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Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats

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ABSTRACT

Oyster (*Crassostrea talienwhanensis Crosse*) proteins were produced from fresh oyster and subsequently digested with pepsin. The separations were performed with a Sephadex LH-20 gel filtration chromatography and a RP-HPLC. A purified peptide with sequence Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe (VVYPWTQRF) was firstly isolated and characterized from oyster protein hydrolysate and its ACE inhibitory activity was determined with IC_{50} value of 66 μmol/L *in vitro*. Stability study for ACE inhibitory activity showed that the isolated nonapeptide had the good heat and pH stability and strong enzyme-resistant properties against gastrointestinal proteases. Kinetic experiments demonstrated that inhibitory kinetic mechanism of this peptide was non-competitive and its K_m and K_i values were calculated. The yield of this peptide from oyster proteins was 8.5%. Furthermore, the oyster protein hydrolysate (fraction II), prepared by pepsin treatment firstly exhibited antihypertensive activity when it was orally administered to spontaneously hypertensive rat (SHR) at a dose of 20 mg/kg. These results demonstrated that the hydrolysate from oyster proteins prepared by pepsin treatment could serve as a source of peptides with antihypertensive activity.

1. Introduction

Hypertension, which affects 15–20% of all adults, is a worldwide problem of epidemic proportions. It is the most common serious chronic health problem and carries a high risk factor for arteriosclerosis, stroke, myocardial infarction and end-stage renal disease (Jung et al., 2006).

ACE (angiotensin-converting enzyme) is a Zn-metallopeptidase and plays an important role in regulating blood pressure. ACE catalyzes the conversion of angiotensin from an inactive decapeptide (angiotensin I) to a potent vasoconstrictor octapeptide (angiotensin II) and also inactivates antihypertensive vasodilator bradykinin (Ondetti, Rubin, & Cushman, 1977). Since the discovery of ACE inhibitor in snake venom, many studies have been carried out in synthesizing ACE inhibitors, such as captopril, enalapril, alacepril and lisinopril, which have been used extensively in the treatment of essential hypertension and heart failure in humans (Ondetti et al., 1977; Patchett et al., 1980). However, the aforementioned ACE inhibitors are believed to have certain side effects, such as cough, taste disturbances and skin rashes (Atkinson & Robertson, 1979). Therefore, in recent years, ACE inhibitors derived from food have been studied.

Food intake is being increasingly considered as not only a source of nutrients but also a source of bioactive compounds, including bioactive peptides. These peptides may already be present in foods as natural components or be produced after enzymatic hydrolysis. These are considered to be milder and safer without side effects contrasting with synthetic drugs. Up to now, large numbers of peptides with ACE inhibitory activity have already been derived from food products, such as milk (Muguerza et al., 2006; Pan, Luo, & Tanokura, 2005), hemoglobin (Yu, Hu, Bai, Du, & Lin, 2006), fish (Byun & Kim, 2001; Hasan et al., 2006; Jung et al., 2006), buckwheat (Ma, Bae, Lee, & Yang, 2006), beef (Jang & Lee, 2005), soybean (Chiang, Tsou, Tsai, & Tsai, 2006; Zhang, Tatsumi, Ding, & Li, 2006) and fermented foods (Je, Park, Byun, Jung, & Kim, 2005; Tsai, Lin, Pan, & Chen, 2006). These foods are considered to be common source of peptides.

Oyster is an abundant resource from ocean, which contains 23.3% proteins on a dry weight basis (Zeng & Zhang, 1998). Je, Park, Jung, Park, and Kim (2005) isolated an ACE inhibitor from

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fermented oyster sauce (FOS) and characterized a purified peptide with ACE inhibitory activity. This suggested that oyster proteins may possess sequence with ACE inhibitory activity. From this point of view, the present study intends to investigate new ACE inhibitor derived from oyster proteins by pepsin treatment.

2. Materials and methods

2.1. Materials

Oyster, *Crassostrea talienwhanensis Crosse*, was purchased from a local shellfish market in May 2005 (Dalian, China). HHL (Hippuryl-histidyl-leucine), pepsin (10 U, from porcine stomach mucosa), chymotrypsin (5 U, from bovine pancreas), trypsin (10 U, from bovine pancreas) and ACE (5 U, from rabbit lung) were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other reagents used in this study were reagent grade chemicals.

