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ACE inhibitory effect of the protein hydrolysates prepared from commercially available nori product by pepsin–
trypsin digestion

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Abstract

We studied angiotensin I converting enzyme (ACE) inhibitory peptides in the protein hydrolysates of commercially available nori products that contain *Pyropia pseudolinearis* as the main ingredient. The water extract of the nori product consisted mainly of phycobiliproteins and RubisCO. The proteins in the aqueous extracts were sequentially hydrolyzed with pepsin and trypsin, and the peptides in the pepsin-trypsin digests were fractionated by reversed-phase HPLC. As a result, twelve ACE inhibitory peptides containing ten novel peptides were identified. These peptides are suggested to have originated from the α - and β -subunits of phycobiliproteins and the large subunits of RubisCO of *P. pseudolinearis*. The interactions of eight peptides (ALR, FAR, FSR, FDR, EVYR, AYR, GRP, and MVT) with ACE were then simulated using the flexible docking tool Auto Dock Vina. The results showed that all peptides interacted with the active center of ACE, and their docking scores ranged from -6.8 to -10.2 kcal/mol. In addition, we synthesized four peptides (AYR, FAR, EVYR, and GRP) and measured the IC₅₀ values of these peptides for ACE. Consequently, FAR and GRP showed considerably low IC₅₀ values (0.29 μ mol and 0.45 μ mol, respectively) in addition to other ACE inhibitory peptides. Moreover, FAR, which is specific to the nori product, was predicted to bind to the S1, S1', and S2' subsites of the catalytic center of ACE. Therefore, it can be expected that daily intake of "nori products" may have a positive effect on the prevention of hypertension.

Keywords Red algae • Nori products • ACE inhibitory activity • Antihypertension • Docking simulation • Phycobiliprotein

Introduction

The World Health Organization (WHO) has reported that 1.13 billion people worldwide have hypertension and one of the global goals for noncommunicable diseases is to reduce the prevalence of hypertension by 25% between 2010 and 2025 [1]. This is because hypertension (higher than 140/90 mmHg) significantly increases the risk of cardiovascular diseases (CVD) such as atherosclerosis, coronary heart disease, stroke, and heart failure [2].

The first drugs introduced for the treatment of hypertension were the inhibitors that target a key enzyme in the regulation of blood pressure: angiotensin I converting enzyme (ACE, EC 3.4.15.1). This enzyme was first reported by Skeggs et al. (1956) and is called a “hypertensin-converting enzyme” [3]. The basic function of ACE is to regulate blood pressure by degrading angiotensin I and bradykinin in the renin-angiotensin and kinin-kallikrein systems, respectively. ACE is a dicarboxypeptidase (molecular weight 147 kDa) with a zinc ion in the active center. In the renin-angiotensin system, angiotensinogen, the precursor of angiotensin I, is produced and secreted by the liver and enlarged fat cells. Angiotensinogen is hydrolyzed by renin, which is a proteolytic enzyme secreted by the paraglomerular cells of the kidney, to produce angiotensin I (DRVYIHPFHL). Angiotensin I is converted to angiotensin II (DRVYIHPF) by ACE mainly in the pulmonary circulation. In other words, ACE hydrolyzes the dipeptide His-Leu on the C-terminal side of angiotensin I, and the resulting angiotensin II causes a strong increase in blood pressure.

On the other hand, since antihypertensive drugs are often associated with side effects, ACE inhibitory peptides derived from food materials have recently been considered desirable for the prevention of hypertension [4–6]. From this viewpoint, many reports on ACE inhibitory peptides derived from food materials have been published [7,8], and some include peptides from macroalgae [9–12]. We have also conducted several investigations on ACE inhibitory peptides from marine red algae, especially the relationship between their structures and the original proteins [13–17]. However, little research has been done on the ACE inhibitory and blood pressure lowering effects of commercially available seaweed products.

Pyropia pseudolinearis is a red alga belonging to Bangiophyceae and thrives on rocks in the tidal zone during winter and spring in Japan. It is called "Uppurui-nori", has long been considered a luxury food and is an industrially important species in Japan [18]. In a previous report, we extracted water-soluble proteins (WSPs) from *P. pseudolinearis* and found that the main components were phycobiliproteins and ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO). Then, we prepared a protein hydrolysate using thermolysin and identified 42 ACE inhibitory peptides, including three novel peptides, in the hydrolysate. These findings suggest that *P. pseudolinearis* has the potential not only as a protein source but also as an ingredient for supplements and functional foods for humans. Therefore, in this study, we assessed the ACE inhibitory effect of a pepsin-trypsin digest of the WSPs prepared from a commercially available nori products.

