Food Chemistry 133 (2012) 315–322

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Primary and secondary structure of novel ACE-inhibitory peptides from egg white protein

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ARTICLE INFO

Article history:
Received 11 October 2011
Received in revised form 25 November 2011
Accepted 16 January 2012
Available online 24 January 2012

Keywords:
ACE inhibitory activity
Peptide
Egg white protein
Secondary structure
Antioxidant property
Anticoagulation activity

ABSTRACT

The primary structure of novel angiotensin converting enzyme (ACE) inhibitory peptide from egg white protein was investigated, and secondary structure of the peptide was explored for the first time. The potential effects of bioactive peptides were submitted to bioactivity screening with ACE inhibitory activity, antioxidant property, and anticoagulation activity. Bioactive peptides from egg white protein were characterized by LC tandem mass spectrometric, and secondary structures of those peptides were investigated by FT-IR. Our results showed that total 11 bioactive peptides with three new and eight known structures were identified with LC/MS/MS, which then were synthesized by Fmoc solid phase method. Peptide Thr-Asn-Gly-Ile-Ile-Arg (TNGIIR) exhibited higher activity against ACE to other two new peptides. The concentration of the peptide TNGIIR, necessary to inhibit 50% the activity of ACE was 70 μM. Results also suggested that the secondary structural differences between peptides could also influence the ACE inhibition capacity. Thus, it appears that primary and secondary structure of peptide plays the potential role inhibiting the ACE activity.

1. Introduction

Angiotensin-converting enzyme (ACE) has a critical role in cardiovascular function by cleaving the carboxy terminal His-Leu dipeptide from angiotensin I to produce a potent vasopressor octapeptide, angiotensin II (Natesh, Schwager, Sturrock, & Acharya, 2003). Inhibitors of ACE are a first line of therapy for hypertension, heart failure, myocardial infarction and diabetic nephropathy. The peptides derived from food proteins are considered to be milder, safer and easily absorbed compared with synthetic drugs (Qu et al., 2010). Thus, the fact that peptides released from food proteins by enzyme hydrolysis may exhibit different biological activities has been generally accepted (Matthews, 1975). The formation of ACE inhibitory peptides by enzymatic hydrolysis (Liu et al., 2010; Nalinanon, Benjakul, Kishimura, & Shahidi, 2011), by simulated gastrointestinal digestion (Yang, Zou, Yu, & Gu, 2011), by fermentation, and by synthesis has been reported.

Egg white is now understood to contain substances with biological functions beyond basic nutrition, and extensive research has been undertaken to identify and characterize these biologically active components. Diverse biological functions have been attributed to egg components and will be discussed, such as antihypertensive activity (Liu et al., 2010), antioxidant property (Huang, Majumder, & Wu, 2010; Majumder & Wu, 2010), anti-diabetic peptides (Yu, Yin, Zhao, & Yu, et al., 2011), antimicrobial activity (Mine, Ma, & Lauriau, 2004), antiadhesive properties (Kassaify, Li, & Mine, 2005), and anticancer activity (Yi, Kim, Kim, & Lee, 2003). It has been suggested that egg white-derived bioactive peptides can exert vasorelaxation effects in the treatment of hypertension (Garcia-Redondo, Roque, Miguel, Lopez-Fandino, & Salaices, 2010). An ACE inhibitory peptide, ovokinin, was firstly isolated from the ovalbumin hydrolysates, and was characterized by MS (Fujita, Usui, Kurahashi, & Yoshikawa, 1995). Several peptides with potent ACE-inhibitory activity were also found, such as Arg-Ala-Asp-His-Pro-Phe-Leu, Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu (Miguel, Recio, Gomez-Ruiz, Ramos, & Lopez-Fandino, 2004), Arg-Ala-Asp-His-Pro-Phe (Matoba, Usui, Fujita, & Yoshikawa, 1999), and Tyr-Gly-Arg-Tyr-Asn-Asp-Leu-Gly-His-Arg (You, Udenigwe, Aluko, & Wu, 2010). Our previous work found that two peptide sequences, RVPSL and QIGLF, exhibited high ACE inhibitory activity with the value IC_{50} 20 and 75 μM, respectively (Liu et al., 2010; Yu, Zhao, Liu, Lu, & Chen, 2011). Although there is vast information on the production and characterization of ACE-inhibitory peptides, little work has been done so far to investigate the information on both primary structure and secondary structure of ACE-inhibitory peptides.

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doi:10.1016/j.foodchem.2012.01.032

In this respect, the aims of the current work were to identify the primary structure of novel ACE inhibitory peptides from egg white protein hydrolysates and to investigate the secondary structure of those peptides. In addition, the further biological activities (anticoagulation activity and antioxidant activity) of peptides have been evaluated, aimed to screen the multifunctional peptide from egg white protein.

