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1 STRUCTURAL CHARACTERISTICS OF PHYCOBILIPROTEINS FROM
2 RED ALGA MAZZAELLA JAPONICA

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21
22 **Short title:** Structural properties of phycobiliproteins from *M. japonica*

ABSTRACT

We determined the primary structures of phycoerythrin, phycocyanin and allophycocyanin from red alga *Mazzaella japonica*. The phycobiliproteins consist of α - and β -subunits like other red algae. *M. japonica* phycobiliproteins all conserved Cys residues for chromophore attachment site. The amino acid sequences of *M. japonica* phycobiliproteins showed considerably high identities with those of other red algae (81 ~ 100%). In addition, the sequences (YRD, LDY, LRY, VY, LF and FY), which were angiotensin I converting enzyme (ACE) inhibitory peptides from other algae, were detected in the primary structures of *M. japonica* phycobiliproteins. Then, we prepared the protein hydrolysates from *M. japonica* and measured its ACE inhibitory activity. Consequently, *M. japonica* protein hydrolysates indicated considerably high ACE inhibitory activity.

PRACTICAL APPLICATIONS

M. japonica is an abundant resource in Japan, which contains a lot of phycobiliproteins. Then, *M. japonica* protein hydrolysates strongly inhibited the activity of ACE. Therefore, it has the potential to be an ingredient of functional food.

KEYWORDS: ACE inhibitory activity; *Mazzaella japonica*; Phycobiliprotein;

Primary-structure; Red algae

1 INTRODUCTION

2
3 Seaweeds have been used extensively as food in Japan since ancient times, and several edible
4 seaweeds are well known worldwide such as Kombu (*Saccharina* sp.), Wakame (*Undaria*
5 *pinnatifida*) and Nori (*Pyropia* sp.). Kombu and Wakame, which belong to brown algae, are
6 rich in polysaccharides such as alginate, laminarin and fucans (Mabeau & Fleurence 1993).
7 In contrast, red algae have high protein levels (Mabeau & Fleurence 1993; Fleurence *et al.*,
8 2012). For example, it was reported that the crude protein content of Nori (*Pyropia tenera*
9 or *Pyropia yezoensis*) is up to 47% of dry weight which is comparable with that of soy
10 Fleurence *et al.* 2012). Although the amino acid composition corresponding to several
11 species of algae is known, the structure and biological properties of algal proteins are still
12 relatively poorly documented (Harnedy & FitzGerald 2011). The main proteins of red algae
13 are phycobiliproteins (up to 50% of the total protein content), and they constitute
14 phycobilisomes attached on the cytoplasmic surface of thylakoid membranes (Cian *et al.*
15 2015). Major phycobiliproteins include phycoerythrin (PE), phycocyanin (PC),
16 allophycocyanin (APC), and phycoerythrocyanin (PEC) (Denis *et al.* 2009).

17 Recently, we have begun exploring the health benefits of dulse (*Palmaria palmata*) to
18 advance its use as a functional food material. Dulse is a red alga distributed mainly in
19 high-latitude coastal areas, and is popular in Ireland, Iceland, Scandinavia and Atlantic
20 Canada as a food and a source of minerals (Mouritsen *et al.* 2013). In Japan, dulse is rarely
21 eaten and is even removed from Kombu farming areas in Hokkaido Prefecture, because it
22 slows the growth of Kombu. Thus, we were going to focus on the protein components from
23 dulse, because dulse was rich in proteins (approximately 40%), which were mainly composed
24 of phycobiliproteins. The principal component of dulse phycobiliproteins was PE followed
25 by PC and APC, so we determined the primary structures of dulse phycobiliproteins and the

3-D structure of dulse PE (Miyabe *et al.* 2017). Moreover, we examined the angiotensin I converting enzyme (ACE) inhibitory activity of dulse protein hydrolysates (Furuta *et al.* 2016). The dulse protein hydrolysates showed high inhibitory activity, and nine ACE inhibitory peptides (YRD, AGGEY, VYRT, VDHY, IKGHY, LKNPG, LDY, LRY, FEQDWAS) were isolated. Then, the sequences of YRD, AGGEY, VYRT, VDHY, LKNPG, LDY and LRY were detected in the primary structures of dulse phycobiliproteins. From these results, it was suggested that the dulse ACE inhibitory peptides were mainly derived from phycobiliproteins.

On the other hand, there are many commercially important red algae in Japan, such as *Pyropia* sp., *Gelidium* sp., *Gracilaria* sp., *Gloiopeltis* sp., *Chondrus* sp., *Mazzaella* sp., *Merisotheca* sp., and so on. Among these, *Mazzaella japonica* distributes in northern part of Japan, especially Hokkaido Prefecture, and it has been used for food since long ago. *M. japonica* also shows considerably high content of protein (about 25%, unpublished data). However, there is no information about gene structures and primary structures of phycobiliproteins from *M. japonica*, which are very important to reveal the mechanisms of these functions. In addition, there are few studies on gene structures of red algal PC and APC comparing with that of PE. Therefore, in this study, we elucidated the gene structures of phycobiliproteins from *M. japonica* and characterized their primary sequences.

MATERIALS AND METHODS

Materials

M. japonica and dulse were collected on the coast of Hokkaido Prefecture, Japan.

ACE (EC 3.4.15.1) from rabbit lung was purchased from Sigma Chemical Co. (Mo, USA). Cetyltrimethylammonium bromide (CTAB), Hyppuryl-L-histidyl-L-leucine (Hip-His-Leu), thermolysin (EC 3.4.24.27) from *Bacillus thermoproteolyticus*, and all other reagents were purchased from Wako Pure Chemical (Osaka, Japan).

Isolation and sequencing of chloroplast DNA

Chloroplast DNA was extracted from the thalli of *M. japonica* according to the CTAB DNA extraction method with some modifications (Wilson 2003). The nucleotide sequences of chloroplast DNA were analyzed by using a next generation sequencer, Ion PGM System (Thermo Fisher SCIENTIFIC, MA, USA). The data were assembled with CLC Genomics Workbench 9.5.4 (QIAGEN, Hilden, Germany). Nucleotide and deduced amino acid sequences of *M. japonica* phycobiliprotein genes were aligned using CLUSTAL W program (Thompson *et al.* 1994). Molecular weight and isoelectric point of *M. japonica* phycobiliproteins were calculated from deduced amino acid sequences by using Compute pI/Mw tool (Bjellqvist *et al.* 1993; Bjellqvist *et al.* 1994; Hoogland *et al.* 2000).

Prediction of 3-D structures of *M. japonica* phycobiliproteins

We predicted the 3-D structures of *M. japonica* phycobiliproteins on the basis of the primary

structural information using SWISS-MODEL server (<http://swissmodel.expasy.org/>) and Swiss-PdbViewer DeepView v4.1 (Hoogland *et al.* 2000; Arnold *et al.* 2006; Kiefer *et al.* 2009; Schwede *et al.* 2003).