2.2. Preparation of oyster proteins Sigma Chemical Company, Louis, USA

Fresh oyster was treated with manual decladding and the remanent component was homogenized using a meat chopper (JYZ-9, Shan Dong Jiu Yang Household Electrical Appliances CO.LTD, Shandong, China). The admixture was marinated in the same volume of PBS (phosphate buffer solution, the volume ratio of 0.2 mol/L monosodium orthophosphate to 0.2 mol/L disodium hydrogen phosphate was 39/61, pH 7.0) as this admixture at 4 °C for 6 h and the solution was continuously stirred. After centrifugation (25 min, 5000*g*, 4 °C) the supernatant fluid was obtained. The proteins were subsequently precipitated from the supernatant fluid by adding ammonium sulphate, when the concentration of ammonium sulphate was 75% (w/v). After removal of supernatant fluid by centrifugation (25 min, 5000*g*, 4 °C) the protein precipitate was dissolved in the same volume of PBS as protein precipitate and subsequently dialyzed with a 10,000 Da molecular weight (MW) membrane over 48 h against distilled water. It was then lyophilized and stored at −20 °C.

2.3. Pepsin treatment of oyster proteins

Oyster proteins were suspended in 0.05 mol/L phosphate buffer and the pH was adjusted to 2.0 by adding 1 mol/L HCl, when the protein concentration was 2.0% (w/v). Oyster protein solution was digested with pepsin at a protein substrate to enzyme ratio of 100:1.5 (w/w) at pH 2.0 and a temperature of 37 °C for 24 h. The hydrolysate was subsequently heated at 100 °C for 10 min to inactive pepsin. After removal of precipitate by centrifugation (25 min, 5000*g*, 4 °C) the supernatant fluid was adjusted to pH 7.0 by the addition of 1 mol/L of NaOH. The hydrolysate passed through a 10,000 Da MW membrane and the portion with MW less than 10,000 Da was lyophilized and stored at −20 °C until used.

2.4. Assay for ACE inhibitory activity

The ACE inhibitory activity was measured by the method of Cushman and Cheung (1971) with slight modifications. A sample solution (5 μL) was mixed with 15 μL of ACE solution (60 munits/mL) and pre-incubated at 37 °C for 5 min before addition of 25 μL of substrate (7.6 mmol/L HHL in 50 mmol/L sodium borate buffer containing 6.8 mmol/L NaCl at pH 8.3). The reaction mixture was incubated for 30 min at the same temperature. The reaction was terminated by the addition of 5 μL of 10% trifluoroacetic acid (TFA), and the hippuric acid liberated by ACE was determined by RP-HPLC (EC2000 system, Dalian Elite Analytical Instruments CO.LTD, Dalian, China) on a Hypersil BDS C_{18} (4.6 mm × 210 mm, 5 μm, Dalian Elite Analytical Instruments CO.LTD, Dalian, China); 20 μL were injected. Isocratic elution was performed with 30% methanol containing 0.1% TFA and 0.05% acetic acid at a flow rate of 1.0 mL/min. The effluent was monitored with an ultraviolet detector (UV230, Dalian Elite Analytical Instruments CO.LTD, Dalian, China) at 228 nm and the ACE inhibitory activity was calculated as peak area. Five concentrations of each sample were performed for the IC_{50} determination. Each concentration was repeated three times. The IC_{50} value was calculated as the concentration of inhibitor required to inhibit 50% of the ACE activity.

2.5. Purification of ACE inhibitor form hydrolysate

2.5.1. Gel filtration system

Two grams of lyophilized hydrolysate (MW less than 10,000 Da) were dissolved in 10 mL of 30% methanol solution, then separated with a Sephadex LH-20 gel filtration chromatography column (2.7 cm × 80 cm, Ammersham Pharmasia Biotech, Tokyo, Japan) which was eluted with 30% methanol at a flow of 0.5 mL/min. Elution curves were obtained by measuring absorbance at 280 nm using an on-line spectrophotometer. The fractions that showed ACE inhibitory activity were collected and subsequently dried with a rotary evaporator and lyophilizer.

2.5.2. Reversed-phase HPLC system

Firstly, 100 mg of lyophilized fraction II were dissolved in 10 mL of milli-Q water. This solution was then separated by RP-HPLC on a Hypersil BDS C_{18} column at a flow rate of 1 mL/min. RP-HPLC (EC2000 system), Hypersil BDS C_{18} column (4.6 mm × 210 mm, 5 μm) and pump (P230) were purchased from Dalian Elite Analytical Instruments CO.LTD, Dalian, China. Separation was performed under linear gradient elution conditions using acetonitrile as the organic modifier. Eluent A consisted of 0.1% TFA in milli-Q water (v/v); Eluent B of acetonitrile. The chromatographic column was conditioned with 100% of eluent A. After 20 μL of fraction II solution (10 mg/mL) were injected into the C_{18} column, eluent B concentrations were increasing: 0–40 min, 0–100% (v/v); 40–50 min, 100% (v/v). The UV absorbance of eluent was monitored at 215 nm. The active fraction was concentrated by lyophilization and applied on a peptide sequencer for sequence determination.

