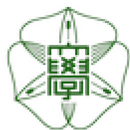
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1 ACE inhibitory effect of the protein hydrolysates prepared from commercially available nori product by pepsin–
2 trypsin digestion
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12 **Abstract**

13

14 We studied angiotensin I converting enzyme (ACE) inhibitory peptides in the protein hydrolysates of
15 commercially available nori products that contain *Pyropia pseudolinearis* as the main ingredient. The water extract
16 of the nori product consisted mainly of phycobiliproteins and RubisCO. The proteins in the aqueous extracts were
17 sequentially hydrolyzed with pepsin and trypsin, and the peptides in the pepsin-trypsin digests were fractionated
18 by reversed-phase HPLC. As a result, twelve ACE inhibitory peptides containing ten novel peptides were
19 identified. These peptides are suggested to have originated from the α- and β-subunits of phycobiliproteins and the
20 large subunits of RubisCO of *P. pseudolinearis*. The interactions of eight peptides (ALR, FAR, FSR, FDR, EVYR,
21 AYR, GRP, and MVT) with ACE were then simulated using the flexible docking tool Auto Dock Vina. The results
22 showed that all peptides interacted with the active center of ACE, and their docking scores ranged from -6.8 to -
23 10.2 kcal/mol. In addition, we synthesized four peptides (AYR, FAR, EVYR, and GRP) and measured the IC_{50}
24 values of these peptides for ACE. Consequently, FAR and GRP showed considerably low IC_{50} values (0.29 μmol
25 and 0.45 μmol, respectively) in addition to other ACE inhibitory peptides. Moreover, FAR, which is specific to
26 the nori product, was predicted to bind to the S1, S1', and S2' subsites of the catalytic center of ACE. Therefore, it
27 can be expected that daily intake of "nori products" may have a positive effect on the prevention of hypertension.

28

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

31

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 dune 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

81 As shown in Fig.1, the WSPs from nori products were prepared according to the same method as in our previous
82 paper [19]. The WSPs were suspended in 0.1 M HCl solution and digested by 1.0 wt% porcine pepsin at 37 °C for
83 3 h. After the reaction, the solution was adjusted to pH 8.0. Then, the pepsin hydrolysate was redigested by 1.0
84 wt% bovine trypsin at 37 °C for 3 h. The enzyme reaction was stopped by heating at 100 °C for 5 min. The reacted
85 solution was centrifuged at 4 °C and 15,000 g for 10 min, and the supernatant was lyophilized into nori peptide
86 (Fig. 1). Visible light absorption spectra of the WSPs were measured in the range of 350-700 nm with a data
87 interval of 2 nm using a UV-1800 spectrophotometer (SHIMADZU, Kyoto, Japan). The amount of
88 phycobiliproteins was determined by the spectra using the following equations: phycocyanin (PC) = [OD620 –
89 0.70 (OD650)]/7.38; allophycocyanin (APC) = [OD650 – 0.19 (OD620)]/5.65; phycoerythrin (PE) = [(OD565) −
90 2.80 (PC) – 1.34 (APC)]/12.7 [20]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS−PAGE) was
91 performed according to Laemmli's method using a 0.1% SDS-13.75% polyacrylamide slab gel [21]. The proteins
92 were stained with 50% methanol-7% acetic acid containing 0.1% CBB R-250, and the background was decolorized
93 with 7% acetic acid. The fluorescence of the phycobiliprotein on the slab-gel was detected by VISIRAYS AE-
94 6935GN (ATTO, Tokyo, Japan).
95
96 Isolation of ACE inhibitory peptides from nori peptide
97
98 ACE inhibitory peptides were identified by reversed-phase HPLC. The nori peptide was dissolved (20 mg/mL) in
99 ultrapure water containing 0.1% TFA and applied to sequential filtration by Millex-GV and Millex-LG. The
100 filtered sample (100 μL) was applied to a reversed-phase column (Mightysil RP-18 Aqua, φ10 mm×250 mm)
101 (Kanto Kagaku, Tokyo, Japan), and peptides were eluted with a linear gradient of acetonitrile (1−35% acetonitrile
102 containing 0.1% TFA at a flow rate of 4.73 mL/min). Absorbance of the eluent was monitored at 228 nm. The
103 eluate per minute (4.73 mL) was collected in a test tube. The fractions eluted between 10 and 40 minutes
104 (designated fraction numbers 10−40) were subjected to an ACE inhibitory assay. The amino acid sequences of the
105 ACE inhibitory peptides were analyzed by MALDI-TOF/MS/MS using a 4700 Proteomics Analyzer mass

106 spectrometer with *DeNovo* Explorer ver. 3.6 (Applied Biosystems, CA, USA). Information on the reported ACE

107 inhibitory peptide was obtained from the BIOPEP-UWM database [22] on 10 September 2021.