Preparation of protein hydrolysates from *M. japonica* and dulse

Phycobiliproteins comprise about 60% of the soluble protein in these cells (Apt *et al.*, 1995). So, we tried to prepare the protein extract from *M. japonica*. Frozen samples were lyophilized and ground into a powder by Wonder Blender WB-1 (OSAKA CHEMICAL Co., Osaka, Japan). Proteins in the powder was extracted by 20 v/w of distilled water at 4 °C for 12 h, and the solution was centrifuged at 4 °C, 10,000 x g for 10 min. However, we hardly obtained a supernatant, since *M. japonica* has a lot of viscous polysaccharides. Therefore, we prepared the protein hydrolysates by the following method.

M. japonica frozen samples were lyophilized and ground into a fine powder by cyclone sample mill, CSM-S1 (Shizuoka Seiki Co., Ltd., Shizuoka, Japan). The powder (1.5 g) was suspended in 25 v/w of distilled water using dialysis membrane (molecular weight cut off: approximately 10 kDa, EIDIA Co., Ltd., Tokyo, Japan), and the suspended solution was dialyzed against distilled water at 4 °C for 4 h to remove low molecular weight contaminants. Then, proteins in the dialysate were hydrolyzed by adding 1.0 wt% of thermolysin at 37 °C for 5 h, and the reaction was ended by heat treatment at 100 °C for 10 min. The solution was dialyzed against distilled water using dialysis membrane (MWCO: approximately 10 kDa) at 4 °C for 12 h. The dialysis outer solution via a dialysis membrane was lyophilized into *M. japonica* protein hydrolysates (56.1 mg).

Preparation of dulse protein hydrolysates was carried out by the same method in our previous paper (Furuta *et al.* 2016).

ACE inhibitory assay

ACE inhibitory assay was carried out according to the method of Cheng and Cushman (1973) with some modifications. Fifteen microliters of sample solution (1.0 mg/mL) were added to 30 μ L of ACE (0.2 units/mL), and the mixture was pre-incubated at 37 °C for 5 min. Thirty microliters of Hip-His-Leu solution (12.5 mM in 0.1 M sodium borate buffer containing 400 mM NaCl at pH 8.3) were added to the mixture. After incubation at 37 °C for 1 hour, the reaction was stopped by adding 75 μ L of 1.0 M HCl. The released hippuric acid was extracted with 450 μ L of ethyl acetate. Four hundred microliters of the upper layer were evaporated, and then the hippuric acid was dissolved in 1.5 mL of distilled water. The absorbance at 228 nm of the solution was measured by a spectrophotometer. The inhibition was calculated from the equation $(1 - (A_s - A_{sb}) / (A_c - A_{cb})) \times 100$, where A_c is the absorbance of the buffer, A_{cb} is the absorbance when the stop solution was added to the buffer before the reaction, A_s is the absorbance of the sample, and A_{sb} is the absorbance when the stop solution was added to the sample before the reaction. The data were expressed as mean $\pm$ SEM from triplicate determination, and statistical analysis was performed using Student's t test.

RESULTS AND DISCUSSION

Nucleotide sequences of phycobiliprotein genes from *M. japonica*

In this study, we obtained 175,332 bp of nucleotide sequences (19 contigs) for *M. japonica* chloroplast DNA, and PE, PC and APC genes were detected in the sequences: PE (*rpeB* and *rpeA*, GenBank accession number AB698819.2) (Fig. 1a), PC (*rpcB* and *rpcA*, GenBank accession number LC222737) (Fig. 1b) and APC (*rapcA* and *rapcB*, GenBank accession number LC222738) (Fig. 1c). The structures of *rpeB* and *rpeA* of *M. japonica* were identical to those of our previous results analyzed by cDNA cloning method (unpublished data). The *M. japonica* PE, PC and APC genes were constituted of α - and β -subunit genes and A/T-rich spacer (Fig. 1). AT contents of the spacers in *M. japonica* PE, PC and APC genes were 79% (49 bp/62 bp), 86% (48 bp/56 bp) and 91% (48 bp/53 bp), respectively. Bernard et al. (1992) reported that *rpeB* gene of *Rhodella violacea* is split by intervening sequence and the sequence has a feature of group II intron which is typical in eukaryotic organisms, however the *M. japonica* phycobiliprotein genes have no introns. The *rpeB* and *rpcB* of *M. japonica* were present in prior to the *rpeA* and *rpcA* (Fig. 1a, b), but the order was opposite in *dulse* APC gene (Fig. 1c). The position of *rapcA* and *rapcB* was the same as those of other Florideophycidae algae, for example *Gracilaria tenuistipitata* (AY673996) (Hagopian et al. 2004) and *Chondrus crispus* (HF562234), but was different from the case of Bangiales (*P. yezoensis*, *Pyropia haitanensis* and *Pyropia purpurea*) (Tajima et al. 2012; Wang et al. 2013). The nucleotide sequences of *M. japonica* phycobiliprotein genes showed considerably high identities (78 ~ 98%) with those of other red algae, especially *C. crispus* (96 ~ 98%) which belongs to Gigartinaceae similar to *M. japonica* (Table 1). The GC contents in *M. japonica* phycobiliproteins were from 34.6% to 38.8% (*rpeB*: 38.8%, *rpeA*: 37.2%, *rpcB*: 36.8%, *rpcA*:

36.4%, *rapcA*: 35.4%, *rapcB*: 34.6%). The GC content of *M. japonica* phycobiliprotein genes (Av=36.5%) showed very high similarity to those of *G. tenuistipitata* (36.8%) and *C. crispus* (36.1%), whereas it was a little lower than those of *P. palmata* (39.3%), *P. haitanensis* (39.2%), *P. yezoensis* (39.2%) and *P. purpurea* (39.4%) (Table 1). The Shine-Dalgarno sequence (5'-AGGAGGT-3') acting as a binding site with 16S rRNA was searched in the *M. japonica* phycobiliprotein genes. As a result, putative motifs were found at upstream regions of the phycobiliprotein genes (*rpeB*: AAGGAGA, *rpeA*: AGGATA, *rpcB*: AAGGAGA, *rpcA*: AAGGAAA, *rapcA*: AAGGAGT, and *rapcB*: AAGGAAA) (dotted underlines in Fig. 1a, b, c)

Primary structures of *M. japonica* phycobiliproteins

The deduced amino acid sequences of α - and β -subunits of *M. japonica* PE, PC and APC are shown in Fig. 1. The α -subunits of PE, PC and APC were composed by 164 (MW: 17,772; pI: 5.19), 162 (MW: 17,566; pI: 6.57) and 161 (MW: 17,465; pI: 5.06) amino acids, respectively, and the β -subunits of them were 177 (MW: 18,333; pI: 5.38), 172 (MW: 18,100; pI: 4.94) and 161 (MW: 17,589; pI: 5.47) amino acids, respectively. It is well known that three kinds of chromophores (phycoerythrobilin, phycocyanobilin and phycourobilin) covalently bind to some Cys residues in red algal phycobiliproteins (Apt *et al.* 1995). The residues for chromophore attachment sites were all conserved in *M. japonica* phycobiliproteins (PE: β Cys50, β Cys61, β Cys82, β Cys158, α Cys82, α Cys139; PC: β Cys82, β Cys153, α Cys84; APC: α Cys81, β Cys81) (Fig. 1). For amino acid sequence, *M. japonica* phycobiliproteins showed remarkably high identities (95 ~ 100%) with those of *C. crispus* (Table 1).

