



Title	Collagen peptides derived from the triple helical region of sturgeon collagen improve glucose tolerance in normal mice
Author(s)	Sasaoka, Yukiho; Kishimura, Hideki; Adachi, Shinji; Takagi, Yasuaki
Citation	Journal of food biochemistry, 42(2), e12478 https://doi.org/10.1111/jfbc.12478
Issue Date	2018-04
Doc URL	http://hdl.handle.net/2115/73422
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Type	article (author version)
File Information	J_Food_Biochem_2018_42_e12478.pdf



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1 **Collagen peptides derived from the triple helical region of**
2 **sturgeon collagen improve glucose tolerance in normal**
3 **mice**

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22

1 **Abstract**

2 We prepared collagen peptides (SCP) from sturgeon by-products: the skin, fin and bone. The
3 materials were pretreated and hydrolyzed by papain. Finally, we obtained 85.1 g of dried SCP
4 powder from 864 g (wet weight) of the materials. In oral glucose tolerance test (OGTT) with
5 ICR mice, blood glucose levels of SCP group were significantly lower than those of control
6 group. Then, we fractionated SCP by Sephadex column chromatography and all fractions
7 showed hypoglycemic effect. Further, we separated each peptide in the fractions by
8 reversed-phase HPLC. Most of the peptides in these peaks consisted of Gly-X-Y (X and Y are
9 optional amino acid residues) repetitive sequences which are common to the triple helical
10 region of the collagen molecules. Moreover, many peptides contained Pro and Ala residues,
11 which promises to serve as a DPP-IV inhibitor. Altogether, these results suggest the
12 hypoglycemic effect of SCP may be caused by these structural properties.

13

14 **Keywords:**

15 Sturgeon; Collagen peptides; Triple helical region; Gly-X-Y; Oral glucose tolerance test
16 (OGTT); Hypoglycemic effect

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1 **1. Introduction**

2 Diabetes is one of the most serious global health problems. The number of patients were
3 estimated 415 million in 2015 and are predicted to reach to 642 million in 2040 (IDF, 2015).
4 About 90% of diabetic patients are type 2 diabetes mellitus and many kinds of oral
5 hypoglycemic drugs have been developed for improving glycemic control to prevent diabetes
6 complication: sulfonylureas, α -glucosidase inhibitors, incretin agonists, DPP-IV inhibitors,
7 and so on (Lorenzati, Zucco, Miglietta, Lamberti, & Bruno, 2010). East Asian, also
8 including Japanese, are innately unable to secrete insulin as much as African and Caucasian
9 (Kodama et al., 2013). A small amount of insulin secretion predisposes East Asian to type 2
10 diabetes mellitus following even a slight deterioration in insulin sensitivity caused by body
11 weight gain. If dairy diet which has therapeutic and medicinal properties routinely enhances
12 an amount of insulin secretion without side-effects, it would lead to better glycemic control
13 and reduce a risk of the onset of type 2 diabetes mellitus. Thus, many studies have been
14 performed to explore foods and/or natural compounds which have hypoglycemic effect
15 (Broadhurst, Polansky, & Anderson, 2000; Mochida, Hira, & Hara, 2010; Yilmazer-Musa,
16 Griffith, Michels, Schneider, & Frei, 2012). Previously, we isolated an acid- and heat-stable
17 trypsin inhibitory peptide from the viscera of Japanese common squid (*Todarodes pacificus*)
18 (Kishimura et al., 2010). The squid trypsin inhibitor showed hypoglycemic effect on blood
19 glucose levels of GK rats (a model of type 2 diabetes mellitus), and its effect was related to an
20 improvement of insulin secretion in GK rats (Adachi et al., 2012; Kishimura et al., 2012).

21 Sturgeon is famous and highly valuable for its salted egg “caviar”. Additionally,
22 people who live in Russia and China have been eaten sturgeon meat traditionally (Bronzi,
23 Rosenthal, & Gessner, 2011). The maximum catch of sturgeon in the world was 32,078 tons
24 in 1977. However, the wild sturgeon population decreased rapidly in 1980s and reached to
25 about 5,000 tons in 1995 (Bronzi et al., 2011) because of overfishing, illegal fishing, changing

1 the water regime by construction of dams and channels, loss of spawning habitats, and water
2 pollution (Billard & Lecointre, 2001). Consequently, sturgeon aquaculture has increased since
3 2003 all over the world and the aquaculture production was no less than 25,000 tons in 2007
4 (Bronzi et al., 2011; Takahashi, 2010). Even in Japan, which is the world's 4-largest caviar
5 importing country, sturgeon aquaculture has been promoting (Takahashi, 2010).

