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Virtual screening for angiotensin I-converting enzyme inhibitory peptides from *Phascolosoma esculenta*

Yalan Liu[1], Lujia Zhang[1], Mingrong Guo[1], Hongxi Wu[2], Jingli Xie[1*] and Dongzhi Wei[1]

Abstract

Background: Many short peptides have proved to exhibit potential anti-hypertensive activity through the inhibition of the Angiotensin I-converting enzyme (ACE) activity and the regulation of blood pressure. However, the traditional experimental screening method for ACE inhibitory peptides is time consuming and costly, accompanied with the limitations as incomplete hydrolysis and peptides loss during purification process. Virtual methods with the aid of computer can break such bottle-neck of experimental work. In this study, an attempt was made to establish a library of di- and tri-peptides derived from proteins of *Phascolosoma esculenta*, a kind of seafood, through BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep), and to screen highly active ACE inhibitory peptides by molecular docking with the help of LibDock module of Discovery Studio 3.5 software.

Results: Two hundred and eighty four (284) di- and tri-peptides, derived from *P. esculenta* proteins after a virtual hydrolysis with pepsin, trypsin and a mixture of pepsin and trypsin, were predicted to possess ACE inhibitory activity, among which there are 99 ACE inhibitory peptides with estimated IC_{50} less than 50 μM. Nine peptides were synthesized for the comparison between the estimated and the experimentally determined IC_{50}. The results indicated that errors between the estimated and measured $\log(1/IC_{50})$ are all less than 1.0 unit.

Conclusions: Virtual method for peptide library construction and ACE inhibitory peptides screening efficiently demonstrated that *P. esculenta* proteins are prospect resource for food-origin ACE inhibitory peptide.

Keywords: Virtual screening; Angiotensin I-converting enzyme (ACE); ACE inhibitory peptide; *Phascolosoma esculenta*

Background

Hypertension is a worldwide health problem, the prevalence of which have affected up to 30% of the adult population according to the World Health Organization. Hypertension carries a high-risk factor for arteriosclerosis, myocardial infarction, and end-stage renal disease [1,2]. It is predicted that by 2025, about 20% of the world population will suffer from hypertension [3].

Although the cause of hypertension currently cannot be well determined, it is understood that the renin-angiotensin system regulates an organism's water, electrolytes, and blood, and the angiotensin I-converting enzyme (ACE) (peptidyldipeptide hydrolase, EC 3.4.15.1) plays an important role in regulating the blood pressure [4]. ACE is a hypertension-responsible glycoprotein distributed in vascular endothelial, absorptive epithelial, and male germinal cells [5,6]. ACE cleaves the carboxyl terminal His-Leu dipeptide from inactive decapeptide angiotensin I to active angiotensin II, a powerful vasoconstrictor which can trigger hypertension [7-10]. ACE also influences the kallikrein-kinin system by promoting the degradation and inactivation of bradykinin, which can lead to reduction of hypertension. Therefore, excessive activity of ACE leads to hypertension. Molecules which can inhibit the activity of ACE are considered useful drugs for hypertension management [11]. Currently, synthetic ACE inhibitors, such as captopril, enalapril and lisinopril, are available on the market [12]; however, they tend to have side effects [13].

Since the discovery of the first anti-hypertensive peptide in snake venom [14], more attention has been paid to natural sources, especially peptides. Peptides derived

* Correspondence: jlxie@ecust.edu.cn
[1]State Key Laboratory of Bioreactor Engineering, Department of Food Science and Engineering, East China University of Science and Technology, 130# Meilong Rd., P.O. Box 283, Shanghai 200237, People's Republic of China
Full list of author information is available at the end of the article

from cheese whey [15], fermented milk [16], mushroom [17], soy bean [18,19], corn gluten [20], insect protein [21], peanut flour [22], and egg [23] have been proven to inhibit the activity of ACE. However, few studies were reported about their side effects [24,25]. Nutritionists claim that peptides found in food are safer than 'traditional' drugs, and they are promising synthetic drug substitutes [26].

