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Research article

The first bioactive (angiotensin-converting enzyme-inhibitory) peptide isolated from pearl matrix protein

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ABSTRACT

In this research, we unveil the medical potential of pearls by identifying a novel bioactive peptide within them for the first time. The peptide, termed KKCHFWPFPW, emerges as a pioneering angiotensin I-converting enzyme (ACE) inhibitor, originating from the pearl matrix of *Pinctada fucata*. Employing quadrupole time-of-flight mass spectrometry, this peptide was meticulously selected and pinpointed. With a molecular weight of 1417.5 Da and a theoretical isoelectric point of 9.31, its inhibitory potency was demonstrated through a half-maximal inhibitory concentration (IC50) of 4.17 μM, established via high-performance liquid chromatography. The inhibition of ACE by this peptide was found to be competitive, as revealed by Lineweaver–Burk plot analysis, where an increase in peptide concentration correlated with an enhanced rate of ACE inhibition. To delve into the interaction between KKCHFWPFPW and ACE, molecular docking simulations were conducted using the Maestro 2022-1 Glide software, shedding light on the inhibitory mechanism. This investigation suggests that peptides derived from the *P. martensii* pearl matrix hold promise as a novel source for antihypertensive agents.

1. Introduction

Hypertension stands as a critical health challenge globally, manifesting through elevated systolic or diastolic blood pressure levels. This condition is closely linked to cardiovascular, cerebral, and vascular diseases, positioning it as a significant risk factor for cardiovascular complications, which are widespread, chronic, and tend to increase with age [1]. Consequently, interventions aimed at lowering blood pressure are pivotal in preventing cardiovascular diseases.

The renin-angiotensin system plays a crucial role in maintaining blood pressure homeostasis in mammals, with angiotensin I-

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converting enzyme (ACE) at its core. ACE removes the C-terminal dipeptide from its substrate precursor peptide angiotensin I to produce angiotensin II, a potent vasoconstrictor that also reduces the vasodilatory effect of bradykinin, thereby raising blood pressure [2]. These functionalities underscore ACE as a prime therapeutic target for managing hypertension, heart failure, type 2 diabetes mellitus, and diabetic nephropathy.

Historically, the initial ACE inhibitors were derived from the venom of the snake *Bothrops jararaca* [3]. This discovery paved the way for the development of synthetic ACE inhibitors like captopril, lisinopril, enalapril, and fosinopril, widely prescribed for hypertension management [4]. Despite their effectives, these synthetic ACE inhibitors can induce side effects including cough, allergic reactions, taste disorders, and skin rashes [5]. This highlights the necessity for discovering bioactive peptides capable of ACE inhibition without toxicity, suitable for lowering blood pressure without affecting individuals with normotension, and offering additional benefits such as immunoregulation and easy absorption. Despite their lower activity compared to synthetic inhibitors, bioactive peptides are regarded as safer, more affordable, and rich in essential amino acids, making them a preferable alternative [6,7].

The marine environment is a rich source of bioactive compounds, including peptides, polysaccharides, and polyunsaturated fatty acids, offering antioxidant, blood pressure regulation, antithrombotic, and immunoregulatory properties. The exploration and utilization of marine resources for ACE-inhibitory compounds, especially from marine proteins, are thus gaining traction.

The bivalve *Pinctada martensii,* also known as *Pinctada fucata*, plays a crucial role in pearl culture and has been recognized for its potential health benefits, including enhancing human immunity, anti-aging properties, skin whitening, and providing a source of calcium. These attributes make pearls highly valuable in medicine, dietary supplements, and cosmetics.

Pearls develop through mineralization in bivalve shells, consisting mainly of calcium carbonate (~95%), and the remaining components (~5%) are a minor yet significant organic matrix of proteins, polysaccharides, and lipids. The protein components are collectively referred to as matrix proteins. They are mainly involved in constructing the organic framework and regulating calcium carbonate nucleation and crystallization [8]. Although the organic matrix proportion is small, it controls the whole shell formation process. Matrix proteins precisely regulate crystal nucleation, morphology, growth, and orientation [9,10]. The nacre of mollusks has a structure similar to that of pearls; the shell matrix proteins from the nacre are considered essential mediators of pearl formation [11,12].

This study focuses on a specific ACE-inhibitory peptide derived from the *P. fucata* pearl matrix, marking a novel approach to exploring antihypertensive agents from pearls. Through employing the classic Lineweaver–Burk model, we examined the *in vitro* ACE-inhibitory activity of pearl matrix peptide KKCHFWPFPW, aiming to elucidate its potential inhibitory mechanism. Our findings offer both theoretical and practical insights into utilizing pearl matrix active peptides as innovative bioactive components.

2. Materials and methods

2.1. Materials and reagents

P. fucata pearls devoid of nuclei were acquired from Kanjiang, Guangdong Province, China. Reagents including ACE, hippuryl-L-histidyl-L-leucine (HHL), hippuric acid (HA), trifluoroacetic acid (TFA), boric acid, sodium chloride (NaCl), acetonitrile, and hydrochloric acid (HCL) were produced from Sigma–Aldrich (Missouri, USA), all of which were of analytical grade.

