 2009;30:1028–33.
[14] Qian ZJ, Je JY, Kim SK. Antihypertensive effect of angiotensin I converting enzyme-inhibitory peptide from hydrolysates of Bigeye tuna dark muscle, *Thunnus obesus*. J Agric Food Chem 2007;55:8398–403.
[15] Suetsuna K, Nakano T. Identification of an antihypertensive peptide from peptic digest of wakame (*Undaria pinnatifida*). J Nutr Biochem 2000;11:450–4.
[16] Sato M, Hosokawa T, Yamaguchi T, Nakano T, Muramoto K, Kahara T, et al. Angiotensin I-converting enzyme inhibitory peptides derived from wakame (*Undaria pinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. J Agric Food Chem 2002;50:6245–52.
[17] Nakagomi K, Fujimura A, Ebisu H, Sakai T, Sadakane Y, Fujii N, et al. Acein-1, a novel angiotensin-I-converting enzyme inhibitory peptide isolated from tryptic hydrolysate of human plasma. FEBS Lett 1998;438:255–7.
[18] Samaranayaka AGP, Kitts DD, Li-Chan ECY. Antioxidative and angiotensin-I-converting enzyme inhibitory potential of a Pacific hake (*Merluccius productus*) fish protein hydrolysate subjected to simulated gastrointestinal digestion and Caco-2 cell permeation. J Agric Food Chem 2010;58:1535–42.
[19] Kuba M, Tanaka K, Tawata S, Takeda Y, Yasuda M. Angiotensin I-converting enzyme inhibitory peptides isolated from tofuyo fermented soybean food. Biosci Biotechnol Biochem 2003;67:1278–83.
[20] Wang JP, Hu J, Cui J, Bai X, Du Y, Miyaguchi Y, et al. Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats. Food Chem 2008;111:302–8.
[21] Meisel H. Biochemical properties of regulatory peptides derived from milk proteins. Biopolymers 1997;43:119–28.
[22] Ferreira SH, Bartelt DC, Greene LJ. Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. Biochemistry 1970;9:2583–93.
[23] Hong F, Ming L, Yi S, Zhanxia L, Yongquan W, Chi L. The antihypertensive effect of peptides: a novel alternative to drugs? Peptides 2008;29:1062–71.
[24] Wu J, Ding X. Characterization of inhibition and stability of soy-protein-derived angiotensin I-converting enzyme inhibitory peptides. Food Res Int 2002;35:367–75.
[25] Tsai JS, Chen JL, Pan BS. ACE-inhibitory peptides identified from the muscle protein hydrolysate of hard clam (*Meretrix lusoria*). Process Biochem 2008;43:743–7.
[26] Yu Y, Hu J, Miyaguchi Y, Bai X, Du Y, Lin B. Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides derived from porcine hemoglobin. Peptides 2006;27:2950–6.
[27] Gobbetti M, Ferranti P, Smacchi E, Goffredi F, Addeo F. Production of angiotensin-I-converting-enzyme-inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT4. Appl Environ Microbiol 2000;66:3898–904.
[28] Ono S, Hosokawa M, Miyashita K, Takahashi K. Inhibition properties of dipeptides from salmon muscle hydrolysate on angiotensin I-converting enzyme. Int J Food Sci Technol 2006;41:383–6.
[29] Li GH, Le GW, Shi YH, Shrestha S. Angiotensin I – converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. Nutr Res 2004;24:469–86.
[30] Ondetti MA, Cushman DW. Enzymes of the renin-angiotensin system and their inhibitors. Annu Rev Biochem 1982;51:283–308.
[31] Choi HS, Cho HY, Yang HC, Ra KS, Suh HJ. Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. Food Res Int 2001;34:177–82.
[32] Lee JK, Hong S, Jeon JK, Kim SK, Byun HG. Purification and characterization of angiotensin I converting enzyme inhibitory peptides from the rotifer, *Brachionus rotundiformis*. Bioresour Technol 2009;100:5255–9.
