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STRUCTURAL CHARACTERISTICS AND ANTIHYPERTENSIVE EFFECTS OF ANGIOTENSIN-I-CONVERTING ENZYME INHIBITORY PEPTIDES IN THE RENIN-ANGIOTENSIN AND KALLIKREIN KININ SYSTEMS

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Abstract

Background: The commercially available synthetic angiotensin-I-converting enzyme (ACE) inhibitors are known to exert negative side effects which have driven many research groups globally to discover the novel ACE inhibitors.
Method: Literature search was performed within the PubMed, ScienceDirect.com and Google Scholar.
Results: The presence of proline at the C-terminal tripeptide of ACE inhibitor can competitively inhibit the ACE activity. The effects of other amino acids are less studied leading to difficulties in predicting potent peptide sequences. The broad specificity of the enzyme may be due to the dual active sites observed on the somatic ACE. The inhibitors may not necessarily competitively inhibit the enzyme which explains why some reported inhibitors do not have the common ACE inhibitor characteristics. Finally, the *in vivo* assay has to be carried out before the peptides as the antihypertensive agents can be claimed. The peptides must be absorbed into circulation without being degraded, which will affect their bioavailability and potency. Thus, peptides with strong *in vitro* IC_{50} values do not necessarily have the same effect *in vivo* and vice versa.
Conclusion: The relationship between peptide amino acid sequence and inhibitory activity, *in vivo* studies of the active peptides and bioavailability must be studied before the peptides as antihypertensive agents can be claimed.

Keywords: Angiotensin-I-converting enzyme, Antihypertensive activity, Bioactive peptides, ACE inhibitor

Introduction

According to Chen et al. (2012), in the year 2000, it was roughly estimated that about 972 million adults had hypertension. This number is expected to swell to a total number of 1560 million in 2025. Hypertension is associated with as a minimum of 7.6 million deaths annually worldwide (13.5% of all deaths), making it the primary risk factor for cardiovascular diseases (Chow et al., 2013). Currently, approximately 35% of the Latin American population, 20-30% of the Chinese and Indian populations and approximately 14% of the Sub-Saharan African population suffer from hypertension (Mittal & Singh, 2010). In a national study of 16,440 subjects in Malaysia, the prevalence of hypertension in subjects aged 15 and above was 27.8%. The prevalence of hypertension among individuals aged 30 and above rose to 40.5% in 2004 compared to 32.9% in 1996. Among subjects aged 60 and above, the prevalence of hypertension was noted to be 69.3% in both sexes (Rampal et al., 2008).

Different biochemical pathways are involved in regulating blood pressure such as the renin-angiotensin system (RAS), kallikrein-kinin system (KKS), and chymase that convert angiotensin I to angiotensin II (Li et al., 2004; Kanemitsu et al., 2006). Typically, RAS is widely associated with the regulation of the blood pressure since it plays a significant role in regulating arterial pressure. In this pathway, angiotensinogen is converted to angiotensin I by renin and later this angiotensin I is further cleaved to angiotensin II by angiotensin-converting-enzyme (ACE), which leads to the vasoconstriction of the artery and then to the elevation of blood pressure (Hong et al., 2008).

ACE, a dipeptidyl carboxypeptidase (EC.3.4.15.1), plays an important role in the regulation of blood pressure. Apart from cleaving angiotensin I to induce vasoconstriction, it also inactivates bradykinin which functions as a vasodilator in KKS (Wijesekara & Kim, 2010; Jung et al., 2006).

Two forms of ACE are present in humans; a somatic ACE of approximately 170 kDa found in endothelial, epithelial and neuronal cells and an approximately 100 kDa size of a smaller testicular isoform that exists in the germinal cells (Michaud et al., 2014). The somatic ACE is necessitated in cardiovascular regulation while germinal ACE is involved in male reproduction. According to Andujar-Sanchez et al. (2003), ACE is also present in the plasma in addition to the somatic and germinal forms. The plasma flowing ACE resulted by cleavage from the somatic form.

The ACE inhibitors, calcium channel antagonists, beta and alpha blockers, natriuretic agents and endothelin receptor antagonists are groups of drugs which fall into the antihypertensive drug categories. For the treatments of high blood pressure and heart failure, the ACE inhibitors are the well-known drugs prescribed to patients. These drugs have also been shown to protect the organs from being damaged by diseases. For example, when administered alone or in

combination, Benazepril produces a renoprotective effect. Besides, when elevated blood pressure can be reduced within a normal range, Benazepril will prevent the cardiovascular disease (Saito, 2008; Barrios & Escobar, 2010).

Despite the effectiveness, these drugs have undesirable effects like coughing, taste disturbance, kidney problem and angioneurotic oedema (Marczak et al., 2003). Hydrochlorothiazide and chlorthalidone can lessen potassium, magnesium, phosphorus, sodium, chloride, folate, B6, zinc, iodine, and CoQ10 while augmenting homocysteine, calcium, creatinine, glucose, insulin resistance, and type-2 diabetes mellitus at a rate of 5% annually (Alexander, 2014).

Higher plasma homocysteine has been suggested as a risk factor for cardiovascular morbidity and mortality (Marcus et al., 2007), while the elevation in serum creatinine concentration within the normal range is an indicator for the augmented risk of cerebrovascular disease in both subjects of normotensive and hypertensive (Wannamethee et al., 1997). The drugs also increase the occurrence of renal deficiency by more than 35% after 10 years of treatment. The administration of the first- and second-generation beta blockers to patients will lower CoQ10 while the ACE inhibitors and angiotensin-receptor blockers will lower zinc (Alexander, 2014). Besides that, the lichenoid reactions, white lesions categorized by linear striations on the buccal mucosa are sometimes noticed in the hypertensive patients resulting from the side effects of the ACE inhibitor drugs, particularly captopril (Kumar et al., 2012). Apart from the above mentioned problems, there is also a need to search for new antihypertensive drugs to overcome drug-resistant hypertension which occurs in approximately one in eight patients with elevated blood pressure (Townsend, 2011).

Unlike these synthetic drugs, antihypertensive peptides do not show any adverse effect (Puchalska et al., 2015) although not all were tested for its toxicity. The inhibition of ACE is without a doubt the utmost studied mechanism to control the high blood pressure with respect to food derived biologically from active peptides. Most peptides have been noticed to inhibit ACE to a certain degree. The food industry has acknowledged the possibility of these natural antihypertensive agents as potential functional ingredients that can assist in the primary prevention and/or management of high blood pressure (Norris & FitzGerald, 2013). In this article, some of the ACE-inhibitory peptides derived from different protein sources, the ACE inhibition type, relationship between peptide amino acid sequence and inhibitory activity, *in vivo* studies of the active peptides, bioavailability and *in silico* bioavailability of the peptides are reviewed.

