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Plant Food-Derived Angiotensin I Converting Enzyme Inhibitory Peptides

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Hypertension is one of the most common worldwide diseases that afflict humans. Angiotensin I converting enzyme (ACE) catalyzes the formation of vasoconstrictor, angiotensin II, and the inactivation of vasodilator, bradykinin. The influences of ACE on blood pressure make it an ideal target clinically and nutritionally in the treatment of hypertension. A number of animal food-derived peptides have been reviewed about their in vitro and in vivo ACE inhibitory activities. The aim of this review is to discuss the plant food-derived angiotensin I converting enzyme (ACE) inhibitory peptides from sources, production, purification, and structure to in vitro and in vivo activities.

KEYWORDS: Hypertension; angiotensin I converting enzyme; plant proteins; bioactive peptides

INTRODUCTION

ACE (EC 3.4.15.1) was originally isolated from horse blood in 1956 as a hypertension-converting enzyme (*1*). It is a monomeric glycoprotein that is distributed in many tissues and biological fluids. There are two isoforms of ACE in humans: somatic ACE (sACE) and germinal ACE (gACE). Somatic ACE is found in many types of endothelial and epithelial cells. Germinal ACE or testicular ACE is present exclusively in germinal cells in the male testis. Somatic ACE and gACE both consist of a hydrophilic C-terminal cytoplasmic domain, a hydrophobic transmembrane domain that anchors the protein in the membrane, and an N-terminal ectodomain (**Figure 1**). The ectodomain of sACE is further divided into two similar domains (N-domain and C-domain), and each domain contains an active His-Glu-X-X-His (HEXXH) sequence (*2*). Somatic ACE is the only known metallopeptidase with two homologous active sites (*3*). Except for a unique sequence constituting its N-terminus, gACE is identical to the C- terminal half of sACE. The three-dimensional structure by X-ray crystallography of gACE (C domain of sACE) reveals a preponderance of α-helices with a zinc ion and two choride ions incorporated. A deep, narrow channel divides the molecule into two subdomains, and the active site is located toward the bottom of this channel. An N-terminal lid on the top of the molecule appears to allow only small peptide substrates access to the active site cleft. In fact, the structure bears little similarity to that of carboxypeptidase A (M14 family) on which the initial drug development of ACE inhibitors was based. Instead, it resembles rat neurolysin (M3 family) and *Pyrococcus furiosus* carboxypeptidase (M32 family), despite sharing little amino acid sequence similarity with these two proteins (*4*). Corradi et al. (*5*) reported the crystal structure of the N- domain of sACE. Similarly, it has an ellipsoid shape and a central groove separates it into two subdomains, one of which has the N-terminal region that covers the central binding channel. But the structure reveals difference in the active site and contains only one chloride ion, equivalent to chloride II of gACE. The availability of 3D structures of C- and N-domains of sACE may make the structure-based design of active site-specific inhibitors possible (*4*, *5*).

ACE is a M2 family metallopeptidase (MA (E), gluzincins) (*6*). Two histidine residues of the functional motif HEXXH and a third distant glutamate positioned 23−24 residues further toward the C-terminus are the ligands for the zinc cofactor (**Figure 1**) (*7*). An activated water molecule complexed to Zn^{2+} acts as the nucleophile to attack the carbonyl group of the targeted peptide bond (*3*). The activity of the C-domain of sACE depends highly on chloride ion concentration and is inactive in its absence, whereas the N-domain can be completely activated at relatively low concentrations of this anion and is still active in the absence of chloride (*8*, *9*). The two active domains of sACE are also subtly different in substrate specificity. They hydrolyze bradykinin almost equally, but the C-domain active site can hydrolyze angiotensin I, substrate P (*8*), and hippuryl-His-Leu (HHL) (*10*) more efficiently, and the N-domain active site preferentially hydrolyzes angiotensin (1−7) (*11*), luteinizing hormone-releasing hormone (LH-RH) (*8*), the hematoregulatory peptide *N*-acetyl-Ser-Asp-Lys-Pro (AcSDKP) (*12*), and Alzheimer amyloid β-peptide (*13*). Fuchs et al. (*14*) proved that the C-terminal catalytic domain was the main site of angiotensin I cleavage in mice. ACE acts as an exopeptidase to cleave dipeptides from the free C-termini of two typical subtrates angiotensin I and bradykinin. For certain substrates such as cholecystokinin (*15*), substrate P (*16*), and LH-RH (*17*) that have amidated C-termini, ACE not only displays exopeptidase activity but also acts as an endopeptidase (*18*). Thus, ACE might have a more general impact on the metabolism of biologically active peptides than previously recognized (*19*).