Among the ACE inhibitory peptides, shorter ones (di- and tri-peptides) usually have significant advantages over longer ones. They easily pass through blood circulation system [27,28] and then reach action sites faster without being hydrolyzed by digestive enzymes during the gastrointestinal digestion [29,30]. For these reasons, the present study focused on di- and tri-peptides.

The discovery of ACE inhibitory peptides with potential anti-hypertensive effect is mostly based on experiments, which require amounts of labors and funds. Besides, the possible active peptides can not be totally harvested due to the incomplete hydrolysis and peptides loss in the purification by the experimental protocols. Recently, as the computation simulation technology for drug design and discovery of molecular interaction are booming, the virtual screening or *in silico* experiment may replace the traditionally experimental screening of anti-hypertensive peptides to some extent. Computational approaches, which are based on computational evaluation of interactions between receptor and ligand, are proved feasible for virtual screening [31]. Molecular docking is a powerful and a widely used tool in molecular simulation, which is approximated to a lock-and-key process. The docking protocol is to 'dock' a ligand into an active site of a receptor; then, the interactions between them were 'scored' to assess the potential bioactivity of candidate compounds. The most advantage of docking is its high-throughput screening in short time with little cost [32].

In this study, an attempt was made to investigate the ACE inhibitory activity of di- and tri-peptides derived from *Phascolosoma esculenta*, a marine deposit-feeding benthonic invertebrates, also a traditional seafood with over 70% protein (dry weight) in Southeast China [33,34]. Database of di- and tri-peptides derived from *P. esculenta* were established, and their ACE inhibitory activities were predicted by virtual hydrolysis and screening method. Finally, di- and tri-peptides which have obvious ACE inhibitory activity were synthesized for verifying the validity of such virtual strategy.

Methods

Materials

There are 22 proteins of *P. esculenta* with the protein messages including entry name and sequence obtained from UniProt (http://www.uniprot.org/) (Table 1). They were used as original materials for database of di- and tri-peptides. With the help of BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep), the 22 proteins were virtually hydrolyzed with pepsin, trypsin, and a mixture of pepsin and trypsin.

Molecular docking experiments

LibDock, a module of Discovery Studio 3.5 software (DS3.5, Accelrys, San Diego, CA, USA), was used for molecular docking experiments. Scoring results (LibDock score) about ligand-receptor combination were used as the final criterion to estimate the ACE inhibitory activity of ligands. Based on a previous study [35], the corresponding relationship between LibDock score and IC_{50} was

$$\text{LibDock score} = 10.063 \log(1/IC_{50}) + 68.08 ,$$

where IC_{50} is 50% inhibitory concentration (in μM) towards ACE. According to the LibDock score, ACE inhibitory activity of ligands could be estimated.

Table 1 Properties of 22 *P. esculenta* proteins in UniProt (http://www.uniprot.org/)

Name	Number of amino acids	MW (kDa)	PI
D2J0B2	226	25.2	6.0
C3PUI4	378	43.2	9.1
D2J288	726	83.6	4.9
C3PUI8	304	34.6	8.5
C3PUI2	267	30.3	6.9
B6CQR3	658	71.6	5.1
A5A2J9	135	15.4	6.5
C3PUI5	121	13.9	6.7
B3TCX0	84	9.3	4.7
C3PUJ0	571	63.3	9.2
C3PUI9	231	25.7	5.9
B6CPA3	120	13.63	5.8
A5A2K2	220	24.4	5.8
C3PUH9	519	57.2	6.2
C3PUI7	450	50.2	9.2
B3TFG2	174	20.2	5.1
C3PUI1	54	6.5	10.8
C3PUI3	157	17.7	9.5
A3EX91	137	14.8	4.7
C3PUJ1	323	36.1	9.1
C3PUI0	231	25.9	4.8
C3PUI6	94	10.5	9.2

MW molecular weight, *PI* isoelectric point.