2. Materials and methods

2.1. Materials and chemicals

Angiotensin converting enzyme (ACE) from rabbit lung, hippuryl-L-histidyl-L-leucine, caporal, hippuric acid, thrombin (1,1)-diphenyl-2-picrylhydrazyl (DPPH), fibrinogen and HPLC grade acetonitrile, trifluoroacetic acid (TFA) were purchased from Sigma. The enzyme Alcalase (a declared activity of 2.4 AU/kg and a density of 1.18 g/ml) was purchased from Novozymes. All the other reagents were analytical grade.

2.2. Egg white protein hydrolysate preparation

Egg white protein that was dispersed in distilled water, to obtain 5% protein slurry (w/v) was hydrolyzed in a 250 ml reactor under the controlled temperature and pH. The mixture was heated to 90 °C, for 10 min, in order to denature the protein, and then cooled down to 50 °C. The pH value was adjusted to 10.0 using 1 M NaOH, and then alkaline proteinase Alcalase was added at 4.0% of protein (w/w). Samples were withdrawn at 180 min reaction and placed in a boiling water bath for 10 min to inactivate the enzyme and centrifuged (12000*g*, 10 min, and 4 °C). Finally, the supernatant was pumped into B-191 BÜCHI Mini Spray Dryer with the following operating conditions: aspirator rate 98%, atomization air flow 400 l/h, drying air inlet temperature 180 °C, pump rate was adjusted to maintain an outlet temperature of 90 °C, the sample was collected from the product collection vessel.

2.3. Assay of ACE activity

The assay was conducted in borate buffer (100 mM, pH 8.3) containing 300 mM NaCl. The same buffer was used for solution preparation of the captopril, substrate, hippuric acid, peptides and enzyme dilutes. A total reaction volume of 60 μl contained 100 mM borate buffer (pH 8.3), 4 mM hippuryl-L-histidyl-L-leucine, 300 mM NaCl, and 10 milliunit ACE. All solutions were incubated at 37 °C, for 30 min, in a thermostatically controlled water bath prior to mixing, and for an additional 30 min at the same temperature after mixing. The reaction was stopped by adding 60 μl 1 M HCl before injecting the sample into the Shimadzu 2010 HPLC (Shimadzu, Kyoto, Japan) sample loop to quantify the hippuric acid produced by the enzymatic hydrolysis of the substrate hippuryl-Lhistidyl-L-leucine. The isocratic mobile phase consisted of 25% acetonitrile in deionized water (v:v) with 0.5% TFA. Mobile phase was filtered through 0.45 μm cellulose filters and degassed by ultrasound for 30 min. An aliquot of 10 μl of the reaction mixture was analyzed on a Shimadzu C18 column. Hippuryl-L-histidyl-L-leucine and hippuric acid were detected at 228 nm when the mobile phase was controlled at 0.5 ml/min (Liu et al., 2010).

The inhibitive activity degree was calculated as follows:

$$\text{ACE inhibitory activity } (\%) = \frac{A - B}{A} \times 100\% \quad (1)$$

where A was the peak area of reaction blank, the reaction blank mixture contained the same volume of the buffer solution instead of the sample; B was the peak area of the reaction in the presence of both ACE and enzymatic peptide sample, and the IC_{50} value was defined as the concentration of inhibitor inhibiting 50% of the ACE activity under the assayed conditions.

Definition of ACE activity: one unit (U) of ACE activity was defined as the amount of enzyme required to catalyze formation of 1 μmol of HA from HHL per minute, at 37 °C.

2.4. Measurement of DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the peptide RVPSL was measured in the borate buffer solution in 300 mM borate buffer at pH 8.3. DPPH was measured according to the previously reported method with some modifications. An aliquot of 100 μl 0.1 mM DPPH (dissolved in 95% ethanol) was mixed with the same volume of the borate buffer solution. After mixing vigorously for 10 s, the solution was transferred into a 96-well microplate. The absorbance was measured at 520 nm after incubating at room temperature for 30 min in the dark. The absorbance of DPPH was measured using a RT 6000 microplate reader (Rayto, China).

The degree of DPPH radical-scavenging was calculated as follows:

$$\text{DPPH} \cdot \text{radical-scavenging} \cdot \text{activity } (\%) = \frac{Ac - As}{Ac} \times 100 \quad (2)$$

where Ac was the absorbance value of the reaction control. The reaction blank mixture contained the same volume of the buffer solution instead of the sample; As was the absorbance value of the reaction sample in the presence of peptide.