Materials and methods

Materials

A commercially available nori products (high class of sheet laver "Iwa-Nori") made in Matsumae-town, Hokkaido, Japan was purchased from a food shop in Kikonai, Hokkaido, Japan in January 2017. ACE from rabbit lungs was purchased from Sigma Chemical Co. (Mo, USA). Millex-GV (pore size: 0.22 μm) and Millex-LG (pore size: 0.20 μm) were purchased from Merck Millipore Ltd. (Darmstadt, Germany). Synthetic peptides (purity: >99%) were purchased from Medical Biological Laboratories Co. (Nagoya, Japan). Hyppuryl-L-histidyl-L-leucine (Hip-His-Leu), pepsin (EC 3.4.23.1) from porcine stomach, trypsin (EC 3.4.21.4) from bovine pancreas, trifluoroacetic acid (TFA), α -cyano-4-hydroxycinnamic acid (α -CHCA), Coomassie Brilliant Blue (CBB) R-250, and all other reagents were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan).

Preparation of nori peptide

As shown in Fig.1, the WSPs from nori products were prepared according to the same method as in our previous paper [19]. The WSPs were suspended in 0.1 M HCl solution and digested by 1.0 wt% porcine pepsin at 37 °C for 3 h. After the reaction, the solution was adjusted to pH 8.0. Then, the pepsin hydrolysate was redigested by 1.0 wt% bovine trypsin at 37 °C for 3 h. The enzyme reaction was stopped by heating at 100 °C for 5 min. The reacted solution was centrifuged at 4 °C and 15,000 g for 10 min, and the supernatant was lyophilized into nori peptide (Fig. 1). Visible light absorption spectra of the WSPs were measured in the range of 350-700 nm with a data interval of 2 nm using a UV-1800 spectrophotometer (SHIMADZU, Kyoto, Japan). The amount of phycobiliproteins was determined by the spectra using the following equations: phycocyanin (PC) = $[\text{OD}_{620} - 0.70 (\text{OD}_{650})]/7.38$; allophycocyanin (APC) = $[\text{OD}_{650} - 0.19 (\text{OD}_{620})]/5.65$; phycoerythrin (PE) = $[(\text{OD}_{565}) - 2.80 (\text{PC}) - 1.34 (\text{APC})]/12.7$ [20]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli's method using a 0.1% SDS-13.75% polyacrylamide slab gel [21]. The proteins were stained with 50% methanol-7% acetic acid containing 0.1% CBB R-250, and the background was decolorized with 7% acetic acid. The fluorescence of the phycobiliprotein on the slab-gel was detected by VISIRAYS AE-6935GN (ATTO, Tokyo, Japan).

Isolation of ACE inhibitory peptides from nori peptide

ACE inhibitory peptides were identified by reversed-phase HPLC. The nori peptide was dissolved (20 mg/mL) in ultrapure water containing 0.1% TFA and applied to sequential filtration by Millex-GV and Millex-LG. The filtered sample (100 µL) was applied to a reversed-phase column (Mightysil RP-18 Aqua, φ10 mm×250 mm) (Kanto Kagaku, Tokyo, Japan), and peptides were eluted with a linear gradient of acetonitrile (1–35% acetonitrile containing 0.1% TFA at a flow rate of 4.73 mL/min). Absorbance of the eluent was monitored at 228 nm. The eluate per minute (4.73 mL) was collected in a test tube. The fractions eluted between 10 and 40 minutes (designated fraction numbers 10–40) were subjected to an ACE inhibitory assay. The amino acid sequences of the ACE inhibitory peptides were analyzed by MALDI-TOF/MS/MS using a 4700 Proteomics Analyzer mass

spectrometer with *DeNovo* Explorer ver. 3.6 (Applied Biosystems, CA, USA). Information on the reported ACE inhibitory peptide was obtained from the BIOPEP-UWM database [22] on 10 September 2021.

Assay of ACE inhibitory activity

The assay was carried out with the same method as described in a previous paper [19]. The inhibition was calculated from the equation: ACE inhibitory activity (%) = $[1 - (As - Asb)/(Ac - Acb)] \times 100$, where Ac is the absorbance of the buffer, Acb is the absorbance when the stop solution was added to the buffer before the reaction, As is the absorbance of the sample, and Asb is the absorbance when the stop solution was added to the sample before the reaction. We defined the IC₅₀ as an absolute quantity of peptide to inhibit 50% of 1.0 U ACE. The ACE inhibitory activities of WSPs, WSPs hydrolysate, and synthetic peptides were measured in triplicate, and each mean $\pm$ standard error was calculated. Statistical analyses were carried out using a Student's t test.

Docking simulation of ACE inhibitory peptide in the active site of human ACE

The structure of the purified peptide was constructed using PyMOL builder software (The PyMOL Molecular Graphics System, Ver. 2. Schrödinger, LLC.). The structure was optimized based on modified molecular mechanics that consider bond stretching, angle bending, internal rotation and van der Waals nonbonded interactions. The structure of human ACE in complex with lisinopril (1O86.pdb) was derived from the RCSB PDB Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>). Before docking, water molecules and lisinopril were removed. Docking was performed using the flexible docking tool AutoDock Vina (TSRI, USA). The docking runs were carried out with coordinates x: 39.3, y: 34.5 and z: 43.4. The best-ranked docking pose of the peptide in the active site of ACE was obtained according to the scores and binding energy value. The 3-D molecular docking result was output by PyMOL, and the 2-D molecular docking result was output by LigPlot⁺ ver. 2.2 in EMBL-EBI [23].