6 Egg and meat are harvested from cultured sturgeon for food, while other parts such as
7 skin, fin and bone are by-products which are not fully utilized in seafood industry. Recently,
8 we purified collagens from Bester sturgeon organs and clarified that the skin and swim
9 bladder are rich in collagen (Zhang et al., 2014). Collagen has Gly-X-Y repetitive sequences
10 which construct a unique triple helix structure because the existence of Gly residue in every
11 third position allows typical triple-chain packing of its molecule (Hulmes, Miller, Parry, Piez,
12 & Woodhead-Galloway, 1973), that is, collagen implicates undenatured triple helix collagen,
13 i.e., atelocollagen, and partially or mostly denatured triple helix collagen. Collagen and
14 collagen peptides have been widely used in food, cosmetics and material of tissue-engineering
15 (Parenteau-Bareil, Gauvin, & Berthod, 2010). There are many reports on health benefits of
16 collagen and collagen peptides, for instance, increasing bone density and improvement skin
17 condition (Koyama, 2010). Furthermore, it was investigated that the improvement of
18 glycemic control in streptozotocin (STZ)-induced diabetic rats by salmon skin gelatin
19 hydrolysate as dipeptidyl-peptidase (DPP)-IV inhibitor (Hsieh, Wang, Hung, Chen, & Hsu,
20 2015). It was also demonstrated that oral administration of fish scale gelatin hydrolysate
21 improved glucose tolerance in normal mice (Iba et al., 2016). These results suggest that the
22 peptide derived from the by-products of sturgeon has a potential as beneficial ingredient for
23 functional foods preventing type 2 diabetes mellitus.

24 Therefore, in this study, we prepared collagen peptides (SCP) from the skin, fin and
25 bone of Bester sturgeon and investigated its hypoglycemic effect. Moreover, we fractionated

1 SCP by gel filtration and analyzed the amino acid sequences of fractionated peptides to
2 discuss the relationship between hypoglycemic effect of SCP and its structural properties.

3

4 **2. Materials and methods**

5 *2.1. Materials*

6 Bester sturgeons (*Huso huso* x *Acipenser ruthenus*) were bred at Nanae Fresh-Water Station,
7 Field Science Center for Northern Biosphere, Hokkaido University. The skin, fin and bone
8 removed notochord (864 g) were dissected from 2 males of live specimens (body weight: 3.40
9 kg and 2.75 kg).

10 Papain (EC 3.4.22.2, 1 : 350) BSA and other reagents were purchased from Wako Pure
11 Chemical Industries (Osaka, Japan). Pepsin from porcine gastric mucosa (EC 3.4.23.1,
12 3,200-4,500 units/mg) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

13

14 *2.2. Preparation of samples*

15 *2.2.1 Preparation of SCP and bovine serum albumin (BSA) hydrolysate*

16 The skin, fin and bone of Bester sturgeon were successively soaked in 1% NaCl solution at 4
17 °C for 2 days, 0.2 M NaOH solution at 4 °C for 24 h, and 0.2 M HCl solution at 4 °C for 24 h
18 to remove other proteins and contaminants. After these treatments, the materials were rinsed
19 with distilled water at 4 °C for 24 h to remove HCl. The pretreated materials were minced and
20 hydrolyzed by 0.2 wt% of papain at 50 °C for 5 h. The hydrolysates were centrifuged at 4 °C,
21 10,000g for 10 min, and the supernatant was lyophilized. Then, the dried powder was soaked
22 in 99.5% ethanol at 4 °C for 3 h to remove lipids. The suspended substance centrifuged at 4 °C,
23 10,000g for 10 min, and the precipitate was dried by incubation at 60 °C into SCP.

24 BSA was hydrolyzed by papain, and delipidated and dried similarly to the preparation of SCP.

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2.2.2 Preparation of A-SCP

Atelocollagen peptides (A-SCP) were prepared as follows. The skin of Bester sturgeon was cut into small pieces. The pieces were successively soaked in 5% H₂O₂ solution at 4 °C for 24 h, 1% NaCl solution at 4 °C for 24 h, 0.2 M NaOH solution at 4 °C for 24 h, 0.2 M HCl solution at 4 °C for 24 h, distilled water solution at 4 °C for 24 h, and 99.5% ethanol at 4 °C for 6 h. After that, the materials were suspended in 0.1 M HCl solution and hydrolyzed by 1 wt% of porcine pepsin at 4 °C for 72 h to extract atelocollagen. After the reaction, NaCl was added to a final concentration of 1 M into the extract and stirred at 4 °C for 24h. The solution was centrifuged at 4 °C, 10,000g for 15 min. The precipitate was suspended in distilled water and dialyzed against distilled water at 4 °C for 24 h. The dialysate was lyophilized into sturgeon atelocollagen. The atelocollagen was hydrolyzed by 0.2 wt% of papain at 50 °C for 5 h. The hydrolysates were centrifuged at 4 °C, 10,000g for 10 min, and the supernatant was lyophilized into A-SCP.