2.5. In vitro anticoagulation activity

Anticoagulation activity was evaluated according to the reported method (Yang, Wang, & Xu, 2007) with some modification, using the microplate reader (Bio-tek). The assay was conducted in Tris–HCl buffer (50 mM, pH 7.4), containing 0.154 mM sodium chloride. The same buffer was used for solution preparation of fibrinogen (w/v 0.1%) and thrombin (10 U/ml). An aliquot of 40 μl peptide solutions or Tris–HCl buffer and 140 μl fibrinogen were added into the plate wells, mixed and incubated for 10 min. Then 10 μl thrombin was added to start the reaction. All solutions were mixed vigorously at 37 °C for 8 s. The absorbance was measured in microplate reader at 405 nm.

$$\text{Antithrombotic activity } (\%) = \frac{A_1 - A_2}{A_1 - A_0} \times 100\%$$

where A_1 was the absorbance for the system with Tris–HCl buffer instead of peptide samples; A_2 was the absorbance for the system in the presence of peptide sample; A_0 was the absorbance for the system with buffer solution instead of thrombin.

Definition of antithrombotic activity: One unit (U) of antithrombotic activity was defined as the amount of peptides inhibiting one unit of thrombin.

2.6. Purification of peptides

The powder of egg white protein hydrolysate was added onto an open column (1.6 × 100 cm) packed with Sephadex G-25 gel that had been pre-equilibrated with distilled water. The hydrolysate was eluted by distilled water at the speed of 60 ml/h. The elution peaks were monitored at 220 nm. Those fractions were vacuum freeze-dried and stored at 20 °C until used.

2.7. Characterization of the purified peptides by tandem MS

The LC/MS/MS system consisted of an Agilent 1200 HPLC system and a 4000 Q-Trap mass spectrometer (Applied Biosystems) with its

Turbo spray. The chromatography was performed on a reverse phase C18 column (column size 250L*4.6, serial No. 6032551). An isocratic mobile phase consisted of 70% acetonitrile (B) and deionized water with 0.1% TFA (A). The gradient elution program was that the elution started from 10% B (70% acetinitral) initially and followed by a linear gradient to 100% B in 10 min. The total mobile