ACE plays an important role in the resin−angiotensin system (RAS), which regulates human blood pressure and fluid

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Table 1. Potent ACE Inhibitory Peptides Derived from Plant Foods

source [a]	parent protein	enzyme	amino acid sequence	IC_{50} (μM)	ref
soybean	whole protein	alcalase	DG	12.3	*45*
			DLP	4.8	
		pepsin	IA	153	*46*
			FFL	37	
			IYLL	42	
			YLAGNQ	14	
			VMDKPQG	39	
		fermentation	HHL[d]	2.2[b]	*47*
			WL	29.9	*48*
			IFL	44.8	
	protein isolate	protease D3	YVVFK	44	*49*
			NWGPLV	21	
			PNNKPFQ	33	
			IPPGVPYWT	64	
	glycinin	protease P	VLIVP	1.69	*50*
	β-conglycinin	*Monascus purpureus*	LAIPVNKP	70	*51*
	glycinin	acid proteiase	WL	65	
mung bean	protein isolate	alcalase	KDYRL	26.5	*52*
			VTPALR	82.4	
			KLPAGTLF	13.4	
sunflower	protein isolate	pepsin-pancreatin	FVNPQAGS	6.9[e]	*53*
rice	protein isolate	alcalase	TQVY[d]	18.2	*54*
corn	gluten	alcalase	AY[d]	14.2	*55*
broccoli	water extract	no enzyme	YPK	10.5[b]	*40*
wheat	germ protein	alcalase	TF	17.8	*56*
			LY	6.4	
			YL	16.4	
			AF	15.2	
			IY	2.1	
			VF	9.2	
			IVY[d]	0.48	
			VFPS	0.46	
			TAPY	13.6	
			TVPY	2	
			TVVPG	2.2	
			DIGYY	3.4	
			DYVGN	0.72	
			TYLGS	0.86	
			GGVIPN	0.74	
			APGAGVY	1.7	
	gliadin	pepsin-protease M	IAP[d]	2.7	*57*
mushroom	water extract	no enzyme	VIEKYP	97[c]	*38*
			GEP[d]	40[c]	*39*
garlic	water extract	no enzyme	FY[d]	3.74	*42*
			NY[d]	32.6	
			NF[d]	46.3	
			SY[d]	66.3	
			GY[d]	72.1	
			SF[d]	130.2	
buckwheat	whole protein	pepsin-chymotrypsin	VK	13	*44*
		-trypsin	FY	25	
			AY	100	
			LF	126	
			YQY	4	
			PSY	16	
			LGI	29	
			ITF	49	
			INSQ	36	
	water extract	no enzyme	GPP	6.25[b]	*41*
spinach	rubisco	pepsin-pancreatin	MRW[d]	0.6	*58*
			MRWRD[d]	2.1	
			LRIPVA[d]	0.38	
			IAYKPAG[d]	4.2	

Table 1. Continued

source [a]	parent protein	enzyme	amino acid sequence	IC_{50} (μM)	ref
wine	wine concentration	no enzyme	AWPF	18.3	*59*
			SWSF	76.3	
			YYAPF	26.4	
			WVPSVY	25.7	
			IPPGVPY	17.5	
			YYAPFDGIL	83	

[a] The content in the blank position is the same as that in the last row of the same column. [b] IC_{50} values quoted are expressed as μg/mL. [c] IC_{50} values quoted are expressed as μg. [d] The in vivo assay has been conducted. [e] captopril had a IC_{50} value of 0.041 μM in the same experiment condition.