2.2. Pearl matrix proteins extraction and enzymatic hydrolysis

Initially, pearls were cleaned and pulverized using a shredder. Subsequently, 1.5 M EDTA was added to the collected pearl powder and agitated for 24 h at 4 °C for decalcification. The resultant supernatant was then separated by centrifugation at 11962*g* and passed through a 0.2-μm microporous membrane. Following this, the filtrate underwent low-temperature dialysis in a chromatography freezer set at 4 °C for 48 h, after which the pearl matrix proteins were isolated through lyophilization.

Trypsin (at 5% of the pearl matrix proteins with activity of 10,000 U/g) was added into the pearl matrix proteins for the purpose of enzymatic hydrolysis. This digestion process was carried out at a pH level of 8.0. The was for For a duration of 2 h at 50 °C, the trypsin underwent hydrolysis and subsequently, its activity was halted through immersion in a boiling water bath. Following this, the supernatant was separated by centrifugal force at 11962 g. Post ultrafiltration, the filtrate with a molecular weight below 3 kDa was collected. The enzyme-treated pearl matrix proteins were then concentrated and lyophilized to obtain the final product.

2.3. Separation and purification of pearl matrix proteins with ACE-inhibitory activity

The enzyme-digested pearl matrix proteins were purified using high-performance liquid chromatography (HPLC). In a C18 column, the mobile phase A consisted of deionized water with 0.1% trifluoroacetic acid (TFA), while the mobile phase B was made up of acetonitrile with 0.1% TFA. The ultraviolet detection was conducted at a wavelength of 280 nm, with a flow rate set to 1 mL/min, and a retention time observed at 14 min. Following this process, the pearl matrix proteins exhibiting ACE-inhibitory activity were concentrated and lyophilized to achieve the final product.

2.4. Assay of ACE-inhibitory activity

In the evaluation of ACE-inhibitory activity, the experimental setup included a reaction mixture of 5 mM Hippuryl-L-histidyl-L-leucine (HHL), the pearl matrix protein as an inhibitor, and 200 U/L of ACE in 0.1 M sodium borate buffer solution with a pH of 8.3 and

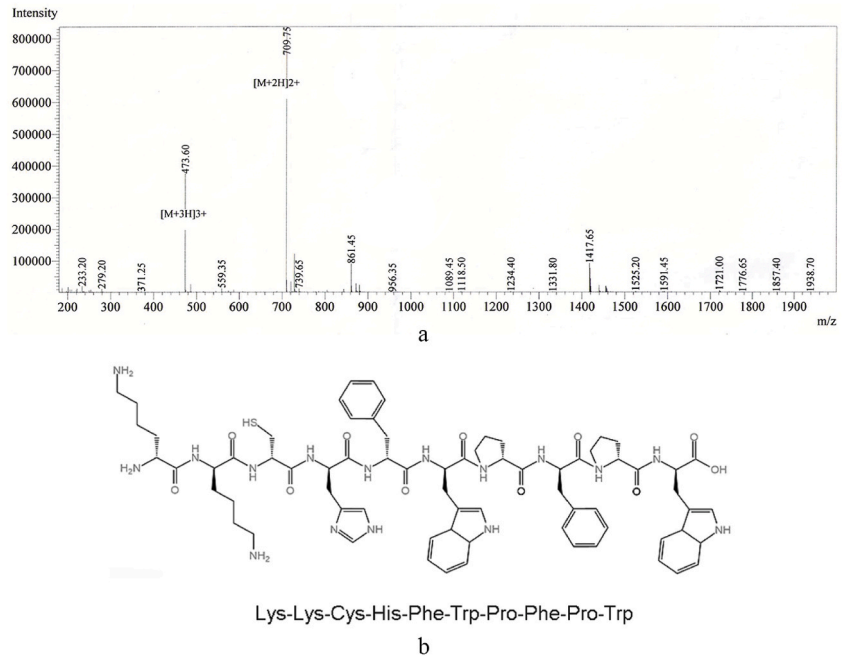

Fig. 1. Identification of KKCHFWPFPW by (a) MS and (b) UPLC-Q-TOF-MS/MS.

0.3 M NaCl concentration. The assay tested six different concentrations of the inhibitor (5, 10, 20, 30, 40, and 50 μL, each volume adjusted to 180 μL with 0.1 M borate buffer). A control was prepared using only the 0.1 M borate buffer, excluding the inhibitor. Initially, HHL and the inhibitor were blended and pre-warmed at 37 °C for 5 min. Following this pre-incubation, 20 μL of ACE was added to the mixture, which then incubated in a shaking water bath at 37 °C with a gentle shaking force of 1.72 g for 30 min. The reaction was halted by adding 0.2 mL of 1 mol/L HCL. The quantification of HHL and its hydrolysis product, Hippuric acid (HA), was performed through high-performance liquid chromatography (HPLC) using a C18 column. The analysis employed isometric elution over a 25-min period, utilizing a mobile phase of 75% acetonitrile (Phase A) and 25% deionized water containing 0.5% TFA (Phase B), at a flow rate of 0.5 mL/min. Ultraviolet detection was executed at a wavelength of 228 nm. Given that ACE facilitates the stable conversion of HHL to HA, a reduction in HA production signifies ACE activity inhibition. The inhibition rate of ACE was calculated with the following formula:

$$\text{Inhibition rate} = (A_d - A_s) \,/\, A_{d,}$$

where A_s represents the peak area of HA in the sample group, and A_d corresponds to the HA peak area in the control group. Through these measurements, the half-maximal inhibitory concentration (IC_{50}) value was derived to quantify the efficacy of the pearl matrix protein inhibitor.