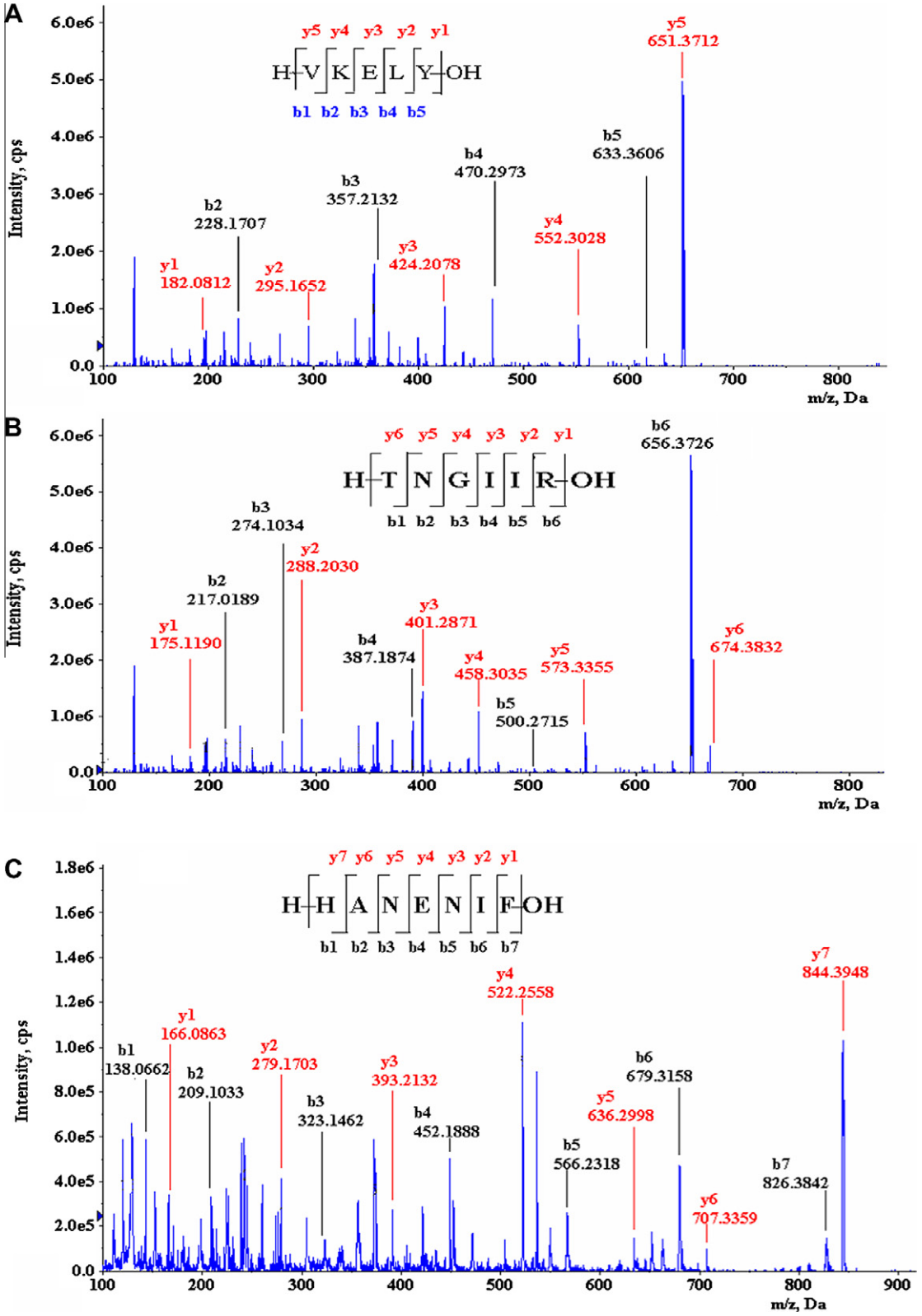

Fig. 1. MS/MS spectra of fraction from egg white hydrolysate. The mass spectrum of the fraction was acquired, and the accurate molecular weight of the fraction was determined. The collision induced fragmentation of 651.4007, 674.4109, and 844.3937 was illustrated. Following sequence interpretation and database searching, the MS/MS spectra of a single charged ion with *m*/*z* at 844.3937, 651.4007, and 674.4109 were determined to be the peptide (A) HANENIF, (B) VKELY, and (C) TNGIIR, which matched 23–29, 122–126,154–159 residue to the ovalbumin of egg white protein.

phase flow was set at 1 ml/min. The acquisition method consisted of an IDA scan cycle including the enhanced mass scan (EMS) as the survey scan, enhanced resolution scan (ER) to confirm charge state and two dependent enhanced product ion (EPI) scans. With the threshold of the ion intensity at 5500 counts per second (cps), the IDA criteria were set to allow the most abundant ions in the EMS scan to trigger EPI scans. To minimize possible fragmentation in the EMS, the collision energy was set at 5 eV and the collision-induced dissociation (CID) gas was set at 'Low'. The scan range of the EMS was set at m/z 275.00–1000.00. The dependent EPIs scanned the range of m/z 275–1000, including setting for CID gas, 55 eV (GS 1) and 60 eV (GS 2) for the collision energy. The speed of EMS and EPI scans were recorded at the speed 4000 Da/s.

2.8. Synthetic peptides

The peptide sequences derived from egg white were synthesized by the solid phase procedure peptide, using Fmoc protected amino acids synthesis methods, with an AAPPTEC 396 Automated Peptide Synthesizer (Advanced Automated Peptide Protein technologies, USA). These peptides were synthesized on Fmoc-Met-Wang Resin and were elongated using standard Fmoc solid-phase peptide conditions on an AAPPTEC Apex 396 peptide synthesizer, as previously described (Yu, Zhao, Liu et al., 2011). These peptides were provided by Shang hai science peptide Biological Technology Corporation. The synthesized peptides was purified by HPLC on an ODS column. Their purity of synthesized peptides was verified by HPLC in our laboratory. The molecular masses of these synthesized peptides were determined by mass spectrometry (Dionex, MSQ).

2.9. Secondary structure of peptides by FT-IR Spectrometer

The IR-spectra were recorded in the transmission mode with a IRPrestige-21 Fourier Transform Infrared Spectroscopy (Shimadzu, Japan) equipped with a KBr Beamsplitter deuterated L-alanined-oped triglycene sulphate (DLaTGS) detector. The measurements were performed in a transmission mode using a demountable thin layer IR-cell, with KBr windows in the frequency region 400–4000 cm^{-1}. Samples of the peptides were placed sample compartment with automatic accessory recognition at the scanning speed of 2.8 mm/s. Each spectrum was recorded at a resolution of 4 cm^{-1}, 200 scans were averaged before Fourier transformation, taking the KBr spectrum as background. After background correction, all spectra were baseline corrected, and then peptides spectra were obtained.

2.10. Statistical analysis

SPSS13.0 was used for the major data processing throughout this work. All results were expressed as the mean ± SD. Differences were considered significantly at $P < 0.05$.