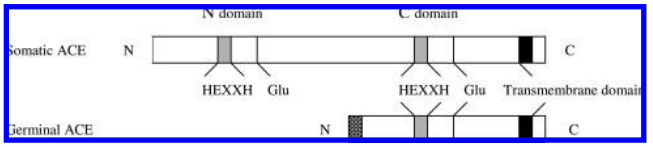

Figure 1. Schematic representation of the primary structures of sACE and gACE (*2*).

homeostasis. The main effector molecule of the RAS, angiotensin II, is produced through an enzymatic cascade consisting of renin, an aspartic protease that first cleaves angiotensinogen to form the decapeptide angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu, 1−10), and ACE, an M2 family metallopeptidase that then further cleaves angiotensin I into the octapeptide angiotensin II (1−8) by removing the C-terminal dipeptide His-Leu (*20*). The resulting angiotensin II is a potent vasoconstrictor, which stimulates the release of aldosterone and antidiuretic hormone or vasopressin and increases the retention of sodium and water and the regeneration of rennin. In addition, ACE, also termed kininase II, inactivates the vasodilators bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-bradykinin) in the kallikrein−kinin system by cleaving the C-terminal dipeptide Phe-Arg. These effects directly act in concert to raise blood pressure. Currently, hypertension is one of the most common worldwide diseases that afflict humans. Overall, approximately 20% of the world's adults are estimated to have hypertension (*21*). It is also reported to affect about 26.7% of persons 20 years of age and over in the United States from 2001 to 2004 (*22*). Hypertension has numerous deleterious effects on the body, significantly increasing the risk of coronary artery disease, stroke, cardiac arrhythmia, heart failure, and abnormal renal function, and producing many other complications related to structural damage to the cardiovascular system (*23*). The influences of ACE on blood pressure have made it an ideal target, and various synthesized ACE inhibitors, such as captopril, enalapril, and lisinopril, have been developed in the clinical treatment of hypertension. Although ACE inhibitory drugs have demonstrated their usefulness, they are not entirely without side effects. A common side effect of ACE inhibitors is a dry cough that appears in 5−20% of patients and may lead to the discontinuation of treatment. Another serious problem is angioedema that has an influence on 0.1−0.5% of patients and can be life threatening (*2*, *24*). ACE inhibitors are also fetotoxic, and fetopathy is characterized by oligohydramnios, intrauterine growth retardation, anuria, hypocalvaria, renal dysplasia, renal failure, congenital malformations, and death (*25*, *26*).

A number of animal food-derived peptides have been shown to have in vitro ACE inhibitory activity, including peptides from milk (*27*), egg (*28*), and muscle proteins (*29*). Some of them display significant antihypertensive activity in rat and human studies (*23*, *30*). Here, we review the current literature on the subject of plant food-derived ACE inhibitory peptides including sources, production, purification, structure, and in vitro and in vivo activities. Compared with ACE inhibitory drugs, these food-derived peptides appear more natural and safer to the consumer. As part of the daily diet, they may represent a much lower cost of health care. Food-derived ACE inhibitory peptides could be applied in the prevention of hypertension and as initial treatment in mildly hypertensive individuals (*31*).

PLANT FOOD SOURCES OF ACE INHIBITORY PEPTIDES

ACE inhibitory peptides have been identified from various plant food sources including soybean, mung bean, sunflower, rice, corn, wheat, buckwheat, broccoli, mushroom, garlic, spinach, and wine (**Table 1**). Although the active peptides have not been sequenced, peanut (*32*), chickpea (*33*, *34*), and potato (*35*) protein hydrolysates also display strong ACE inhibitory activity. In silico gastrointestinal digestion of vicilin and albumin PA2 in pea directly releases a number of potent peptides, indicating that pea protein is a rich source of ACE inhibitory peptides (*36*). A database survey shows that rye, barley, and oats possess most of the known active peptides in their storage protein structure. Thus, cereal storage proteins can be potential sources of ACE inhibitory peptides (*37*).