Results and discussion

Protein composition of nori WSPs and ACE inhibitory activity of nori peptide

Spectral analysis was carried out for WSPs prepared from the nori product. As shown in Fig. 2a, the maximum absorption peak was indicated at approximately 560 nm, followed by 500 nm, 615 nm, and 655 nm. The absorption at 560 nm comes from phycoerythrobilin, which binds to PE. The absorptions at 500, 615 and 655 nm are due to phycourobilin of PE, phycocyanobilin of PC and phycocyanobilin of APC, respectively [24]. From this absorption spectrum, it was calculated that 49% of the phycobiliproteins were PE, 34% were PC, and 17% were APC (Fig. 2b). The nori WSPs were then subjected to SDS–PAGE, and the molecular weights of the main components were approximately 20 kDa and 50 kDa (Fig. 2c). Phycobiliproteins of red algae commonly contain α - and β -subunits. and each subunit binds one or several phycobilin chromophores at the specific Cys residues through thioether linkage (PE: α Cys82, α Cys139, β Cys50, β Cys61, β Cys82, β Cys158; PC: α Cys84, β Cys82, β Cys153; APC: α Cys81, β Cys81) [25–27]. In the previous study, we determined the primary structures of phycobiliproteins from *P. pseudolinearis* and clarified the molecular weights of α - and β -subunits of them (PE α : 1,7697.9; PE β : 1,8423.1; PC α : 1,7463.8; PC β : 1,8170.6; APC α : 1,7509.0; APC β : 1,7484.0). In addition, the α - and β -subunits of these phycobiliproteins retained the Cys residues, which bind phycobilin chromophores, at the corresponding positions. In addition, our previous study revealed that the protein with 55,000 MW in the WSPs of red algae was the RubisCO large subunit [17]. Based on these findings, it is considered that the 20 kDa component consists of the α - and β -subunits of phycobiliprotein because of its fluorescence, and the 50 kDa component is the large subunit of RubisCO. These protein compositions of the nori WSPs are almost the same as those of the WSPs from *P. pseudolinearis* in our previous report [19].

On the other hand, no protein bands were detected in the nori peptide, confirming that it was degraded into small peptides by pepsin-trypsin digestion (Fig. 2c). The ACE inhibitory activity of the nori WSPs was increased

by pepsin-trypsin treatment (Fig. 2d). The results suggest that ACE inhibitory peptides derived from phycobiliproteins and RubisCO are probably generated in the gastrointestinal tract when we eat the nori product.

Identification of ACE inhibitory peptides in the nori peptide

To identify ACE inhibitory peptides derived from the nori products by pepsin-trypsin digestion, the nori peptide was subjected to reversed-phase HPLC (Fig. 3a), and the inhibitory activities of the eluted fractions (Nos. 10–40) were measured (Fig. 3b). The peptide structures in these fractions showing inhibitory activity were examined by MALDI-TOF/MS/MS, and twelve sequences (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, AYR, FVSGQR, LSNCELQAINGR, GRP, MVT, and DGEILR) were obtained (Table 1). These twelve sequences were checked in the BIOPEP-UWM database [22], and ten of these (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, FVLSGQR, LSNCELQAINGR, MVT, DGEILR) were confirmed to be novel peptides that had not been registered. As shown in Table 1, all these sequences were found in the primary structures of the α - and β -subunits of phycobiliproteins and large subunits of RubisCO from *P. pseudolinearis*. From the results, it is strongly suggested that ACE inhibitory peptides are generated from the α - and β -subunits of phycobiliproteins and large subunits of RubisCO, which are the major proteins in the nori product after pepsin-trypsin digestion. In this study, the nori peptide was prepared by sequential digestion of WSPs with pepsin and trypsin. Thus, peptides with Arg or Lys residues at the C-terminus may be produced. In fact, ten of the twelve ACE inhibitory peptides had Arg residues at their C-termini. It is well known that the effectiveness of ACE inhibitory peptides is closely related to chain length, amino acid composition, and sequence [28]. For example, the presence of positive charges of Arg (guanidino group) and Lys (ϵ -amino group) in the C-terminal residues has been reported to contribute to the inhibitory efficacy [29,30]. It has also been reported that the removal of the C-terminal Arg residue decreases ACE inhibitory activity [31,32]. Therefore, the peptides mentioned above were also expected to exhibit high ACE inhibitory activity. Then, we examined the ACE inhibitory activity of these peptides.