In 1992, Ficner *et al.* reported the three-dimensional structure of $(\alpha\beta)_6$ hexamer of PE

isolated from red alga *Porphyridium sordidum*. They revealed that the intersubunit pairs of α Ser3- β Asp3, α Asp13- β Arg93, α Asp13- β Tyr94, α Asp13- β Arg110 and α Arg17- β Tyr97 are formed in $(\alpha\beta)$ heterodimer. The specific hydrogen bonds of neighboring $(\alpha\beta)$ in $(\alpha\beta)_3$ trimeric disc are also formed: 1 α Arg93-2 β Tyr76, 1 α 111 main chain carbonyl oxygen-2 β Asn78, 1 β Asp13-2 β Tyr76. In addition, $(\alpha\beta)_3$ trimer aggregates each other by electrostatic interactions (1 α Lys2-6 α Asp23, 1 α Arg17-6 α Asp108). Moreover, α Asn47, α Ala74, α Lys80, α Lys83, α Arg86, α Asp87, α Arg139, α Arg140d, β Asn35, β Asp39, β Asp54, β Thr77, β Arg79, β Arg80, β Arg86, β Asp87, β Arg131, β Thr148a, β Ala151, β Gly153 are important in polar and ionic chromophore-protein interactions. In the α - and β -subunits of *M. japonica* PE, these amino acid residues were highly conserved (α Lys2, α Ser3, α Asp13, α Arg17, α Asp23, α Asn47, α Ala72, α Lys78, α Lys81, α Arg84, α Asp85, α Arg91, α Asp106, α Arg137, α Arg142, β Asp3, β Asp13, β Asn35, β Asp39, β Tyr74, β Thr75, β Asn76, β Arg77, β Arg78, β Ala81, β Arg84, β Asp85, β Arg91, β Tyr92, β Tyr95, β Arg108, β Arg129, β Gly156) (Fig. 2a).

Similar to PE, it has been clarified that several amino acids in PC molecule are related to the specific intersubunit co-relation (Apt *et al.*, 1995; Schirmer *et al.* 1986). That is to say, the residues for interaction between α - and β -subunits were conserved in *M. japonica* PC (α Asp13, α Arg93, α Tyr97, β Asp13, β Arg91, β Tyr92, β Tyr95) (Fig. 2b). In addition, α Arg17 involving $(\alpha\beta)_3$ trimer-trimer interaction was exist in PC α -subunit of *M. japonica*. Moreover, the residues related to chromophore interaction (α Arg86, α Asp87, β Asn72, β Arg77, β Arg78, β Ala81) were found in *M. japonica* PC (Fig. 2b).

The crystal structure of APC from red alga *P. yezoensis* was determined by molecular replacement method (Liu *et al.* 1999). In the crystal, two $(\alpha\beta)_3$ trimers associate face to face into a $(\alpha\beta)_6$ hexamer. In the $(\alpha\beta)$ heterodimer, the ionic- and polar-interacting pairs between the two subunits are α Ser2- β Asp3, α Asp13- β Tyr94, α Asp13- β Arg110, α Arg17- β Tyr97,

α Tyr18- β Arg93, α Asp89- β Tyr18, α Arg93- β Asp13 and α Tyr97- β Asp13. In addition, the polar and charged interactions between the two $(\alpha\beta)_3$ trimers are 1 α Asp25-6 α Arg37, 1 α Gly22-6 α Arg26, 1 α Asp25-6 α Glu151, 1 α Asp25-6 α Tyr155 and 1 α Lys28-6 α Asp147. The chromophores at α Cys84 and β Cys84 have similar hydrophobic environment; i.e., there are three aromatic residues close to α Tyr90, α Tyr91, α Tyr119, β Tyr90, β Tyr91, and β Tyr119. In the α - and β -subunits of *M. japonica* APC, these amino acid residues were highly conserved (α Ser2, α Asp12, α Arg16, α Tyr17, α Gly21, α Asp24, α Arg25, α Lys27, α Arg36, Asp86, α Tyr87, α Tyr88, α Arg90, α Tyr94, α Tyr116, α Asp145, α Glu148, α Tyr152, β Asp3, β Asp13, β Tyr18, β Tyr87, β Tyr88, β Arg90, β Tyr91, β Tyr94, β Arg107, β Tyr116) (Fig. 2c).

The 3-D structures of α - and β -subunits of *M. japonica* phycobiliproteins were predicted on the basis of their primary structures. As shown in Fig. 3, the predicted backbone conformations of all subunits are highly similar, and they have nine α -helixes (X, Y, A, B, E, F, F', G, and H) as a dominant secondary structure element. These properties are similar to the known 3-D structures (Miyabe *et al.* 2017; Ficner *et al.* 1992; Schirmer *et al.* 1986; Jiang *et al.* 2001). From the results, it is suggested that the α - and β -subunits of *M. japonica* phycobiliproteins would form an $(\alpha\beta)_3$ trimer in the phycobilisome.

ACE inhibitory activity of *M. japonica* protein hydrolysates

ACE is a key enzyme in the regulation of peripheral blood pressure catalyzing the production of angiotensin II in the renin-angiotensin-aldosterone system and the destruction of bradykinin in the kinin-kalikrein system (Cheung *et al.* 1980). The specific inhibitors of the enzyme therefore have been considered with effective antihypertensive drugs. In addition to the drugs, ACE inhibitory peptides from daily food also useful for maintaining blood pressure at a healthy level. Although the potency of peptide is lower than drug, it does not have side

effect (Balti *et al.* 2015). It is well known that the peptides containing hydrophobic amino acid residues with aromatic (Y, F, W) or branched (L, I, V) side chains possess a strong ACE inhibitory effect (Saiga *et al.*, 2008). For instance, Suetsuna (1998) obtained four ACE inhibitory peptides (IY, MKY, AKYSY and LRY) from red alga *P. yezoensis*. In 2004, Suetsuna *et al.* also isolated ten ACE inhibitory peptides (YH, KW, KY, LF, FY, VW, VF, IY, IW and VY) from brown alga *U. pinnatifida* (Wakame), and they clarified both single and repeated oral administrations of synthetic peptide (YH, KY, FY or IY) significantly decreased the blood pressure in spontaneously hypertensive rats. Recently, we found that the dulse phycobiliprotein hydrolysates possess strong inhibitory effect to ACE (Furuta *et al.*, 2016). The dulse protein hydrolysates showed high inhibitory activity, and nine ACE inhibitory peptides (YRD, AGGEY, VYRT, VDHY, IKGHY, LKNPG, LDY, LRY, FEQDWAS) were isolated. Then, the sequences of YRD, AGGEY, VYRT, VDHY, LKNPG LDY and LRY were detected in the primary structures of dulse phycobiliproteins. From these results, it was suggested that the dulse ACE inhibitory peptides were mainly derived from phycobiliproteins.

