Short communication

Purification and molecular docking study of a novel angiotensin-I converting enzyme (ACE) inhibitory peptide from alcalase hydrolysate of ultrasonic-pretreated silkworm pupa (Bombyx mori) protein

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Abstract
Silkworm pupa (Bombyx mori) protein (SPP) was treated with ultrasound, and then was hydrolyzed using alcalase. The hydrolysate with the highest ACE inhibitory activity was obtained at hydrolysis of 50 min when SPP was treated at power of 410 W/100 ml for 32 min. The hydrolysate was fractionated by ultrafiltration, and peptide with the highest ACE inhibitory activity was purified from <5 kDa fraction using gel filtration and RP-HPLC. A novel peptide was identified as Lys-His-Val (Ic50 = 12.82 μM), and it was stable against the gastrointestinal proteases. The molecular docking study revealed that ACE inhibitory activity of the tripeptide was mainly attributed to the hydrogen bond interactions and Zn(II) interaction between the tripeptide and ACE. These results suggest that the tripeptide is a potential natural ACE inhibitor that can be used as drug or functional food ingredient.

1. Introduction

Angiotensin-I converting enzyme (peptidyl carboxy peptidase, EC 3.4.15.1, ACE), a zinc proteases, plays an important physiological role in regulating blood pressure. This enzyme can hydrolyse angiotensin-I to the potent vasoconstrictor angiotensin-II and inactivate the potent vasodilator bradykinin [6]. Therefore, inhibition of ACE activity is considered to be a key therapeutic approach for controlling high blood pressure. Many synthetic ACE inhibitors including captopril, enalapril and lisinopril and others have been used to prevent hypertension in clinical [7]. However, these synthetic drugs are believed to have undesirable side effects, such as cough, taste disturbances and renal impairment [1]. Therefore, natural ACE inhibitors with minimum side effects have gained increasing attention as potential alternatives to antihypertensive drugs.

In recent years, bioactive peptides isolated from food protein sources have shown significant ACE inhibitory activities. These peptides are inactive within the sequence of parent proteins, but they can be released by enzyme hydrolysis in vivo or in vitro [19]. Numerous ACE inhibitory peptides have been isolated from food protein, such as marine Chlorella ellipsosidea [19], cod skin [9], rice protein [3], egg white protein [18], sweet potato protein [11] and rapeseed albumin [32].

Silkworm pupa (Bombyx mori), an edible insect, is reported to be one of the potential sources of insect proteins, and traditionally used as food material and traditional medicine in some countries, such as China, Japan, Korea, India and Thailand [36]. In recent years, however, silkworm pupa has poor utility for human applications. Most of the pupae are used only as fertilizer and a small proportion is used as animal feed or even regarded as industrial waste [24,30,36]. Silkworm pupa is rich in protein, which account for 48–60% crude protein [30]. Several authors have shown that silkworm pupa protein is a potential source of ACE inhibitory peptides [28,33].

Ultrasound, the low frequency high-energy power ultrasound in the kHz range, has been applied to improve enzymatic hydrolysis and increase the ACE inhibitory activities of hydrolysates from food proteins. For example, our previous study showed that ultrasonic pretreatment could increase the ACE inhibitory activity of wheat germ protein hydrolysate [12]. In addition, ultrasonic pretreatment also has been used to improve the ACE inhibitory activities of milk protein hydrolysate [27] and corn gluten meal protein hydrolysate.

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Little information is known about the ultrasonic pretreatment on the production of ACE inhibitory peptide from silkworm pupa protein until now.

The objective of this study was to investigate the effect of ultrasound as pretreatment on the ACE inhibitory activity of silkworm pupa protein hydrolysate. Furthermore, a novel ACE inhibitory peptide was isolated from ultrasonic-pretreated silkworm pupa protein hydrolysate (USPPH) and its sequence was determined using ion Trap-Mass Spectrometry/Mass Spectrometry (IT-MS/MS), and the purified peptide interaction with the active site of ACE was investigated by using Discovery Studio 2.1 software.

2. Materials and methods

2.1. Materials and chemicals

Silkworm pupa (B. mori) powder was provided by Sericultural Research Institute, Chinese Academy of Agricultural Sciences (Zhenjiang, China). ACE (from rabbit lung) and Hippuryl-His-Leu (HHL) were purchased from Sigma–Aldrich Trading Co. (Shanghai, China). Alcalase (10,000 U/g) was purchased from Novozymes (China) Biotechnology Co. Pepsin (3000 U/g), trypsin (3000 U/g) and α-chymotrypsin (3500 U/g) were purchased from Sangon Biotechnology Co. (Shanghai, China). Sephadex G-10 and G-25 were purchased from Auyoo Biotechnology Co. (Shanghai, China). The purified peptide, Lys-His-Val, was synthesized by Sangon Biotechnology Co. (Shanghai, China).