108

109 Assay of ACE inhibitory activity

110

111 The assay was carried out with the same method as described in a previous paper [19]. The inhibition was

112 calculated from the equation: ACE inhibitory activity (%) = [1- (As-Asb)/(Ac-Acb)]×100, where Ac is the

113 absorbance of the buffer, Acb is the absorbance when the stop solution was added to the buffer before the reaction,

114 As is the absorbance of the sample, and Asb is the absorbance when the stop solution was added to the sample

115 before the reaction. We defined the IC_{50} as an absolute quantity of peptide to inhibit 50% of 1.0 U ACE. The ACE

116 inhibitory activities of WSPs, WSPs hydrolysate, and synthetic peptides were measured in triplicate, and each

117 mean ± standard error was calculated. Statistical analyses were carried out using a Student's t test.

118

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

120

121 The structure of the purified peptide was constructed using PyMOL builder software (The PyMOL Molecular

122 Graphics System, Ver. 2. Schrödinger, LLC.). The structure was optimized based on modified molecular

123 mechanics that consider bond stretching, angle bending, internal rotation and van der Waals nonbonded

124 interactions. The structure of human ACE in complex with lisinopril (1O86.pdb) was derived from the RCSB PDB

125 Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Before docking, water molecules and lisinopril were

126 removed. Docking was performed using the flexible docking tool AutoDock Vina (TSRI, USA). The docking runs

127 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

128 active site of ACE was obtained according to the scores and binding energy value. The 3-D molecular docking

129 result was output by PyMOL, and the 2-D molecular docking result was output by LigPlot^{+} ver. 2.2 in EMBL-EBI

130 [23].

131

132 Results and discussion

133

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

135

136 Spectral analysis was carried out for WSPs prepared from the nori product. As shown in Fig. 2a, the maximum
137 absorption peak was indicated at approximately 560 nm, followed by 500 nm, 615 nm, and 655 nm. The absorption
138 at 560 nm comes from phycoerythrobilin, which binds to PE. The absorptions at 500, 615 and 655 nm are due to
139 phycourobilin of PE, phycocyanobilin of PC and phycocyanobilin of APC, respectively [24]. From this absorption
140 spectrum, it was calculated that 49% of the phycobiliproteins were PE, 34% were PC, and 17% were APC (Fig.
141 2b). The nori WSPs were then subjected to SDS−PAGE, and the molecular weights of the main components were
142 approximately 20 kDa and 50 kDa (Fig. 2c). Phycobiliproteins of red algae commonly contain α- and β-subunits.
143 and each subunit binds one or several phycobilin chromophores at the specific Cys residues through thioether
144 linkage (PE: αCys82, αCys139, βCys50, βCys61, βCys82, βCys158; PC: αCys84, βCys82, βCys153; APC:
145 αCys81, βCys81) [25−27]. In the previous study, we determined the primary structures of phycobiliproteins from
146 *P. pseudolinearis* and clarified the molecular weights of α- and β-subunits of them (PEα: 1,7697.9; PEβ: 1,8423.1;
147 PCα: 1,7463.8; PCβ: 1,8170.6; APCα: 1,7509.0; APCβ: 1,7484.0). In addition, the α- and β-subunits of these
148 phycobiliproteins retained the Cys residues, which bind phycobilin chromophores, at the corresponding positions.
149 In addition, our previous study revealed that the protein with 55,000 MW in the WSPs of red algae was the
150 RubisCO large subunit [17]. Based on these findings, it is considered that the 20 kDa component consists of the
151 α- and β-subunits of phycobiliprotein because of its fluorescence, and the 50 kDa component is the large subunit
152 of RubisCO. These protein compositions of the nori WSPs are almost the same as those of the WSPs from *P.*
153 *pseudolinearis* in our previous report [19].