In this study, the primary structures of *M. japonica* phycobiliproteins were determined, and they showed high amino acid sequence identities (81 ~ 100%) with those of other red algae (Table 1). It was clarified that *M. japonica* phycobiliproteins are rich in hydrophobic amino acid residues (46.0 ~ 52.3%), especially aromatic (Y and F) or branched (V, L and I) side chain residues. In addition, similar sequences of above ACE inhibitory peptides (YRD: PE α -subunit; LDY: PE α -subunit, PC α -subunit, APC α -subunit and APC β -subunit; LRY: PE β -subunit, PC β -subunit and APC α -subunit; VY: PE α -subunit, PC α -subunit and PC β -subunit; LF: PC β -subunit and APC α -subunit; FY: APC α -subunit; TKYSY: PE α -subunit; VYRA: PE α -subunit) were detected in the primary structures of *M. japonica* phycobiliproteins (Fig. 2). So, it was suggested that ACE inhibitory peptides are also derived from *M. japonica* phycobiliproteins. Then, we prepared the protein hydrolysates

1 from *M. japonica* and measured its ACE inhibitory activity. As a result, *M. japonica* protein
2 hydrolysates showed significantly higher inhibitory activity than that of dulse (Fig. 4).
3 Therefore, we concluded that *M. japonica* also has the potential to be an ingredient of
4 functional food.

6 CONCLUSION

8 We determined the primary structures of phycoerythrin, phycocyanin and
9 allophycocyanin from red alga *Mazzaella japonica*. The amino acid sequences of *M.*
10 *japonica* phycobiliproteins showed considerably high identities with those of other red algae.
11 In addition, the sequences (YRD, LDY, LRY, VY, LF and FY), which were angiotensin I
12 converting enzyme (ACE) inhibitory peptides from other algae, were detected in the primary
13 structures of *M. japonica* phycobiliproteins. Then, we prepared the protein hydrolysates
14 from *M. japonica* and measured its ACE inhibitory activity. Consequently, *M. japonica*
15 protein hydrolysates indicated considerably high ACE inhibitory activity. Therefore, we
16 concluded that *M. japonica* has the potential to be an ingredient of functional food.

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(Captions to figures)

FIG. 1 NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES OF *M. JAPONICA* PHYCOBILIPROTEIN GENES.

a: *M. japonica* phycoerythrin gene.

b: *M. japonica* phycocyanin gene.

c: *M. japonica* allophycocyanin gene.

PE, PC, and APC represent phycoerythrin, phycocyanin and allophycocyanin, respectively. Asterisks show stop codon. Dotted underline is putative RNA polymerase-binding motif (Shine-Dalgarno Sequence).

FIG. 2 ALIGNMENT OF AMINO ACID SEQUENCES OF PHYCOBILIPROTEIN SUBUNITS FROM SOME RED ALGAE.

a: Phycoerythrin.

b: Phycocyanin.

c: Allophycocyanin.

PE β , PE α , PC β , PC α , APC α and APC β represent phycoerythrin β -subunit, phycoerythrin α -subunit, phycocyanin β -subunit, phycocyanin α -subunit, allophycocyanin α -subunit and allophycocyanin β -subunit, respectively. Asterisks show characteristic amino acid residues in the molecule.

M.japonica: *Mazzaella japonica* (in this study).

P.palmata: *Palmaria palmata* (AB625450.2, AB679831.1, AB742300.1).

G.tenuistipitata: *Gracilaria tenuistipitata* (NC_006137.1).

C.crispus: *Chondrus crispus* (NC_020795.1).

P.yezoensis: *Pyropia yezoensis* (KC517072.1).

1 **FIG. 3** PREDICTED 3-D STRUCTURES OF *M. JAPONICA* PHYCOBILIPROTEIN
2 SUBUNITS.

3 PE β : Phycoerythrin β -subunit.

4 PE α : Phycoerythrin α -subunit.

5 PC β : Phycocyanin β -subunit.

6 PC α : Phycocyanin α -subunit.

7 APC α : Allophycocyanin α -subunit.

8 APC β : Allophycocyanin β -subunit.

9

10

11 **FIG. 4** ACE INHIBITORY ACTIVITY OF PROTEIN HYDROLYSATES PREPARED
12 FROM *M. JAPONICA*.

13 *P. palmata*: protein hydrolysates prepared from *Palmaria palmata*.

14 *M. japonica*: protein hydrolysates prepared from *Mazzaella japonica*.

15 Data are the mean $\pm$ SEM from triplicate determination.

16 *: $P < 0.05$ (the data of *M. japonica* compared with that of *P. palmata* by Student's t test).

TABLE 1

CHARACTERISTICS OF GENE AND PRIMARY STRUCTURES OF PHYCOBILIPROTEINS FROM *M. JAPONICA*.

Organism	Gene name	GC content (%)	Nucleotide identity to <i>M.japonica</i> (%)	Amino acid identity to <i>M.japonica</i> (%)	Accession No.
<i>Mazzaella japonica</i>	PE β -subunit	38.8	-	-	AB698819.2
	α -subunit	37.2	-	-	AB698819.2
	PC β -subunit	36.8	-	-	LC222737
	α -subunit	36.4	-	-	LC222737
	APC α -subunit	35.4	-	-	LC222738
	β -subunit	34.6	-	-	LC222738
<i>Chondrus crispus</i>	PE β -subunit	37.2	96	95	NC_020795.1
	α -subunit	39.1	97	96	NC_020795.1
	PC β -subunit	35.6	98	100	NC_020795.1
	α -subunit	35.6	97	100	NC_020795.1
	APC α -subunit	34.6	98	99	NC_020795.1
	β -subunit	34.2	98	100	NC_020795.1
<i>Gracilaria tenuistipitata</i>	PE β -subunit	38.8	80	81	NC_006137.1
	α -subunit	37.0	84	86	NC_006137.1
	PC β -subunit	37.6	83	92	NC_006137.1
	α -subunit	36.8	81	93	NC_006137.1
	APC α -subunit	36.2	82	96	NC_006137.1
	β -subunit	34.6	84	92	NC_006137.1
<i>Palmaria palmata</i>	PE β -subunit	40.5	79	83	AB625450.2
	α -subunit	40.2	82	87	AB625450.2
	PC β -subunit	38.9	79	91	AB679831.1
	α -subunit	39.3	79	91	AB679831.1
	APC α -subunit	40.7	79	95	AB742300.1
	β -subunit	36.0	81	94	AB742300.1
<i>Pyropia haitanensis</i>	PE β -subunit	41.4	81	88	HM008260.1
	α -subunit	41.2	81	85	HM008260.1
	PC β -subunit	39.9	80	91	HM008261.1
	α -subunit	38.2	80	91	HM008261.1
	APC α -subunit	38.7	81	94	AY372218.1
	β -subunit	35.6	81	93	AY372218.1
<i>Porphyra yezoensis</i>	PE β -subunit	41.2	80	88	KC517072.1
	α -subunit	42.2	81	85	KC517072.1
	PC β -subunit	38.9	81	91	KC517072.1
	α -subunit	39.3	79	91	KC517072.1
	APC α -subunit	37.2	83	95	KC517072.1
	β -subunit	36.4	80	93	KC517072.1
<i>Porphyra purpurea</i>	PE β -subunit	42.0	81	87	NC_000925.1
	α -subunit	41.8	80	85	NC_000925.1
	PC β -subunit	40.7	80	91	NC_000925.1
	α -subunit	38.7	78	90	NC_000925.1
	APC α -subunit	36.8	83	95	NC_000925.1
	β -subunit	36.2	81	93	NC_000925.1