2.5. Determination of molecular weight and amino acid sequence

The molecular weight and amino acid composition of the purified peptides derived from pearl matrix proteins were ascertained through the utilization of a quadrupole time-of-flight mass spectrometer (Q-TOF MS; Micromass, Cheshire, UK), which was equipped with an electrospray ionization (ESI) source. For the analysis, the purified peptide was dissolved in a methanol/water mixture at a 1:1, *v/v* volume ratio and then infused into the ESI source. The molecular weight of the peptide was accurately determined by examining its doubly charged (M+2H)2+ state analysis within the mass spectrum. Subsequent to the determination of the molecular weight determination, the peptide was automatically selected for fragmentation within the instrument, facilitating the acquisition of its sequence information was obtained through tandem MS analysis.

2.6. Determination of ACE-inhibitory kinetics

The inhibition mechanism of the peptide KKCHFWPFPW on angiotensin-converting enzyme (ACE) was investigated through the construction of Lineweaver–Burk, which graphically represent the inverse of velocity ($1/V$) against the inverse of substrate concentration (1/HHL). This analysis was carried out under varying concentrations of the substrate HHL (0.5, 1, and 2 mM) and the peptide (at the concentrations of 0, 17.63, 35.26, 70.52, 105.78, 141.04, and 176.3 μM) in combination with the ACE solution. Through the Lineweaver–Burk diagrams, both the Michaelis constant (K_m) and maximum velocity (V_{max}) were graphically derived, facilitating the identification of the peptide's mode of inhibition against ACE. The intercepts on the Y-axis and X-axis of the plot provided the values of

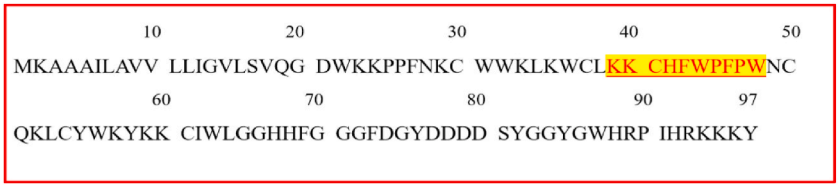

Fig. 2. Location of KKCHFWPFPW in lysine-rich matrix protein 6 (KP940480).

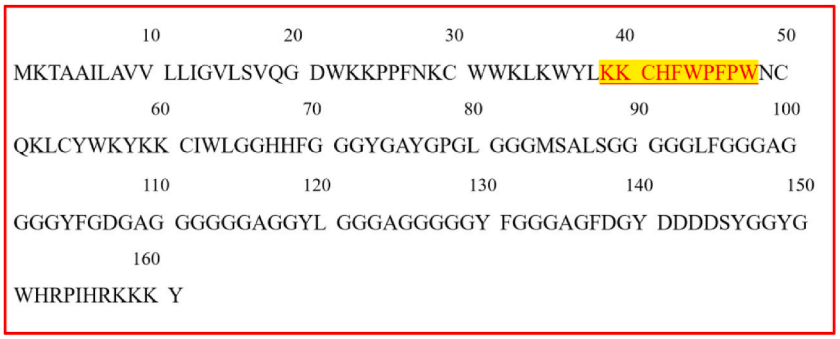

Fig. 3. Location of KKCHFWPFPW in lysine-rich matrix protein 9 (KP940483).

V_{max} and K_m, respectively. To ensure reliability, all experiments were performed in triplicate.

2.7. Molecular docking

The molecular docking of the pearl-derived peptide with angiotensin-converting enzyme (ACE) was executed utilizing the Maestro 2022-1 Glide software suite. The ACE crystal structure, denoted by the Protein Data Bank (PDB) ID: 1O8A, was acquired from the PDB database to serve as the docking receptor. Prior to the docking procedure, the structure and minimum energy conformations of the target peptide were determined automatically based on its amino acid sequence. This preparatory step ensured that the most relevant conformation of the peptide was utilized for docking. Following the docking process, the nature of the interactions between the receptor (ACE) and the ligand (the pearl peptide) was meticulously analyzed to understand how they bind, and the specific interaction dynamics involved.

3. Results and discussion

3.1. Identification of peptides from pearl matrix proteins

In the present study, UPLC-Q-TOF-MS/MS and UniProt search were used to analyze and detect the fragment spectra so as to identify the peptides in the pearl matrix proteins.