ACE inhibitory activity of representative nori peptides

In general, ACE inhibitory peptides are often low molecular weight peptides with two to six amino acids. For instance, crystallographic studies have shown that large peptides are unable to bind to the active site of ACE. [33]. In research on yak milk casein hydrolysates, hepta- and octa-peptides did not show ACE inhibitory activity, and the authors speculated that the spatial structure of these peptides may have been too large to fit into the active site [34]. Based on these findings, we selected eight peptides (ALR, FAR, FSR, FDR, EVYR, AYR, GRP, and MVT) from the twelve peptides identified in the preceding paragraph, and the binding energy (docking score) between these peptides and human ACE was then simulated using an AutoDock Vina. The results showed that all peptides docked with the active site of ACE, and their docking scores were relatively low (ranging from -7.6 to -10.2 kcal/mol) except for MVT (-6.8 kcal/mol) (Table 2). This result is presumably due to the C-terminal Arg residue as described before. In addition, the C-terminus of GRP is a Pro residue. There are also many reports of peptides with high ACE inhibitory effects that contain aromatic amino acids and Pro at the C-terminus [29–31,35]. However, few of the peptides with high ACE inhibitory effects reported thus far had Thr residues at the C-terminus, and the docking score of MVT was higher than those of other peptides. In addition, the underlined parts of the peptides in Table 2 indicate the sequences that are registered as ACE inhibitory peptides in the BIOPEP-UWM database [22]. Therefore, considering this information, we synthesized AYR, EVYR, FAR, and GRP and measured the ACE inhibitory activities of these peptides. Consequently, FAR had the lowest IC₅₀ value (0.29 μmol), followed by GRP (0.45 μmol), EVYR (5.9 μmol), and AYR (8.7 μmol), and the ACE inhibitory effects of these nori peptides were comparable to those of the peptides from *P. pseudolinearis* and dulse, except for LRY (IC₅₀: 0.044 μmol) (Table 3). The sequence of LRY is also present in the primary structures of the β-subunits of PE, PC, and APC from *P. pseudolinearis*, and LRY was isolated from the WSPs hydrolysate of *P. pseudolinearis* by thermolysin digestion [19]. However, LRY was not detected in this study, which may be due to pepsin-trypsin digestion.

Predicted binding mode between the ACE inhibitory peptide and the active site of human ACE

ACE is a dipeptidyl carboxypeptidase and is a type of metalloproteinase with a zinc binding motif (HExxH) in its active center, where His383, His387 and Glu411 residues form zinc binding ligands [28,36]. There are two different types of ACE, somatic ACE (sACE) and testicular ACE (tACE) [28]. sACE is widely distributed in cells throughout the body and is composed of two catalytic domains (N- and C-domains) and each domain has different properties such as reactivity to substrates [28,36]. Angiotensin I is mainly hydrolyzed by the C-domain of sACE, whereas the N-domain is responsible for the degradation of bradykinin [36]. When the catalytic activity of the N-domain is inhibited and bradykinin accumulates, side effects such as dry cough occur [37]. Considering the risk of this side effect, an ACE inhibitor (peptide) that specifically inhibits the catalysis of the C-domain without the accumulation of bradykinin would be ideal. On the other hand, tACE, which is expressed in germinal cells of the testis, has only one domain that is almost the same primary structure as that of the C-domain of sACE, except for a unique 36-residue sequence constituting its amino terminus [28,36]. Therefore, tACE is often used to analyze the interaction between ACE inhibitory peptides and the active center of human ACE. In this study, two peptides, FAR and GRP, with appreciably high ACE inhibitory activity were detected in the nori peptide. However, as shown in Table 2, GRP was found in sardine muscle hydrolysate and has already been registered in the database as an ACE inhibitory peptide [38]. Hence, we analyzed the binding mode of FAR, specific in the nori product, to the catalytic center of tACE.

As shown in Fig. 4, the N-terminal amino group of FAR was predicted to hydrogen bond with the carboxyl group of Asp453 residue of tACE: their interatomic distance was calculated to be 3.11 Å, and the carbonyl group of the N-terminal Phe residue interacted with the amide group of Asn281 residue (interatomic distance: 3.24 Å). In addition, the C-terminal carboxyl group of FAR was related to the amide group of Asn281 (2.93 Å), the ε-amino group of Lys511 (2.87 Å), and the hydroxy group of Tyr520 (2.89 Å). Furthermore, the guanidino group of the C-terminal Arg connected with the hydroxy group of Tyr523 (3.07 Å), the carboxyl group of Glu384 (3.12 Å) and the carbonyl group of Ala354 (3.34 Å), and the 2-amino group of the C-terminal Arg also interacted with the carboxyl group of Glu384 (3.11 Å) and the carbonyl group of Ala354 (3.16 Å). The guanidino group of the C-

terminal Arg also formed a salt bridge with the carboxyl group of Glu384. On the other hand, tACE has three major catalytic subsites: S1, S1', and S2'. Glu143, Ser355, Ser516, and Val518 have been reported to be present in S1, Glu162, Thr166, Asn277, Ser284, His353, Glu372, Asn374, Glu376, Glu376, Asp377, Val380, and His513 in S1', Gln281, Thr282, Ser284, Glu376, Val379, Val380, Asp415, Ala418, Asp453, Lys454, Phe457, Lys511, Tyr520, Tyr523, Phe527, and Gln530 in S2' of tACE [39,40]. On the C-domain of sACE, Ala354, His353, Ser355, Val518, Pro519, and Arg522 are present in S1, Glu162, Ala354, Trp357, Asp377, Val380, Phe512, His513, and Val518 in S1', and Val379, Val380, Asp415, Asp453, Phe457, Phe519, Tyr523, Phe527, and Phe547, in S2' [41]. Based on these findings, the N-terminal Phe residue of FAR can be inferred to be located in the S2' subsite, the Ala residue in S1', and the C-terminal Arg residue in S1.