ACE was used as receptor in docking simulation, whose crystal structures was available in the Protein Data Bank (PDB) (http://www.pdb.org), from where the three-dimensional structure of ACE was imported [PDB:1O8A]. Before the docking procedure, water molecules were removed and zinc ions were retained. The 284 di- and tri-peptides derived from *P. esculenta* were used as ligands, of which structures and energies were generated with ChemBioDraw software [36] and minimized with the CHARMM program [37], respectively. Parameters used in the docking process are shown in Table 2.

Synthesis of peptides

Five tri-peptides (GYF, WAL, AYF, GLR, and ILK) and four di-peptides (FK, QF, EL, and HK) generated through *in silico* hydrolysis of *P. esculenta* protein, with purity of 95%, were synthesized by GL Biochem Co. Ltd. (Shanghai, China) for IC_{50} testing.

Measurement of ACE inhibitory activity

The ACE inhibitory activity was measured according to the method of Cushman and Cheung [38] with slight modifications. Ten milligram of the sample was dissolved in 1 mL distilled water and then diluted to seven different concentrations for ACE inhibitory measurements. Fifteen microliters of the sample solution in certain concentration (Seven different concentrations) were needed, which were determined by the pre-experiment about ACE inhibition ratio. The whole principle is that the concentration which ACE inhibition ratio reaches 50% is included within the concentration range. The concentrations for GYF, FK, WAL, QF, and AYF are 10, 20, 30, 40, 50, 60, and 70 μg/mL, and for EL, GLR, HK, and ILK are 20, 40, 60, 80, 100, 120, and 140 μg/mL, respectively) and 15 μL substrate hippuryl-L-histidyl-L-leucine (HHL) (8.3 mM Hip-His-Leu in 50 mM sodium borate buffer containing 0.5 M NaCl at pH 8.3) were mixed together and then pre-incubated at 37°C for 5 min. The reaction was initiated by adding 5 μL of ACE solution (310 mU/mL) and incubated for 60 min at the same temperature. The reaction was terminated by the addition of 1.0 M HCl (200 μL). Ten microliters of the reaction solution was injected directly onto a Thermo BDS-C18 column (3.0 mm × 250 mm, 5 μm, Thermo Scientific Co. Ltd., Waltham, MA, USA). The mobile phase consisted of 10% acetonitrile and 90% water with 0.1% trifluoroacetic acid (TFA). The flow rate was 0.7 mL/min and the absorbance was monitored at 228 nm. All determination was carried out at least in triplicate. The inhibition activity was calculated using the following equation:

$$\text{ACE inhibition } (\%) = [1-(A_{\text{inhibitor}}/A_{\text{control}})] \times 100 \ ,$$

where $A_{\text{inhibitor}}$ is the absorbance with ACE, HHL, and sample, and A_{control} is the absorbance of hippuric acid (HA) with ACE and HHL without the sample. Dose-dependent ACE inhibition was investigated using at least five different concentrations of peptides. The concentration of peptides that inhibited ACE activity by 50% (IC_{50}) was calculated using a non-linear regression from a plot of ACE inhibition versus sample concentrations.

Table 2 Parameters for molecular docking experiments performed with the LibDock of DS3.5

Parameter name	Parameter value
Docking sphere	10 Å
Input site sphere	
x	48.65
y	82.55
z	54.04
Number of HPTPot	100
Docking tolerance	0.25
Docking preference	User specified
Max hits to save	10
Max number of hits	100
Minimum LibDock score	100
Final score cutoff	0.5
Max BFGS steps	50
Rigid optimization	False
Max conformation hits	30
Max start conformations	1,000
Steric fraction	0.10
Final cluster radius	0.5
Apolar SASA cutoff	15.0
Polar SASA cutoff	5.0
Surface grid steps	18
Conformation method	Best
Minimization algorithm	Do not minimize
Parallel processing	True

Study on structural-active relationship of ACE inhibitory peptides

The chemical properties of C-terminal and N-terminal amino acids of 99 peptides with estimated IC_{50} less than 50 μM were summarized to deduce the structural-active relationship of ACE inhibitory peptides.