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

156 by pepsin-trypsin treatment (Fig. 2d). The results suggest that ACE inhibitory peptides derived from
157 phycobiliproteins and RubisCO are probably generated in the gastrointestinal tract when we eat the nori product.
158
159 Identification of ACE inhibitory peptides in the nori peptide
160
161 To identify ACE inhibitory peptides derived from the nori products by pepsin-trypsin digestion, the nori peptide
162 was subjected to reversed-phase HPLC (Fig. 3a), and the inhibitory activities of the eluted fractions (Nos. 10−40)
163 were measured (Fig. 3b). The peptide structures in these fractions showing inhibitory activity were examined by
164 MALDI-TOF/MS/MS, and twelve sequences (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, AYR, FVSGQR,
165 LSNGELQAINGR, GRP, MVT, and DGEIILR) were obtained (Table 1). These twelve sequences were checked
166 in the BIOPEP-UWM database [22], and ten of these (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, FVLSGQR,
167 LSNGELQAINGR, MVT, DGEIILR) were confirmed to be novel peptides that had not been registered. As shown
168 in Table 1, all these sequences were found in the primary structures of the α- and β-subunits of phycobiliproteins
169 and large subunits of RubisCO from *P. pseudolinearis*. From the results, it is strongly suggested that ACE
170 inhibitory peptides are generated from the α- and β-subunits of phycobiliproteins and large subunits of RubisCO,
171 which are the major proteins in the nori product after pepsin-trypsin digestion. In this study, the nori peptide was
172 prepared by sequential digestion of WSPs with pepsin and trypsin. Thus, peptides with Arg or Lys residues at the
173 C-terminus may be produced. In fact, ten of the twelve ACE inhibitory peptides had Arg residues at their C-termini.
174 It is well known that the effectiveness of ACE inhibitory peptides is closely related to chain length, amino acid
175 composition, and sequence [28]. For example, the presence of positive charges of Arg (guanidino group) and Lys
176 (ε-amino group) in the C-terminal residues has been reported to contribute to the inhibitory efficacy [29,30]. It has
177 also been reported that the removal of the C-terminal Arg residue decreases ACE inhibitory activity [31,32].
178 Therefore, the peptides mentioned above were also expected to exhibit high ACE inhibitory activity. Then, we
179 examined the ACE inhibitory activity of these peptides.
180

181 ACE inhibitory activity of representative nori peptides

182

183 In general, ACE inhibitory peptides are often low molecular weight peptides with two to six amino acids. For
184 instance, crystallographic studies have shown that large peptides are unable to bind to the active site of ACE. [33].
185 In research on yak milk casein hydrolysates, hepta- and octa-peptides did not show ACE inhibitory activity, and
186 the authors speculated that the spatial structure of these peptides may have been too large to fit into the active site
187 [34]. Based on these findings, we selected eight peptides (ALR, FAR, FSR, FDR, EVYR, AYR, GRP, and MVT)
188 from the twelve peptides identified in the preceding paragraph, and the binding energy (docking score) between
189 these peptides and human ACE was then simulated using an AutoDock Vina. The results showed that all peptides
190 docked with the active site of ACE, and their docking scores were relatively low (ranging from -7.6 to -10.2
191 kcal/mol) except for MVT (-6.8 kcal/mol) (Table 2). This result is presumably due to the C-terminal Arg residue
192 as described before. In addition, the C-terminus of GRP is a Pro residue. There are also many reports of peptides
193 with high ACE inhibitory effects that contain aromatic amino acids and Pro at the C-terminus [29–31,35]. However,
194 few of the peptides with high ACE inhibitory effects reported thus far had Thr residues at the C-terminus, and the
195 docking score of MVT was higher than those of other peptides. In addition, the underlined parts of the peptides in
196 Table 2 indicate the sequences that are registered as ACE inhibitory peptides in the BIOPEP-UWM database [22].
197 Therefore, considering this information, we synthesized AYR, EVYR, FAR, and GRP and measured the ACE
198 inhibitory activities of these peptides. Consequently, FAR had the lowest IC_{50} value (0.29 μmol), followed by GRP
199 (0.45 μmol), EVYR (5.9 μmol), and AYR (8.7 μmol), and the ACE inhibitory effects of these nori peptides were
200 comparable to those of the peptides from *P. pseudolinearis* and dulse, except for LRY (IC_{50}: 0.044 μmol) (Table
201 3). The sequence of LRY is also present in the primary structures of the β-subunits of PE, PC, and APC from *P.*
202 *pseudolinearis*, and LRY was isolated from the WSPs hydrolysate of *P. pseudolinearis* by thermolysin digestion
203 [19]. However, LRY was not detected in this study, which may be due to pepsin-trypsin digestion.