PRODUCTION OF PLANT FOOD-DERIVED ACE INHIBITORY PEPTIDES

ACE inhibitory peptides can be produced by solvent extraction, enzyme hydrolysis, and microbial fermentation of food proteins (**Table 1**). Water-soluble extracts from pulverized mushroom (*38*, *39*), and sonicated broccoli powder (*40*), contain higher ACE inhibitory activity than the organic solvent soluble extracts. Ma et al. (*41*) used water at pH 9.0 to extract defatted buckwheat flour and produced an active tripeptide, Gly-Pro-Pro. Water extracts of garlic also provides several active peptides (*42*). The most common way to produce ACE inhibitory peptides is through enzymatic hydrolysis of food proteins. The specificity of the proteolytic enzyme and process conditions influence the peptide composition of hydrolysates and thus their ACE inhibitory activities (*43*).The combination of pepsin−pancreatin or pepsin−chymotrypsin−trypsin is usually used to simulate the gastrointestinal degradation of food proteins in human. Pepsin treatment cannot effectively elicit ACE inhibitory peptides from buckwheat protein, while pepsin treatment followed by chymotrypsin and trypsin leads to a significant increase in ACE inhibitory activity (*44*). For the pea protein, the highest ACE activity is reached early in the simulated stomach phase using pepsin treatment, and the level is maintained during the simulated small intestine phase using trypsin−chymotrypsin treatment (*36*). In several other studies, the plant protein hydyolysates generated during pepsin digestion have greater ACE inhibitory activities

than those after subsequent digestion with pancreatin, which suggests that pepsin-produced inhibitory peptides are subsequently hydrolyzed during pancreatic hydrolysis (*53*, *58*, *60*, *61*). Commercially available bacterial and fungi proteases are also widely used in producing potent hydrolysates. Alcalase generates more potent ACE inhibitory hydrolysates than other studied proteases from sources such as corn gluten (*55*), wheat germ (*56*), potato tubers (*35*), soy (*62*), and peanut (*32*) proteins. However, esperase and neutrase produced stronger hydrolysates than alcalase from potato pulp fraction (*35*). GC 106 (an acid protease from *Aspergillus niger* and commercialized by Genencor Co.) hydrolysates of wet- and dry-milled corn germ both reveal stronger ACE inhibitory activity than those from trypsin and thermolysin degradations, while Flavourzyme treatment cannot enhance the activity of either corn germ protein source (*63*). In addition to the adequate match of the enzyme and protein sources, for a complete optimization of the hydrolysis process the influence of other parameters, such as pH, temperature, enzyme to substrate ratio, hydrolysis time, and their interactive effects on ACE inhibitory activity should also be considered (*43*). Research has been conducted to immobilize proteolytic enzymes. Compared to the soluble enzyme, Flavourzyme immobilized on highly activated glyoxyl-agarose support shows more thermal stability and produces less free amino acids in chickpea hydrolysis (*64*). Batch-type operations represent the most common mode for enzymatic hydrolysis of food proteins. However, there have been recent developments of protein digestion in a membrane reactor, in which hydrolysis of isolated soy protein is combined with partial purification of ACE inhibitory peptides from the reaction mixture through the use of membranes with varying molecular weight cut-offs. A continuous membrane reactor can achieve higher productivities and more uniform products than batch approaches (*62*).

ACE inhibitory activity has been found in traditional fermented soybean products, such as natto (*65*), tempeh (*66*), and Douchi (*67*). Active peptides have been purified and sequenced from the fermented tofu Tofuyo (*48*), soy paste (*47*), and soy sauce (*68*). However, fermentation cannot fully hydrolyze soybean proteins to oligopeptides. Phosphoproteins, glycoproteins, and other post-translationally modified species that have a higher number of disulfide bridges are more difficult to cleave. The proteases in *Rhizopus* and *Bacillus* strains can only partly hydrolyze soybean protein (*66*). Further enzymatic hydrolysis is needed to produce peptides with higher activities (*66*, *67*). The same is true of pea proteins where ACE inhibition of *Lactobacillus helverticus* and *Saccharomyces cervisiae* fermented pea protein was increased by subsequent pepsin/trypsin−chymotrypsin digestion (*69*).