Results and discussion

Pool of di- and tri-peptides derived from *P. esculenta* proteins

Pepsin, trypsin, and the mixture of pepsin and trypsin were used to virtually hydrolyze the 22 proteins from *P. esculenta*, with the help of BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep). In total, 2,667 peptides were virtually produced, and among them, 1,084 were di-

and tri-peptides, which accounted for about 40.6% (Figure 1). After excluding the repeated ones, there were 1,017 non-repeated peptides, among which 284 were di- and tri-peptides. The sequences and the frequencies of these 284 short peptides are shown in Table 3. These 284 peptides were used as the ligands for docking experiment with ACE.

Estimated IC_{50} distribution of ACE inhibitory di- and tri-peptides

The estimated ACE inhibitory IC_{50} of the 284 di- and tri-peptides derived from *P. esculenta* proteins were obtained according to LibDock scores, which were summarized in Figure 2. Ninety-nine (99) peptides had an estimated IC_{50} less than 50 μM (34.9% of 284 peptides), 100 peptides had an estimated IC_{50} between 50 and 100 μM (35.2% of 284 peptides), and 37 peptides had an estimated IC_{50} less than 500 μM, accounting for 13.0%. Most reported ACE inhibitory peptides with IC_{50} less than 100 μM showed potent *in vivo* anti-hypertensive activity [39]. Therefore, *P. esculenta* is a prospective anti-hypertensive peptide-containing resource since more than two thirds di- and tri-peptides theoretically possess obvious ACE inhibitory activity. The sequences and estimated IC_{50} of di- and tri-peptides with estimated IC_{50} less than 50 μM are shown in Table 4.

Short peptides were usually used for predicting potent ACE inhibitory activity. Pripp docked 58 di-peptides into protein target using the Molegro Virtual Docker version 1.1.1 software and found significant relationship between docking results and experimental IC_{50} values [32]. Several tri-peptides consisting of I or L and positive charged amino acids and aromatic amino acids were synthesized, and their ACE inhibitory activities were measured to clarify the amino acid sequence for inhibition of ACE [40]. Larger peptides, for instance, the sequence length more than 5, were also focused in some work [41]; however, such works were reported with lower R^2 (coefficient of variation) because of the complexity in the modeling due to the bigger peptide [42,43].

Confirmation of virtual screening method

In order to confirm the validity of virtual screening method of the present work, nine peptides were synthesized for IC_{50} testing. The sequences of these peptides were obtained from *P. esculenta* protein through virtual hydrolysis and screening by docking experiments. The estimated $\log(1/IC_{50})$ and measured $\log(1/IC_{50})$ of the nine peptides were compared (Table 5). The error between the estimated $\log(1/IC_{50})$ and measured $\log(1/IC_{50})$ is less than 1.0 unit. Desirable limit for model is that the error between estimated $\log(1/IC_{50})$ and measured $\log(1/IC_{50})$ is less than 1.5 units [30]. A reported quantitative structure-activity relationship (QSAR) model was constructed on 168 di-peptides and 140 tri-peptides collected from literatures, and the model verification was made on seven reported di-peptides and tri-peptides (not included in 168 di-peptides and 140 tri-peptides), of which the error was between 0.07 and 1.39 [29]. On the ground of such criterion, the present model is efficient and credible.