204

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

206

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

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

231 terminal Arg also formed a salt bridge with the carboxyl group of Glu384. On the other hand, tACE has three
232 major catalytic subsites: S1, S1', and S2'. Glu143, Ser355, Ser516, and Val518 have been reported to be present in
233 S1, Glu162, Thr166, Asn277, Ser284, His353, Glu372, Asn374, Glu376, Glu376, Asp377, Val380, and His513 in
234 S1', Gln281, Thr282, Ser284, Glu376, Val379, Val380, Asp415, Ala418, Asp453, Lys454, Phe457, Lys511,
235 Tyr520, Tyr523, Phe527, and Gln530 in S2' of tACE [39,40]. On the C-domain of sACE, Ala354, His353, Ser355,
236 Val518, Pro519, and Arg522 are present in S1, Glu162, Ala354, Trp357, Asp377, Val380, Phe512, His513, and
237 Val518 in S1', and Val379, Val380, Asp415, Asp453, Phe457, Phe519, Tyr523, Phe527, and Phe547, in S2' [41].
238 Based on these findings, the N-terminal Phe residue of FAR can be inferred to be located in the S2' subsite, the
239 Ala residue in S1', and the C-terminal Arg residue in S1.
240
241 Conclusions
242
243 Since antihypertensive drugs are often associated with side effects, ACE inhibitory peptides derived from food
244 materials have recently been considered desirable for the prevention of hypertension. In this study, we assessed
245 the ACE inhibitory effect of a pepsin-trypsin digest (nori peptide) of WSPs prepared from a commercially available
246 nori product. As a result, we identified novel peptides (ALR, FAR, FSR, FDR, EVYR, SLTNNAQR, FVLSGQR,
247 LSNGELQAINGR, MVT, and DGEIILR) that are derived from phycobiliproteins and RubisCO. Among these,
248 FAR showed considerably low IC_{50} values (0.29 μmol) and was predicted to bind to the S1, S1', and S2' subsites
249 of the catalytic center of tACE. Therefore, it was expected that daily intake of "nori products" may have a positive
250 effect on the prevention of hypertension. We are convinced that the results obtained in this study will be useful
251 towards verifying the effects of nori products on human blood pressure (human intervention trial).
252
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254
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256

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351

(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 (Å). 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	PEα [b)]
20	409.18	FSR	PEβ [b)]
20	437.18	FDR	PCβ [c)]
20	566.26	EVYR	PEα [b)]
20	903.42	SLTNNAQR	PCα [c)]
28	409.20	AYR	RubisCO L [a)]
28	806.44	FVLSGQR	APCα [d)]
28	1,271.60	LSNGELQAINGR	PCα [c)]
30	329.08	GRP	RubisCO L [a)]
30	349.91	MVT	PCα [c)]
30	815.40	DGEIILR	PEβ [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	ALR	-7.6	9213
20	EVYR	-10.2	3492
20	FAR	-8.5	7742
20	FDR	-9.2	
20	FSR	-8.2	
28	AYR	-8.6	3563
30	GRP	-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.

408 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
P. pseudolinearis (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