2.6. LC–MS Method

For RP-HPLC separations a Waters 2690 system (Waters Corporation, Milford, USA) equipped with an automatic sample injector was used. Separations were performed at 1 mL/min with eluent A consisted of 1% formic acid in milli-Q water (v/v); eluent B of acetonitrile. LC/UV traces were recorded on-line with a Waters 2690 PDA detector with detection at 280 nm. The sample was filtered through a 0.22 mm syringe filter and injected into a Hypersil BDS C_{18} (4.6 mm × 210 mm, 5 μm, Dalian Elite Analytical Instruments CO.LTD, Dalian, China) column. The chromatographic column was conditioned with 100% of eluent A before 10 μL of the fraction II (10 mg/mL) were applied on the chromatographic column. It was subsequently eluted with eluent A for 10 min and with following increasing eluent B concentrations: 0–5 min, 0%; 5–25 min, 0–100%; 25–35 min, 100%. The chromatographic analysis was linked with on-line MS analysis on a triple-quadrupole mass spectrometer (TSQ, Finnigan MAT, San Jose, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) interface. The heated capillary temperature was set at 200 °C, vaporizer temperature at 400 °C, auxiliary gas at 10 arbitrary units and sheath gas at 40 psi. Argon was used as target gas (3 mTorr) in the collision. Corona discharge voltage was set at 4 kV and corona discharge current was set at 4 μA. The precursor isolation window was set 1 u and collision energy was set at 20–30 V.

2.7. Peptide sequence

The purified peptide showing ACE inhibitory activity was subjected to the automated Edman degradation using a Perkin Elmer (Model 491, Applied Biosystems, Foster, CA, USA) protein sequencer. It was performed by Professor Weiqun Shen in School of Life Science, Beijing University, Beijing, China.

2.8. Peptide synthesize

The peptide with sequence VVYPWTQRF was synthesized by Hangzhou Zhongtai Company, Hangzhou, China. The purity of peptide synthesized was more than 95.7% by HPLC analysis.

2.9. Stability study for ACE inhibitory activity

2.9.1. Effect of temperature to ACE inhibitory activity

The nonapeptide synthesized was dissolved in milli-Q water (0.08 mg/mL) and subsequently incubated at different temperatures (4, 20, 40, 60, 80, and 100 °C) for 2 h. After the temperature was acclimated to room temperature (25 °C) and the pH value was adjusted to 8.3, the ACE inhibitory activity was determined.

2.9.2. Effect of pH to ACE inhibitory activity

The nonapeptide synthesized was dissolved in milli-Q water (0.08 mg/mL) and subsequently incubated at 37 °C and under pH values varying from 2 to 12 for 2 h. After the pH values were adjusted to 8.3, the ACE inhibitory activity was determined.

2.9.3. Effect of gastrointestinal protease to ACE inhibitory activity

The stability of ACE inhibitory peptide against gastrointestinal proteases was assessed *in vitro*. The nonapeptide solution (0.08 mg/mL) was successively digested with pepsin, chymotrypsin and trypsin as previously described (Kuba, Tanaka, Tawata, Takeda, & Yasuda, 2003) and the digests (0.08 mg/mL) were performed for the determination of ACE inhibitory activity.

2.10. Inhibitory kinetics study

Various concentration substrates (HHL) (1, 1.4, 2.4, and 7.6 mmol/L) were incubated with ACE solution in the absence and presence of 0.075, 0.15, 0.3 mg/mL of nonapeptide/water solution at 37 °C. The inhibitory kinetics was investigated by the Lineweaver–Burk plots. The inhibitor constant K_i and the Michaelis-constant K_m for the binding of inhibitor to ACE were also calculated according to the formulation as

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

where v is the velocity of generating product; K_m is Michaelis-constant; $[S]$ is substrate concentration; V_{max} is maximum velocity; $[I]$ is inhibitor concentration; K_i is inhibitor constant.