Previous studies suggested that the structural-active relationship of ACE inhibitory peptides largely depended on their amino acid composition, sequence, and configuration, though the full mechanism of interaction between peptides and ACE is not established so far [44,45]. For the short peptides as di- and tri-peptides, the amino acid composition and configuration are more significant. The di- and tri-peptides which have an estimated IC_{50} within 50 μM were used to study the structural-active relationship of these ACE inhibitors.

There are four kinds of C-terminal residues for 99 sequences (Figure 3) due to the cutting specificity of pepsin

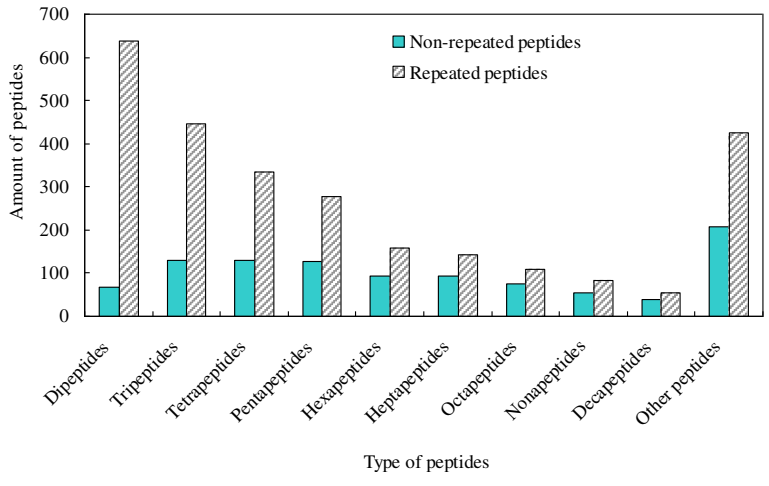

Figure 1 Length distribution of peptides derived from *P. esculenta* proteins by virtual hydrolysis. The enzymes used in virtual hydrolysis are pepsin, trypsin, and a mixture of pepsin and trypsin.

Table 3 Sequence and frequency of di- and tri-peptides derived from *P. esculenta* proteins by virtual hydrolysis

Peptide	Frequency	Peptide	Frequency	Peptide	Frequency	Peptide	Frequency
ADL	1	GYF	2	NDK	2	STL	2
AEF	2	HAQ	2	NF	10	SVF	2
AEK	1	HER	1	NGK	1	SVL	4
AEL	2	HF	9	NIL	2	SWK	2
AF	19	HGL	2	NK	2	SWL	2
AGF	2	HIK	2	NL	13	SYL	1
AIF	4	HK	4	NLK	1	TAL	2
AK	2	HKF	1	NMR	1	TDK	1
AL	35	HL	17	NPF	6	TDR	1
ALR	1	HSL	2	NR	2	TEF	2
AMF	2	HTK	1	NRF	2	TF	7
APF	2	HTL	1	NSF	2	TGF	2
AQF	2	IAR	2	NTL	3	TGL	4
AR	2	ICL	4	NVL	5	TIL	2
ASK	1	IF	20	NVR	2	TK	4
AVK	2	IGR	1	NWL	2	TL	31
AVR	2	IHR	2	PCK	1	TLK	1
AYA	2	IIL	4	PDL	2	TML	2
AYF	3	IK	7	PF	19	TNR	1
CF	2	IL	46	PGF	2	TPF	1
CK	1	ILK	1	PIL	2	TSL	4
CL	9	IMF	2	PK	7	TTK	1
CVF	2	IMK	2	PKL	1	TVK	1
DF	4	IPK	2	PL	30	TVL	2
DK	16	IPL	5	PNK	2	TVR	1
DL	11	IQK	2	PPL	2	TWK	1
DMF	2	IR	4	PRL	1	TYF	2
DNR	1	IRF	1	PSF	2	VAL	6
DPK	1	ISF	2	PSK	1	VDL	2
DR	4	ISL	2	PSL	2	VEK	3
DSK	2	ISR	1	PTL	4	VER	2
DSL	4	ITK	1	PTR	2	VF	9
DWL	1	ITL	2	PVK	1	VGF	6
EAF	2	IVL	1	PVL	2	VGL	3
EAR	1	IWL	4	QAL	2	VIR	3
EDK	2	KAL	1	QDF	2	VK	2
EEF	1	KDL	2	QEL	2	VKL	1
EEL	2	KF	2	QF	5	VL	14
EER	1	KGF	1	QGL	2	VMK	2
EF	2	KIL	1	QIR	1	VML	2
EGL	1	KL	4	QK	2	VNL	4
EIF	1	KPL	1	QL	7	VPK	1
EIL	1	KRF	1	QR	1	VPL	4
EK	7	KSL	1	QYK	1	VSF	2