409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424

Fig. 1

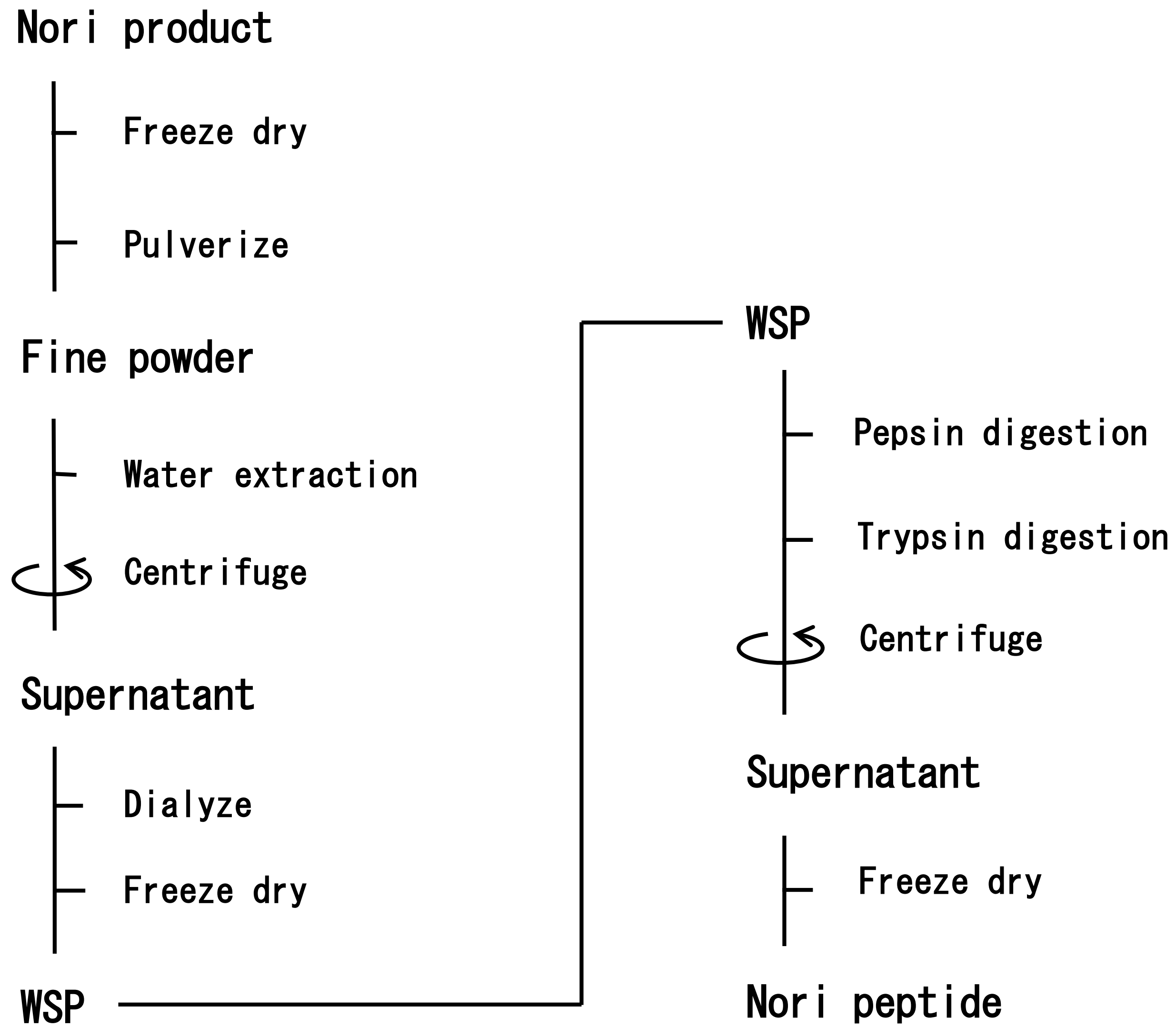
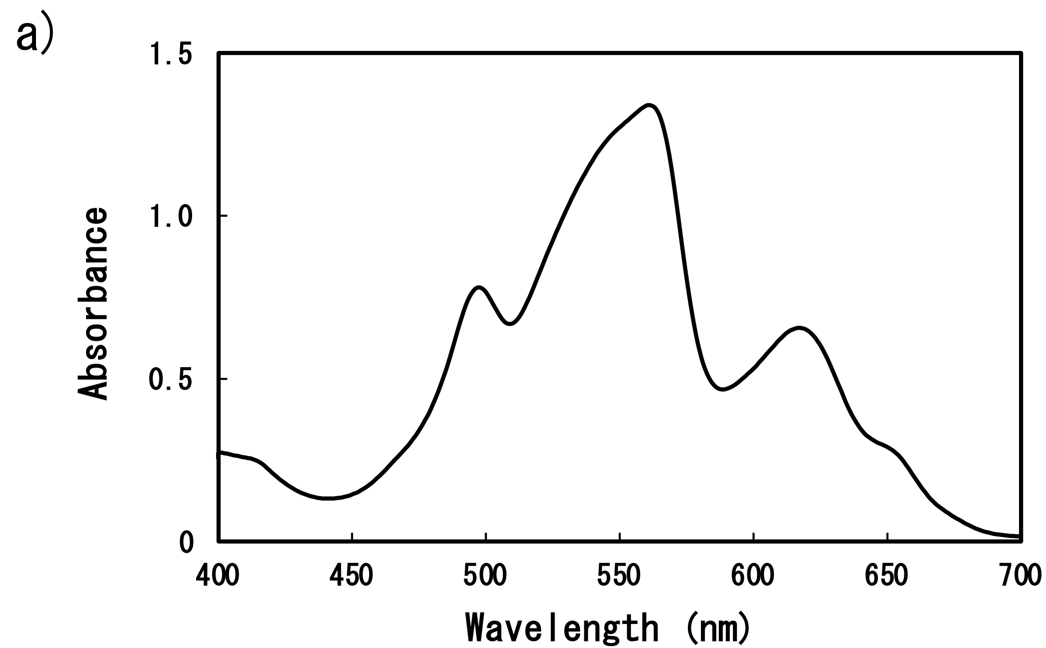


Fig. 2

a)



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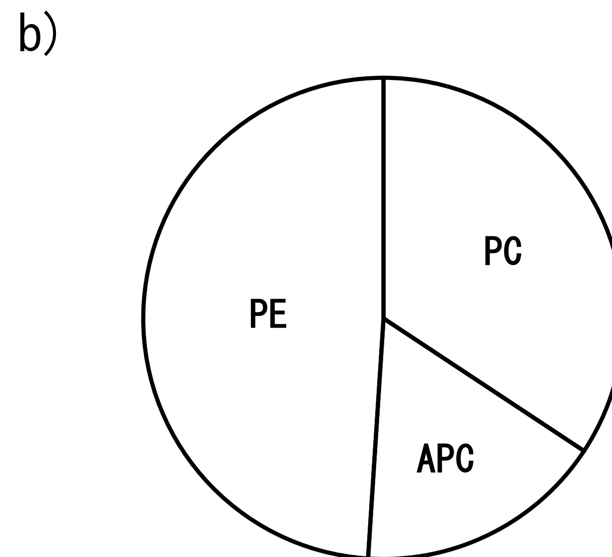