PURIFICATION AND SEQUENCE OF PLANT FOOD-DERIVED ACE INHIBITORY PEPTIDES

ACE inhibitory peptides can be separated from a hydrolysate mixture by various kinds of membrane-based separation and chromatography techniques. Prior to the separation process, a peptide mixture is often subjected to precipitation, salting out, and solvent extraction (*70*). Enzymatic hydrolysate of fermented soybean products can be treated using a mixture of water, acetonitrile, and trifluoroacetic acid to extract active peptides (*66*). After centrifugation, the supernatant is filtered, usually by microfiltration under vacuum conditions, in order to remove enzymes and other insoluble components in the source material (*53*). The supernatant can also be applied to a column containing a cation exchange resin such as Dowex 50 (polystyrene-divinylbenzene beads with sulfonic acid groups), which is washed with deionized water to remove impurities, and the desired peptides are eluted with ammonia solution (*42*, *46*). Resin can be adopted to fractionate protein hydrolysates and peptides extracts before (*51*) or after (*45*) membrane separation. Kuba et al. subjected soybean protein hydrolysates (*51*) or peptide extracts from fermented tofu (*48*) to different resins and then eluted with a stepwise gradient of ethanol to get strong ACE inhibitory fractions. Single- or multimembrane separations have been used to isolate ACE inhibitory peptides. It is not always true that permeates from membranes with smaller molecular weight cut-offs (MWCO) have stronger ACE inhibitory potency. Compared to permeates from studied smaller MWCO membranes, permeates of potato liquid fraction (*35*) and soy protein hydrolysates (*45*, *62*) from 10 kDa membrane had no significant difference in ACE inhibitory activities and therefore were selected for further purification.

On the basis of different properties of peptides, different chromatography techniques have been adopted. Among them, reversed-phase HPLC is the most commonly used separation method. Frequently, reversed-phase columns are packed with a chemically bonded octadecylsilyl coated silica; such columns are referred to as C-18 and are very nonpolar. Other popular bonded columns have dodecylsilyl, octasilyl, or phenylsilyl packings. Gradient elution is usually practiced with gradually increased organic solvent (acetonitrile, methanol, or propanol) concentration. The result is that the more polar components of the peptide mixture elute first. Trifluoroacetic acid (TFA) with a concentration of 0.1% (v/v) is often added to the eluting solvents to improve the chromatographic peak shape. Changing the concentration of the TFA modifier can affect the resolution of peaks. RP-HPLC is usually coupled with a quantitative/qualitative analyzing instrument such as a UV detector or mass spectrometer. Among UV detectors, the photodiode array detector (PDA) is often used (*32*, *66*) and shows obvious advantages when compared to the conventional UV detectors that are single channel detectors. PDA is a multichannel detector and can measure a spectrum of wavelengths simultaneously. It also gives more reproducible results than a conventional UV detector because the latter is operated by a stepper motor for selecting a specific wavelength, and the moving part can affect the reproductibility (*71*). Recording a spectrum rather than a single absorbance provides, among other data, abundant comparisons within a symmetrical peak and may reveal the presence of two or more closely eluting components.

Other chromatography techniques include ion-exchange chromatography (IEC), capillary electrophoresis (CE), capillary isoelectric focusing (CIEF), and size-exclusion chromatography (SEC). IEC, CE, and CIEF separate peptides based on their charge properties, while SEC is a separation method based on molecular size. SEC is also named gel filtration chromatography when operated in an aqueous mobile phase or gel permeation chromatography when performed in organic mobile phases (*70*, *72*). Li et al. (*44*) obtained the di- and tripeptide fraction having an average peptide length of 2.31 from the buckwheat digest though gel-permeation chromatography with a Superdex Peptide HR 10/30 column. More recently, affinity purification has been developed to separate ACE inhibitory peptides from alcalase hydrolysate of sunflower protein using immobilized ACE on an activated glyoxyl-agarose support (*73*).