Table 3 Sequence and frequency of di- and tri-peptides derived from *P. esculenta* proteins by virtual hydrolysis *(Continued)*

EL	16	KTL	1	RAL	1	VSL	3
EML	1	KVF	1	REL	1	VVF	2
ENK	2	LDK	1	RL	3	VVK	2
ENL	6	LEK	1	RPF	1	WAF	2
ER	2	LK	2	RSF	2	WAL	2
ESK	1	LMK	1	RTL	1	WCF	2
ETL	3	LR	1	SAL	4	WF	6
EVK	1	LSK	1	SEK	1	WGK	2
EVL	4	MAL	8	SF	20	WL	8
FFK	1	MF	16	SGF	4	WML	2
FK	1	MFK	1	SGL	2	WNF	2
FWR	1	MFR	1	SHL	2	WPF	2
GAL	2	MGF	2	SIF	2	WQK	2
GF	12	MGL	2	SIK	2	WR	1
GGL	4	MIK	2	SIL	4	WTR	1
GGR	2	MIL	2	SK	6	WWF	2
GK	6	MK	10	SL	50	YAL	1
GKF	1	MKF	2	SNL	3	YF	7
GL	29	ML	16	SPF	2	YIF	2
GLR	1	MPL	1	SPL	2	YIK	1
GNL	2	MR	4	SQL	2	YK	4
GR	3	MSK	2	SR	2	YL	8
GSL	2	MSL	10	SS	2	YPL	2
GTL	4	MTK	2	SSF	6	YS	2
GTR	2	MTL	2	SSL	2	YSK	3
GVK	1	MVK	2	STF	2	YTL	2
GWL	2	NAL	2	STK	1	YVR	1

The enzymes used in virtual hydrolysis are pepsin, trypsin, and a mixture of pepsin and trypsin.

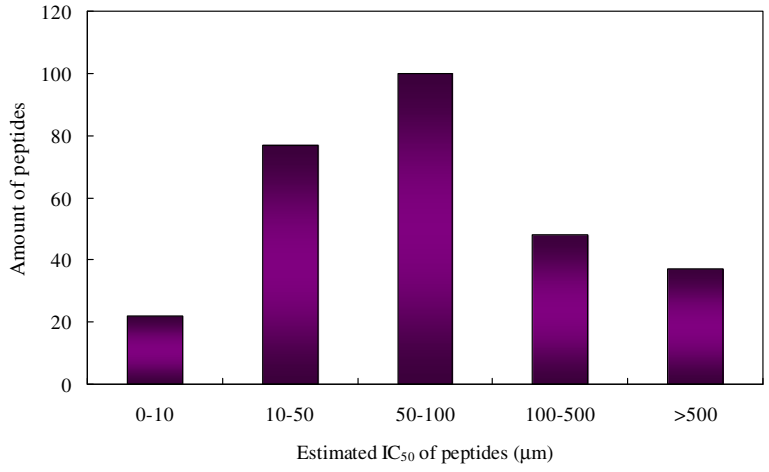

Figure 2 Distribution of estimated IC_{50} of di- and tri-peptides with ACE inhibitory activity. The peptides were derived from *P. esculenta* proteins. All IC_{50} values were predicted by LibDock scores according to the equation, LibDock score = 10.063 log(1/IC50) + 68.08.