2.3. Animals

Six weeks old male ICR mice were purchased from Charles River Laboratories (Yokohama, Japan). They were maintained under controlled temperature (23±1 °C), humidity (50±10%), and 12 h-light/dark cycle (light: 8:00 ~ 20:00) with free access to deionized water and MF diet (Oriental Yeast Co., Tokyo, Japan). All procedure for the use and care of the mice were approved by Institutional Animal Care and Use Committee of National University Corporation Hokkaido University.

2.4. Measurement of blood glucose levels in mice

1 All the oral glucose tolerance tests (OGTT) were performed as follows. ICR mice were fasted
2 for 16 h and performed an OGTT. The mice were divided into a control group and sample
3 groups (SCP, BSA hydrolysate, the f1, f2, f3 or A-SCP) matched for body weight in each test.
4 After basal blood collection (0 min), glucose solution (2 g/kg body weight, 15 mL/kg body
5 weight) mixed with sample (1.5 g/kg body weight) (sample group) or with distilled water
6 (control group) were orally injected with a disposable probe (FUCHIGAMI, Kyoto, Japan). A
7 single drop of blood was periodically sampled (15, 30, 60 and 120 min after injection) from
8 tail vein and glucose levels were measured using a glucose sensor (GLUCOCARD MyDIA,
9 ARKRAY Inc., Kyoto, Japan). Area under the glucose curves between 0 and 120 min (AUC)
10 were calculated by the trapezoidal rule based on the blood glucose levels up to the time of
11 final measurement for each group.

12

13 *2.5. Gel filtration*

14 SCP was fractionated by gel filtration on Sephadex G-50 and Sephadex G-25 (GE Healthcare
15 Bio-Science AB, Uppsala, Sweden). Both columns (G-50: 4.0 x 77 cm, G-25: 6.5 x 100 cm)
16 were equilibrated with distilled water, and peptides were eluted with the same solution at a
17 flow rate of 0.7 mL/min and detected by the absorbance at 230 nm.

18

19 *2.6. Reversed-phase HPLC*

20 The samples were dissolved to a final concentration of 0.5 mg/mL in ultrapure water
21 containing 0.1% trifluoroacetic acid (TFA). The solutions applied to filtration by Millex-LG.
22 Peptides in the filtrate were separated by reversed-phase HPLC with a Mightysil RP-18 GP
23 Aqua (4.6 x 250 mm) (KANTO CHEMICAL CO., INC., Tokyo, Japan). Elution was
24 performed with a linear gradient of acetonitrile from 5 to 80% in 0.1% TFA at a flow rate of
25 1.0 mL/min.

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2.7. Analysis of amino acid sequence

Amino acid sequences of peptides separated by reversed-phase HPLC was analyzed by using a protein sequencer Procise 493 (Applied Biosystems, CA, USA).

2.8. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was performed on a 0.1% SDS-7.5% polyacrylamide slab-gel according to the method of Laemmli (Laemmli, 1970). The atelocollagen was dissolved in 0.1 M HCl (3 mg atelocollagen / mL) and centrifuged at 4 °C, 15,000g for 5 min. The supernatant was mixed at a ratio of 1:1 (v/v) with the sample buffer (0.0625 M Tris-HCl buffer (pH 6.8), 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 2% bromophenol blue as the dye) and boiled for 5 min. Ten milligrams of protein were loaded in each lane. Electrophoresis was carried out at 10 mA. After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R-250 in 50% methanol-7% acetic acid, and then destained in 7% acetic acid. Protein Molecular Weight Markers (GE Healthcare Life Sciences, Tokyo, Japan) were used to estimate the molecular weight.

The triple helical structure of the prepared atelocollagen was validated with reference to the denaturation temperature of Bester sturgeon collagen (Zhang et al., 2014). The supernatants were preincubated at 15 °C and 35 °C for 30 min, respectively, and then hydrolyzed by 5 wt% of porcine pepsin. After 30 min reaction, NaOH was added to a final concentration of 0.1 M into the digest solutions to stop the enzymatic reaction. Then, SDS-PAGE was performed as described above.