FIG. 1

a

5' -

GATACAATATATTATATTGTGCGACACTAATACATCGGCTTGTCAAAAAATGACAGCTGAAACAGCTAAGTCCTTTACAAAAAATACAAGGAGAGTTCC	100
ATGCTTGATGCATTTTCTAGAGTTGTAGTTAATTCAGACGCTAAAGCTGCTTACGTAGGTGGCAGTGATCTACAAGCTCTTAAACTTTTATCGCAGATG	200
M L D A F S R V V V N S D A K A A Y V G G S D L Q A L K T F I A D	
GTAACAAACGTTTAGATGCTGTAAATTCTATTGTTTCTAATGCTAGCTGCATCGTATCAGACGCTGTTTCTGGTATGATTTGCGAAAATTCTGGACTAAT	300
G N K R L D A V N S I V S N A S C I V S D A V S G M I C E N S G L I	
TGCTCCAGGAGGCAATTGCTATACTAATCGTCGTATGGCTGCTTGTTCACGTATGGAGAGATTATTTACGCTATATCTCTTATGCTTTACTTGCAGGA	400
A P G G N C Y T N R R M A A C L R D G E I I L R Y I S Y A L L A G	
GATGCTTCTGTATTAGAAGATCGCTGCCTAAATGGTTTAAAGAACTTATATTGCTCTTGGCGTACCTAATAATTCTTCTGCTAGATCTGTTGCTATAA	500
D A S V L E D R C L N G L K E T Y I A L G V P N N S S A R S V A I	
TGAAATCTGCAGCTGTTGCATTTGTTAGTAATTCAGCATCTCAACGTACAATGCCTACAACCTAGTGGTGATTGTTCTGCATTATCTGCAGAAGTTGCAAG	600
M K S A A V A F V S N S A S Q R T M P T T S G D C S A L S A E V A S	
CTACTGTGATAGAGTTACCGCTGCACCTGGCTAAACTTTACAGCAATAAGACAATAATATTTAAATAGGTACTACAATCTAATAAGGATATAAAATGA	700
Y C D R V T A A L G * M	
AATCAGTTATTACTACTATAATCAGCGCTGCTGATGCTGCTGGTCGTTTTCTGACTAGCTCAGATCTTGAATCAGTACAAGGAAATATTCAACGTGCAGG	800
K S V I T T I I S A A D A A G R F L T S S D L E S V Q G N I Q R A G	
TGCAAGATTAGAAGCAGCTGAGAACTTGCAGATAATCATGAAGCTGTTGTAAGAAGCAGGAGATGCTTGTTTTACTAAATATTCATACTTAAAAAT	900
A R L E A A E K L A D N H E A V V K E A G D A C F T K Y S Y L K N	
TCAGGTGAAGCTGGAGACAGTCAAGAAAAAGTTAATAAATGTTATAGAGATATAGATCATTATATGCGTCTAATTAATGCTTTAATTGTTGGTGGAA	1,000
S G E A G D S Q E K V N K C Y R D I D H Y M R L I N Y A L I V G G	
CAGGACCTCTTGATGAGTGGGGTATTGCTGGTGCACGTGAAGTTTACAGAGCATTAAATCTACCATCTGCTTCTTATATTGCTGCATTTGCATTTACTCG	1,100
T G P L D E W G I A G A R E V Y R A L N L P S A S Y I A A F A F T R	
TGATAGATTGTGTGTCCTAGAGATATGTCAGCGCAAGCAGGCTTAGAATACGGTGTCTCTAGATTATGTTATCAATCACTATCTTAATATTATAAA	1,200
D R L C V P R D M S A Q A G L E Y G A A L D Y V I N S L S *	
CTTCAACACAATAATATTAAATAGATTTAAATAAAATAAAACCAAACTATATATAGTTTTGGTTTTATTTTAACTTGTACTTATTACTGTATT	1,300

-3'

b

5' -

AATTCAATATTTTCTTATCGTATTTGAAATGAAGATAAAATCTAAATCTTAGTTCTTAATAAACTTTGGTATATAAATCCATGTAAGGAGAAAAATCAAT	100
ATGTTAGATGCGTTTGCAAAAATCGTAGCTCAAGCTGATGCTAGAGGTGAATTCCTAAGTAATACACAATTAGATGCTTTAGTAGCAATGGTTGCAGAAG	200
M L D A F A K I V A Q A D A R G E F L S N T Q L D A L V A M V A E	
GTAATAAAAGATTAGATGTGGTAAATAAAATTAATTCTAATGCCTCAGCTATTGTAACAACTCTGCACGTGCTTTATTTGCAGAACCAACCACAATTAGT	300
G N K R L D V V N K I N S N A S A I V T N S A R A L F A E Q P Q L V	
TCAACCTGGAGGAAATGCTTATACGAGTAGAAGAATGGCTGCATGCTTGAGAGACATGGAAATCGTTTTAAGATATGTAAGCTATGCTATGATTGCTGGA	400
Q P G G N A Y T S R R M A A C L R D M E I V L R Y V S Y A M I A G	
GATTCTAGTGTTTTAGATGACAGATGCTTAATGGTTTAAGAGAACTTATCAAGCATTAGGCACACCAGGAACCTTCTGTAGCAGTAGCTGTACAAAAAA	500
D S S V L D D R C L N G L R E T Y Q A L G T P G T S V A V A V Q K	
TGAAAGAGGCTTCAATTGCACTAGCTAATGACTTAAATGGAGTACCTTTAGGAGACTGTAGTTCATTGACTGCTGAACTAGTTGTATATTTGACCGTGC	600
M K E A S I A L A N D L N G V P L G D C S S L T A E L V V Y F D R A	
TGCAGTTGCAGTCGTATAATTACACTAATAATACCAAGTTAAGAAATAAATATTATTATATAAGGAAAATTTATTATGAAAACACCTATCACAGAAGCTA	700
A V A V V * M K T P I T E A	
TTGCATCTGCTGACAGTCAAGGAAGATTTTTGAGCAATGGTGAATTACAATCAATAAATGGTAGATATCAAAGAGCTAACGCTAGTATAGAAGCAGCAAA	800
I A S A D S Q G R F L S N G E L Q S I N G R Y Q R A N A S I E A A K	
ATCTTTAACTAATAACGCACAAAGATTAATTACTGGAGCTGCTCAAGCCGTATATACAAAATATCCATTGTAACACAAATGCCAGGCCCACTTATGCA	900
S L T N N A Q R L I T G A A Q A V Y T K Y P F V T Q M P G P T Y A	
TCAAGTGCTATTGGAAAAGCCAAGTGTGCACGTGATATTGGATATTATTTAAGAATGACTACGTATTGCTTAGTTGTTGGAGCAACAGGACCGATGGACG	1,000
S S A I G K A K C A R D I G Y Y L R M T T Y C L V V G A T G P M D	
AGTATTTGGTGGCTGGTTTAGAAGAAATTAACCGCAGCTTTGAACTTTACCAAGTTGGTACATTGAAGCTTTACAGTATATTAAGTAAGTCATGGATT	1,100
E Y L V A G L E E I N R S F E L S P S W Y I E A L Q Y I K V S H G L	
AGCTGGACAAGCAGCAAAATGAAGCAAATACTTACTTAGATTATGCAATTAACACATTAAGTTAAGACTTCTATGTAACTTATATAAGTATAACAAATAA	1,200
A G Q A A N E A N T Y L D Y A I N T L S *	
CTAGAAAACGCATTATTTACATATTAATTGATGCTTACTTCTAGTTTCTTATTTTAATATTTGCAATAAATTAATATAAATTCCTTTTCTATATATTCTT	1,300