The mechanism of action of angiotensin

In order to induce constriction, angiotensin I has to be cleaved by the ACE to produce angiotensin II. This angiotensin II has the ability to bind to the angiotensin receptors AT_1 and AT_2, which are two principal angiotensin II receptor subtypes heterogeneously distributed in the peripheral tissues and brain (Chung et al., 1998). In the cardiovascular system, the AT_1 receptor is ubiquitously expressed and facilitates most of the physiological and pathophysiological actions of Angiotensin II. The AT_2 receptor is vastly expressed in developing foetus, and its expression is very little in the normal adult cardiovascular system. According to Lemarié and Schiffrin (2010), the angiotensin II physiological actions induced through the AT_2 receptor opposes that of mediated by the AT_1 receptor. While activation of the AT_1 receptor by angiotensin II promotes the constriction of vascular beds, activation of the AT_2 receptors causes vasodilation.

Relationship between the structure and activity of the ACE inhibitory peptide

An understanding of the correlation between the peptide sequence and its bioactivity as an inhibitor is crucial in generating the potentially potent peptide ACE inhibitor. With the knowledge, it would reduce the need for long conventional ACE inhibitor peptide discovery approach. Unfortunately, there is inadequate information on the structure-activity relationship of antihypertensive peptides because current researches in bioactive peptide field are mainly focusing on the generation and characterization of the antihypertensive peptides.

The first structural characteristic of the ACE-inhibitory peptides is the chain length. Out of many peptide sequences reported, potent ACE inhibitor peptides are typically short with 2-12 amino acids long (Norris & FitzGerald, 2013). The short peptide sequences are easily absorbed into the blood circulation and retain their activity. This is most probably because oligopeptides may not be further digested into shorter fragments in the gastrointestinal tract and are readily absorbed into the circulation, and thus having good bioavailability. It is the best that the ACE inhibitory peptides should not have any enzymatic cleavage site for a gastrointestinal enzyme. However, depending on factors affecting the catalysis ability of these enzymes, it is possible that the peptides can escape from being degraded in the gastrointestinal tract. For example, proline and hydroxyproline comprising peptides are unaffected by the action of digestive proteases especially tripeptides with C-terminal Pro-Pro, which are resistant towards the proline-specific peptidases (Jao et al., 2012).

Some oligopeptides (parent chain) may be cleaved into several smaller fragments. The outcome of the cleavage depends on the fragments produced where it may increase or reduce the overall ACE inhibition activity. The ACE inhibitory activity may increase if suitable amino acid such as proline is present at the C-terminal of the fragments. Thus, the degradation in the gastrointestinal tract may be beneficial in enhancing the activity of the inhibitor and similarly will cause the loss in the activity if the degradation causes the loss of a suitable structure needed to inhibit ACE activity.

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Yet, several inhibitory peptides with longer sequences have been reported (Norris & FitzGerald, 2013). However, the biological efficacy of long peptide sequences cannot be determined through *in vitro* tests. In some cases, the good inhibitory activity observed *in vitro* is lost when *in vivo* assays are carried out and vice versa (Iwaniak et al., 2014). Long peptides which are able to produce good ACE inhibitory effects most probably carry potent amino acids in the sequence, particularly within the four amino acids at the C-terminal of the sequence (Norris & FitzGerald, 2013). Depending on the nature of the peptide whether it is water or lipid soluble, these peptides may pass through the tight junction between cells of the paracellular or transcellular route, which explains the bioavailability of long peptides (Vermeirssen et al., 2004).

Studies have shown that the C-terminal tripeptide sequence of an inhibitory peptide highly influences its binding by ACE. The published ACE inhibitory peptide sequences are listed in Table 1. Looking at the sequence, it seems there is a correlation between the IC_{50} values with a sequence of the peptides, where it could be predicted if a given sequence will be a good ACE inhibitor or not. Potent ACE inhibitors comprised of hydrophobic (aromatic or branched side chains) amino acid residues at the C-terminus where the utmost preferred is the presence of proline (Norris & FitzGerald, 2013). The presence of proline at C-terminal and antepenultimate position results in a good binding to the ACE, while the enzyme binds weakly if proline is present at the penultimate position of the peptide (Miguel et al., 2012). In many potent ACE inhibitor peptide sequences, tyrosine, phenylalanine, and tryptophan residues are also present at the C-terminus particularly for the di- and tripeptide inhibitors. The presence of hydrophobic amino acid leucine at the C-terminus also suggests that it may contribute to the ACE inhibition. In addition, the presence of positive charges on the side chains of arginine and lysine residues at the C-terminus have been identified to contribute to the peptide ACE inhibitory effectiveness (Norris & FitzGerald, 2013).

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Table 1: Peptide sequences of ACE inhibitor from different sources and their IC_{50} value

Source	Preparation	IC_{50}	Peptide	Reference
Agaricus bisporus	Ammonium sulphate precipitation	63	AHEPVK	Lau et al., 2013
Alaskan pollack	Alcalase, pronase E, and collagenase	2.6	GPL	Byun and Kim, 2001
Alaskan pollack	Pepsin	14.7	FGASTRGA	Je et al., 2004
Alaskan pollack	Alcalase → pronase E	0.72	LGP	Byun and Kim, 2002
Algae	Pepsin	29.6	VECYGPNRPQF	Sheih et al., 2011
Beef	Thermolysin + proteinase A	23.2[a]	VLAQYK	Jang and Lee, 2005
Blue mussel	Fermentation with 25% NaCl	19.34[a]	EVMAGNLYPG	Je et al., 2005
Bonito	Thermolysin; boiling water extract	5.1 3.7	IWHHT IY	Yokoyama et al., 1992
Bonito	Thermolysin; ACE	2.4 0.32	LKPNM LKP	Fujita and Yoshikawa, 1999
Bonito	Autolysis	1.0	LRP	Matsumura et al., 1993
Bovine	Alcalase → Pronase E	2.55	GPL	Kim et al., 2001
Bovine lactoferrin	Pepsin	0.47	LIWKL	Ruiz-Gimenez et al., 2012
Broccoli	Water extraction	10.5[a]	YPK	Lee et al., 2006a
Buckwheat	Water extraction	6.25[a]	GPP	Ma et al., 2006
Bullfrog	Alcalase	0.95	GAAELPCSADWW	Qian et al., 2007b
Canola meal	Alcalase	0.15	VSV	Wu et al., 2008b
Catfish	Thermolysin	0.86	GPPP	Ghassem et al., 2012
Chicken	*Aspergillus oryzae* protease	29	GAXGLXGP	Saiga et al., 2008
Common fig	Latex collection from tree	4.5	LYPVK	Maruyama et al., 1989
Corn	Pescalase	0.1[b]	PSGQYY	Suh et al., 1999
Corn	Alcalase	14.2	AY	Yang et al., 2007
Cotton leaf worm larvae	Subsequent hydrolysis using pepsin, trypsin and chymotrypsin	2123	AVF	Vercruysse et al., 2008
Cuttlefish	Digestive proteases	6.1	VYAP	Balti et al., 2010a
Cuttlefish	Bacterial proteases	11.6	AHSY	Balti et al., 2010b
Dwarf gulper shark	Thermoase	3.3[a]	VW	Ikeda et al., 2015
Egg white	Alcalase	20	RVPSL	Liu et al., 2010
Egg white lysozyme	Combination of pepsin, α-chymotrypsin and trypsin	2.86	VAW	Rao et al., 2012