Table 4 Di- and tri-peptides derived from *P. esculenta* with estimated IC_{50} less than 50 μM

Peptide	Estimated IC_{50}	Peptide	Estimated IC_{50}	Peptide	Estimated IC_{50}
WNF	0.12	YIF	13.1	DSL	27.9
HKF	0.42	RPF	13.3	IR	28.9
WCF	0.47	APF	13.4	TGF	29.5
WPF	0.85	REL	13.7	NWL	30.7
WWF	1.18	YK	13.9	FFK	30.9
YVR	1.45	AEF	14.1	GYF	32.2
NRF	1.71	AYF	14.7	ISF	32.3
TYF	1.75	KTL	14.8	FK	33.2
IHR	2.06	WGK	14.9	SPF	33.4
WML	2.15	DWL	14.9	VML	33.7
WAF	3.27	SSF	15.0	KPL	36.1
IWL	5.22	NSF	15.4	ISL	36.5
RSF	5.44	VKL	16.1	QF	36.7
IRF	5.72	MAL	16.3	TML	36.9
CVF	7.15	NR	17.0	QEL	37.0
YIK	7.33	MTL	17.1	MKF	38.0
MGF	7.38	IMF	17.5	YAL	38.6
YF	7.48	WR	17.7	EEF	40.4
KRF	8.11	ER	18.1	IGR	40.5
SR	9.05	AEL	19.4	TEF	41.1
GNL	9.09	QDF	19.5	TTK	41.6
GKF	9.51	YTL	19.7	SGF	41.6
WTR	10.2	PIL	19.9	KVF	42.0
PRL	10.2	KDL	20.2	SIL	43.0
SYL	10.4	SVF	20.8	EVL	43.7
WF	10.6	YSK	21.5	DMF	46.6
IIL	10.8	VVF	22.5	RTL	46.9
WAL	11.3	ITK	22.6	QAL	47.0
AQF	11.5	DR	23.4	TPF	47.0
RAL	11.8	WQK	23.9	HSL	47.1
PGF	12.2	PKL	24.9	NAL	47.1
NVL	12.7	EIF	25.2	SHL	48.0
HTL	13.1	VGF	27.8	STF	49.4

and trypsin. Leu and Phe are C-terminal residues formed by pepsin hydrolysis, and C-terminal Lys and Arg are formed by trypsin reaction. Hydrophobic C-terminal (Phe and Leu) is dominant in amount and accounts for more than 80% peptides (44.4% and 36.4%, respectively). There are some accepted concepts about the structure-activity relationship of ACE inhibitory peptides, such as that peptides with hydrophobic amino acid in C-terminus showed a highly potent ACE inhibitory activity [46]. Highly active peptide in general should be composed of large, hydrophobic, and aromatic amino acid with a polar functional group in C-terminus [47]; and the physicochemical attributes of amino acids such as hydrophobicity, bulkiness, and electronic properties had impacts on the bioactivity of peptides [48]. Accordingly, benzene ring in Phe can also increase the bulkiness and bring about the stability of binding between ACE and peptide and sequentially result in high ACE inhibitory activity.

Table 5 Estimated and measured log(1/IC_{50}) of the nine synthesized peptides derived from *P. esculenta*

Peptide	Estimated log(1/IC_{50})	Measured log(1/IC_{50})	Error
GYF	4.49	4.31 ± 0.02	0.18
FK	4.79	4.27 ± 0.01	0.52
WAL	4.95	4.38 ± 0.04	0.57
QF	4.44	4.21 ± 0.03	0.23
AYF	4.83	4.18 ± 0.01	0.65
EL	3.19	3.43 ± 0.02	−0.24
GLR	4.07	3.61 ± 0.02	0.46
HK	3.53	3.86 ± 0.01	−0.33
ILK	4.09	3.72 ± 0.03	0.37