For unknown peptides, mass spectrometry methods are adopted to determine molecular mass and amino acid sequence. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are two main techniques for measuring molecular mass. For the former, the sample solution is pumped

through a narrow, stainless steel capillary; a high voltage is applied to the tip of the capillary, which is located within the ionization source of the mass spectrometer, and a stream of nebulizing gas is introduced. As a consequence of the strong electric field, the sample emerging from the tip is sprayed into highly charged droplets. These droplets are then evaporated at the interface by the drying gas. Charged sample ions, free from solvent, are finally released from the droplets and detected by the analyzer. For the latter, the dried sample is introduced into the mass spectrometer where a laser is fired to desorb and ionize the sample. The matrix is used to absorb the energy needed for sample desorption and ionization from the laser, and the analyzer separates ions according to their mass-to-charge ratio. A tandem mass spectrometer has more than one analyzer and generates structural information for a compound by fragmenting specific peptide ions and identifying the resulting fragment ions. This information can be then used to produce sequence information about the intact peptide (*74*). For example, Ma el al. (*41*) used the ESI mode to get the molecular mass of ACE inhibitory peptides from mushrooms. A HPLC coupled online to ESI mass spectrometry system was shown to be effective to sequence peptides with ACE inhibition activity purified from hydrolyzed corn gluten meal (*55*). The soy protein database (NCBI and SWISS-PROT) search combined with ESI mass/mass spectrometry (MS/MS) efficiently determined the amino acid sequence of peptides from soy protein hyrolysates (*49*). MALDI is usually coupled to a time-of-flight (TOF) mass spectrometer because of its pulsed nature (*75*). MALDI-TOF/TOF tandem mass spectrometry can effectively provide mass spectra and tandem mass spectra for analyzing and sequencing the purified ACE inhibitory peptides from mung bean protein hydroysates (*52*).

STRUCTURAL CHARACTERISTICS OF PLANT FOOD-DERIVED ACE INHIBITORY PEPTIDES

Table 1 presents the source, hydrolyzing enzyme, sequence, and other information about a large number of plant food-derived ACE inhibitory peptides. ACE inhibitory peptides are generally short sequences, which is in agreement with the results of Natesh et al. (*4*) who showed that the active site of ACE cannot accommodate large peptide molecules. The C-terminal tripeptide strongly influences the binding of substrate or inhibitor to ACE. ACE appears to have a preference to a substrate or a competitive inhibitor containing hydrophobic (aromatic or branched side-chains) amino acids in the C-terminal tripeptide. ACE inhibition studies with dipeptides show that C-terminal phenylalanine, proline, tryptophan, or tyrosine residues are the most effective in enhancing substrate binding (*76*). C-terminal lysine, leucine, isoleucine, and valine may also contribute significantly to increasing the ACE inhibitory activity of peptides (*23*). It is suggested that arginine and phenylalanine residues in RSFCA are essential for a specific interaction with ACE and ACE inhibition (*77*). In **Table 1**, several peptides have arginine in the penultimate position, which is in agreement with the suggestion of Rohrbach et al. (*78*) that a positively charged amino acid in the penultimate position has a positive influence for peptide−enzyme binding. Structure−activity data suggest that a C-terminal arginine or lysine, with a positive charge on the guanidine or ε-amino group, respectively, seems to contribute substantially to ACE inhibitory potency. A possible interaction, thus, may exist between the inhibitor and an anionic binding site of ACE that is distinct from the catalytic site. The removal of the arginine residue at the C-terminus can lead to essentially inactive peptide analogues (*23*, *76*). ACE appears to require the L-configuration of amino acids at position three from the C-terminal (*23*). That peptide conformation, i.e., the structure adopted in a specific environment, is also expected to contribute to ACE inhibitory potency. Because of the substrate specificity difference between the two active sites of ACE, hydrophobic peptides have superior binding to the N-terminal catalytic site, while hydrophilic peptides can only bind to the C-terminal catalytic site (*31*, *70*).