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Garlic	Aqueous extract	3.74	FY	Suetsuna, 1998.
Grass carp	Alcalase	5.34[a]	VAP	Chen et al., 2012
Grifola frondosa	Water extract	2.6	KYTFAVTTVKTWV	Ohtsuru et al., 2000
Hard clam	Protamex	51	YN	Tsai et al., 2008
Hypsizygus marmoreus	Water extract	190[a]	LSMGSASLSP	Kang et al., 2013
Larva	Alcalase	17[a]	YAN	Dai et al., 2013
Lizard fish	Neutral protease	41	SPRCR	Wu et al., 2012
Lysozyme	Papain–trypsin	30[a]	FESNFNTQATNR	Asoodeh et al., 2012
Microalgae	Alcalase	128.4	VEGY	Ko et al., 2012
Milk	Proteinase, aminopeptidas, x-prolyl-dipeptidyl aminopeptidase	9.13 5.15	VPP IPP	Pan et al., 2005
Mung bean	Alcalase	13.4	KLPAGTLF	Li et al., 2006
Ostrich egg white	Trypsin	80.2	AFKDEDTEEVPFR	Tanzadehpanah et al., 2013
Oyster	Pepsin	66	VVYPWTQRF	Wang et al., 2008a
Pleurotus cornucopiae	Water extraction	460[a]	RLPSEFDLSAFLRA	Jang et al., 2011
Pleurotus cystidiosus	Ammonium sulphate precipitation	62.8	AHEPVK	Lau et al., 2013
Porcine	Thermolysin	549.0	ITTNP	Arihara et al., 2001
Porcine	Pepsin	6.1	KRVIQX	Muguruma et al., 2009
Porcine haemoglobin	Pepsin	4.92	LGFPTTKTYFPHF	Yu et al., 2006
Porcine skeletal troponin	Pepsin	26.2	KRQKYDI	Katayama et al., 2008
Porcine troponin C	Pepsin	34	RMLGQTPTK	Katayama et al. 2003
Rainbow trout muscle	Pepsin	63.9	KVNGPAMSPNAN	Kim and Byun, 2012
Rice	Hydrolysate	4.5	VWP	Chen et al., 2013
Rice	Alcalase	18.2	TQVY	Li et al., 2007
Rice wine	Concentrates	340[a]	QFYAV	Kang et al., 2012
Salmon	Alcalase	7.72	FNVPLYE	Ahn et al., 2012
Salmon skin	Alcalase → papain	60[a]	AP	Gu et al., 2011
Sardine	Alkaline protease	1.63	KW	Matsufuji et al., 1994
Sea bream	Protease	7.5	VIY	Fahmi et al., 2004
Sea cucumber	Bromelain and alcalase	15.9	MEGAQEAQGD	Zhao et al., 2009

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Sea cucumber	Bromelain, alcalase	14.2[a]	EDPGA	Zhao et al., 2007
Seaweed pipefish	Alcalase	620[a]	TFPHGP	Wijesekara et al., 2011
Sesame	Thermolysin	0.78	LKY	Nakano et al., 2006
Shark	Protease	1.45	FE	Wu et al., 2008a
Shrimp	Fermentation by *Lactobacillus fermentum* SM 605	2.15	DP	Wang et al., 2008b
Silkworm albumin	Acid protease (*Asperqiius usamii* NO. 537)	47[a]	APPPKK	Wang et al., 2011
Sour milk	Fermentation by *Lactobacillus helveticus*, *Saccharomyces cerevisiae*	9 5	VPP IPP	Nakamura et al., 1995
Soy bean	Acid proteinase	70 65	LAIPVNKP WL	Kuba et al., 2005
Soy bean	Homogenization with dH_2O	29.9	WL	Kuba et al., 2003
Soy bean	Fermentation	2.2[a]	HHL	Shin et al., 2001
Soy bean	Rapid fermentation	43.7	LVQGS	Rho et al., 2009
Soybean	Protease P	1.69	VLIVP	Gouda et al., 2006
Spinach rubisco	Pepsin-pancreatin	0.38	LRIPVA	Yang et al., 2003
Spirulina platensis	Alcalase	5.77	IQP	Lu et al., 2010
Squid	Esperase	47.78	GRGSVPAXGP	Alemán et al., 2013
Styela plicata	Protamex	24.7	MLLCS	Ko et al., 2011
Sunflower	Pepsin and pancreatin	6.9	FVNPQAGS	Megias et al., 2004
Tricholoma giganteum	Water extraction	40[a]	GEP	Lee et al., 2004
Tuna	Pepsin	11.28	GDLGKTTTVSNWSPPKXKDTP	Lee et al., 2010a
Tuna	Pepsin	21.6	WPEAAELMMEVDP	Qian et al., 2007a
Wakame	Pepsin	21	YNKL	Suetsuna and Nakano, 2000
Wakame	Protease S “Amano”	1.5	IW	Sato et al., 2002
Walnut	Hydrolysate	25.67[a]	WPERPPQIP	Liu et al., 2013
Walnut	Hydrolysate	128.98[a]	LPGRPPIKPWPL	Wang et al., 2014
Yellowfin sole	α-chymotrypsin	28.7[a]	MIFPGAGGPEL	Jung et al., 2006

a= µg/mL; b= mM; while others are expressed in µM.