3. Results and discussion

3.1. Amino acid sequences of purified peptides

The hydrolysate for 180 min was purified by Sephadex G 25 and the fraction has the high activity with the value of IC_{50} 0.18 mg/ml. The ACE inhibitory activity of fraction purified by Sephadex G 25 in the work was comparable to that of oligopeptides from cooked egg, amaranth grain or ovine milk (Gomez-Ruiz, Ramos, & Recio, 2007; Majumder & Wu, 2009). The fraction purified by Sephadex G 25 was utilized for amino acid sequence analysis. To increase throughput, the application of information-dependent data acquisition (IDA) becomes very important. IDA is a procedure that combines two or more different scan modes in a sequential fashion for the same LC/MS run. The present paper illustrated the potential of the Q TRAP in combination with LC for the analysis of small peptides in the food technology. Since the egg white protein mainly consisted of ovalbumin (54%), ovotransferrin (12%), lysozyme (3.4%), Ovomucin (3.5%) and those proteins were identified, the amino acid sequences are found from egg white databases on ExPASy. Some researchers have discovered that FRADHPFL, RADHPF, FGRCVSP, NIFYCP, FFGRCVSP, LW, FCF, ERKIKVYL, exhibit the ACE inhibitory activity (Miguel, Alvarez, Lopez-Fandino, Alonso, & Salaices, 2007; Miguel, Lopez-Fandino, Ramos, & Aleixandre, 2005; Miguel, Lopez-Fandino, Ramos, & Aleixandre, 2006). In this paper, the fraction was analyzed by HPLC-MS/MS. The fraction was subjected to Turbo spray mass spectrometry. 19 ions scan mass spectrum were obtained by full scan EMS model, and ion charged were confirmed that those ions were single charge by ER model. The charged ions with different m/z were obtained by MS/MS spectrum (Liu et al., 2010). Following amino acid sequence analysis and Expasy database search (http://www.expasy.org/), the previous work have identified as RVPSLM, TPSPR, DLQGK, AGLAPY, RVPSL, DHPFLF, HAEIN, and QIGLF, five of which were fragments of ovotransferrin and three of which was fragments of ovalbumin (Liu et al., 2010; Yu, Zhao, Liu et al., 2011). In the current work, another three peptides HANENIF, VKELY, and TNGIIR were identified. The MS/MS spectra for HANENIF, VKELY, and TNGIIR were shown in Fig.1. Peptide information shown in Table 1 contains the complete series of fragments b and y ion model.

3.2. Primary structure and ACE inhibitory activity of synthesized peptides

Synthetic peptides RVPSLM, TPSPR, DLQGK, AGLAPY, RVPSL, DHPFLF, HAEIN, QIGLF, HANENIF, VKELY, and TNGIIR were identified by analytical RP-HPLC and Dionex MSQ mass spectrometry. Peptide purity was estimated by the amount of peak area of correct peptide relative to all the integrated peak areas at 214 nm. The HPLC result shows that the purity of the peptides RVPSLM, TPSPR, DLQGK, AGLAPY, RVPSL, DHPFLF, HAEIN, QIGLF, HANENIF, VKELY, and TNGIIR were 98.73%, 98.77%, 96.68%, 98.92%, 98.60%, 98.73%, 98.77%, 96.68%, 95.21%, 92.30% and 94.26%. Mass spectrum of those peptides shows the correct MW of synthetic peptides, as the calculated MW of which was correspond to theirs theory MW. The chemical structures of HANENIF, VKELY, and TNGIIR were illustrated in Fig. 2. In order to further confirm the ACE inhibitory activity of these peptides, the peptides were measured by Section 2.3 mentioned. The peptides RVPSL, QIGLF, and TNGIIR have the high activity and the values of IC_{50} were 20 μM, 75 μM, and 70 μM. However, peptides RVPSLM, TPSPR, DLQGK, AGLAPY, DHPFLF, HAEIN, HANENIF, and VKELY have no significant activity against ACE. Some research revealed that C-terminal tetrapeptide residues of long-chain peptides were more important to their ACE-inhibitory activity. The most likely preferred amino acid

Table 1
Peptide information.

Time	Sequence	Prec m/z	Theo MW	Prec MW	b ion	y ion	m
8.922	TNGIIR	674.4109	673.3759	673.4036	6	6	0.0277
11.766	VKELY	651.4007	650.3639	650.3934	5	5	0.0295
12.324	HANENIF	844.3937	843.3875	843.3864	7	7	0.0011

residues starting from C-terminus are tyrosine and cysteine for the first position (Wu, Aluko, & Nakai, 2006). Although RVPSL, QIGLF, and TNGIIR contain no cysteine (C) amino acid residue at its C terminal. It was also observed that these three peptides have higher ACE inhibitory activity compared to LF and VVYPWTQRF (Wilson, Hayes, & Carney, 2011). Several other ACE inhibitory peptides from