Among the peptides obtained by enzymolysis from pearl matrix proteins, one peptide, KKCHFWPFPW, was predicted to be an ACE-inhibitory peptide according to the affinity analysis by molecular docking. Therefore, the KKCHFWPFPW was purified and isolated to clarify the mechanism of ACE-inhibitory activity. The molecular weight of the ACE inhibitor (ACEI) was 1417.5 Da, as determined by MS/MS (Fig. 1a). Also, its amino acid sequence was Lys-Lys-Cys-His-Phe-Trp-Pro-Phe-Pro-Trp (Fig. 1b). The theoretical isoelectric point was 9.31, and the total average hydrophilicity was −0.790. It was an alkaline hydrophilic protein.

The present study identified the peptide KKCHFWPFPW from the pearl matrix protein hydrolyzed by trypsin for 2 h at 50 °C and pH 8.0. This was the first attempt to extract ACE-inhibitory peptides from mollusk shell pearls. The result of UniProt search showed that the peptide KKCHFWPFPW could be matched with the peptides in two kinds of *Pinctada fucata* matrix proteins, which were named as lysine-rich matrix protein 6 and lysine-rich matrix protein 9. The location of KKCHFWPFPW (marked in red) in lysine-rich matrix protein 6 and lysine-rich matrix protein 9 from the UniProt database is shown in Figs. 2 and 3, respectively.

Moreover, several studies have reported on the peptides of hydrolyzed proteins from various natural sources with ACE-inhibitory activity. The sources of ACE inhibitors are divided into four groups: plant sources, animal sources, microbial fermentation sources, and marine sources. The animal sources include milk, fish, and eggs. The first ACE inhibitors were isolated from the venom of *B. jararaca* in 1965 [3]. Subsequently, eggs, which are rich in proteins and many essential amino acids, were found to be an ideal raw material for ACE inhibitors. Examples include WESLSRLLG [11] from ostrich albumen and LKYAT and TNGIIR from egg white [12]. Milk is another vital source of ACE inhibitors because of its balanced nutrition and a wide variety of proteins [13].

Plant sources of ACE-inhibitory peptides include legumes [14], cereals [15], and seeds [16]. ACE inhibitors from plant sources have