-3'

C

5' -

TAGCTAATTAATTGTATTGTATAATAGTATTATATATATAATTAGATTATCGTGTAAATTAATTTTTATTTAATACAAAATAGCTAAGGAGTTTAATAA	100
ATGAGTATAGTAACTAAATCAATTGTAATGCTGATGCAGAAGCTAGGTATTTAAGTCCAGGCGAATTAGATCGTATTAAAAGTTTTGTTTTATCTGGTA	200
M S I V T K S I V N A D A E A R Y L S P G E L D R I K S F V L S G	
AACGGAGATTAAGAATAGCTCAGGTATTAACAGATAATCGTGAACCTATTGTAAAACAAGGTGGTCAGCAATTATTTCAAAAGCGTCCAGATGTTGTTTC	300
K R R L R I A Q V L T D N R E L I V K Q G G Q Q L F Q K R P D V V S	
TCCAGGAGGAAATGCCTATGGTGAAGAAATGACAGCTACTTGTTTACGTGATTTAGACTATTATTTACGCTTAGTAACATATGGTATAGTTGCAGGGGAT	400
P G G N A Y G E E M T A T C L R D L D Y Y L R L V T Y G I V A G D	
GTTACACCCATCGAAGAAATTGGACTAGTCGGTGTAAAAGAAATGTATAATTCATTAGGTACTCCAATTTCTGGTGTAGCAGAAGGTATTCGTTCTATGA	500
V T P I E E I G L V G V K E M Y N S L G T P I S G V A E G I R S M	
AAAGTGCAGCTTGTTTCATTATTGTCTAGGAGAAGACTCTGCAGAAGCAGGTTTTTTATTTGATTATACTTTAGGAGCAATGCAGTAAAAATATACTAAAGA	600
K S A A C S L L S G E D S A E A G F Y F D Y T L G A M Q *	
TATATTTTATATTAATATAAAGTAAGGAAATTAATATTATGCAAGATGCTATTACTTCTGTAATTAATACAGCCGATGTACAAGGAAAATATTTAGACG	700
M Q D A I T S V I N T A D V Q G K Y L D	
ATAACTCATTAGAAAAATTACGTGGTTATTTTCAAACCTGGTGAACCTAAGAGTACGTGCAGCTGCTACTATCGCAGCGAATGCAGCGACTATCATTAAAGA	800
D N S L E K L R G Y F Q T G E L R V R A A A T I A A N A A T I I K E	
ATCAGTGGCTAAAGCTTTGTTGTATTCCGATATTACTAGACCTGGTGGTAATGTATACAACAAGACGCTATGCAGCATGTATTAGAGATTTAGATTAT	900
S V A K A L L Y S D I T R P G G N M Y T T R R Y A A C I R D L D Y Y	
TATTTAAGATATGCTACTTATGGTATGTTAGCAGGAGATCCATCTATCTTAGATGAACGTGTCTTGAATGGTTTAAAGAAACATATAATTCATTAGGTG	1,000
L R Y A T Y G M L A G D P S I L D E R V L N G L K E T Y N S L G V	
TACCAATCGGAGCAACGATTACAGACTATACAAGCAATGAAAGAAGTTACGGCTAAACTTGTAGGCTCTGATGCTGGTAAAGAAATGAGCCTATATTTTGA	1,100
P I G A T I Q T I Q A M K E V T A K L V G S D A G K E M S L Y F D	
TTATATTTGTTCTGGATTAAGTTAATATTAGATTAATTATAATAAAATCATAAGGAAGCTGAATAACCTCCTTATGATAATTGTTTAAAGCTTTAATTATT	1,200
Y I C S G L S *	
TAATACATTTATAGCCTGTATTCTAGCACGTGCTTTCTCAAATTTTGGGTTGCTTCTATCTTATCCTTATTACTTTTCGCTTCTGATAAAGCAATAGTT	1,300

-3'

FIG. 2

a

PE β	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MLDAFSRVVVNSDAKAAVVGGS DLQALKTFIADGNKRLDAVNSIVSNASCIVSDAVSGMICENSGLIAPGGNCYTNRMA								
<i>C. crispus</i>	MLDAFSRVVVNSDAKAAVVGGS DLQALKTFIADGNKRLDAVNSIVSNASCIVSDAVSGMICENPGLIAPGGNCYTNRMA								
<i>G. tenuistipitata</i>	MLDAFSRVVIDSDTKAAVVGGS NLQALKTFISEGNQRLDAVNSIVSNASCIVSDAVSGMICENPGLTSPGGNCYTNRMA								
<i>P. palmata</i>	MLDAFSRVVVNSDAKAAVVGGS DLQALKKFI TDGNKRLDSVSVSNASCIVSDAVSGMICENPGLIAPGGNCYTNRMA								
<i>P. yezoensis</i>	MLDAFSRVVVNSDAKAAVVGGS DLQALKKFIADGNKRLDSVNAIVSNASCIVSDAVSGMICENPGLIAPGGNCYTNRMA								
	*	*		*	*	*	*	*	*****
	90	100	110	120	130	140	150	160	170
	ACLRDGEIILRYISYALLAGDASVLED RCLNGLKETYIALGVPNNSSARSVAIMKSAAVAFVNSASQRTMPTTSGDCSALSAEVASYCDRVTAALG								
	ACLRDGEIILRYISYALLAGDASVLED RCLNGLKETYIALGVPNNSSIRSVMKAAAVFVNNTASQRKMATTSGDCSALSAEVASYCDRVGAALS								
	ACLRDGEIILRYISYALLAGDPSVLED RCLNGLKETYIALGVPITSSARAVNIMKASVAAFILNTAPGRKMDTASGDCTALASEVGSYFDRVCAAIS								
	ACLRDGEIILRYASYALLAGDPSVLED RCLNGLKETYIALGVPTNSSVRVAVSIMKASATAFVSGTASDRKMACPDGDCSALASELGSYCDRVAAAIS								
	ACLRDGEIILRYVSYALLAGDPSVLED RCLNGLKETYIALGVPTNSSVRVAVSIMKAAVAFITNTASQRKMATADGDCSALASEVASYCDRVAAAIS								
	** **	** *	*		*			* *	
PE α	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MKSVITTTIISAADAAGRFLTSSDLESVQGNIQRAGARLEAAEKLADNHEAVVKEAGDACFTKYSYLKNSGEAGDSQEKVN								
<i>C. crispus</i>	MKSVITTTIISAADAAGRFLTSSDLESVQGNIQRAGARLEAAEKLANNHEAVVKEAGDACFAKYSFLKNSGEAGDSQEKVN								
<i>G. tenuistipitata</i>	MKSVITTTIISAADAAGRFPSSDLESIQGNIQRASARLEAAEKLADNHDVAVVKEAGDACFGKYSYLKNAGEAGENQEKVN								
<i>P. palmata</i>	MKSVMTTTIISAADAAGRFPSSDLESVQGNIQRAAARLEAAEKLASNHEAVVKEGGDACFAKYSYLKNPGEAGDSQEKVN								
<i>P. yezoensis</i>	MKSVITTTIISAADAAGRFPSSDLESVQGNIQRAAARLEAAEKLASNHEAVVKEAGDACFAKYSYLKNPGEAGDSQEKVN								
	**	*	*	*		*		*	*
	90	100	110	120	130	140	150	160	
	KCYRDIDHYMRLINYALIVGGTGPLDEWGIAGAREVYRALNLPASYSIAAFATRDRLCVPRDMSAQAGLEYGAALDYVINSL								
	KCYRDIDHYMRLINYALIVGGTGPFDEWGIAGAREVYRALNLPASYLAAFVFTRDRLCVPRDMSAQAGLEYGAALDYVINSL								
	KCYRDIDHYMRLVNYSLVGGTGPLDEWAIAGAREVYRTLNLPTSAYVAAFATRDRLCVPRDMSAQAGVEYTTALDYIINSL								
	KCYRDVDHYMRLVNYSLVGGTGPLDEWAIAGAREVYRTLNLPTSAYVAAFATRDRLCVPRDMSAQAGGEYVAALDYIVNALT								
	KCYRDVDHYMRLVNYCLVGGTGPDWGIAGAREVYRTLNLPTSAYVASFAFARDRLCVPRDMSAQAGVEYAGNLDYIINSLC								
	** **	*		*		* * *			