Conclusions

Since antihypertensive drugs are often associated with side effects, ACE inhibitory peptides derived from food materials have recently been considered desirable for the prevention of hypertension. In this study, we assessed the ACE inhibitory effect of a pepsin-trypsin digest (nori peptide) of WSPs prepared from a commercially available nori product. As a result, we identified novel peptides (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, FVLSGQR, LSNGLQAINGR, MVT, and DGEIILR) that are derived from phycobiliproteins and RubisCO. Among these, FAR showed considerably low IC₅₀ values (0.29 μ mol) and was predicted to bind to the S1, S1', and S2' subsites of the catalytic center of tACE. Therefore, it was expected that daily intake of "nori products" may have a positive effect on the prevention of hypertension. We are convinced that the results obtained in this study will be useful towards verifying the effects of nori products on human blood pressure (human intervention trial).

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(Captions to figures)

Fig. 1 Preparation of nori peptide.

WSPs: water-soluble proteins.

Fig. 2 Protein composition of WSPs and the ACE inhibitory effect of nori peptide.

a) Visible light absorption spectrum of the WSPs. b) Composition of phycobiliprotein in WSPs. c) SDS-PAGE.

1: Molecular weight marker, 2: WSPs (CBB R-250 staining), 3: WSPs (fluorescent photography), 4: nori peptide (CBB R-250 staining), 5: nori peptide (fluorescent photography). d) ACE inhibitory activity.

Fig. 3 Separation of ACE inhibitory peptides in the nori peptide.

a) nori peptide was separated by reversed-phase HPLC. The eluate per minute (4.73 mL) was collected in a test tube. b) ACE inhibitory activity of each fraction.

Fig. 4 Predicted binding model of FAR to the catalytic site of tACE (1O86.pdb).

a) 3-D molecular docking result. FAR are shown as a stick model, with backbone, nitrogen, oxygen, and hydrogen shown in magenta, blue, red, and white, respectively. Side chains of amino acid residues that form hydrogen bonds with FAR are shown as light purple sticks. Side chains of amino acid residues that form the catalytic pocket are shown as gold sticks. Zn^{2+} is shown as a gray sphere. Hydrogen bonds are shown as light blue dotted lines. b) 2-D molecular docking result. FAR is shown as a solid purple stick, with carbon, nitrogen, and oxygen shown in black, blue, and red, respectively. Side chains of amino acid residues that form hydrogen bonds with FAR are shown as orange sticks. Hydrogen bonds are shown as green dotted lines with their interatomic distance ($\text{\AA}$). The salt bridge is shown as a red dotted line.

Table 1 Identified ACE inhibitory peptide sequences in Nori peptide

Fraction No.	m/z	Peptide	Original protein
13	359.33	ALR	RubisCO L ^{a)}
20	393.19	FAR	PE α ^{b)}
20	409.18	FSR	PE β ^{b)}
20	437.18	FDR	PC β ^{c)}
20	566.26	EVYR	PE α ^{b)}
20	903.42	SLTNNAQR	PC α ^{c)}
28	409.20	AYR	RubisCO L ^{a)}
28	806.44	FVLSGQR	APC α ^{d)}
28	1,271.60	LSNGELQAINGR	PC α ^{c)}
30	329.08	GRP	RubisCO L ^{a)}
30	349.91	MVT	PC α ^{c)}
30	815.40	DGEHLR	PE β ^{b)}

a) DDBJ accession No. LC638845, b) DDBJ accession No. LC599086, c) DDBJ accession No. LC599087, d)

DDBJ accession No. LC599088.

Table 2 interactions of representative Nori peptides with human ACE

Fraction No.	Peptide	Docking score (Kcal/mol)	ID of BIOPEP-UWM
13	<u>ALR</u>	-7.6	9213
20	<u>EVYR</u>	-10.2	3492
20	<u>FAR</u>	-8.5	7742
20	FDR	-9.2	
20	FSR	-8.2	
28	<u>AYR</u>	-8.6	3563
30	<u>GRP</u>	-8.4	3378
30	MVT	-6.8	

The underlined sequences indicate ACE inhibitory peptides: this information was obtained from the BIOPEP-UWM database [22] on 10 September 2021.