2.11. SHRs and measurement of blood pressure

Spontaneously hypertensive rats (10-week-old, male, SPF, 200–280 g body weight) with tail systolic blood pressure (SBP) over 180 mmHg were obtained from Shanghai Center of Experiment Animal, Chinese Academy of Sciences, Shanghai, China. SHR were housed individually in steel cages in a room kept at 25 °C with a relative humidity of 50% and a 12 h light-dark cycle, and fed with standard laboratory diet. Tap water was freely available. The SBP was measured by tail-cuff method with a tail measurement device (ALC–NIBP system, Shanghai Alcott Biotech CO.LTD, Shanghai, China).

In short-term experiments, the lyophilized fraction II was dissolved with saline water at different concentrations and administrated orally using a metal gastric zoned in SHR at multiple doses of 2, 20, 100 mg/kg. Captopril, a kind of antihypertensive drug, was administrated at a dose of 2 mg/kg. Control rats were administrated with saline water. All the volumes of oral administration were at a dose of 5 mL/kg. The SBP was measured in rats by the tail-cuff method before administration, and also 2, 4, 6 and 8 h post-administration.

In long-term experiments, the lyophilized fraction II was dissolved with saline water at different concentrations and administrated orally at a dose of 20 mg/kg. Positive control rats were administrated with captopril at a dose of 2 mg/kg and control rats were administrated with saline water. All the volumes of oral administration were at a dose of 5 mL/kg. Oral administration was performed once a day between 9:00 am and 12:00 noon for 28 days. The SBP was measured on 0, 1, 3, 5, 9, 14, 21, 28 day.

Data are shown as mean values ± standard deviation for a minimum of eight rats, and the significance levels contrasting with control rats were assessed using software OriginLab (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Purification and identification of the peptide

As shown in Fig. 1a, the hydrolysate was separated with a Sephadex LH-20 gel filtration chromatography and three fractions were obtained. The IC_{50} values of the three fractions were 1.2, 0.49 and 0.53 mg/mL and these yields were 4.7%, 55.8% and 13.5% from 6 g of oyster proteins, respectively. The fraction II was then further separated by RP-HPLC on a Hypersil BDS C_{18} column. As shown in Fig. 1b, fraction II was divided into seven major peaks and only peak C7 displayed ACE inhibitory activity.

The MW of peak C7 was speculated to be 1195 Da as shown in Fig. 1c. The sequence of peak C7 was VVYPWTQRF determined by Edman Degradation Method and its ACE inhibitory activity was determined with IC_{50} value of 66 μmol/L. The IC_{50} values and yields of these fractions obtained on hydrolysate, Sephadex LH-20 gel filtration chromatography and RP-HPLC from 6 g of proteins are shown in Table 1.

3.2. Stability study for ACE inhibitory activity

As shown in Fig. 2a and b, the nonapeptide retained ACE inhibitory activity after treatment at different temperatures and under different pH treatments. This result indicated that ACE inhibitory activity of the nonapeptide displayed heat and pH stability.

The stability of the nonapeptide against gastrointestinal proteases was assessed *in vitro*. As shown in Fig. 2c, the ACE inhibitory activity of the nonapeptide hardly showed any change after treatment with gastrointestinal proteases, which suggested that the nonapeptide had strong enzyme-resistant properties against gastrointestinal proteases.

3.3. Inhibitory kinetics study

Lineweaver-Burk plots of ACE inhibitory activity are shown in Fig. 3. Kinetic experiments demonstrated that inhibitory kinetic mechanism of this peptide was non-competitive, which means the combination between substrate and ACE do not affect the inhibitory activity of this peptide against ACE. The calculated K_m and K_i values for the nonapeptide were 13 and 52 mmol/L, respectively.