There are 40 peptides among 99 peptides (40.4%) with hydrophobic amino acid at N-terminal, 38 peptides with neutral amino acid at N-terminal (38.4%), and 21.9% peptides with positively or negatively charged amino acid at N-terminal (Figure 4). N-terminal amino acid of ACE inhibitory peptides also favors the hydrophobic interactions with ACE [7,30]. The peptides with hydrophobic amino acid at N-terminal showing higher ACE inhibitory activity have some superiority in amount in the present study, which verified such view.

Conclusions

A virtual method of hydrolysis and screening of ACE inhibitory peptides with high activity such as IC_{50} value < 50 μM was constructed in this work. Ninety-nine (99) peptides were obtained from 22 proteins of *P. esculenta*. Besides, the efficiency and the validity of such method were verified by comparing the predicted IC_{50} and measured

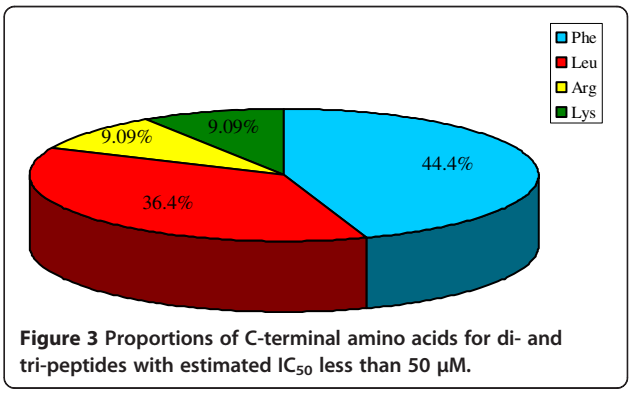

Figure 3 Proportions of C-terminal amino acids for di- and tri-peptides with estimated IC_{50} less than 50 μM.

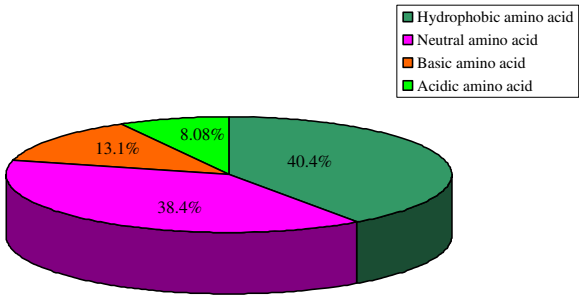

Figure 4 Proportions of N-terminal amino acids for di- and tri-peptides with estimated IC_{50} less than 50 μM.

IC_{50} of some synthesized peptides among the 99 peptides. The results demonstrated that the virtual hydrolysis and screening method is an efficient way that greatly cuts down the experimental labor to get highly active ACE inhibitory peptides. Moreover, *P. esculenta* proteins were proved as a good resource of ACE inhibitory peptides, which could be a beneficial ingredient for functional foods or pharmaceuticals against hypertension. Further research on larger anti-hypertension peptides derived from *P. esculenta* and *in vivo* activity testing will be carried out.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
YL carried out the establishment of pool of di- and tri-peptides derived from *P. esculenta* proteins and molecular docking experiments. MG carried out the measurement of ACE inhibitory activity. JX designed the study and revised the manuscript. HW performed the statistical analysis and partly revised the manuscript. LZ participated in part of the method establishment. DW conceived of the study and participated in the design and coordination. All authors read and approved the final manuscript.

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Author details
[1]State Key Laboratory of Bioreactor Engineering, Department of Food Science and Engineering, East China University of Science and Technology, 130# Meilong Rd., P.O. Box 283, Shanghai 200237, People's Republic of China. [2]Zhejiang Key Lab of Exploitation and Preservation of Coastal Bio-resource, Wenzhou 325005, People's Republic of China.

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