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In the case of tetrapeptides, the best promising amino acids (beginning from C-terminus) are tyrosine and cysteine (Iwaniak et al., 2014). At the tetrapeptide second position, residues like histidine, tryptophan, and methionine are commonly found. In the peptide chain third position, residues such as isoleucine, leucine, valine, or methionine are positioned and tryptophan is located as the fourth residue (Iwaniak et al., 2014). However, this is not the only structure required for the inhibition. For example, the peptide GPPP with IC_{50} of 0.86 µM, apart from its shorter sequence which allows easy absorption, also shows significant inhibition due to the presence of proline at C-terminal and antepenultimate position. It can be concluded that the presence of proline can also play a significant role for the tetrapeptide inhibitor. The location of proline in this sequence best describes why it can significantly inhibit ACE.

Two peptides (TKVIP and AYFYP) with proline residue present at the C-terminus studied by Maeno et al., (1996) did not produce strong ACE inhibitory activity. Although the proline residue is important in inhibiting ACE, this study has shown that the presence of proline alone at the C-terminal does not promise a good inhibition of the enzyme. In the same research, when KVLPVPQ was digested to a shorter KVLPVP, IC_{50} was improved from 1000 µM to 5 µM. This is possibly due to the presence of proline at C-terminal and also at the antepenultimate position of the peptide, which causes the peptide to bind stronger to the ACE active site as compared to the presence of proline at the penultimate position. The deletion of glutamine produced a better inhibition by placing proline at C-terminal and also the antepenultimate position of the peptide.

When YKVPQL was partially hydrolysed by pancreatin to YKVP, IC_{50} of the peptide increased from 22 µM to more than 1000 µM (Maeno et al., 1996). The YKVP sequence was not a significant sequence for the ACE inhibition based on the amino acids arrangement. For a tetrapeptide sequence, tyrosine and cysteine are the best promising amino acids at the C-terminal and the sequence arrangement for tetrapeptide was explained by Iwaniak et al., (2014). As mentioned above, the GPPP sequence does not fit in this explanation and it is hypothesized that the presence of few prolines in tetrapeptides especially at the antepenultimate and C-terminal, will yield a good ACE inhibition.

Generally, proline and hydroxyproline comprising peptides are unaffected by the digestive proteases (Jao et al., 2012).

Compared to the original sequence of YKVPQL, the presence of proline at the antepenultimate position and leucine at the C-terminal are considered as a good sequence for increased potency. For a longer or oligopeptide sequence, the arrangement of the C-terminal amino acids is important so that the peptide can bind strongly to the ACE active site (Norris & FitzGerald, 2013).

In another study of the long peptide, once the phenylalanine at the C-terminus of the GFXGTXGLXGF peptide was removed, IC_{50} was altered to 25000 µM from the original IC_{50} of 46 µM. This indicates that the presence of phenylalanine at C-terminus is also an utmost importance for the ACE inhibitory activity (Saiga et al., 2006). According to Ni et al., (2012), the peptides with phenylalanine at the C-terminal have high ACE inhibitory activity. Similar characteristic was observed for the peptide sequence of VECYGPNRPQF, with proline at the antepenultimate and phenylalanine at the C-terminal.

Compared to the peptides described above, the ACE inhibitory activity of peptide LPGRPPIKPWPL was reported with lower IC_{50} value of 128.98 µg/mL. Although leucine is located at the C-terminal of the peptide, it does not necessarily make peptide a good inhibitor. Similarly, even though peptide has several proline residues, the position of this residue at the end of the C-terminal is really crucial. The presence of proline at the penultimate position will cause the peptide to bind weakly to the ACE active site (Miguel et al., 2012).

The proline penultimate and antepenultimate positions are not really applicable for the tripeptide and dipeptide. Since the sequences are already short, the presence of proline may be exceptional. As mentioned above, the proline and hydroxyproline comprising peptides are unaffected to the action by the digestive proteases. Referring to Table 1, the results of tripeptides with proline at various positions such as VPP, IPP, GPP, GPL, LKP, and YPK give a good IC_{50} for ACE inhibition. Likewise, VSV produced a good IC_{50} of 0.15 µM. This probably suggests that the presence of valine at C-terminal for a tripeptide produces significant ACE activity reduction. To support this statement, Loponen, (2004) demonstrated that the VSP peptide sequence produced higher IC_{50} of 10 µM. Another characteristic of a good ACE inhibitor is the presence of glutamic acid at the C-terminal of the peptide. The good activity of such peptide can be explained by the ability of the peptide to chelate zinc by glutamic acid, which is a segment of the ACE active centre (Iwaniak et al., 2014). For example, the peptide FNVPLYE with a significant IC_{50} value consists of glutamic acid at the C-terminal.

Kinetic study of the peptides

In explaining the competitive inhibition of the inhibitors, the C-terminal tripeptide amino acids possibly will interact with the subsites S1, S1', and S2' at the active site of ACE. The zinc ion of ACE is correctly positioned between S1 and S1' to contribute in the hydrolytic cleavage of the substrate peptide bond causing the release of the dipeptide product. ACE active sites S1, S1'and S2' have high attractions for the side chains of tryptophan, alanine, and proline, respectively (Byun & Kim, 2002). Therefore, it can be concluded that ACE seems to favour substrates or competitive inhibitors that comprise hydrophobic amino acid residues at the three locations of the C-terminal which will interact with these subsites (Hong et al., 2008). Thus, Tovar-Pérez et al. (2009) recommended that active naturally-occurring ACE inhibitory peptides should comprise proline or aromatic amino acids. They also suggested that the

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broad substrates or inhibitor specificity of the enzyme may be due to the two active enzymatic sites in ACE. The somatic ACE comprises two homologous domains, both bearing a functional active site.

To prove the *in vivo* involvement of each active site in releasing angiotensin II and the inactivation of bradykinin, radiolabeled angiotensin I and bradykinin act as the ACE physiological substrates were deployed in *in vitro* and *in vivo* studies to expand understandings into the functional roles of these dual active sites of somatic ACE. *In vitro* experiments showed that a complete inhibition of the angiotensin I and bradykinin cleavage needs a blockade of the dual ACE active sites. Oppositely, the *in vivo* studies in mice revealed that the selective inhibition of either the N-domain or the C-domain of ACE by these inhibitors reduced the conversion of angiotensin I to angiotensin II; however, bradykinin protection involves the inhibition of the dual ACE active sites (Georgiadis et al., 2003).