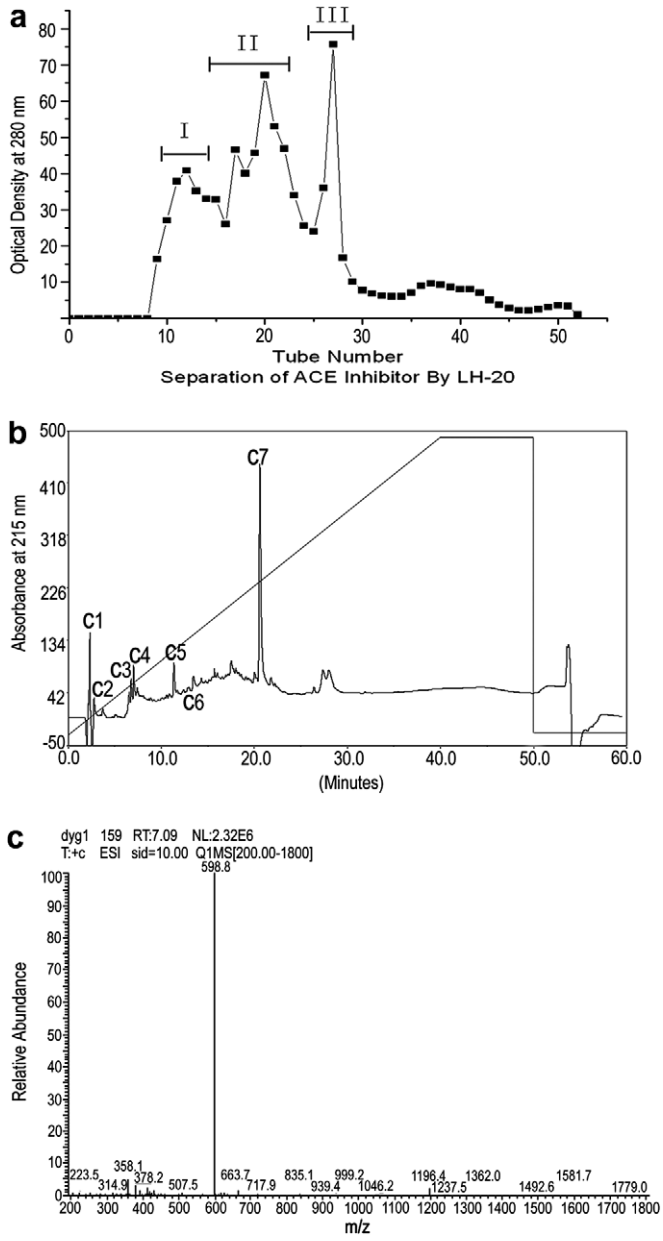

Fig. 1. (a) The hydrolysate (MW less than 10,000 Da) was separated using a Sephadex LH-20 gel filtration chromatography column with 30% methanol solution at a flow rate of 0.5 mL/min. (b) Fraction II was separated with RP-HPLC on a Hypersil BDS C_{18} column at a flow rate of 1 mL/min. Eluent A consisted of 0.1% TFA in milli-Q water (v/v); Eluent B of acetonitrile. Eluent B concentrations were increasing: 0–40 min, 0–100%; 40–50 min, 100%. (c) (Negative) LC–MS spectrum of peak C7.