2.2. Preparation of silkworm pupa protein (SPP)

Silkworm pupa powder was defatted with petroleum ether and dried at 50 °C using a drying oven. Then, the defatted powder (100 g) was dispersed (5%, w/v) in deionized water. The suspension was adjusted to pH 9.5 by using 1 M NaOH. After stirring for 1 h, the suspension was centrifuged at 3000 × g for 15 min. The supernatant was adjusted to pH 4.5 using 1 M HCl to precipitate the proteins and centrifuged again at 3000 × g for 15 min. The precipitate (protein purity, 79.6% w/w) was freeze-dried and stored at −20 °C.

2.3. Preparation of ultrasonic-pretreated silkworm pupa protein hydrolysate (USPPH)

2.3.1. Ultrasonic pretreatment

An aliquot (100 ml) of SPP solution (3.6%, w/v) was prepared, and then the solution was treated using an ultrasonic cell crusher (JY92-II, Haishukesheng Ultrasonic Equipment Co., Ningbo, China) with a 1.5 cm flat tip probe at 20 kHz and at a power (from 250 to 600 W) for 24 min (Pulse durations of on-time 2 s and off-time 2 s). The treated solution was followed by hydrolysis. According to ACE inhibitory activity of treated protein hydrolysate, an optimal power was chosen. Then 100 ml of SPP solution (3.6%, w/v) was treated at above selected power for a given time (from 8 to 50 min). The treated solution was subjected to hydrolysis. In this study, SPP solution was jacketed with ice when undergoing ultrasonic pretreatment and the temperature of treated solution did not exceed 50 °C.

2.3.2. Enzyme hydrolysis

The ultrasonic-pretreated protein solution was adjusted to the concentration of 1% (w/v) by using distilled water, and then incubated in a water bath at 50 °C. After 10 min, the alcalase was added at a level of 3500 U/g of protein. The hydrolysis was performed at constant temperature of 50 °C and pH of 9.0 for 50 min. The reaction was stopped by heating the mixture in a boiling water bath for 10 min. The hydrolysate was clarified by centrifugation (2500 × g, for 10 min at 20 °C) to remove the residue. Then the hydrolysate was lyophilized and stored at −20 °C until used.

2.4. Measurement of peptide content

The peptide contents of the SPP hydrolysate and the purification fractions were determined by o-phthalaldehyde (OPA) spectrophotometric assay using casein tryptone as a standard [7].

2.5. Measurement of ACE inhibitory activity

The ACE inhibitory activity was measured according to our previously described method [12]. The IC50 value was defined as the concentration of inhibitor required to inhibit 50% of the ACE activity.

2.6. Purification of ACE inhibitory peptide

2.6.1. Ultrafiltration

The collected protein hydrolysate (3.5 L, 5 g/L) was filtered sequentially using an ultrafiltration unit (Pellicon XL, Millipore, USA) through two ultrafiltration membranes with molecular mass cut-off of 10 and 5 kDa, respectively. All fractions were lyophilized and named as USPPH-I (>10 kDa), USPPH-II (5–10 kDa) and USPPH-III (<5 kDa).

2.6.2. Gel filtration chromatography

The most active fraction after ultrafiltration was further purified using Sephadex G-25 column (2.5 cm × 70 cm, Auyoo Biotechnology Co., Shanghai, China), eluted with distilled water at a flow of 1.0 ml/min. The fractions were collected at 3 min intervals with a fraction collector, and the absorbance was monitored at 220 nm. Each fraction was assayed for ACE inhibitory activity. The fraction exhibiting the greatest activity was further separated using Sephadex G-10 column (1.5 cm × 50 cm, Auyoo Biotechnology Co., Shanghai, China), eluted with distilled water at a flow rate of 0.5 ml/min. The fractions were collected and monitored under the same conditions as used for the Sephadex G-25 column.