According to Chen et al. (2010), the somatic angiotensin-I-converting enzyme (sACE) isolated from the pig lung had a molecular weight of 180 kDa. Upon the proteolytic cleavage, two fragments with the size of about 90 kDa were attained and identified by amino-terminal sequence analysis and later known as N- and C-domains of sACE where each domain comprises a functional Zn^{2+} binding active centre (Danilov et al., 2014). The germinal ACE which is smaller than sACE composes only a single catalytically active domain alike to the C-domain. To some extent, N- and C-domains of sACE have a different enzymatic activity where the C-domain is better in converting Ang I into Ang II but having similar efficiency in cleaving bradykinin (Michaud et al., 2014).

Some peptides do not have clear amino acid sequences for example GDLGKTTTVSNWSPPKXKDTP from the tuna frame and yet had a significant ACE inhibitory activity with IC_{50} of 11.28 µM and noncompetitively inhibited ACE (Lee et al., 2010a). It is possible that these peptides are non-competitive inhibitors where they did not bind to the active site of ACE. Several research groups have determined the type of inhibition of their peptides. To our knowledge, the reported ACE inhibitors are either competitive or non-competitive inhibitor based on the Lineweaver-Burk graph plotted (Table 2).

Interestingly, Ni et al., (2012) have shown that the yeast-derived hexapeptide, TPTQQS, non-competitively inhibited ACE by binding N-terminal helices of ACE while its serine residue attracts the zinc residue in the active site, resulting in the conformational change of the active site. Thus, even though substrates can bind to the active site catalysis cannot be carried out.

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Table 2: ACE inhibitory peptides and its inhibition mode

Source	Peptide	Type of inhibitor	References
Alaska pollack	FGASTRGA	Non-competitive	Je et al., 2004
Algae	VECYGPNRPQF	Non-competitive	Sheih et al., 2009
Bigeye tuna dark muscle	WPEAAELMMEVDP	Non-competitive	Qian et al., 2007a
Bovine lactoferrin	LRPVAA	Non-competitive	Lee et al., 2006b
Bullfrog muscle protein	GAAELPCSADWW	Non-competitive	Qian et al., 2007b
Cuttlefish	AHSY	Non-competitive	Balti et al., 2010b
Dwarf gulper shark	VW	Competitive	Ikeda et al., 2015
Freshwater zooplankton	AQGERHR	Competitive	Lee et al., 2010b
Hen egg white lysozyme	MKR, RGY, VAW	Competitive	Rao et al., 2012
Manchego cheese	VRYL	Competitive	Ruiz et al., 2004
Marine *Chlorella ellipsoidea*	VEGY	Competitive	Ko et al., 2012
Mushroom	GEP	Competitive	Lee et al., 2004
Mushroom	AHEPVK	Competitive	Lau et al., 2013
Mushroom	RIGLF PSSNK	Competitive Non-competitive	Lau et al., 2014
Oyster	VVYPWTQRF	Non-competitive	Wang et al., 2008a
Porcine	VVYPWT LGFPTTKTYFPHF	Competitive	Yu et al., 2006
Rainbow trout muscle	KVNGPAMSPNAN	Competitive	Kim and Byun., 2012
Rice	VNP, VWP	Competitive	Chen et al., 2013
Salmon	VWDPPKFD	Non-competitive	Ahn et al., 2012
Soybean	VLIVP	Competitive	Gouda et al., 2006
Spirulina platensis	IQP	Non-competitive	Lu et al., 2010
Tofuyo	IFL, WL	Non-competitive	Kuba et al., 2003
Tuna frame	GDLGKTTTVSNWSPPKXKDTP	Non-competitive	Lee et al., 2010a
Turtle egg white	IVRDPNGMGAW	Competitive	Rawendra et al., 2013
Wakame	VY, VW IY, LW AW, FY, IW	Competitive Non-competitive Un-competitive	Sato et al., 2002
Yeast	TPTQQS	Non-competitive	Ni et al., 2012

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***In vivo* studies and bioavailability of ACE inhibitory peptides**

The ACE inhibitory percentages observed *in vitro* by a peptide do not always correlate with the *in vivo* effect when administered in spontaneously hypertensive rats (SHR). There are many factors contributing to the effectiveness of the peptides once they are administrated *in vivo*. The digestion by gastrointestinal enzymes would be the first factor causing the loss of the peptide availability where the degraded peptides may no longer have a structure suitable to inhibit ACE. Secondly, as mentioned previously, the size of peptides may affect the absorption and thus, their availability. On top of that, *in vitro* analysis typically only focuses on one inhibition of ACE and yet there are many pathways involved in regulating blood pressure. Once administered into the body, there is a possibility that the peptide may also interact with enzymes other than ACE or that the inhibition of ACE alone would not be sufficient to reduce blood pressure. These activities will lead to the incomparable outcome between the *in vivo* and *in vitro* study. The blood pressure lowering effects by the ACE inhibitory peptide on SHR are listed in Table 3.

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Table 3: Blood pressure lowering effect by ACE inhibitory peptide in SHR

Source	Peptide	Dose	mmHg reduction	Reference
Bigeye tuna dark muscle	WPEAAELMMEVDP	10 mg/kg	~17 mmHg	Qian et al., 2007a
Blue mussel	EVMAGNLYPG	10 mg/kg	19 mmHg	Je et al., 2005
Bullfrog	GAAELPCSADWW	10 mg/kg	10 mmHg	Qian et al., 2007b
Chlorella ellipsoidea	VEGY	10 g/kg	22.8 mmHg	Ko et al., 2012
Corn gluten meal	AY	50 mg/kg	9.5 mmHg	Yang et al., 2007
Dried bonito	LKPNM	100 µg/kg	30 mmHg	Fujita and Yoshikawa, 1999
Dried bonito	IKP	10 mg/kg	70 mmHg (IV route)	Fujita et al., 2000
Egg white	RVPSL	50 mg/kg	24 mmHg (compared to negative control)	Yu et al., 2014
Garlic	SY GY FY NY SF GF NF	200 mg/kg	~50 mmHg ~52 mmHg ~32 mmHg ~35 mmHg ~39 mmHg ~25 mmHg ~54 mmHg	Suetsuna, 1998
Oyster	VVYPWTQRF	20 mg/kg	~32 mmHg	Wang et al., 2008a
Porcine myosin B	KRVIQX	10 mg/kg	23 mmHg	Muguruma et al., 2009
Porcine skeletal muscle troponin	KRQKYDI	10 mg/kg	9.9 mmHg	Katayama et al., 2008
Rice	VNP VWP	5 mg/kg	29 mmHg 38 mmHg	Chen et al., 2013
Rice	TQVY	30 mg/kg	~40 mmHg	Li et al., 2007
Sea cucumber	MEGAQEAQGD	3 µM/kg	~17 mmHg	Zhao et al., 2009
Skimmed milk	VPP	1.80 mg/kg 3.60 mg/kg	~17 mmHg ~25 mmHg	Pan et al., 2005
	IPP	1.20 mg/kg 1.80 mg/kg	~25 mmHg ~29 mmHg	
Soybean	HHL	5 mg/kg	32 mmHg (single injection) 61 mmHg (triple injection)	Shin et al., 2001