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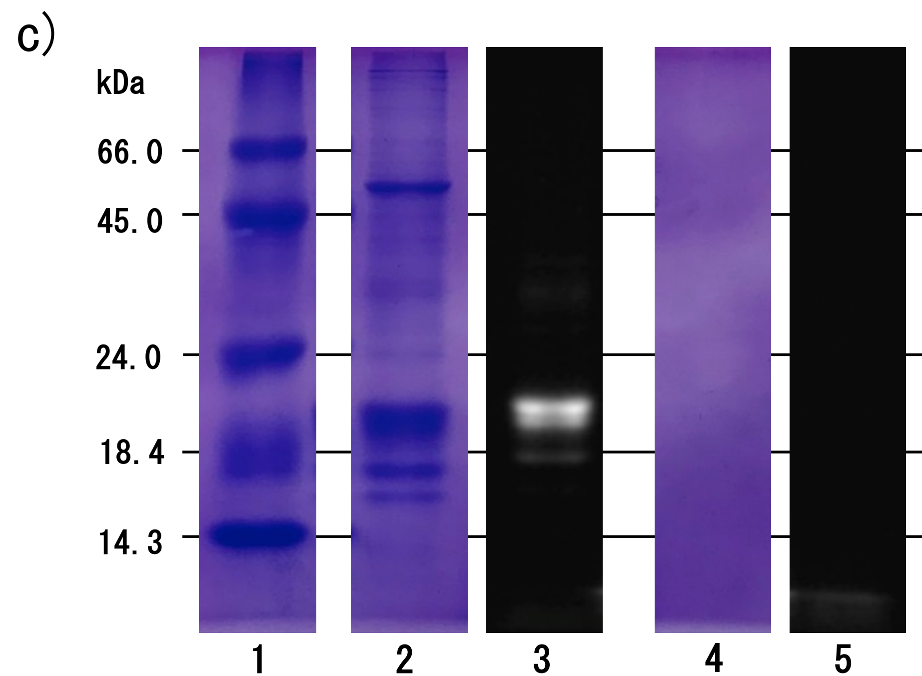