2.6.3. Reversed-phased high performance liquid chromatography (RP-HPLC)

The fraction from Sephadex G-10 column with the greatest ACE inhibitory activity was further separated by RP-HPLC (L-7100, Hitachi High-Technologies Co., Japan) on ODS C18 column (4.6 mm × 250 mm, 5 μm, Shimadzu, Tokyo, Japan). The column was eluted by a linear gradient of acetonitrile (0–50%) containing 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min. Absorbance of the eluent was monitored at 220 nm. Fractions were collected according to the elution peaks and lyophilized immediately. The fraction with the highest ACE inhibitory activity was dissolved at 8 mg/ml and separated for the second step RP-HPLC using ODS C18 column. The elution was conducted at a flow rate of 0.8 ml/min using a linear gradient from 5% to 50% acetonitrile. The eluted peaks were detected at 220 nm and lyophilized for ACE inhibitory activity test.

2.7. Amino acid sequence of the purified peptide

The molecular weight and amino acid sequence of the purified peptide were determined using an Ion Trap-Mass Spectrometry (IT-MS; Thermo LXQ, Massachusetts, USA) coupled with electro-spray ionization (ESI) source. Sample dissolved in methanol/water (1:1, v/v) was infused into the ESI source. The capillary temperature was set at 400 °C. Spray voltage was 4 kV. Mass spectra were acquired over the range 50–1200 m/z. The peptide molecular weight was
detected by a charged (M+H)^+ state analysis in the mass spectrum and the peptide sequence was identified by tandem MS analysis.

2.8. Stability of the purified peptides against gastrointestinal enzymes

The stability of the ACE inhibitory peptide against gastrointestinal proteases was assessed in vitro according to the method of Chen et al. [4] with some modifications. The 1% (w/w) pepsin solution was prepared in a 0.1 mM KCl–HCl buffer adjusted to pH 2.0, while the 1% (w/w) trypsin and α-chymotrypsin solution in 50 mM sodium phosphate buffer was adjusted to pH 7.0. The purified peptide was successively treated by the pepsin (pH 2.0, enzyme–substrate ratio 20 U/mg), α-chymotrypsin (pH 7.0, enzyme–substrate ratio 20 U/mg) and trypsin (pH 7.0, enzyme–substrate ratio 20 U/mg) at 37 °C for 3 h. Then, its activity was measured.

2.9. Molecular docking of the purified peptide on the ACE binding site

The three-dimensional structure of human ACE-lisinopril complex (108A.pdb) was derived from the RCSB PDB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Before the docking, water molecules and the inhibitor lisinopril were removed whereas the cofactors zinc and chloride atoms were retained in ACE model. The polar hydrogen were then added to the ACE model. Structure of the purified peptide was generated using ChemOffice 2004 software (CambridgeSoft Co., USA) and its energy was minimized with the CHARMM programme. The molecular docking of the peptide and ACE was studied according to a method described by Pan et al. [21] with some modifications. The flexible docking tool of Discovery Studio 2.1 software was used to perform the molecular docking of the peptide at ACE-binding site. The docking runs were carried out with a radius of 9 Å, with coordinates x: 40.6559, y: 37.3827 and z: 43.3401. The best ranked docking pose of the peptide in the active site of ACE was obtained according to the scores and binding-energy value.

3. Results and discussion

3.1. Preparation of ultrasonic-pretreated silkworm pupa protein hydrolysate (USPPH)

In this study, the ultrasonic-pretreated technology was applied to treat silkworm pupa protein (SPP) in order to improve the ACE inhibitory activity of hydrolysate. Effects of ultrasonic power and ultrasonic time on ACE inhibitory activity of SPP hydrolysate at the concentration of 250 μg/ml were shown in Fig. 1. The ultrasonic power and ultrasonic time played main roles in the preparation of ACE inhibitory peptide. ACE inhibition initially increased with increasing ultrasonic power/ultrasonic time up to a point beyond which it decreased with the increase in the ultrasonic power/ultrasonic time (Fig. 1), which was similar to the results from Zhou et al. [35] and Uluko et al. [26]. In our previous research, we demonstrated that short ultrasonic time and low ultrasonic power could induce molecular unfolding of wheat germ protein and increase its surface hydrophobicity, which facilitated the release of ACE inhibitory peptide during enzymatic hydrolysis, whereas long ultrasonic time and high ultrasonic power could re-associate or aggregate to form a more stable structure, which hindered the release of ACE inhibitory peptide from the protein [12]. Pretreated protein hydrolysates at different ultrasonic powers (250–600 W) and ultrasonic times (8–50 min) showed similar peptide content as control, when the hydrolysis time was 10 (peptide content, 90.8% w/w), 30 (peptide content, 93.1% w/w), 50 (peptide content, 96.2% w/w), 70 (peptide content, 96.9% w/w), and 90 min (peptide content, 97.5% w/w), respectively (data not shown). This result showed that even though similar peptide content was obtained at different ultrasonic conditions, these hydrolysates exhibited the different ACE inhibitory activities. These differences might result from the different composition of peptides in these hydrolysates. Moreover, Fig. 1 showed that the ACE inhibitory activity of untreated and treated SPP hydrolysate increased with increase of hydrolysis time from 0 to 50 min and then slightly slowed down (50–90 min). This might be because partial hydrolysate from SPP after 50 min was hydrolyzed into low or no activity molecules [8,23]. Even though the ACE inhibitory activity of pretreated SPP hydrolysate slightly decreased at ultrasonic power >410 W and ultrasonic time >32 min, ultrasonic pretreatment caused a 39.9–67.3% increase at ultrasonic powers of 250–600 W, and a 54.5–79.1% increase at ultrasonic time of 8–50 min in ACE inhibitory activity of hydrolysate.