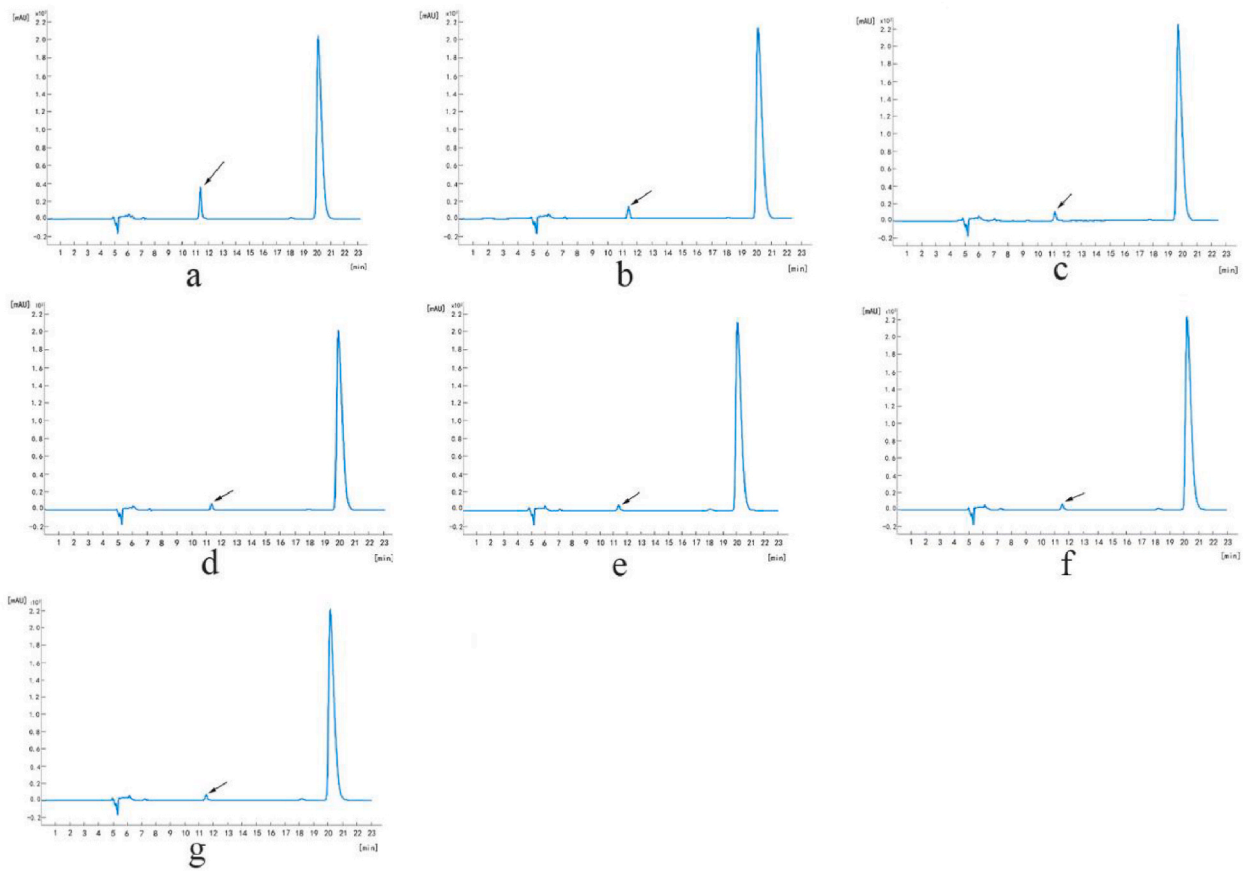

Fig. 4. ACE inhibition of small-molecule active peptides by HPLC. Concentration of polypeptides: (a) 0 μM (control group); (b) 17.63 μM; (c) 35.26 μM; (d) 70.52 μM; (e) 105.78 μM; (f) 141.04 μM; and (g) 176.30 μM.

three to eight amino acid residues and special structures that make them more hydrophobic, conferring strong ACE-inhibitory activity. Microbial fermentation sources include Lactobacillus and other species. For example, *Lactobacillus plantarum* secretes extracellular protease to hydrolyze soybean proteins during the fermentation process, producing many ACE inhibitors [17]. Marine sources include fish [18], mollusks [19,20], aquatic products [21,22], and seaweed [23].

Many ACE-inhibitory peptides have been isolated and identified from mollusks, which have the potential for lowering blood pressure. For example, Liu et al. [24] isolated and identified VVCVPW and found that it bound to ACE via interactions with His383, His387, and Glu411 residues in *Mactra veneriformis*. Li et al. [25] studied the activity of ACE with razor clam (*Sinonovacula constricta*) hydrolysates using five proteases. Wang et al. [26] isolated and characterized a purified peptide (VVYPWTQRF) from oyster (*Crassostrea talienwhanensis*) protein hydrolysate. Wu et al. [27] used alcalase, followed by papain, to hydrolyze abalone (*Haliotis discus hannai*) gonads to produce ACE-inhibitory peptides. *P. martensii* tissues were hydrolyzed by protease, and ACE-inhibitory peptides were obtained after the separation and extraction of the hydrolysate [28].

Various studies showed that the amino acid composition, structure, and length of the antihypertensive peptide significantly affected ACE-inhibitory activity. Daskaya-Dikmen et al. [29] explored the structure–activity relationship of ACE-inhibitory peptides and reported that peptides with a rich hydrophobic amino acid content, such as Ala, Val, Leu, Ile, Phe, Pro, Trp, and Met, at the C-terminal residues had a strong effect on ACE binding. Kumar et al. [30] built a platform for predicting, screening, and designing antihypertensive peptides. They reported that residue Pro was highly abundant in antihypertensive peptides. Cushman and Cheung [31] reported that Trp, Tyr, Pro, and Phe at the C-terminal, as well as branched aliphatic amino acids at the N-terminal, were suitable for ACE binding as competitive inhibitors. Additionally, Norris et al. [7] found that shorter peptides might have stronger ACE-inhibitory activity. The molecular weight of KKCHFWPFPW was 1417.5 Da, and the number of amino acids was 10. Hence, the identified peptide was consistent with the basic characteristics of the aforementioned antihypertensive peptides. Based on the aforementioned conclusions, the sequence of pearl matrix proteins was found to contain Pro, Trp/Pro, and Phe, which helped improve its ACE-inhibitory ability. Moreover, the affinity of peptides to ACE protein was evaluated by molecular docking. KKCHFWPFPW had a relatively high affinity toward ACE, which was evaluated as −10.6691 using the Maestro 2022-1 Glide module. As our previously inference, the concentration of matrix protein in pearl powder is relatively low, and the polypeptide mentioned in this paper represents only a fraction of them. Based on this problem, we believe that if we want to reduce the cost of obtaining this peptide, we can take

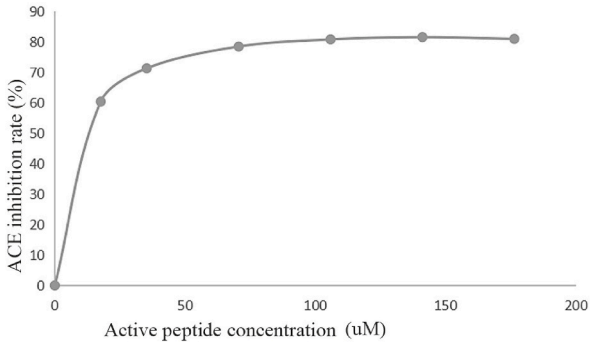

Fig. 5. ACE inhibition activity diagram.