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Spinach rubisco	MRW MRWRD IAYKPAG LRIPVA	20 mg/kg 30 mg/kg 100 mg/kg 100 mg/kg	20 mmHg 13.5 mmHg 15 mmHg 0 mmHg	Yang et al., 2003
***Tenebrio molitor* (L.) larvae**	YAN	400 mg/kg	27 mm Hg	Dai et al., 2013
Tuna frame	GDLGKTTTVSNWSPPKXKDTP	10 mg/kg	21 mmHg	Lee et al., 2010a
Wakame	AIYK YKYY KFYG YNKL	50 mg/kg	~38 mmHg ~48 mmHg ~42 mmHg ~52 mmHg	Suetsuna and Nakano, 2000
Yellowfin sole	MIFPGAGGPEL	10 mg/kg	22 mmHg	Jung et al., 2006

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Digestion of ACE inhibitory peptides

In the digestive tract, proteinases and peptidases may degrade or reduce the activity of peptide and thus, it is utmost importance that the peptide needs to be resistant to the enzymes if an intact peptide is required to inhibit ACE. In the stomach, the acidic pH favours pepsin to digest proteins and peptides. Later, the action of pancreatic proteases, trypsin, α-chymotrypsin, elastase, and carboxypeptidases A and B further shorten polypeptides at more alkaline pH. In the *in vitro* model, the bioavailability of bioactive peptides is commonly confirmed by successive hydrolysis with pepsin and pancreatic enzymes so as to mimic the gastrointestinal condition.

It has been reported that during the simulated gastrointestinal digestion, the peptide inhibitory activity reduces (Norris & FitzGerald, 2013; Jao et al., 2012). After the simulated digestion on a potent ACE inhibitory peptide YLVPQL, a peptide YLVP with low ACE inhibitory activity was produced which resulted in a non-significant antihypertensive effect in SHR (Jao et al., 2012). Casein-derived ACE inhibitory peptide KVLPVPQ was cleaved to KVLPVP in the simulated gastrointestinal digestion and produced similar ACE inhibitory activity (Maeno et al., 1996). When these peptides were administered orally to SHR at the dose of 2 mg/kg, a reduction in the systolic blood pressure was recorded at 32.2 and 31.5 mmHg, respectively. Thus, a similar pattern could be observed between the *in vitro* and *in vivo* analysis.

Therefore, a prediction on how stable a peptide is against gastrointestinal digestion may be helpful in deducing the availability of peptide sequence after oral administration. In Table 4, with the aid of BIOPEP database, the stability of peptides listed in Table 1 was tested against the gastrointestinal enzymes trypsin, chymotrypsin, and pepsin. The BIOPEP application comprises a database of biologically active peptide sequences and a program facilitating the creation of profiles of the potential biological activity of protein fragments, calculation of quantitative descriptors as measures of the value of proteins as the potential precursors of bioactive peptides, and the prediction of bonds susceptible to the hydrolysis by endopeptidases in a protein chain (Minkiewicz et al., 2008). Out of 75 ACE inhibitory peptide sequences, only 12 were predicted to be stable against the action of these three enzymes. There are many more enzymes present in the gastrointestinal tract and the fate of these 12 peptides are not known although several peptides like IPP and VPP can be absorbed into circulation without being cleaved (Ryan et al., 2011). From Table 4, the reason why several peptides lost their activity *in vivo* although yielded significant result *in vitro* or vice versa could be hypothesized. For example, the peptide TQVY from rice produced an IC_{50} value of 18.2 µM but based on the prediction, pepsin can cleave this sequence and release TQ-VY. From the BIOPEP database, it was suggested that TQ and VY are two dipeptides with ACE inhibitory characteristic. As predicted, when TQVY was administered to SHR, a significant reduction in blood pressure of 40 mmHg was observed (Li et al., 2007). It is possible the reduction of blood pressure was due to these two dipeptides, rather than the intact peptide. In another study, a peptide AVF yielded the IC_{50} value of 2123 µM and from the IC_{50} value (Vercruysse et al., 2008), it was predicted that this peptide will not give a significant reduction of elevated blood pressure *in vivo*. Interestingly, AVF was predicted to be cleaved to A-VF by pepsin. The peptide VF was suggested as the ACE inhibitor by the database. Although the inhibitory activity of the intact peptide was weak *in vitro*, it is possible that peptide could have a better activity *in vivo* due to the digestion.

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Table 4: Hydrolysis of ACE inhibitor peptides by trypsin, pepsin and chymotrypsin as predicted by BIOPEP database

Original sequence	Trypsin	Pepsin (pH > 2)	Chymotrypsin (A)
		Low IC_{50}	
AHEPVK	AHEPVK	A - HE - **PVK**	AHEPVK
AHSY	AHSY	A - **HSY**	AHSY
AP	AP	A-P	AP
AY	AY	A-Y	AY
DP	**DP**	**DP**	**DP**
EDPGA	EDPGA	E - DPGA	EDPGA
EVMAGNLYPG	EVMAGNLYPG	E - VMA - GNL - Y - **PG**	EVMAGNL - Y - **PG**
EVSQGRP	EVSQGR - P	E - VSQ - **GRP**	EVSQGRP
FNVPLYE	FNVPLYE	F - NVPL - Y - E	F - NVPL - Y - E
FVNPQAGS	FVNPQAGS	F - VNPQ - A - GS	F - VNPQAGS
FY	FY	F-Y	F-Y
GEP	GEP	GE - P	GEP
GPL	**GPL**	**GPL**	**GPL**
GPP	**GPP**	**GPP**	**GPP**
GPPP	**GPPP**	**GPPP**	**GPPP**
HHL	**HHL**	**HHL**	**HHL**
IPP	**IPP**	**IPP**	**IPP**
IW	**IW**	**IW**	**IW**
IVY	**IVY**	**IVY**	**IVY**
KRQKYDI	K - R - QK - YDI	KRQ - KY - DI	KRQKY - DI
KVNGPAMSPNAN	K - VNGPAMSPNAN	KVNGPA - MSPNA - N	KVNGPAMSPNAN
LAIPVNKP	LAIPVNK - P	L - A - IPVNKP	L - AIPVNKP
LGFPTTKTYFPHF	LGFPTTK - TYFPHF	L - **GF** - PTTKTY - F - **PHF**	L - **GF** - PTTKTY - F - **PHF**
LIWKL	LIWK - L	L - **IW** - KL	L - **IW** - KL
LKPNM	LK - PNM	L - **KPNM**	L - **KPNM**
LKY	LK - Y	L - KY	L - KY
LRIPVA	LR - IPVA	L - RIPVA	L - RIPVA
LVQGS	LVQGS	L - VQ - **GS**	L - VQGS
LW	LW	L - W	L - W

