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.

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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
the liver into angiotensin I, which subsequently undergoes proteolytic cleavage, in the presence of angiotensin I-conv-
erting 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 bra-
dykinin 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 inhibi-
tory peptides have also been isolated [14–17]. Although some
studies have demonstrated the relationship between ACE
inhibitory activities and peptide structures, only a 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 inhibi-
tory 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 physio-
logical 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 alterna-
tives 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],
LGFPITTTFYFHF [26], VVYPWT [26], LNVGEIV [27],
NPLLTQTPV [27], DKHPF [27], LF [28], WA [28], WM [28], were
reported to be competitive inhibitors. A previous study has re-
ported 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 phys-
iological 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 differ-
ences 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
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]. 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.

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Preparation</th>
<th>Peptide</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>Alcalase</td>
<td>NLP</td>
<td>4.8</td>
<td>[23]</td>
</tr>
<tr>
<td>Hard clam meat</td>
<td>Protamex</td>
<td>YN</td>
<td>51</td>
<td>[24]</td>
</tr>
<tr>
<td>Porcine hemoglobin</td>
<td>Pepsin</td>
<td>LGFPKKTTPYPFHF</td>
<td>4.92</td>
<td>[25]</td>
</tr>
<tr>
<td>Milk</td>
<td>Fermentation</td>
<td>LNVPGEIVE</td>
<td>300.1</td>
<td>[26]</td>
</tr>
<tr>
<td>Mushroom</td>
<td>Hot water extraction</td>
<td>VIEKYYP</td>
<td>129.7</td>
<td>[30]</td>
</tr>
<tr>
<td>Chum salmon muscle</td>
<td>Thermolysin</td>
<td>LF</td>
<td>383.2</td>
<td>[27]</td>
</tr>
<tr>
<td>Marine rotifer</td>
<td>Alcalase</td>
<td>NNTGHNFENTGEAM</td>
<td>9.64</td>
<td>[31]</td>
</tr>
<tr>
<td>Hen ovotransferrin</td>
<td>Chymotrypsin</td>
<td>KVREGTTY</td>
<td>102.8</td>
<td>[33]</td>
</tr>
<tr>
<td>Oyster</td>
<td>Pepsin</td>
<td>VVYPWTQRF</td>
<td>66</td>
<td>[34]</td>
</tr>
<tr>
<td>Oyster</td>
<td>Trypsin</td>
<td>DLTDY</td>
<td>143</td>
<td>[35]</td>
</tr>
<tr>
<td>Algae protein waste</td>
<td>Pepsin</td>
<td>VECYGPNRPQF</td>
<td>29.6</td>
<td>[36]</td>
</tr>
<tr>
<td>Yellowfin sole frame</td>
<td>α-Chymotrypsin</td>
<td>MIFPGAGGPEL</td>
<td>26.4</td>
<td>[37]</td>
</tr>
<tr>
<td>Tuna frame</td>
<td>Pepsin</td>
<td>GNLKTTTVSNWSPKWKNTT</td>
<td>11.28</td>
<td>[12]</td>
</tr>
<tr>
<td>Chum salmon muscle</td>
<td>Thermolysin</td>
<td>FL</td>
<td>13.6</td>
<td>[27]</td>
</tr>
<tr>
<td>Human plasma</td>
<td>Trypsin</td>
<td>YLYEIARR</td>
<td>86</td>
<td>[17]</td>
</tr>
</tbody>
</table>

ACE = angiotensin I-converting enzyme; IC<sub>50</sub> = median inhibitory concentration.

Fig. 1 – Active site of angiotensin I-converting enzyme (ACE) showing the interactions between ACE inhibitory peptides and ACE [23].
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, a-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, NIPPLTQTV, DHIHPF (from fermented milk) [27], WFPSV, YTVF, VVYPW (from porcine hemoglobin) [41], and IPP, VFP (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 YAEEYRPL and RADHFPF were hydrolyzed to other active forms after simulated GI digestion [43]. There was also a study in which the peptide KVLVPVQ derived from β-casein showed low ACE inhibitory activity (IC_{50} = 1000 μM); however, the shorter peptide KVLVPF obtained after pancreatic digestion showed greater ACE inhibitory activity at the IC_{50} value of 5 μM [44]. In the same study, YKVPQ 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 KVLVPVQ derived from casein was degraded to KVLVPF 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 KVLVPF and KVLVPQ got a systolic blood pressure (SBP) reduction of 32.2 and 31.5 mmHg, respectively [44]. In the same study, YLVPQ 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 YAEERYPI 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 WVPVSY, 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 WVPVSY 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