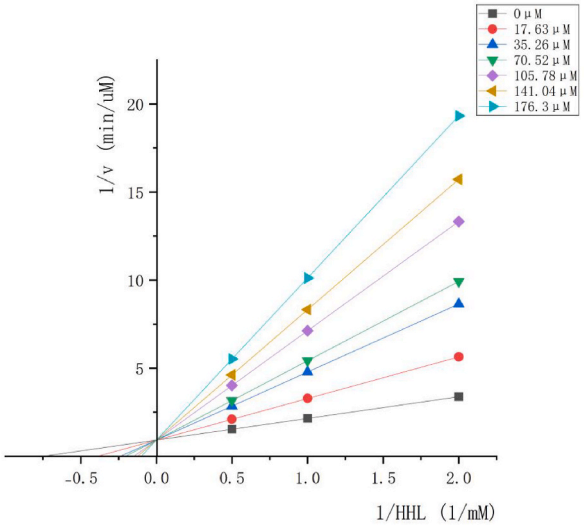

Fig. 6. Lineweaver–Burk plot of ACE inhibition by the pearl matrix protein. 1/[HHL] and 1/[*v*] represent the reciprocal substrate concentration and velocity, respectively.

synthetic or genetic recombination technologies to obtain the peptide. Currently, numerous scholars [32–34] both domestically and internationally have successfully expressed proteins through gene recombination technology, which has reached a relatively advanced stage of development. In the case of requiring large-scale production of peptides, gene recombination technology can be employed to obtain recombinant vectors containing gene fragments that can be translated into KKCHFWPFPW. These vectors can then be transferred into host cells capable of expressing the target protein, such as *Escherichia coli*, enabling the generation of a substantial quantity of peptides. Therefore, the KKCHFWPFPW can be selected for further investigation.

3.2. *ACE-inhibitory activity determination*

Fig. 4 depicts the HPLC chromatograms of the peptide KKCHFWPFPW at concentrations of 17.63, 35.26, 70.52, 105.78, 141.04, and 176.3 μM and a blank control group. The 11- to 12-min peak in the figure is HA, and the 20- to 21-min peak is the substrate HHL.

The ACE-inhibitory activity was calculated using the following formula: ACE inhibition rate = $(A_s - A_d)/A_d$ (A_s is the peak area of HA detection in the sample group, and A_d is the peak area of HA detection in the blank group). In the present study, the ACE-inhibitory activity of the peptide KKCHFWPFPW released by trypsin digestion was evaluated, as shown in Fig. 5. The ACE inhibition rates were 53.2%, 65.9%, 74.4%, 77.2%, 78.1%, and 79.1%.

The IC_{50} value was determined using the regression equation: $Y = -(4.7 \times 10^{-3})X^2 + 1.1352X + 18.908$ ($R^2 = 0.8055$). The IC_{50} value of the peptide was 4.17 μM. According to the previous studies on ACE-inhibitory peptides, the peptide obtained in this study showed higher activity than most reported food-derived peptides which exhibited IC_{50} values ranging from 32.9 to 128 μM [7,35,36]. Moreover, comparing with peptides isolated from marines animals like bonito (Tyr-Arg-Pro-Tyr, IC_{50} = 320 μM) [37] and sea cucumber (Met-Glu-Gly-Ala-Gln-Glu-Ala-Gln-Gly-Asp, IC_{50} = 4.5 μM) [38], the peptide obtained in this study showed higher activity but was less effective than the peptide isolated from freshwater clam (Val-Lys-Pro, IC_{50} = 3.7 μM) [39].Therefore, the peptide KKCHFWPFPW could be considered as a potential ACEI.

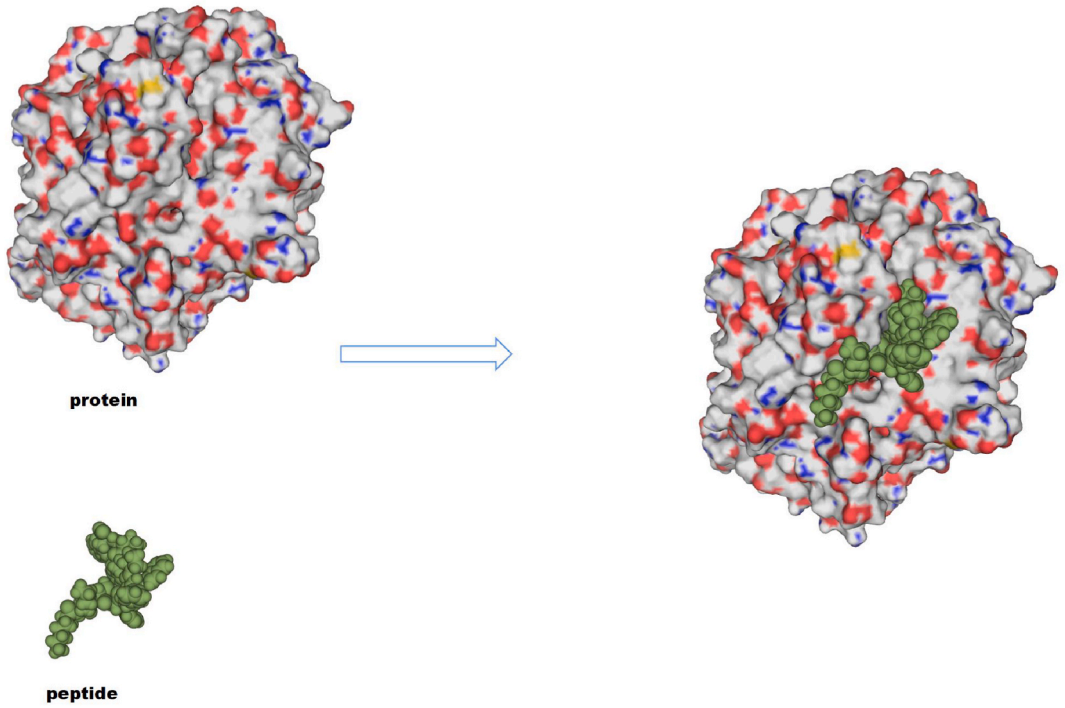

Fig. 7. Structures of the peptide and the peptide–ACE complex.