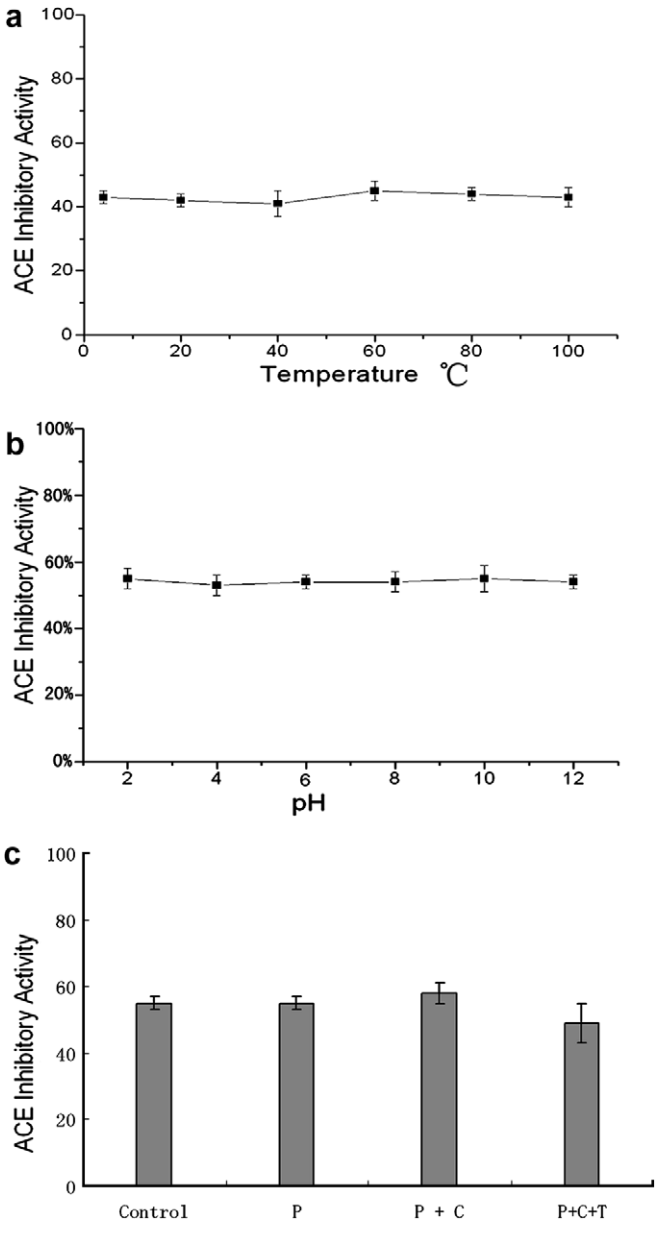

Fig. 2. (a) Stability on ACE inhibitory activity of the nonapeptide (0.08 mg/mL) after 2 h incubation at different temperatures. (b) Stability on ACE inhibitory activity of the nonapeptide (0.08 mg/mL) after 2 h incubation at different pH. (c) Stability on ACE inhibitory activity of the nonapeptide after digestion with gastrointestinal proteases. Control: the nonapeptide solution (0.08 mg/mL); P: the nonapeptide solution (0.08 mg/mL) was digested with pepsin for 2 h; P + C: the nonapeptide solution (0.08 mg/mL) was successively digested with pepsin for 2 h and chymotrypsin for 1 h; P + C + T: the nonapeptide solution (0.08 mg/mL) was successively digested with pepsin for 2 h, chymotrypsin for 1 h and trypsin for 2 h. Then the digests (0.08 mg/mL) were performed for ACE inhibitory activity determination, respectively. Data are expressed as mean ± SEM for a minimum of three experiments.

Table 1
The weight, recovery yield and ACE inhibitory activity of these fractions obtained on hydrolysate, Sephadex LH-20 gel filtration chromatography column and RP-HPLC from 6 g of proteins

Fraction	Weight (g)	Recovery yield (%)	IC_{50} (μmol/mL)
Hydrolysate	4.44	74	9.5
Fraction II(LH-20)	3.35	55.8	0.49
C7(HPLC)	0.51	8.5	0.066

3.4. Antihypertensive activity of the fraction II

In the short-term experiments, antihypertensive activity of fraction II and captopril were evaluated by measuring the SBP in SHR at 2, 4, 6 and 8 h after oral administration as shown in Fig. 4a. There was no change in SBP in the control group during the investigation period. The maximum decrease in SBP caused by 2 mg/kg of captopril was observed 2 h post-administration. The value of SBP 8 h post-administration of captopril was similar to that measured before oral administration. Three concentrations of fraction II could all decrease the SBP in SHR but only the administrations of 20 and 100 mg/kg of fraction II could result in significant decreases of the SBP contrasting with control group. The maximum decreases in SBP caused by fraction II were observed

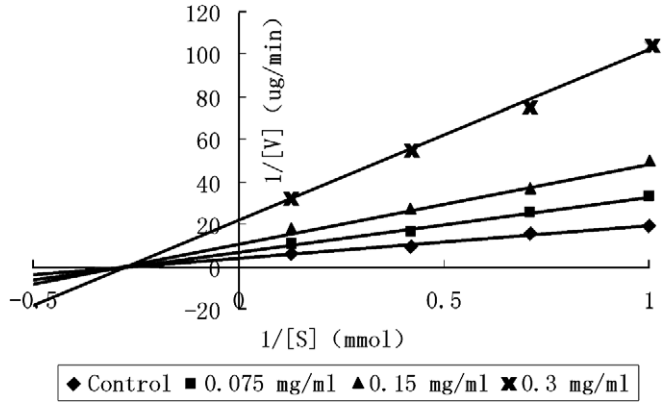

Fig. 3. Lineweaver-Burk plots of ACE inhibitory activity in the presence of the nonapeptide.

4 h post-administration, and these decreases were less pronounced than that caused by 2 mg/kg of captopril contrasting with control group. However, the decreases in SBP caused by 20 and 100 mg/kg of fraction II were even greater than that caused by 2 mg/kg of captopril 8 h post-administration. Heart rates of these SHR were up to the standard in the course of experiment with the value of 400 ± 50 beats/min.

In the long-term experiments, a significant decrease of the SBP, caused by 20 mg/kg of fraction II, was observed from 3rd day, and moreover, the decrease in SBP was even greater than that caused by 2 mg/kg of captopril after 9 days as shown in Fig. 4b. Heart rates of these SHR were up to the standard in the course of experiment with the value of 400 ± 50 beats/min.

As shown in Table 2, the body weight of control group increased more than that of groups administrated with fraction II and captopril. The relationship between adiposity and hypertension should be studied further.