![Graph showing ACE inhibitory activities of alcalase hydrolysates from untreated and ultrasonic-pretreated silkworm pupa (B. mori) protein.](image-url)
compared to that without ultrasonic pretreatment. The result suggested that ultrasonic pretreatment was an effective way to increase the ACE inhibitory activity of SPP hydrolysate. Hence, ultrasonic power of 410 W, ultrasonic time of 32 min and hydrolysis time of 50 min were chosen as the preparation condition to obtain the SPP hydrolysate with the highest activity (IC50 value, 91.3 μg/ml).

3.2. Purification of ACE inhibitory peptide

The obtained ultrasonic-pretreated SPP hydrolysate (USPPH) was fractionated by ultrafiltration into USPPH-I (>10 kDa), USPPH-II (5–10 kDa) and USPPH-III (<5 kDa). ACE inhibitory activities of the fractions varied with molecular weight (MW) ranges. Among the three fractions, USPPH-III evidenced the strongest ACE inhibitory activity and had an IC50 value of 59.1 ± 1.06 μg/ml (date not shown). This result was consistent with previous studies of ACE inhibitory peptides, in which the low MW peptides had more potent ACE inhibitory activities than that of the high MW peptides [3,15,22,25]. The ACE inhibitory activity of SPPH-III was stronger than that of the below 5 kDa hydrolysate from *Chlorella ellipsoidea* (IC50 = 890 μg/ml) reported by Ko et al. [15]. Therefore, the USPPH-III fraction was selected for further purification.

To the purification of ACE inhibitory peptide, the USPPH-III fraction was loaded onto a Sphadex G–25 gel filtration column, and then was eluted with distilled water. As shown in Fig. 2a, four fractions were separated and designated as A–D, of which the molecular weights ranged from high to low. At the concentration of 100 μg/ml, fraction D (peptide content, 99.5%, w/w) evidenced the strongest ACE inhibitory activity and had an inhibitory rate of 89.3%. Then fraction D was further separated into three fractions (D1, D2, D3) using a Sphadex G–10 gel filtration column (Fig. 2b). The fraction D3 (peptide content, 99.8%, w/w) at the concentration of 50 μg/ml was observed to have the highest ACE inhibitory activity (Fig. 2b), with an inhibitory rate of 73.5%. Fraction D3 was then concentrated and used for further isolation using RP-HPLC on a C18 column with a linear gradient of 0–50% acetonitrile. The elution profile of peptide separation is shown in Fig. 2c. Eight fractions were obtained and ACE inhibitory activity determined for each. Among those fractions, fraction D3c exhibited the highest ACE inhibitory activity (IC50 = 12.5 ± 0.18 μg/ml). Fraction D3c was further subjected to the second step of RP-HPLC fractionation (Fig. 2d). Fraction D3c-2 (IC50 = 4.8 ± 0.09 μg/ml) was identified as the most potent fraction. After the second step RP-HPLC separation, the chromatogram showed a major peak (Fig. 2e) suggesting the fraction D3c-2 had a purity satisfactory for amino acid sequencing. The ACE inhibitory activity of USPPH was improved more than 19 times after five steps of purification.

The amino acid sequences of fraction D3c-2 were identified by IT-MS/MS (Fig. 3a and b). As shown in Fig. 3a, fraction D3c-2 mainly contains a peptide. The molecular mass of the peptide, deduced from the m/z value of (M+H)+ by subtraction of one mass unit for the attached proton, is 382.36 Da. According to this molecular mass and IT-MS/MS spectrum (Fig. 3b), the sequence of the peptide was identified to be a tripeptide, Lys-His-Val (KHV). This is the first time that the Lys-His-Val (KHV) with high ACE inhibitory activity is isolated from food protein.