3.3. Inhibition pattern of the ACE-inhibitory peptide

The inhibition of ACE by ACE-inhibitory peptide was estimated using Lineweaver–Burk plot analysis to evaluate the mechanism of action of the peptide. The V_m values decreased with the increase in the pearl matrix peptide concentration (Fig. 6), confirming that the peptide likely blocked the substrate from binding to the ACE active site. When the ACEI peptide was added to the reaction, K_m values were higher than that of the control, indicating that a higher substrate concentration was essential for the ACE-catalyzed reaction. The regression curves of different concentrations of the pearl matrix peptide intersected at the $1/[v]$ axis, indicating that the inhibition mode was competitive inhibition.

The ACE-inhibitory peptides are classified based on different inhibition mechanisms. They are divided into three groups: substrate-type peptide, prodrug-type peptide, and inhibitor-type peptide [40]. The activity of the substrate-type peptide is increased by pre-incubation with ACE. The prodrug-type peptide is converted by ACE or gastrointestinal enzymes into a true inhibitor with higher activity. The inhibitor-type peptide is a true inhibitor, and its activity does not change after treatment with ACE or other enzymes. The main inhibition models of ACE-inhibitory peptides are classified into competitive and noncompetitive inhibition. Competitive inhibition is the interaction of the inhibitor with active enzyme sites to prevent substrate binding [41]. Noncompetitive inhibition occurs when the inhibitor molecule has a binding affinity for free enzyme and enzyme–substrate complex [42].

According to the Lineweaver–Burk plot, the peptide KKCHFWPFPW identified in this study was a true inhibitor because it competed with the substrate HHL for the binding sites of ACE. Only a few peptides exhibited noncompetitive inhibition (i.e., the peptide did not compete with the substrate and only bound to the enzyme–substrate complex). Jang et al. [43] reported that all of the purified ACE inhibitors from the mushroom *Pleurotus cornucopiae* were noncompetitive inhibitors. Most peptides were competitive inhibitors. Competitive inhibitors have a stronger affinity for ACE than for substrates. They can preferentially bind to ACE active centers and occupy sites such that substrates cannot bind to ACE and are degraded. Peptides with competitive inhibition modes usually bind to the ACE active sites through hydrogen bonding [44,45] and even coordinate with Zn^{2+} of ACE [46]. Previous studies showed that the amino acids of ACE-inhibitory peptides at the C-terminal bound to ACE. In contrast, the hydrophobic amino acids at the N-terminal interacted with Zn^{2+} ions at the metal-centered active site of ACE or certain active amino acid residues of ACE, thereby inactivating ACE active sites and inhibiting ACE activity [47,48]. The Lineweaver–Burk plot in the present study showed that the regression curves of pearl matrix peptides with different concentrations intersected at the $1/[v]$ axis, indicating that the peptides were in competitive inhibition mode like most inhibitory peptides.

3.4. Activity mechanism of KKCHFWPFPW

Molecular docking is a general method used for predicting the interactions of the inhibitor as a donor and the enzyme as a receptor by calculating the affinity energies, indicating the binding sites and interactive bonds of the donor and the receptor. The molecular