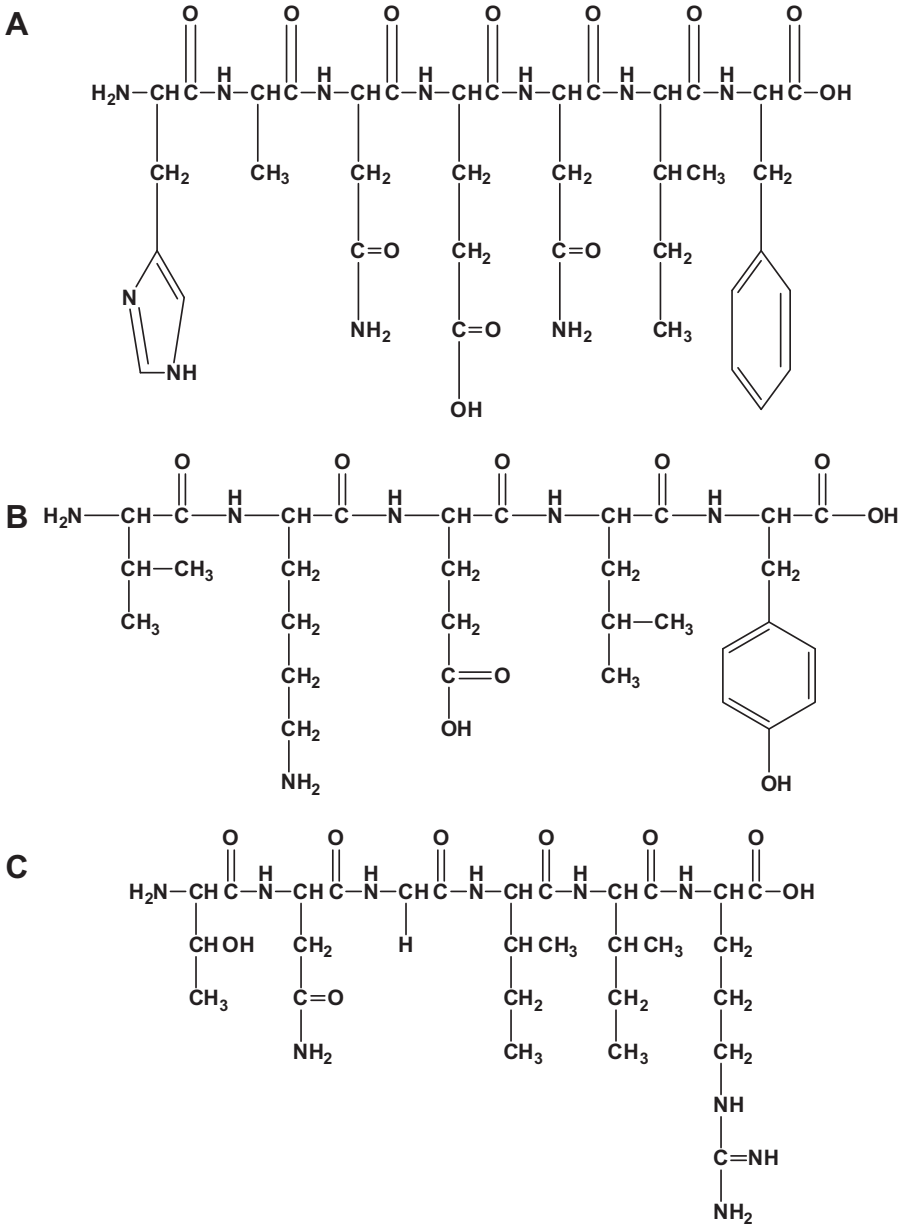

Fig. 2. Chemical structures of peptides sequence (A) TNGIIR, (B) HANENIF, and (C) VKELY, which derived from egg white protein.

Table 2
The DPPH radical scavenging activity and anticoagulation activity of peptides.

Peptide sequences	DPPH radical scavenging activity (%)				Anticoagulation activity (%)			
	0.01 (mM)	0.1 (mM)	1 (mM)	100 (mM)	0.01 (mM)	0.1 (mM)	1 (mM)	100 (mM)
RVPSLM	3.1 ± 0.8	5.0 ± 0.9	6.6 ± 1.5	7.0 ± 2.1	1.1 ± 0.4	1.0 ± 0.3	2.6 ± 0.5	13.0 ± 5.9
TPSPR	2.7 ± 0.5	3.8 ± 1.2	5.5 ± 2.2	11.0 ± 5.6	0.7 ± 0.5	0.8 ± 0.6	3.5 ± 1.1	16.0 ± 0.6
DLQGK	0.9 ± 0.5	1.2 ± 0.6	3.0 ± 1.3	5.6 ± 1.5	0.9 ± 0.2	0.9 ± 0.5	2.2 ± 0.3	12.0 ± 0.5
AGLAPY	2.0 ± 0.9	3.1 ± 1.6	4.2 ± 1.4	8.1 ± 2.6	1.0 ± 0.5	1.1 ± 0.6	3.0 ± 0.4	12.1 ± 0.6
RVPSL	11.4 ± 4.1	15.8 ± 4.2	18.3 ± 3.2	34.2 ± 5.1	1.0 ± 0.5	1.1 ± 0.6	15.5 ± 8.2	100.2 ± 5.1
DHPFLF	2.1 ± 0.8	3.0 ± 0.6	4.6 ± 1.1	7.6 ± 2.3	1.0 ± 0.8	1.5 ± 0.8	3.1 ± 0.4	13.0 ± 0.6
HAEIN	4.1 ± 1.2	5.0 ± 1.3	7.6 ± 1.5	15.0 ± 3.3	0.9 ± 0.2	1.0 ± 0.4	2.9 ± 0.5	12.8 ± 1.4
QIGLF	3.7 ± 1.6	5.8 ± 1.9	7.5 ± 0.1	16.0 ± 2.6	1.0 ± 0.6	0.9 ± 0.6	1.2 ± 0.8	5.9 ± 0.5
VKELY	1.9 ± 0.6	2.6 ± 1.5	3.2 ± 2.0	7.2 ± 3.5	0.5 ± 0.8	0.8 ± 0.5	1.0 ± 0.5	3.1 ± 0.6
HANENIF	2.4 ± 0.7	3.1 ± 1.2	4.0 ± 2.0	6.8 ± 2.6	0.9 ± 0.5	1.0 ± 0.3	1.2 ± 0.7	1.9 ± 0.5
TNGIIR	1.0 ± 0.5	2.1 ± 0.8	3.5 ± 1.4	8.1 ± 3.6	1.0 ± 0.1	1.1 ± 0.5	2.0 ± 0.5	11.1 ± 0.6