Based on the tripeptide sequence, a synthetic peptide was obtained from Sangon Biotechnology Corporation (Shanghai, China) and its IC50 value is 12.82 μM. The ACE inhibitory activity of the purified peptide was stronger than VEGY (IC50 = 128.4 μM) from *Chlorella ellipsoidea* [15], DWMKGQ (IC50 = 422.92 μM) and YVNDAAATLPKR (IC50 = 105.85 μM) from defatted koumiss roe [10] but weaker than VNP (IC50 = 6.4 μM) and VWV (IC50 = 4.5 μM) from rice. According to previous studies on ACE inhibitory peptides, the preferred ACE inhibitors are those peptides that contain hydrophobic amino acids on the C-terminal [16,21]. Thus, it is evident that the C-terminal Val residue (a hydrophobic amino acid) of Lys-His-Val plays an important role in binding the ACE active site. The Val residue was also the amino acid most frequently observed in the C-terminal of other ACE inhibitory peptides, such as MLVFAV (IC50 = 3.07 μM) from skipjack tuna roe [10], RCSAGV from goby muscle [20], VSV (IC50 = 0.15 μM) from defatted canola meal [31] and YQDPRLGPTGELDPATQIVAVHPVIV (IC50 = 14.53 ± 0.21 μM) from koumiss [5].

3.3. Stability of the purified peptide against gastrointestinal proteases

In order to predict the antihypertensive effect in vivo, the purified peptide Lys-His-Val was incubated under simulated gastrointestinal conditions using pepsin, trypsin and α-chymotrypsin. The ACE inhibitory rates are summarized in Table 1. The similar inhibitory rates showed no impacts of gastrointestinal proteases on ACE inhibitory activity of Lys-His-Val. The finding was in agreement with other reports evaluating gastrointestinal enzymatic degradation of ACE inhibitory peptides [3,15]. Our results showed that Lys-His-Val was stable against gastrointestinal proteases of pepsin, trypsin and α-chymotrypsin. This could be attributed to the fact that the short-chain peptides were less susceptible to degradation by gastrointestinal proteases [7].

3.4. Insight into molecular docking simulation

In order to explore the molecular mechanism of the interactions between Lys-His-Val and ACE, the docking simulation was made using flexible docking tool of Discovery Studio 2.1 software. The docking simulation of the peptide Lys-His-Val at the ACE active site has shown the best docking pose (Fig. 4a) in the presence of Zn(II) with binding energy of −52.415 kJ/mol. After docking, Lys-His-Val is buried deep inside the active site channel of ACE (3D figure not shown), and makes hydrogen bond interaction with ACE residues Asn277, Gln281, Thr282, His383, Asp415, Lys454, Ser526, Phe527 and Gln530 (Fig. 4a). According to our research, more than 18 hydrogen bonds were formed between Lys-His-Val and ACE. The hydrogen bond interaction force stabilizes the docking complex and plays an important role for the enzyme catalytic reactions [2]. This result suggested that the peptide could effectively interact with the active site of ACE [2]. Similar to lisinopril [13], the new potent Lys-His-Val also shared interactions at Gln281 and His383, showing that the two amino acids might play major roles in ACE binding. Besides Gln281 and His383, Lys-His-Val can also interact with Asn277, Thr282, Asp415, Lys454, Ser526, Phe527 and Gln530. This result

### Table 1

ACE inhibitory rates of Lys-His-Val after treatment by gastrointestinal proteases.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACE inhibitory rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.3 ± 1.01</td>
</tr>
<tr>
<td>Pepsin</td>
<td>66.0 ± 1.15</td>
</tr>
<tr>
<td>Pepsin + α-chymotrypsin</td>
<td>66.7 ± 0.92</td>
</tr>
<tr>
<td>Pepsin + α-chymotrypsin + trypsin</td>
<td>65.5 ± 1.32</td>
</tr>
</tbody>
</table>

ACE inhibitory rates were tested at a concentration of 7.5 μg/ml. Values are presented as means ± standard deviations from three replications (n=3).

a) Pepsin hydrolysis for 3 h
b) Pepsin hydrolysis for 3 h followed by α-chymotrypsin hydrolysis for 3 h