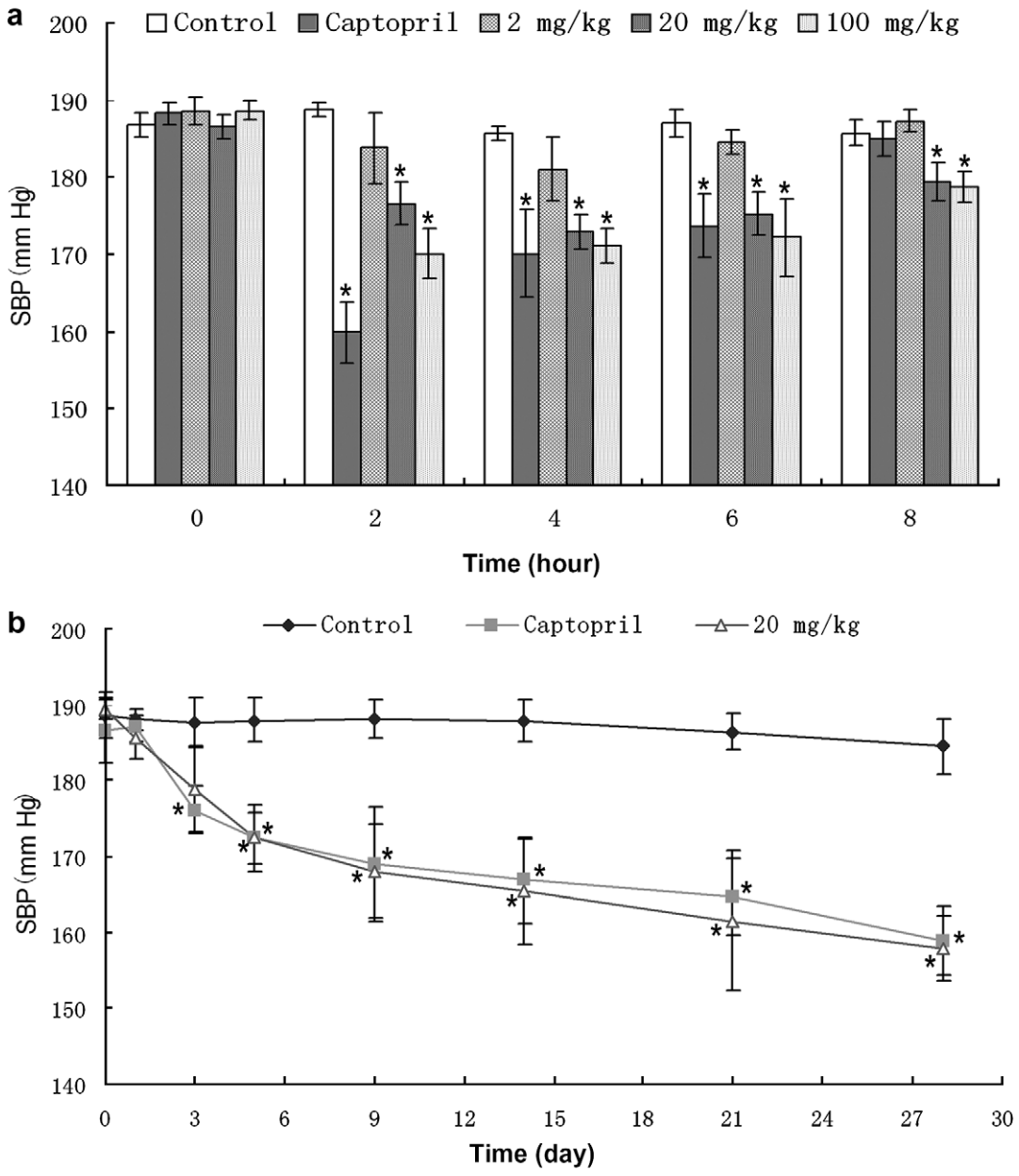

Fig. 4. (a) Decreases of SBP caused by different products where control: 5 mL/kg of saline water; captopril: 2 mg/kg; low, middle and high dose: 2, 20 and 100 mg/kg of fraction II. (b) Decreases of SBP caused by captopril and fraction II where control: 5 mL/kg of saline water; captopril: 2 mg/kg; fraction II: 20 mg/kg. Data are expressed as mean ± SEM for a minimum of eight rats. Asterisk indicates significant differences where: $P < 0.01$ vs control.