b

PC β

	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MLDAFAKIVAQADARGEFLSNTQLDALVAMVAEGNKRLDVVNKINSNASAIVTNSARALFAEQPQLVQPGGNAYTSRRMA								
<i>C. crispus</i>	MLDAFAKIVAQADARGEFLSNTQLDALVAMVAEGNKRLDVVNKINSNASAIVTNSARALFAEQPQLVQPGGNAYTSRRMA								
<i>G. tenuistipitata</i>	MLDAFAKVVAQADARGEFLSNTQLDALANMVAEGNKRLDIVNKINSNASAIITNSARSLFAEQPQLVQPGGNAYTNRRMA								
<i>P. palmata</i>	MLDAFAKVVAQADARGEFLSNTQLDALSTMVNEGKKRLDVVNKINANASAIVTNSARALFAEQPQLVQPGGNAYTSRRMA								
<i>P. yezoensis</i>	MLDAFAKVVAQADARGEFLSNTQLDALSSMVAEGNKRLDVVNKINSNASAIVTNSARALFAEQPQLIQPGGNAYTSRRMA								
		*						*	**
	90	100	110	120	130	140	150	160	170
ACLRDMEIVLRYVSYAMIAGDSSVLDDRCLNGLRETYQALGTPGTSVAVAVQKMKEASIALANDLNGVPLGDCSSLTAELEVYFDRAAVAVV									
ACLRDMEIVLRYVSYAMIAGDSSVLDDRCLNGLRETYQALGTPGTSVAVAVQKMKEASIALANDLNGVPLGDCSSLTAELEVYFDRAAVAVV									
ACLRDMEIVLRYVSYAMIAGDSSVLDDRCLNGLRETYQALGTPGTSVAVAIQKMKEASVSLANDSNGTPIGDCSSLTAELEVYFDRAAVAVV									
ACLRDMEIVLRYVSYSMVAGDSSVLDDRCLNGLRETYQALGTPGTSVAVAIQKMKEASVALANDLNNVPLGDCSALTAELEGSYFDRAAIAVV									
ACLRDMEIVLRYVSYAMIAGDSSVLDDRCLNGLRETYQALGTPGSSVSVAVQKMKEASVALANDLTGITQGDCSALIAELGSYFDRAAVSVV									
**	**	*					*		

PC α

	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MKTPITEAIASADSQGRFLSNGELQSINGRYQRANASIEAAKSLTNNAQRLITGAAQAVYTKYPFVTQMPGPTYASSAIG								
<i>C. crispus</i>	MKTPITEAIASADSQGRFLSNGELQSINGRYQRANASIEAAKSLTNNAQRLITGAAQAVYTKYPFVTQMPGPTYASSAIG								
<i>G. tenuistipitata</i>	MKTPITEAIASADSQGRFLSNGELQSINGRYQRASASLEAARSLSNAQRLITGAAQSVYTKFPFTTQMPGPTYASSAIG								
<i>P. palmata</i>	MKTPITEAIASADSQGRFLSNAELQSINGRYERASSSLEAAASLTNSAQRLITGAAQAVYTKFPFTTQMPGPTYASSAIG								
<i>P. yezoensis</i>	MKTPITEAIASADSQGRFLSNGELQAINGRYQRAAASLGAARSLTNNAQRLITGAAQSVYTKFPYVTQMPGPTYASSAIG								
		*							
	90	100	110	120	130	140	150	160	
KAKCARDIGYYLRMTTYCLVVGATGPMDEYLVAGLEEINRSFELSPSWYIEALQYIKVSHGLAGQAANEANTYLDYAINTLS									
KAKCARDIGYYLRMTTYCLVVGATGPMDEYLVAGLEEINRSFELSPSWYIEALQYIKVSHGLAGQAANEANTYLDYAINTLS									
KAKCARDIGYYLRMTTYCLVVGATGPMDEYLIAGLEEINRSFELSPSWYIEALQYIKNSHLSGQAANEANTYLDYAINALS									
KAKCARDIGYYLRMTTYCLVVGATGPMDEYLVAGLEEINRSFELSPSWYIEALQYIKSSHLSGQVGNEANTYVDYAINTLS									
KAKCARDIGYYLRMTTYCLVVGATGPMDEYLVAGLEEINRSFELSPSWYVEALQYIKGSHLSGQIGNEANVYLDYAINTLS									
* **	*	*							

C

APC α

	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MSIVTKSIVNADAEARYLSPGELDRIKSFVLSGKRRLRIAQVLTDNRELIVKQGGQQLFQKRPDVVSPGGNAYGEEMTAT								
<i>C. crispus</i>	MSIVTKSIVNADAEARYLSPGELDRIKSFVLSGKRRLRIAQVLTDNRELIVKQGGQQLFQKRPDVVSPGGNAYGEEMTAT								
<i>G. tenuistipitata</i>	MSIITKSIVNADAEARYLSPGELDRIKSFVLSGQRRLRIAQILTNDRELIVKQGGQQLFQKRPDVVSPGGNAYGEEMTAT								
<i>P. palmata</i>	MSIVTKSIVNADAEARYLSPGELDRIKSFVLSGQRRLRIAQILTENRERIVKQGGQQLFQKRPDVVSPGGNAYGEEMTAT								
<i>P. yezoensis</i>	MSIVTKSIVNADAEARYLSPGELDRIKSFVLSGQRRLRIAQILTNDRELIVKQGGQQLFQKRPDVVSPGGNAYGEEMTAT								
	*	*	**	*	**	*			*