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LY	LY	L - Y	L - Y
LYPPP	LYPPP	L - Y - **PPP**	L - Y - **PPP**
MEGAQEAQGD	MEGAQEAQGD	**ME** - **GA** - Q - E - A - Q - **GD**	MEGAQEAQGD
MIFPGAGGPEL	MIFPGAGGPEL	MIF - PGA - GGPE - L	MIF - PGAGGPEL
MLLCS	MLLCS	ML - L - CS	ML - L - CS
MVGSAPGVL	MVGSAPGVL	MVGSA - PGVL	MVGSAPGVL
RVPSL	R - **VPSL**	RVPSL	R VPSL
SPRCR	**SPR** - CR	SPRCR	SPRCR
TQVY	TQVY	**TQ** - **VY**	TQVY
VAP	VAP	**VA**-P	VAP
VAW	VAW	**VA** - W	VAW
VECYGPNRPQF	VECYGPNR - PQF	**VE** - CY - GPNRPQ - F	VECY - GPNRPQF
VELYP	VELYP	**VE** - L - Y - P	VEL - Y - P
VLIVP	VLIVP	**VL** - **IVP**	**VL** - **IVP**
VPP	**VPP**	**VPP**	**VPP**
VSV	**VSV**	**VSV**	**VSV**
VVYPWTQRF	VVYPWTQR - F	**VVY** - **PW** - **TQ** - RF	VVY - PW - TQRF
VW	**VW**	**VW**	**VW**
VWP	VWP	**VW** - P	**VW** - P
VYAP	VYAP	**VY** - A - P	**VY** - AP
WL	WL	W-L	W-L
WPERPPQIP	WPER - PPQIP	W - PE - RPPQ - **IP**	W - PERPPQIP
YAN	YAN	Y - A - N	Y-AN
YI	YI	Y - I	Y - I
YN	YN	Y-N	Y-N
YNKL	YNK - L	Y - **NKL**	Y - **NKL**
YPK	YPK	Y-**PK**	Y - **PK**
LKP	LK-P	L-KP	L-KP
FE	FE	F-E	F-E
GAAELPCSADWW	GAAELPCSADWW	**GA** - A - E - L - PCSA - DW - W	GAAEL - PCSADW - W
APPPKK	APPPK - K	A - PPPKK	APPPKK
IQP	IQP	IQ-P	IQP

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FESNFNTQATNR	FESNFNTQATNR	F - E - SNF - NTQ - A - TNR	F - ESNF - NTQATNR
		Moderate IC_{50}	
AFKDEDTEEVPFR	AFK - DEDTEEVPFR	A - F - KDE - DTE - E - **VPF** - R	AF - KDEDTEEVPF - R
AWLHPGAPKVF	AWLHPGAPK - VF	A - W - L - HPGA - PKVF	AW - L - HPGAPKVF
GMNNLTP	GMNNLTP	GMNNL - TP	GMNNL - TP
HLFGPPGKKDPV	HLFGPPGK - K - DPV	**HL** - F - GPPGKKDPV	HL - F - GPPGKKDPV
LPGRPPIKPWPL	LPGR - PPIK - PWPL	L - PGRPPIKPW - **PL**	L - PGRPPIKPW - **PL**
LSMGSASLSP	LSMGSASLSP	L - SMGSA - SL - SP	L - SMGSASL - SP
VEGY	VEGY	**VE** - **GY**	VEGY
		High IC_{50}	
AVF	AVF	A-**VF**	AVF
ITTNP	**ITTNP**	**ITTNP**	**ITTNP**
QFYAV	QFYAV	Q - F - Y - A - V	QF - Y - AV
RLPSEFDLSAFLRA	R - LPSEFDLSAFLR - A	RL - PSE - F - DL - SA - F - L - RA	RL - PSEF - DL - SAF - L - RA
TFPHGP	TFPHGP	**TF** - PHGP	TF - PHGP
YAHYSYA	YAHYSYA	Y - A - **HY** - **SY** - A	Y - AHY - SY - A

Peptide sequences in green colour are the peptides which can exert ACE inhibitory activity as predicted by BIOPEP database.
Peptide sequences in red colour are the peptides which can exert ACE inhibitory activity based on its characteristics as predicted by the authors. Some of the sequences in red have been reported earlier.
BIOPEP database does not obey the Keil rule. Generally, Keil rule is defined as trypsin cleaves next to amino acid arginine (R) or lysine (K), but not before proline (P) (Rodriguez et al., 2008).

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Due to the complexity of the blood pressure regulation system the *in silico* digestion should not be taken as a definitive outcome; there were *in vivo* data which cannot simply be explained by the problem of digestion. For example, YGGY did not produce any reduction in the elevated blood pressure when administered orally to SHR even at the dose of 100 mg/kg despite its IC_{50} value of 3.4 µM (Saito et al., 1994). Its digested product also has a good inhibitory activity. YGGY can be cleaved by pepsin pH >2 to Y-GGY. The tripeptide, GGY was suggested as an ACE inhibitor by BIOPEP database where this tripeptide was reported to have the IC_{50} value of 1.3 µM (Shamloo et al., 2015). To a lesser extent, the released amino acid tyrosine can also exert ACE inhibitory activity on its own as shown by Sved et al. (1979). When tyrosine was given intraperitoneally at the dose of 50 mg/kg and 200 mg/kg, about 12 mm Hg and 40 mm Hg reductions were observed, respectively. Thus, other factors may explain the loss of its ACE inhibitory activity (Section 5.4).