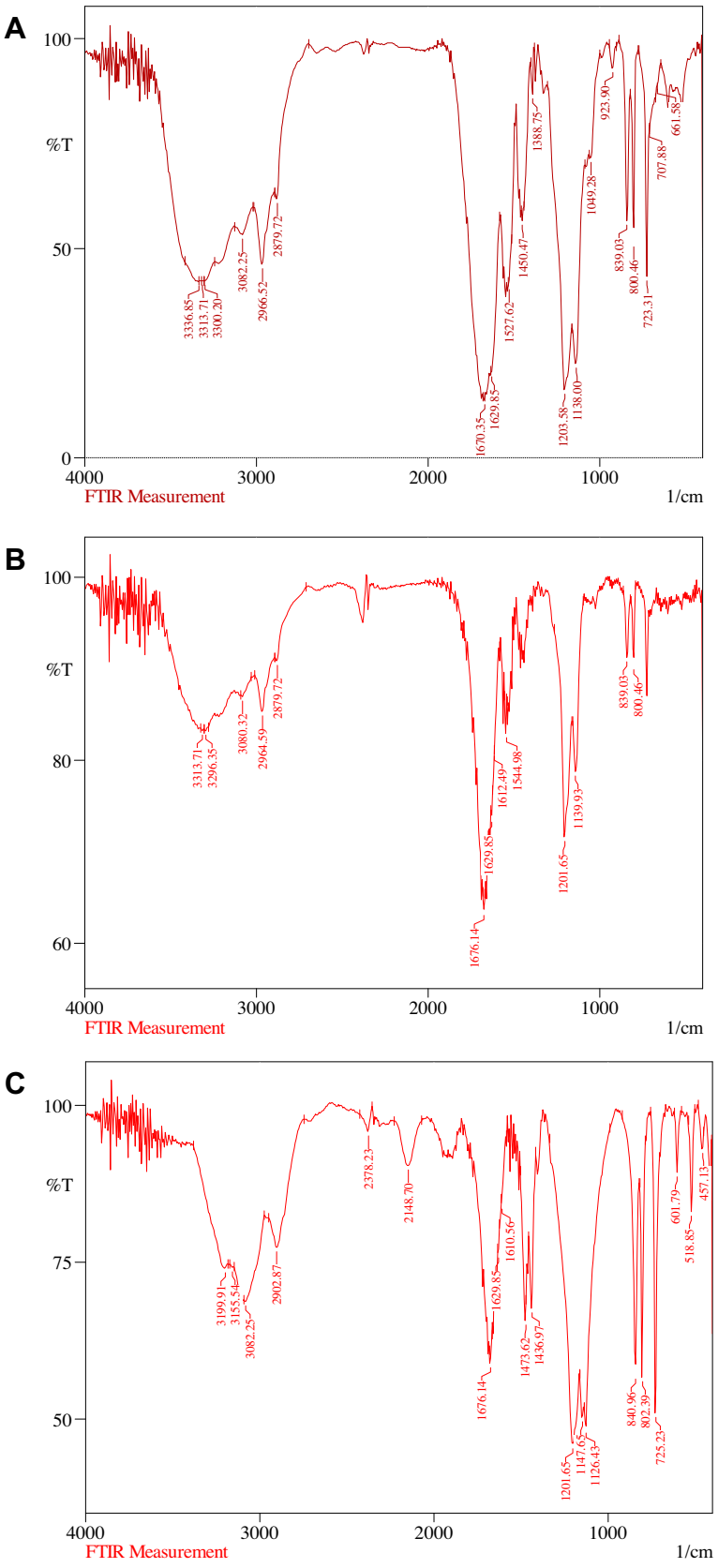

Fig. 3. FT-IR spectra of peptides (A) RVPSL, (B) QIGLF, and (C) TNGIIR.

egg white protein have been reported, such as IQWCA (Majumder & Wu, 2010), LVYPFTGPIPN, and HLPLP (Miguel, Gomez-Ruiz, Recio, & Aleixandre, 2010). Among the peptides RVPSL, IQWCA, LVYPFTGPIPN, HLPLP, QIGLF, and TNGIIR, there are no similar amino acid residues in C or N terminal. It is necessary to evaluate the structure activity relationship model of ACE inhibitory peptides.