	90	100	110	120	130	140	150	160	
	CLRDLDYYRLRLVTYGI VAGDVTPIEEIGLVGVKEMYNSLGTPISGVAEGIRSMKSAACSLLSGEDSAEAGFYFDYTLGAMQ								
	CLRDLDYYRLRLVTYGI VAGDVTPIEEIGLVGVKEMYNSLGTPISGVAEGIRSMKGAACSLLSGEDSAEAGFYFDYTLGAMQ								
	CLRDLDYYRLRLVTYGI VAGDVTPIEEIGLVGVKEMYNSLGTPISGVAEGVRSMKNVACSLLAGEDSAEAGFYFDYTLGAMQ								
	CLRDLDYYRLRLVSYGI VAGDVTPIEEIGLVGVKEMYNSLGTPISGVAEGVRSMKNVACSLLSGEDSAEAGFYFDYTLGAMQ								
	CLRDLDYYRLRLVTYGI VAGDVTPIEEIGLVGVKEMYNSLGTPISGVAEGVKCMKSVACSLLAGEDSAEAGFYFDYTLGAMQ								
	*	***	*	*			*	*	*

APC β

	1	10	20	30	40	50	60	70	80
<i>M. japonica</i>	MQDAITSVINTADVQGGKYLDNLSLEKLRGYFQTGELRVRAAATIAANAATIIKESVAKALLYSDITRPGGNMYTTRYAA								
<i>C. crispus</i>	MQDAITSVINTADVQGGKYLDNLSLEKLRGYFQTGELRVRAAATIAANAATIIKESVAKALLYSDITRPGGNMYTTRYAA								
<i>G. tenuistipitata</i>	MQDAITSVINAADVQGGRYLDNLSLKLRLGYFQTGELRVASATIAANAATIIKDSVAKALLYSDITRPGGNMYTTRYAA								
<i>P. palmata</i>	MQDAITSVINAADVQGGKYLDSSVEKLRGYFQTGELRVRAAATIAANAATIIKESVAKSLLYSDITRPGGNMYTTRYAA								
<i>P. yezoensis</i>	MQDAITSVINAADVQGGKYLDSSVEKLRGYFQTGELRVRAAATIAANAATIIKESVAKSLLYSDITRPGGNMYTTRYAA								
	*	*	*						

	90	100	110	120	130	140	150	160
	CIRDLDYLLRYATYGMLAGDPSILDENVLNGLKETYNSLGVPIGATIQTIQAMKEVTAKLVGSDAGKEMSLYFDYICSGLS							
	CIRDLDYLLRYATYGMLAGDPSILDENVLNGLKETYNSLGVPIGATIQTIQAMKEVTAKLVGSDAGKEMSLYFDYICSGLS							
	CIRDLDYLLRYSTYGMLAGDPSILDENVLNGLKETYNSLGVPIGATIQAQAMKEVTSSLVGPDAGKEMGVYFDYICSGLS							
	CIRDLDYLLRYATYGMLAGDPSILDENVLNGLKETYNSLGVPIGATIQAQAMKEVTIGLVGPDAGKEMGLYFDYICSGLS							
	CIRDLDYLLRYATYGMLAGDPSILEENVLNGLKETYNSLGVPIGATIQAAILAMKEVTISLVGPDAGKEMGLYFDYICSGLS							
	*	**	**	*	*			

FIG. 3

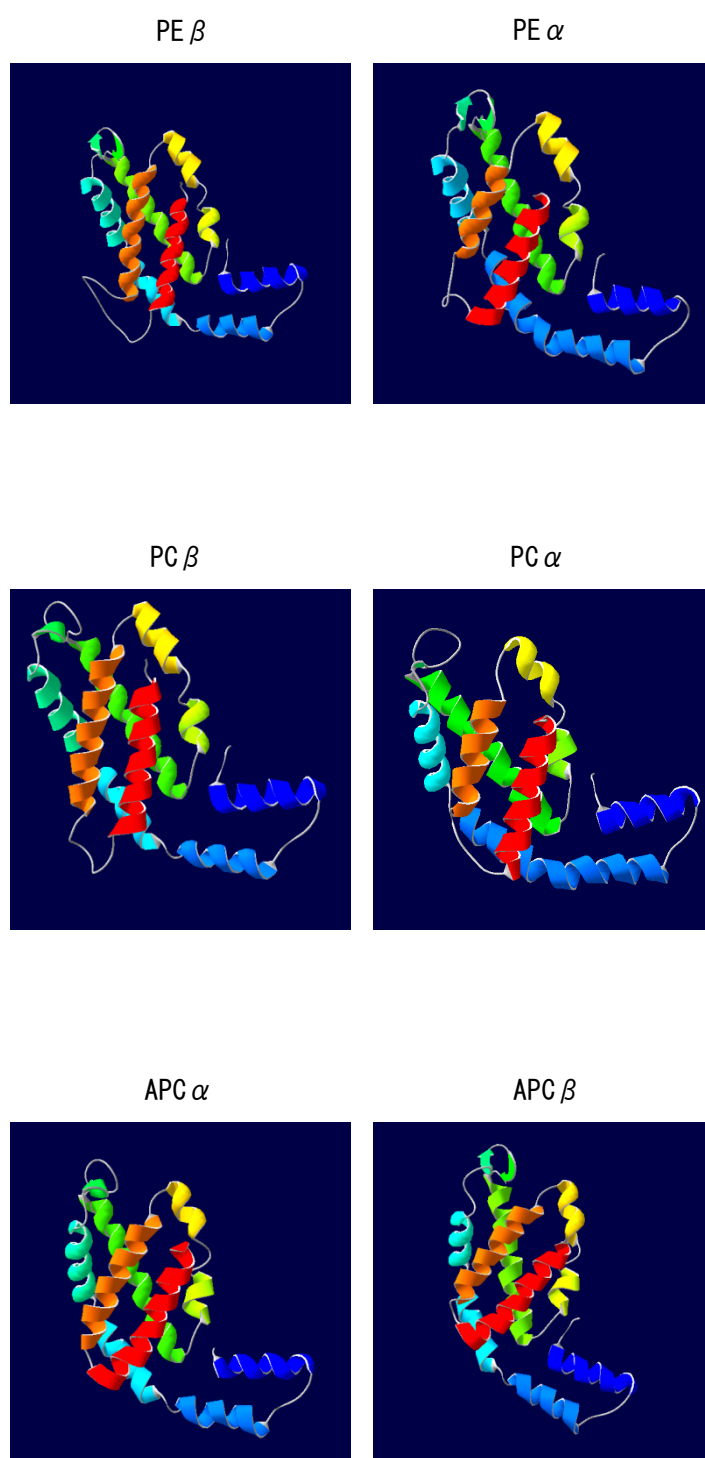


FIG. 4