Table 3 ACE inhibitory activities of synthetic peptides

Sample	Peptide	IC ₅₀ (μmol)	Original protein
Nori (pepsin-trypsin digestion)	AYR	8.7	RubisCO L
	EVYR	5.9	PEα
	FAR	0.29	PEα
	GRP	0.45	RubisCO L
<i>P. pseudolinearis</i> (thermolysin digestion) [19]	ARY	1.3	APCα
	YLR	5.8	PCα, APCα, APCβ
	LRM	0.15	PCα
Dulse (thermolysin digestion) [13]	VYRT	0.14	PEα
	LDY	6.1	PEα, PCα, APCα, APCβ
	LRY	0.044	PEβ, PCβ, APCβ
	FEQWAS	>2.8	RubisCO L

Fig. 1

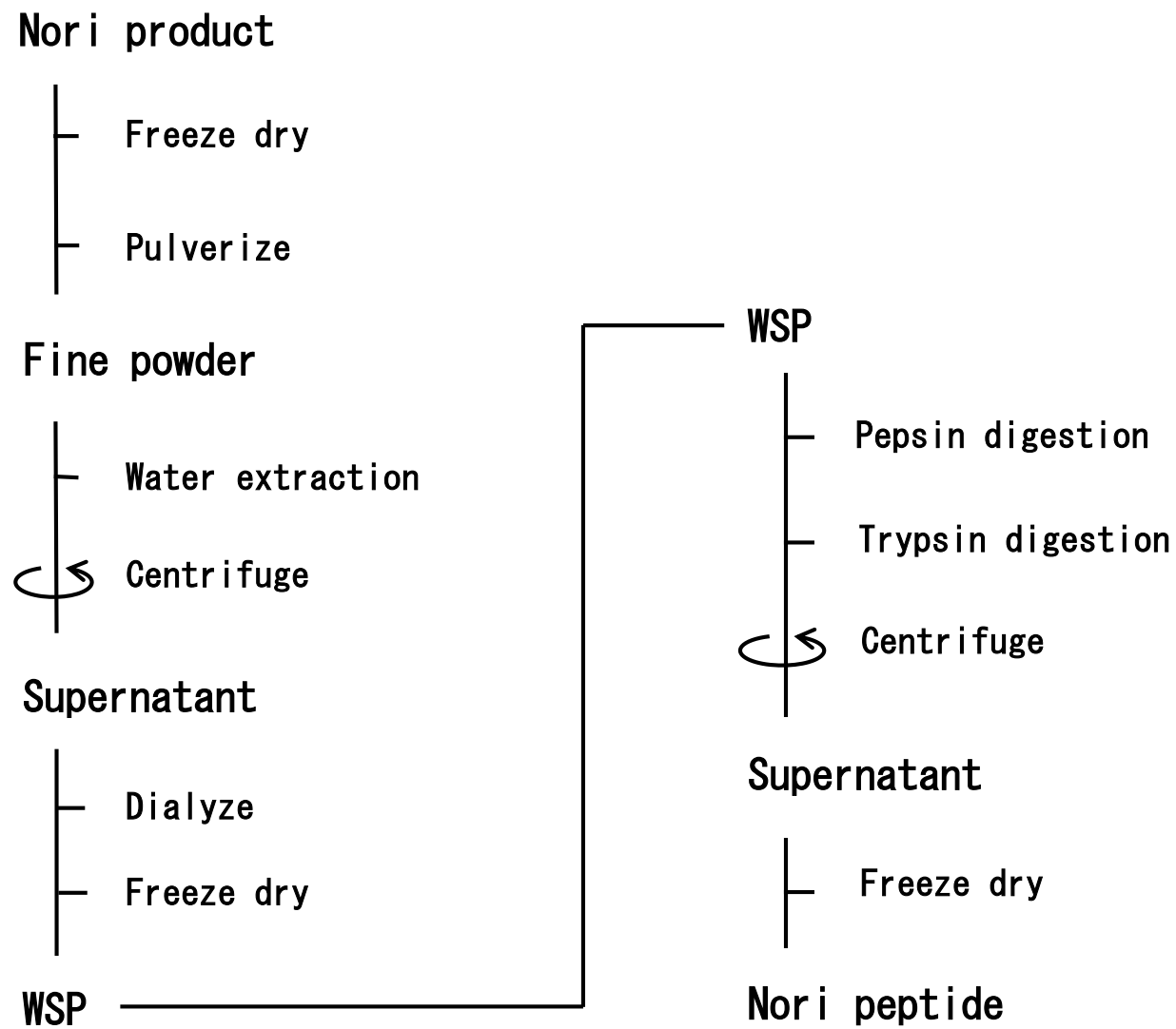


Fig. 2

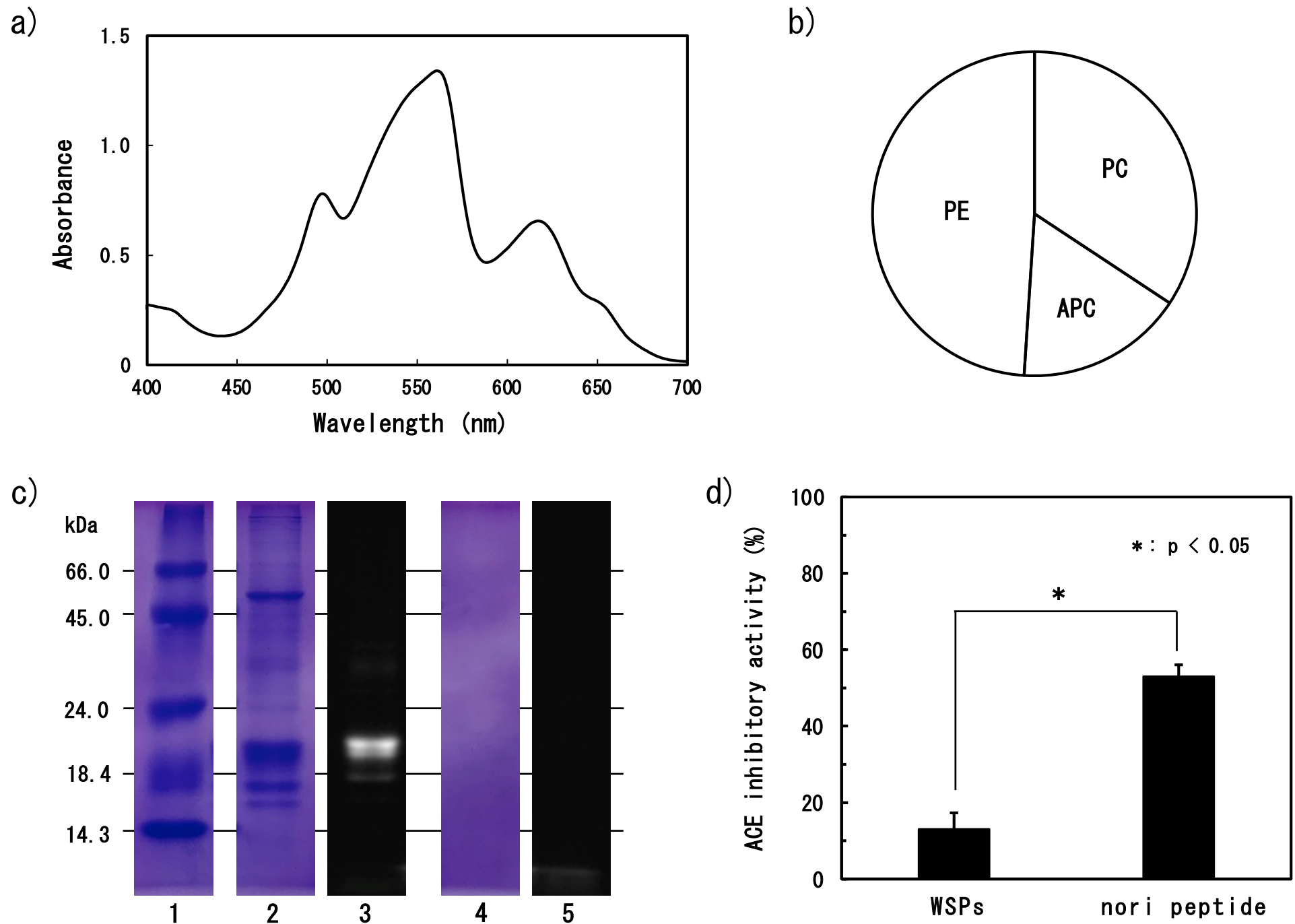


Fig. 3

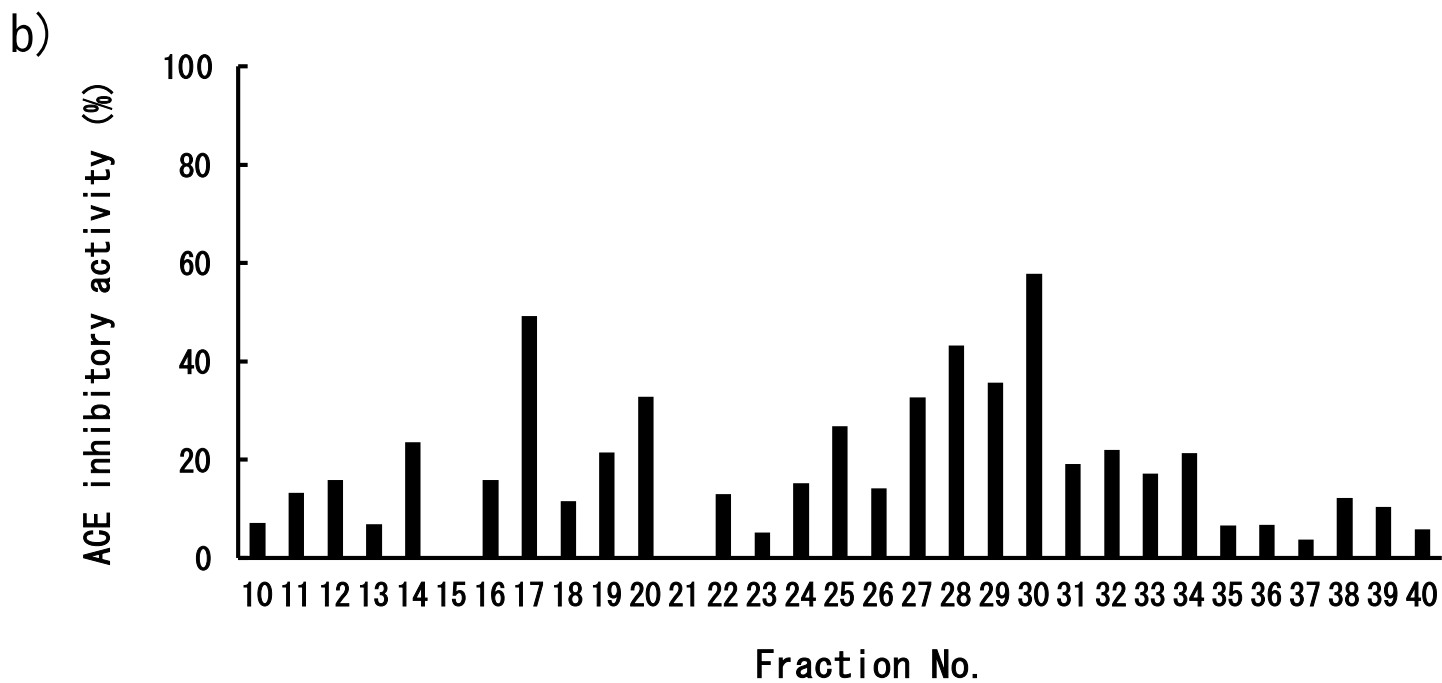
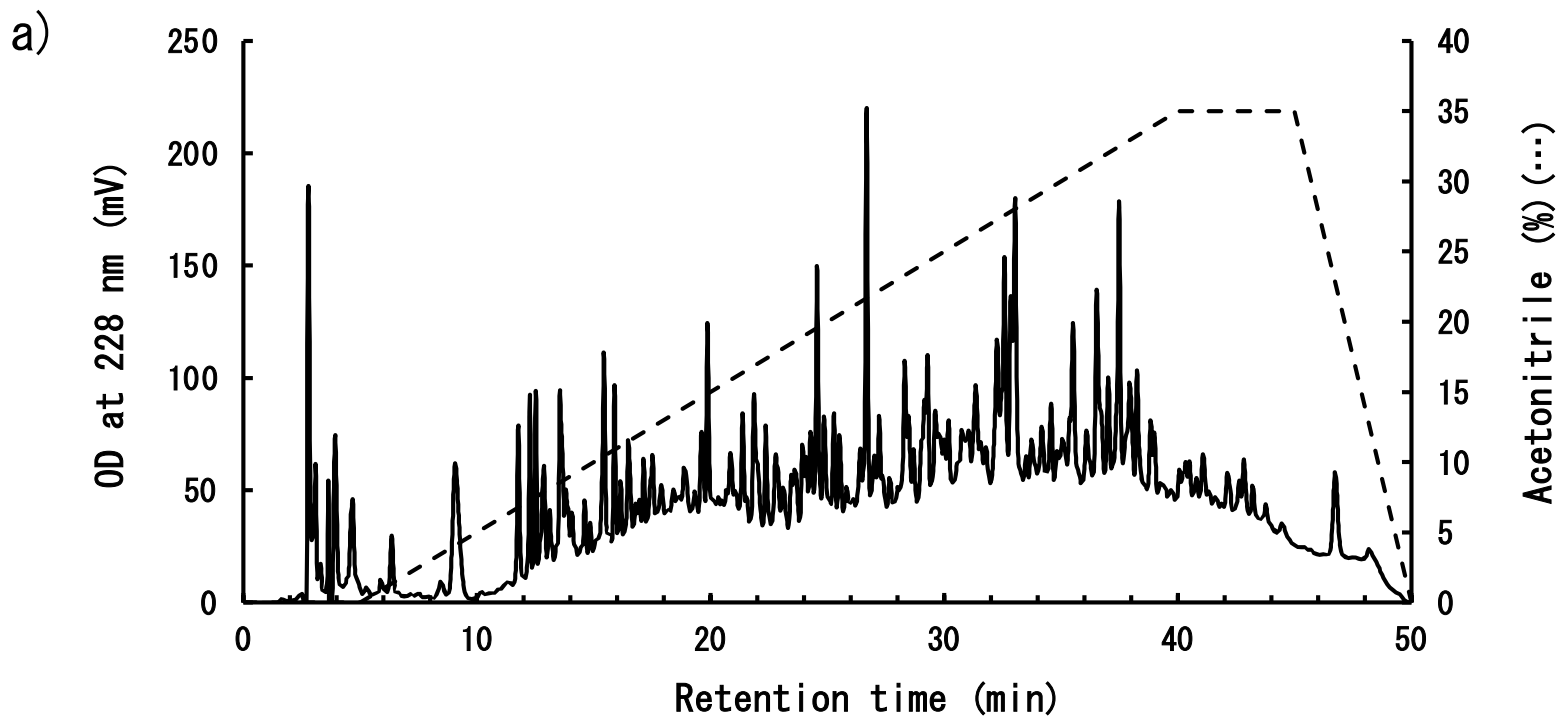


Fig. 4