IN VITRO ACTIVITY OF PLANT FOOD-DERIVED ACE INHIBITORY PEPTIDES

Various methods have been used to quantify the activity of plant food-derived ACE inhibitory peptides. In all cases, the enzyme is presented with a peptide substrate, the hydrolysis of which is measured by detecting the formation of products. The extent to which inhibitors interfere with this reaction is a measure of their inhibitory power. The original method developed by Cushman and Cheung (*79*) is the one most widely adopted by later authors with minor modifications. The major development for this method lies in the selected product and the method of quantifying the product. The release of two products, hippuric acid (HA) and L-His-L-Leu (HL) from the substrate hippuryl-L-histydyl-L-leucine (Hip-His-Leu, HHL) hydrolysis by ACE, is directly related to ACE activity. Instead of measuring the absorbance of extracted HA at 228 nm as in the original method, an aliquot of product mixture may be directly injected to the HPLC system to quantify the release of HA (*45*). Alternatively, the released HA is determined spectrophotometrically on the basis of the specific colorimetric reactions of HA with 2,4,6-trichloro-*s*-triazine (TT) in dioxane (*53*) or with benzene sulfonyl chlorine in the presence of quinoline (*52*). In other modifications, the released HL is quantified spectrophotometrically on the basis of the reaction of HL with 2,4,6-trinitrobenzene sulfonate (TNBS) (*56*) or spectrofluorometrically by the fluorescent adduct between *o*-phthaldialdehyde (OPA) and HL (*57*). The inhibition mode of ACE-catalyzed hydrolysis of HHL is determined by Lineweaver−Burk plots. Competitive ACE inhibitory peptides are most frequently reported and have been identified from mushroom extracts (*38*, *39*), chickpea (*33*), and soy (*45*) protein hydrolysates. These inhibitors can bind to the active site to block it or to the inhibitor binding site that is remote from the active site to alter the enzyme conformation such that the substrate no longer binds to the active site. A noncompetitive mechanism is also observed in chickpea peptides (*33*). Noncompetitive ACE inhibitory peptides have been isolated from sunflower protein hydrolysate (*80*) and fermented soybean foods (*48*). The spectrophotometic assay using another synthetic tripeptide substrate *N*-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG) blocked at the amino-terminus is gaining popularity because it is simple and well suited to automation. The substrate has been used in quantifying the ACE inhibitory activity of pea (*81*) and peanut (*32*) protein hydrolysates. In addition to the common assay of using soluble ACE, ACE immobilized on glyoxyl-agarose has been developed to determine ACE inhibition by a sunflower peptide inhibitor. The immobilized ACE can be reused and has an increased thermal stability compared to that of the soluble enzyme (*80*).

The potency of an ACE inhibitory peptide is usually expressed as IC_{50}, which is equivalent to the concentration of peptide inhibiting 50% of ACE activity (*23*). An IC_{50} value is determined by regression analysis of ACE inhibition (%) versus peptide concentration (*53*) or ACE inhibition (%) versus log(peptide concentration) (*52*, *54*). It can also be determined by fitting inhibitor concentration−ACE activity data to a four parametric

Table 2. Bioactivity of Plant Food-Derived ACE Inhibitory Peptides

peptides	administration	dose (mg/kg rat weight)	activity	ref
HHL	intravenous	5	decrease SBP[a] 32 mmHg at 30 min	(*47*)
TQVY	oral	30	maximum decrease of about 40 mmHg in SBP at 6 h	(*54*)
AY	oral	50	maximum decease of 9.5 mmHg in SBP at 2 h	(*55*)
GEP	oral	1	decrease SBP about 36 mmHg at 2 h	(*39*)
FY, NY, NF,SY,GY,SF	oral	200	qualitatively similar to that of captopril	(*42*)
IVY	intravenous	5	decrease arterial BP 19.2 mmHg at 8 min	(*82*)
IAP	intraperitoneal	50	decrease SBP significantly at 1.5 h and 3 h	(*57*)
MRWRD	oral	30	maximum decrease of 13.5 mmHg in SBP at 4 h	(*58*)
MRW	oral	20	maximum decrease of 20 mmHg in SBP at 2 h	(*58*)
LRIPVA	oral	100	no antihypertensive effect	(*58*)
IAYKPAG	oral	100	maximum decrease of 15 mmHg in SBP at 4 h	(*58*)

[a] SBP: systolic blood pressure.

logistic model using the Marquardt−Levenberg algorithm (*32*, *81*). The use of a variety of methods for measuring ACE inhibition and thus calculating IC_{50} has made it difficult for the exact comparison of IC_{50} values. The substrates and units of ACE activity within the assay may influence the determination of IC_{50} (*23*). It should be pointed out that the unit shift from μg/mL to μM for the final pure peptides further complicates direct comparisons. Parris et al. (*63*) got negative ACE inhibition values for Flavourzyme hydrolysates of corn germs according to their equation, which might be due to the failure to consider background sample absorbance. The peptide WL from soy glycinin protein shows two quite different IC_{50} values of 29.9 μM (*48*) and 65 μM (*51*); another peptide FVNPQAGS from sunflower protein is also reported to have two distinct IC_{50} values of 6.9 μM (*53*) and 30.56 μM (*80*).