Table 2
Difference of the body weight in SHR between 28 days in long-term experiments where control: 5 mL/kg of saline water; captopril: 2 mg/kg; fraction II: 20 mg/kg

Dosage	Body weight (g)		
	0 day	28 day later	Difference
Control	232 ± 21	275 ± 27	43 ± 19
Captopril	249 ± 29	279 ± 28	30 ± 9
Fraction II	252 ± 18	278 ± 20	26 ± 7*

Data are expressed as mean ± SEM for a minimum of eight rats.
Asterisk indicates significant differences where *: $P < 0.05$ vs control.

4. Discussion

The nonapeptide with sequence Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe has firstly been isolated from bovine brain in 1994 as an opioid peptide with IC_{50} value of 34.3 μmol/L, which corresponds to the sequence 33–41 of bovine hemoglobin beta-chain (Karelin, Philippova, Karelina, & Ivanov, 1994). Spinorphin (LVVYPWT) was found to be an endogenous factor that modulated enkephalin-degrading enzymes and was purified from bovine spinal cord based on its inhibitory activity toward various enkephalin-degrading enzymes containing neutral endopeptidase, aminopeptidase, ACE and dipeptidyl peptidase III (DPP III) (Nishimura & Hazato, 1993). Yamamoto, Hashimoto, Shimamura, Yamaguchi, and Hazato (2000) have synthesized spinorphin analogues and assayed their inhibitory activity toward DPP III. They found that the tynorphin (VVYPW), an N-terminal and C-terminal truncated form of spinorphin, exhibited more potent inhibitory activity and an IC_{50} value of 86 mg/L, whereas sequence smaller than four amino acid residues exhibited almost no or less activity, suggesting that a five amino acid sequence containing a Tyr-Pro residue is essential for the inhibition.

At present, more and more studies come to pay attention to this multifunctional nonapeptide (VVYPWTQRF) (Cohena, Arnaudin, Balandier, & Piot, 2003; Karelin et al., 1998; Poljak, Lahnstein, Mason, Smythe, & Duncan, 1997; Szikra et al., 2001). Blishchenko et al. (2002) have synthesized this nonapeptide and found that it could suppress the growth of transformed murine fibroblasts L929 tumor cell culture. In addition, Lammerich et al. (2003) have confirmed that stimulation of hBRS-3 (The human orphan G-protein coupled receptor bombesin receptor subtype 3) by this nonapeptide leads to an elevation of $[Ca^{2+}]_i$ from IP3 (inositol(1,4,5)trisphosphate)-sensitive stores, a subsequent influx of $[Ca^{2+}]_{ex}$ via CRAC (Ca^{2+}-release-activated Ca^{2+}-channels), an induction of PLC (phospholipase C) and PKC (protein kinase C) activity and phosphorylation of MAPK (mitogen-activated protein kinase) 42/44. All the above research reports suggest that this nonapeptide correlates with neural regulation and tumorimmunity potentially, which should be studied further. This paper is the first report showing release of this nonapeptide with ACE inhibitory activity from oyster proteins by pepsin treatment. The IC_{50} value, stability and inhibitory kinetics study were firstly investigated by using this peptide. The yield of the nonapeptide from oyster proteins was 8.5%, which suggests the oyster proteins can serve as another raw material for producing this nonapeptide.

The protein hydrolysate (fraction II) firstly displayed antihypertensive activity in SHR. Fraction II was divided into seven peaks with a RP-HPLC and only peak C7 (this nonapeptide) displayed ACE inhibitory activity. So we speculate that the antihypertensive activity of the fraction II mostly owe to antihypertensive activity of this nonapeptide. Some antihypertensive drugs are known to produce side effects after administration. Captopril has stronger ACE inhibitory activity than this nonapeptide with IC_{50} value of 0.022 μmol/L determined by the method 2.4. However, we could not find any side effect after administration of fraction II, which suggests that ACE inhibitory peptide derived from oyster proteins could be utilized as nutraceuticals and pharmaceuticals. In addition, it is expected that this study will contribute developing interest in basic research and potential applications of bioactive peptides.

Up to now, there are many reports that the peptides with ACE inhibitory activity are obtained from the hydrolysates of food proteins. These peptides have been purified and their sequences have been investigated, which is important for researching the mechanism of ACE inhibitory activity. However, most of these studies always paid attention to the level of ACE inhibitory activity, but ignored the practical application of active peptides, such as the yield, effect of oral administration and costcutting. Oyster is an abundant resource from Oceans and pepsin treatment for oyster proteins is an effective cheap means of production contrasting with synthesis of pure peptide.

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