[33] Si D, Wang Y, Zhou YH, Guo Y, Wang J, Zhou H, et al. Mechanism of CYP2C9 inhibition by flavones and flavonols. Drug Metab Dispos 2009;37:629–34.
[34] Lee NY, Cheng JT, Enomoto T, Nakano Y. One peptide derived from hen ovotransferrin as pro-drug to inhibit angiotensin converting enzyme. J Food Drug Anal 2006;14: 31–5.
[35] Shiozaki K, Shiozaki M, Masuda J, Yamauchi A, Ohwada S, Nakano T, et al. Identification of oyster-derived hypotensive peptide acting as angiotensin-I-converting enzyme inhibitor. Fish Sci 2010;76:865–72.
[36] Sheih IC, Fang TJ, Wu TK. Isolation and characterization of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste. Food Chem 2009;115: 279–84.
[37] Jung WK, Mendis E, Je JY, Park PJ, Son BW, Kim HC, et al. Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (*Limanda aspera*) frame protein and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 2006;94:26–32.
[38] Ni H, Li L, Liu G, Hu SQ. Inhibition mechanism and model of an angiotensin I-converting enzyme (ACE)-inhibitory hexapeptide from yeast (*Saccharomyces cerevisiae*). PLoS One 2012;7:e37077.
[39] Fujita H, Yokoyama K, Yoshikawa M. Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. J Food Sci 2000; 65:564–9.
[40] Li GH, Qu MR, Wan JZ, You JM. Antihypertensive effect of rice protein hydrolysate with *in vitro* angiotensin I-converting enzyme inhibitory activity in spontaneously hypertensive rats. Asia Pac J Clin Nutr 2007;16(Suppl. 1): 275–80.
[41] Ren Y, Wan D-G, Lu X-M, Chen L, Zhang T, Guo J- L. Isolation and characterization of angiotensin I-converting enzyme inhibitor peptides derived from porcine hemoglobin. Sci Res Essays 2011;6:6262–9.
[42] Ohsawa K, Satsu H, Ohki K, Enjoh M, Takano T, Shimizu M. Producibility and digestibility of antihypertensive β-casein tripeptides, Val-Pro-Pro and Ile-Pro-Pro, in the gastrointestinal tract: analyses using an *in vitro* model of mammalian gastrointestinal digestion. J Agric Food Chem 2008;56:854–8.
[43] Miguel M, Aleixandre MA, Ramos M, López-Fandiño R. Effect of simulated gastrointestinal digestion on the antihypertensive properties of ACE-inhibitory peptides derived from ovalbumin. J Agric Food Chem 2006;54:726–31.
[44] Maeno M, Yamamoto N, Takano T. Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. J Dairy Sci 1996;79:1316–21.
[45] Vanhoof G, Goossens F, De Meester I, Hendriks D, Scharpé S. Proline motifs in peptides and their biological processing. FASEB J 1995;9:736–44.

[46] FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 2000;84(Suppl. 1):S33–7.
[47] Sipola M, Finckenberg P, Vapaatalo H, Pihlanto-Leppälä A, Korhonen H, Korpela R, et al. α-Lactorphin and β-lactorphin improve arterial function in spontaneously hypertensive rats. Life Sci 2002;71:1245–53.
[48] Kouno K, Hirano S, Kuboki H, Kasai M, Hatae K. Effects of dried bonito (*katsuobushi*) and captopril, an angiotensin I-converting enzyme inhibitor, on rat isolated aorta: a possible mechanism of antihypertensive action. Biosci Biotechnol Biochem 2005;69:911–5.
[49] Maes W, Van Camp J, Vermeirssen V, Hemeryck M, Ketelslegers JM, Schrezenmeir J, et al. Influence of the lactokinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells. Regul Pept 2004; 118:105–9.
[50] Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension 2004;44:248–52.