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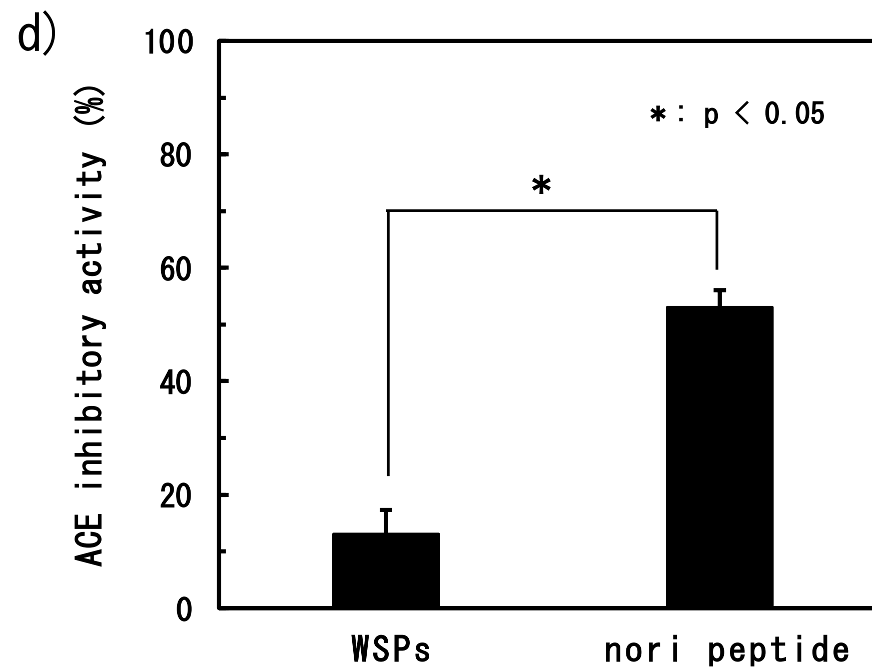
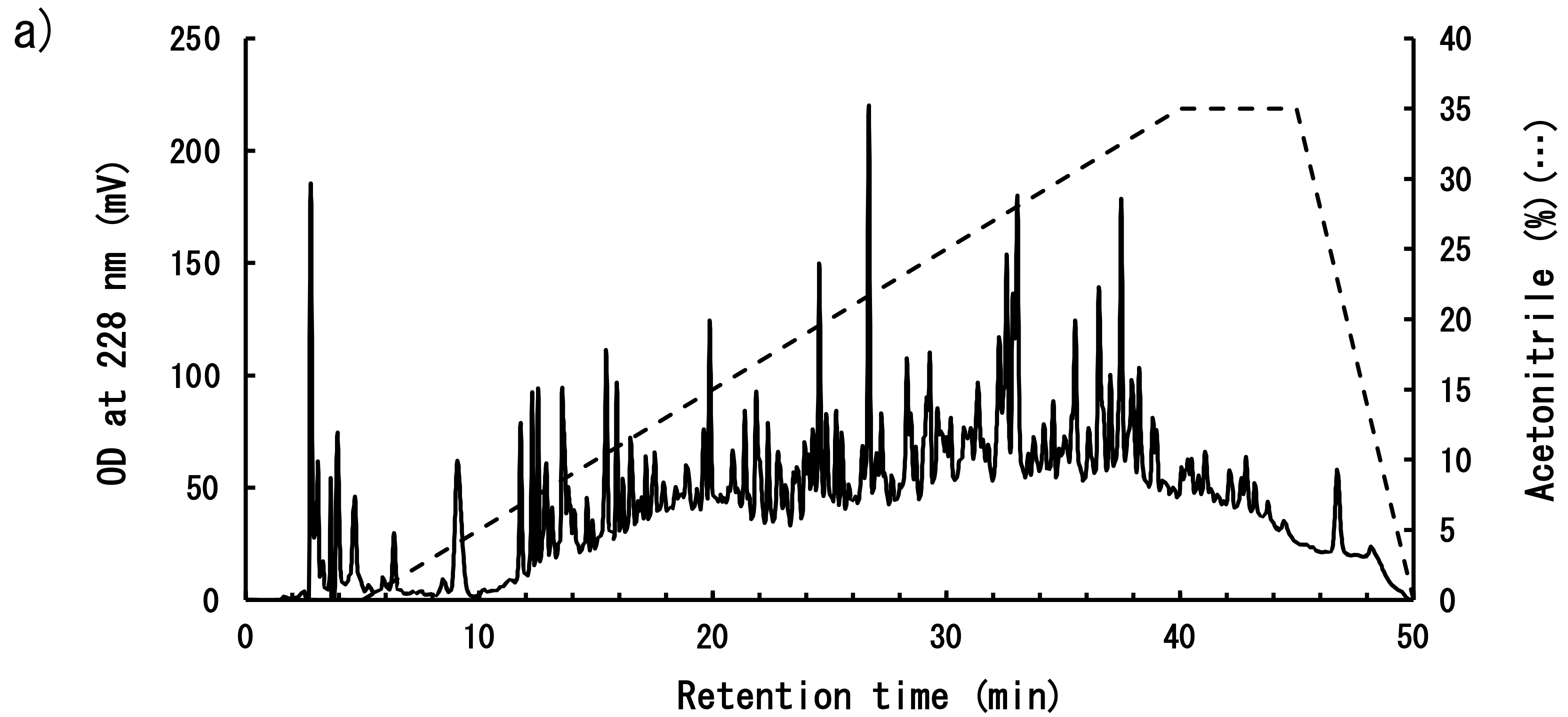