Another interesting example is the peptides YPI and RADHP with the IC_{50} value of more than 1000 µg/mL and 153 µg/mL respectively before and after the digestion. These peptides were derived from YAEERYPIL and RADHPFL respectively, where the parent peptides possess the *in vitro* ACE inhibitory effect. However, these peptides (YPI and RADHP) can significantly reduce the elevated blood pressure in SHR at the dose of 2 mg/kg, even though both peptides are considered as weak inhibitors based on the *in vitro* studies. The authors concluded that the peptides possibly did not exert the antihypertensive effect via ACE inhibitory mechanism (Miguel et al., 2006).

Absorption of ACE inhibitory peptides

When given orally, peptides should be absorbed in the intestine and induce the effect once they reach the target site. Several proteins or peptide structures are noted to be resistant to the action of gastrointestinal digestion, depending whether they have sequences recognized by the digestive enzymes. Peptides with proline and hydroxy proline residues have been noted being resistant towards hydrolysis. Similarly, modified peptides such as glycopeptides and Maillard reaction products were shown to be resistant towards the GI tract enzyme cleavage (Norris & FitzGerald, 2013).

Peptides must also be transported through the brush border membrane intact and be resistant towards the serum peptidases. By mean of a specific peptide transport system for example peptide transporter PepT1, shorter peptides with two or three amino acids are absorbed undamaged through the brush border membrane (Vermeirssen et al., 2004). Peptide IPP and VPP are two well-documented peptides. They are derived from milk which possesses the ACE inhibitory activity. Using various models, it was found that these two peptides were readily transported through the intestinal epithelium. It has been described that IPP escapes gastrointestinal digestion and can pass in the human circulatory system undamaged (Ryan et al., 2011).

As for longer intact peptides, paracellular and transcellular routes were suggested although debates are still going on regarding the relative importance of the routes. Via the paracellular route, large water-soluble peptides pass through the tight junctions between cells. On the other hand, highly lipid soluble peptides seem capable of diffusing through the transcellular route. Apart from these, peptides also possibly enter the enterocytes through endocytosis. Biologically active peptides produced in food can be absorbed intact via the intestine and yield biological effects at the tissue level. Yet, the effectiveness of the given peptides drops as the chain-length increases. In the case of infants, the gastrointestinal barrier is not yet fully developed, thus, intact peptides and proteins are much well absorbed in infants compared to adults (Vermeirssen et al., 2004).

An interesting study was carried out by Ryan et al. (2011) to study the peptide resistance towards peptidases and its transportation at the brush boarder where the ACE inhibitory peptide LHLPLP from β-casein was used. Due to partial hydrolysis by brush border peptidases, HLPLP was formed from the parental sequence. With a concentration dependent manner, the hydrolysed peptide was promptly absorbed through the Caco-2 cell monolayer. By the *in vitro* approach, the hydrolysed peptide was also shown to be resistant towards serum proteases and remained intact after one-hour incubation in the human plasma.

Mode of administration *in vivo*

Other than oral route, peptides can be administered intravenously (IV). Compared to the oral route, injection of the peptide through IV will produce 100% bioavailability. Large peptide easily entered the circulation but comparing the easiness to administer the drug in patients, the oral route is much preferred. The antihypertensive activity of peptide LRPVAA with an IC_{50} value of 4.14 µM was studied using SHR where the peptide was administered via IV. The most significant reduction of the elevated blood pressure was recorded at the rate of 1 nmol/ml/kg. The reduction of the blood pressure by this peptide was calculated as 210% of captopril (10 pmol/ml/kg) where this drug was used as a positive control (Lee et al., 2006b).

Other blood lowering pathways

Apart from ACE, chymase can convert angiotensin I to angiotensin II. Both ACE and chymase are present in the RAS and kallikrein-kinin system and thus, inhibiting ACE alone may not be sufficient to reduce blood pressure. Thus, even though some peptides are very potent for ACE inhibition, due to the complex system of regulating blood

pressure, their ACE inhibition activity cannot be observed *in vivo*. According to Iwaniak et al., (2014), they also agreed that sometimes the results of *in vitro* and *in vivo* produced by peptides do not correlate with each other possibly due to the regulation of blood pressure by factors other than ACE.

RAS is not the only pathway that regulates blood pressure. It is possible that these peptides inhibit other mechanisms *in vivo* and cause a significant reduction in blood pressure. Apart from the ACE inhibition, a few other antihypertensive mechanisms induced by a peptide derived from food have been reported as endothelin converting enzyme (ECE) and endothelin 1 (ET-1) release inhibition, arginine-nitric oxide pathway inhibition, renin inhibition, calcium channel blocking and angiotensin receptor blocking (Udenigwe & Mohan, 2014). Thus, this may explain why some peptides with high IC_{50} value for ACE inhibition *in vitro* have good blood pressure lowering property *in vivo.*

Another reason why *in vivo* analyses have to be carried out is that the pattern of blood pressure lowering could be monitored, which may be unsuitable for patients even if the peptide is very potent. For example, after administered orally in SHR, dipeptide with tyrosine at the C-terminal will produce a slow but prolonged systolic blood pressure reduction. However, when phenylalanine is present in dipeptides at the C-terminal, it resulted in a more rapid reduction and the duration of action becomes shorter (Vermeirssen et al., 2004). Since phenylalanine is hydrophobic and aromatic while tyrosine is aromatic amino acid, the interaction of these peptides to the ACE's S1, S1', and S2' subunits may differ according to the nature of the amino acids.

Conclusion

Knowledge of the structure-activity relationship of the ACE-inhibitory peptides is still limited. Apart from the proline residue positions in the peptide sequence, other residues have not been completely studied which lead to the difficulties in predicting the potent sequence. Compared to the N-terminal of the peptide, C-terminal of the peptide amino acids sequence is utmost importance in order for the peptide to bind to the ACE active site. Although the ACE inhibitory activity of a peptide can be predicted from the amino acids residue arrangement, it does not necessarily give a positive outcome, especially when *in vivo* test is carried out. To retain its activity, it is important for the peptide not to be hydrolysed by gastrointestinal system and readily absorbed into the blood circulation. Since some of the peptides will be hydrolysed by the action of trypsin, pepsin, pancreatin based on the cleavage site in the sequences, their activity *in vivo* is questionable. Peptide administered through IV route produced good ACE inhibition. However, although this route can provide 100% bioavailability, this is not a practical way of a nutraceutical administration if care is to be taken on the easiness of nutraceutical administration in patients for treatment purposes.

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