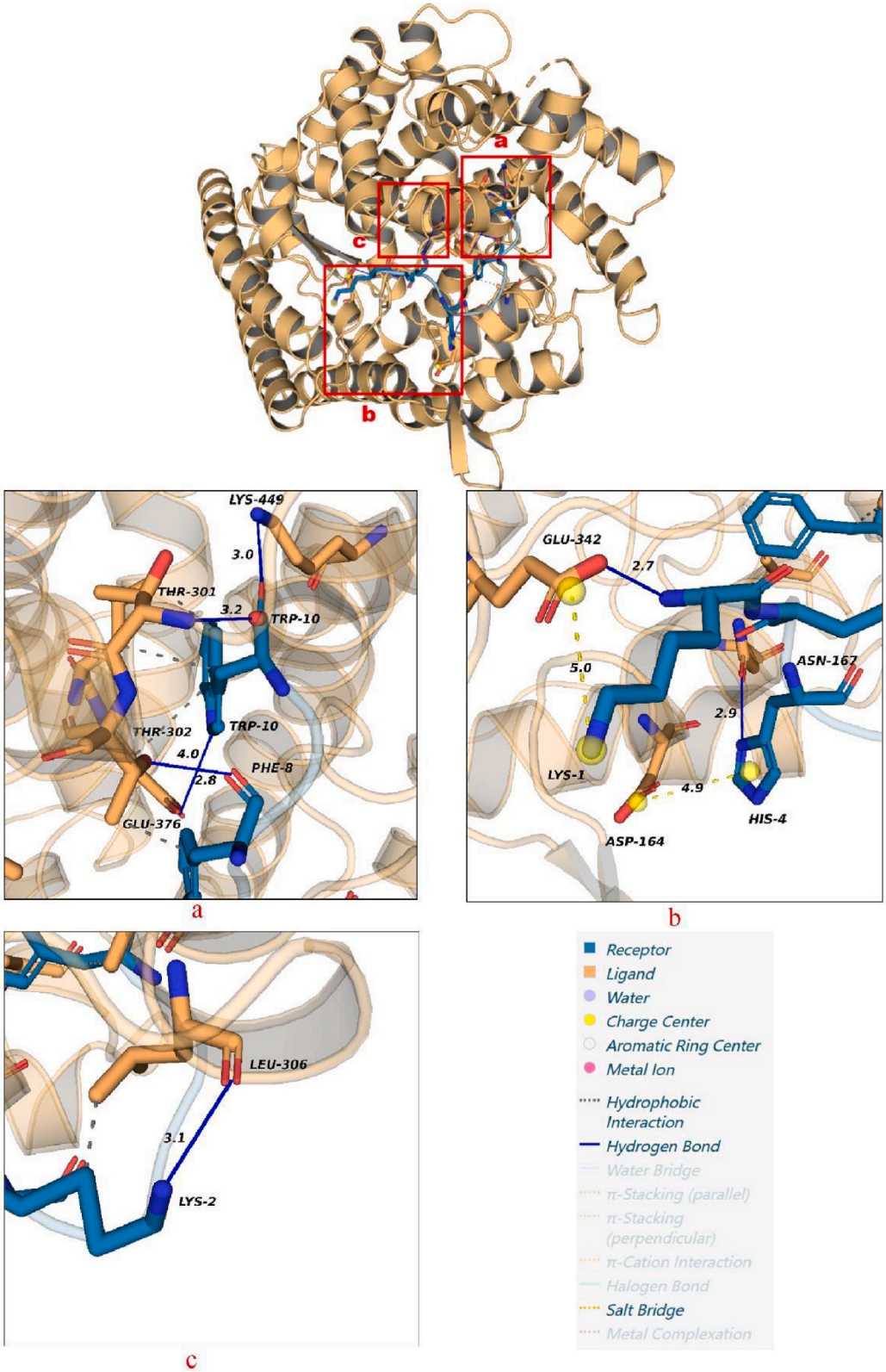

Fig. 8. The interaction of KKCHFWPFPW against ACE (PDB: 1O8A) analyzed by PLIP.

docking against ACE was performed by the Maestro 2022-1 Glide module to clarify the ACE-inhibitory mechanism of KKCHFWPFPW.

The structures of the peptide and the peptide–ACE complex are shown in Fig. 7, indicating that the peptide was a linear peptide. Fig. 8 shows the interaction between the protein and the polypeptide analyzed by Protein-Ligand Interaction Profiler (PLIP), using protein as the reference chain for interaction analysis, with gold representing the protein and blue representing the polypeptide. As shown in Fig. 8A, hydrogen bonds were formed between PH-8 of the polypeptide and THR-302 of the protein (solid blue line) between TRP-10 of the polypeptide and GLU-376 of the protein and between TRP-10 of the polypeptide and THR-301 and LYS-449 of the protein. As shown in Fig. 8B, both hydrogen bonds and salt bridges were formed between LYS-1 of the polypeptide and GLU-342 of the protein (yellow dashed line). Also, hydrogen bonds and salt bridges were formed between HIS-4 of the polypeptide and ASP-164 and ASN-167 of the protein. As shown in Fig. 8C, hydrogen bonds were formed between LYS-2 of the polypeptide and LEU-306 of the protein. In addition, several sets of hydrophobic interactions were present (gray dashed line).

4. Conclusions

The novel ACE-inhibitory peptide KKCHFWPFPW is a peptide located in the pearl matrix protein with an IC_{50} value of 4.17 μM, which is competitively inhibited. Potential ACE inhibition mechanisms are mainly attributed to hydrogen bonding and hydrophobic and electrostatic interactions. Therefore, the peptide can be used in developing health-care products or drugs for treating hypertension and has broad application prospects in these fields.

Data availability statement

No data was used for the research described in the article.

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CRediT authorship contribution statement

Chaoyi Wu: Writing – original draft, Methodology, Investigation, Data curation. **Zehui Yin:** Writing – original draft, Software, Methodology, Investigation. **Yayu Wang:** Software, Methodology, Formal analysis, Data curation. **Xinjiani Chen:** Software, Data curation. **Bailei Li:** Software, Data curation. **Qin Wang:** Validation. **Liping Yao:** Visualization, Project administration. **Zhen Zhang:** Validation, Project administration. **Xiaojun Liu:** Writing – review & editing, Supervision, Software, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Rongqing Zhang:** Validation, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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