BIOACTIVITY OF PLANT FOOD-DERIVED ACE INHIBITORY PEPTIDES

The in vivo assay of ACE inhibitory activity is generally conducted by measuring the blood pressure response in spontaneously hypertensive rats (SHRs) following intravenous injection and intraperitoneal or oral administration of the synthesized peptides (**Table 2**). Direct administration of angiotensin I has also been used to assess ACE inhibitory activity of peptides. For example, 15 mg/kg of IAP from wheat gliadin can inhibit the hypertensive activity of 50 mg/kg of angiotensin I with intravenous injection in SHRs (*57*). The wide variation in blood pressure responses may be due to variations in sample type, the dosage and administration, the mode of delivery, and the method for the measurement of blood pressure (*23*). For example, triple injections of a total of 5 mg/kg of HHL (His-His-Leu) from soy paste with an interval of 20 min can result in a significantly larger decrease in systolic blood pressure (SBP) of SHRs than a single injection of the same total amount of HHL. The lowering efficacy of triple injections on SBP is comparable to that of the synthetic antihypertensive drug captopril. Unlike injections, the efficacy of orally administrated HHL might be changed by the accompanying digestive modification (*47*). Oral administration can convert certain prodrug-type peptides to true ACE inhibitors. For example, the antihypertensive effect of orally administrated IAYKPAG from spinach in SHR is probably a result of the antihypertensive activity of IAYKP, IAY, and KP (*58*). Conversely, hydrolysis of in vitro inhibitory peptides by peptidases in the brush border may inactivate them (*31*).

Interestingly, there appears to be some difference between the observed blood pressure reduction and the in vitro IC_{50} value. An example is that the peptide LRIPVA shows no antihypertensive effect after oral administration in SHRs at a dose of 100 mg/kg despite its potent ACE inhibitory activity in vitro (IC_{50} = 0.38 μM). This may be due to the conversion of LRIPVA into peptides with very low ACE inhibitory activities (*58*). The IC_{50} values of food-derived ACE inhibitory peptides are about 1000-fold higher than that of the synthetic captopril, but there is no significant difference observed in the antihypertensive effect. Captopril at a dose of 10 mg/kg results in a reduction of blood pressure in SHR of about 50 mmHg, while oral administration of 200 mg/kg of dipeptide purified from garlic exerts an antihypertensive effect of about 30 mmHg. The durations of the effect are not substantially different (*31*, *42*). The profiles of SBP versus oral administration time are similar for 1 mg/kg of the peptide GEP from mushrooms and captopril in SHR (*39*). These findings indicate that plant food-derived ACE inhibitory peptides have higher in vivo activity than would be expected from their in vitro activity when compared with the antihypertensive drug captopril. It has been suggested that food-derived peptides might act via different antihypertensive mechanisms, possess higher affinities for tissues, and be more slowly eliminated than the synthetic captopril (*31*).

The transport of bioactive peptides affects their intestinal absorption and bioavailability. It has been proven that small peptides (di- and tripeptides) generated in the diet can be absorbed across the brush border membrane by a specific peptide transport system and thus produce biological effects (*83*). The caco-2 monolayer is generally used as a model to investigate intestinal transport. Two dipeptides, AF and IF, from salt-free soy sauce are transportable across the caco-2 cell monolayers and display ACE inhibitory activity. Kinetic studies show that IF possesses greater affinity toward the transport than AF (*68*).

Given the discrepancy between in vivo and in vitro results, further investigation into the in vivo and clinical antihypertensive effect of plant food-derived ACE inhibitory peptides is necessary. However, since it is based on a biological mechanism, evidence of in vitro ACE inhibitory activity is a good starting point (*31*). ACE inhibitory peptides derived from plant foods that are consumed frequently can be used as components for functional foods (*29*). As ACE possesses different functions in the human body, ACE inhibition may have additional implications than antihypertensive effects (*31*).

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