- Table 1 provided the ACE inhibitory rates of Lys-His-Val after treatment by gastrointestinal proteases. Each treatment condition was tested for 3 replications to ensure the reliability of the results. The ACE inhibitory rates were calculated using the IC50 values obtained from previous studies.
- The results showed that Lys-His-Val was stable against gastrointestinal proteases of pepsin, trypsin and α-chymotrypsin. This stability can be attributed to the presence of hydrophobic amino acids in the C-terminal of the peptide.
- The ACE inhibitory activity of the purified peptide was stronger than other known peptides, indicating its potential as an effective ACE inhibitor.
- The molecular docking simulation revealed the binding modes of Lys-His-Val with the ACE active site, providing insights into the mechanism of action.
Fig. 2. Purification profiles of ACE inhibitory peptide from USPPH. (a) Sephadex G-25 gel chromatography of fraction USPPH-III from ultrafiltration. The inserted table showed the ACE inhibitory activity of each fraction at the concentration of 100 μg/ml. (b) Sephadex G-10 gel chromatography of fraction D. The inserted table showed the ACE inhibitory activity of each fraction at the concentration of 50 μg/ml. (c) RP-HPLC chromatography on ODS C18 column of fraction D3 obtained from Sephadex G-10 gel. (d) RP-HPLC chromatography on ODS C18 column of fraction D3c from the first step RP-HPLC purification. (e) RP-HPLC chromatography on ODS C18 column of fraction D3c-2 from the second step RP-HPLC purification. In the first step (c), the elution was performed with linear gradient of acetonitrile from 0% to 50% at a flow rate of 1.0 ml/min and monitored at 220 nm. In the second step (d) and the third step (e), the elution was performed with linear gradient of acetonitrile from 5% to 50% at a flow rate of 0.8 ml/min and monitored at 220 nm. Values are presented as mean ± standard deviations from three replications (n = 3).
differed from some published studies. Le et al. [17] reported that Ala-Cys-Leu-Glu-Pro (IC50 = 126 μM) from pistachio hydrolysates could interact with ACE residues His387, Glu384, Arg522, Asp358, Ala356 and Asn70. Wang et al. [29] reported that Lys-Val-Leu-Ile-Leu-Ala (IC50 = 20.85 μM) from milk casein could interact with ACE residues Lys118, Asp121, Glu123, Met223, Ala354, Ala356, Glu403, Arg522 and Tyr523. Our result indicated that the binding mode might be different from the previously reported ACE inhibitory peptides.

Zn(II) at the ACE active site usually plays a significant role and is bound to ACE residues His383, His387, Glu411 and ACT700 (Fig. 4b). The bond lengths between Zn(II) and coordinating atoms from ACE residues (NE2 of His383, NE2 of His387, OE1 of Glu411 and OXT of ACT700) before and after docking were shown in Fig. 4c. After docking, the distances between the Zn(II) and its surrounding atoms were changed, suggesting that the tetrahedral geometry of Zn(II) at the ACE active site was distorted. The phenomenon was in agreement with the findings of Pan et al. [21], who observed that a distorted Zn(II) tetrahedral geometry was formed in the interaction of ACE with Leu–Leu. Many studies have shown that interactions between the ACE inhibitors and Zn(II) at the ACE active site usually play a significant role and therefore deactivate ACE [14,21]. The results may offer an explanation why Lys-His-Val exhibits strong ACE inhibitory activity.
Fig. 4. The best ranked docking pose of the purified peptide Lys-His-Val (grey) in the active site of ACE (PDB: 108A). (a) Details of Zn(II) (purple) coordination with ACE residues before docking. (b) Details of Zn(II) (purple) coordination with ACE residues after docking. Green dotted line indicates hydrogen bond formation. (c) Distances between Zn(II) and coordinating atoms from ACE residues before and after docking. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

This study demonstrated that ultrasonic pretreatment was a good means for improving the ACE inhibitory activity of silkworm pupa protein hydrolysate. Furthermore, a new peptide, Lys-His-Val, was successfully purified from this hydrolysate using ultrafiltration, gel filtration chromatography and RP-HPLC. The purified tripeptide exhibited a potent ACE inhibitory activity with an IC\textsubscript{50} value of 12.82 \mu M, and was stable against gastrointestinal proteases. Molecular docking indicated that the peptide could effectively interact with the active site of ACE. This is the first report about novel ACE inhibitory peptide from silkworm pupa protein hydrolysate. Our research may provide an efficient utilization of silkworm pupa protein.

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