3.3. DPPH radical-scavenging activity and anticoagulation activity of synthesized peptides

Firstly, DPPH radical-scavenging activity of each peptide was evaluated. Radical scavenging was a primary mechanism of antioxidants to inhibit oxidative processes, and used extensively to evaluate reducing substances. As the concentration of RVPSLM, TPSPR, DLQGK, AGLAPY, RVPSL, DHPFLF, HAEIN, QIGLF, HANENIF, VKELY, and TNGIIR increased from 0.01 to 100 mM, the DPPH radical scavenging activity of those peptides increased subsequently. Results showed that Peptide RVPSL had the higher DPPH radical scavenging activity as 34.2 ± 5.1% to other peptides identified (Table 2). Although the molecular weight of those peptides was very similar, DPPH radical-scavenging activity of peptides was difference. The result revealed that the radical scavenging activity of peptides was dependent not only on the size of peptide, but also on amino acid sequences. Consequently, through the screening of the anticoagulation activity of those peptides, RVPSL was significantly enhanced when its concentration increased from 1 to 100 mM (Table 2). TNGIIR and QIGLF with high ACE inhibitory activity exhibited no significant DPPH radical-scavenging activity. Previous work also suggested that RVPSL have the potential as multiple bioactive peptide (Yu, Yin, Zhao, Wang, et al., 2011). Furthermore, a clear relationship between ACE inhibitory properties, antioxidant activity, and anticoagulation activity of peptides was valuable to fully established.

3.4. Secondary structure of peptides

FT-IR spectra of ACE inhibitory peptides with high activity, RVPSL, QIGLF and TNGIIR, were illustrated in Fig. 3, shown with similar spectra for the first glance. The COOH was present at 3199 cm^{-1} and 3300–3500 cm^{-1}; C-H stretch was observed at 2902–2964 cm^{-1}. The fraction of peptide bonds in α-helical, β-sheet, and aperiodic conformations can be accurately estimated by analysis of the amide I band (1600–1700 cm^{-1}) in the mid-IR region (Carbonaro & Nucara, 2010). The amide I band was the most intense absorbance band for all the investigated peptides, and had a characteristic shape for each peptide. RVPSL displayed a band range from 1629 to 1690 cm^{-1}, with the maximum of absorption at 1670 cm^{-1}. Peptide, QIGLF, showed high absorption in the band at 1676 cm^{-1}. Indeed, the secondary structures of the three peptides were found to be different, as shown in Fig. 3. Some researchers also found the similar observation, which was explained by the existence of various secondary structures of different peptides, and suggested that the β-sheet structure may appear in the same range from 1625 to 1640 cm^{-1} (Murariu, Dragan, & Drochioiu, 2009). Thus, these results revealed that the secondary structure depended much on amino acid sequence. In addition, circular dichroism spectra and nuclear magnetic resonance will be applied for the further study of secondary structure of peptides.

4. Conclusions

The current work identified the purified fraction derived from egg white protein hydrolysate containing RVPSLM, TPSPR, DLQGK, AGLAPY, RVPSL, DHPFLF, HAEIN, QIGLF, HANENIF, VKELY, and TNGIIR. Subsequently, the angiotensin I-converting enzyme inhibitory activity, antioxidant property, and anticoagulation activity of those peptides was investigated. The present results have shown that RVPSL, QIGLF, and TNGIIR exhibited the high ACE inhibitory activity *in vitro*, with the IC_{50} value 20 μM, 75 μM, and 70 μM, respectively. FT-IR spectra of RVPSL, QIGLF, and TNGIIR revealed that the secondary structure difference was contributed to amino acid sequence. The result indicated that the secondary structure of peptides played an important role in inhibiting ACE activity. Although the structure activity relationship of ACE inhibitory peptides has been conducted, most studies should pay attention to research on the Structure–activity relationship of ACE inhibitory peptides.

Acknowledgements

This paper was supported by Grant from The Chinese National Natural Science Funds (No. 31071577). The authors would like to thank WenHai Jin, KeRong Zhang and YaoYu Liu from Applied Biosystems and professor YingWu Wang from College of Life Science Jilin University for his technical assistance with the MS/MS analysis.

The authors have declared no conflict of interest.

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