Fig. 3



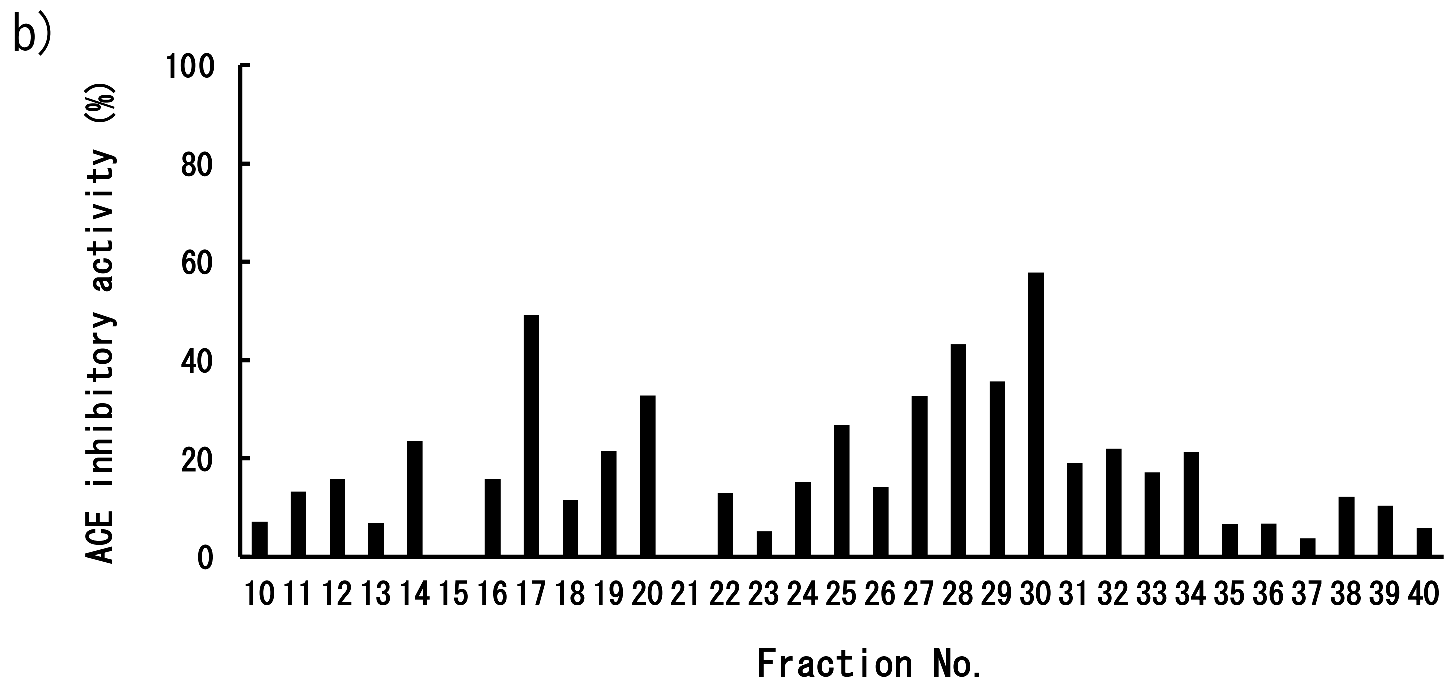
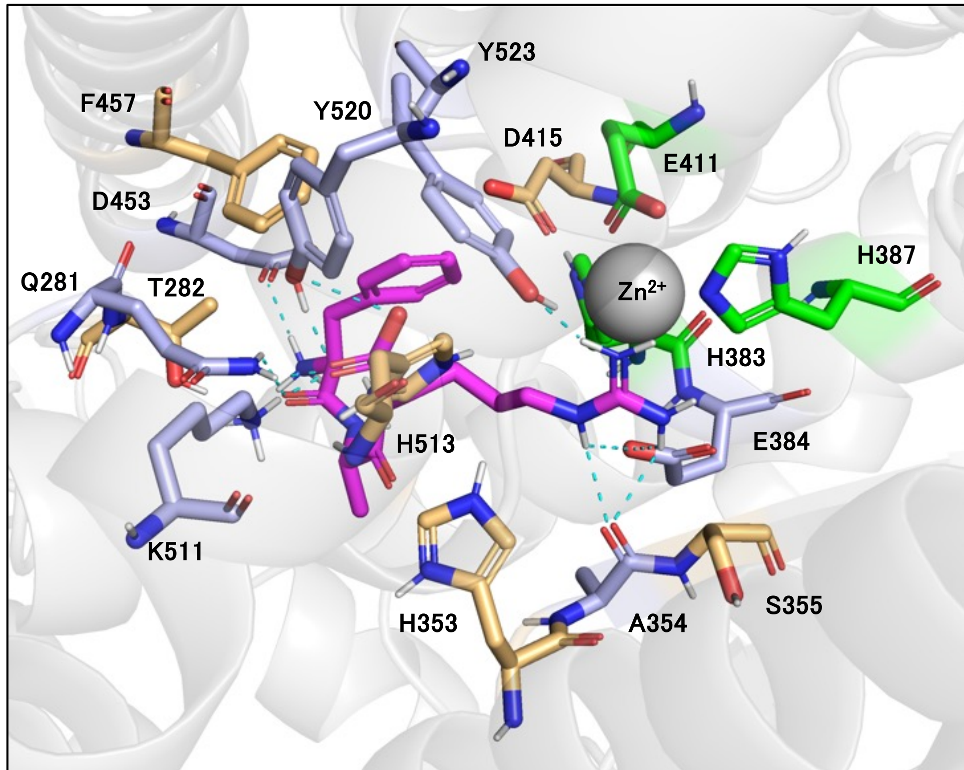


Fig. 4

a)



b)