2.9. Statistical analysis

The data of OGTT were expressed as mean \pm standard error of the mean (SEM). Statistical

1 analyses were performed using Student's t test for single comparisons, and Kruskal-Wallis test
2 or one-way ANOVA followed by Steel-Dwass's test or Tukey-Kramer's test for multiple
3 comparisons. All statistics were completed with Statcel - The Usefull Addin Forms on Excel
4 3rd ed. (OMS Publishing Inc., Saitama, Japan).

5

6 **3. Results**

7 *3.1. Preparation of SCP*

8 We obtained 85.1 g (dry weight) of SCP from 864 g (wet weight) of the skin, fin and bone of
9 Bester sturgeon (yield: approximately 100 g SCP/kg by-products). As the papain hydrolysates
10 contained a small amount of lipid (mainly triglyceride), we removed them by ethanol
11 treatment because the lipids cause fish odor and oxidation that lead quality degradation. The
12 final of SCP was white, odorless, and easily soluble in water by the treatment.

13

14 *3.2. Hypoglycemic effect of SCP*

15 Blood glucose levels rapidly increased after glucose injection to 15 min in all the groups (Fig.
16 1a). In the control group, the levels kept increasing to 247 mg/dL at 30 min, and then returned
17 to the basal level at 120 min. However, in the SCP group, the levels slightly increased to 198
18 mg/dL at 30 min, and gradually declined to the basal level. The level was significantly lower
19 than that of the control group at 15 min. In the BSA hydrolysate group, the levels also
20 increased to 210 mg/dL at 15min, and then turned to decrease differing from the control and
21 SCP groups. Although the levels were lower than those of the control group at 15 and 30 min,
22 the levels did not go below those of the SCP group. The AUCs of the SCP and BSA
23 hydrolysate groups were lower than that of the control group. However, there was no
24 difference in the AUCs among all the groups (Fig. 1b).

25

1 *3.3. Investigation of the relationship between hypoglycemic effect of SCP and its structure*

2 Using gel filtration with Sephadex G-50, we obtained a single fraction as F1 (Fig. 2a). Thus,
3 we fractionated F1 with Sephadex G-25 and detected three fractions; f1, f2 and f3 (Fig. 2b).
4 In OGTT, the F1 group lowered blood glucose levels (The data was not shown). All the three
5 fractions showed hypoglycemic effect, and the levels of the f2 and f3 groups were
6 significantly lower than those of the control group at 15 and 30 min (Fig. 3a). Also, the levels
7 of the f1 and f2 groups were significantly lower than those of the control group at 30 and 60
8 min. The AUCs of the f1 and f2 groups were also significantly lower than that of the control
9 group, and there was no difference in the AUCs between the f1 and f2 groups (Fig. 3b).

10 Furthermore, we performed reverse-phased HPLC analysis and clarified amino acid
11 sequences of the peptides in the three fractions. In the analysis, we obtained 6 peptide
12 sequences from the f1 fraction, 5 peptide sequences from the f2 fraction, and 7 peptide
13 sequences from the f3 fraction, respectively (Fig. 4a, 4b and 4c). As shown in Table 1, the
14 peptides collected from the f1 and f2 fractions basically consisted of Gly, Ala, Pro and Hyp,
15 which composed Gly-X-Y repetitive sequences, while several peptides collected from the f3
16 fraction did not construct the repetitive sequences. The mean values of molecular weight of
17 the peptides in the f1, f2 and f3 fractions were 859 (774.93 ~ 907.08), 498 (454.56 ~ 556.69)
18 and 576 (314.38 ~ 1001.20), respectively.

19

20 *3.4. Hypoglycemic effect of A-SCP*

21 *3.4.1. Structure of A-SCP*

22 We prepared an atelocollagen from the skin of Bester sturgeon. As shown in Fig. 5a, the
23 prepared atelocollagen consisted of the β -chain (ca. 220kDa) which was formed by
24 intramolecularly cross-linking between two α -chains, the α 1-chain (ca. 120kDa) and the
25 α 2-chain (ca. 110kDa), and the density of the α 1-chain band was higher than that of the

1 α 2-chain band. This result suggests the atelocollagen seems to be classified as type I collagen.
2 In Fig. 5b, although the density of the bands of the β -chain and α -chains were clearly
3 observed in the undigested atelocollagen, the band intensity became lower at 15 °C and lots of
4 bands were observed in low molecular weight. It means that part of the prepared atelocollagen
5 is denatured and digested by pepsin at 15 °C. Moreover, the bands of the chains were
6 completely abolished by pepsin digestion at 35 °C. These results affirm previous study (Zhang
7 et al., 2014), and demonstrate the prepared atelocollagen formed a triple helix structure.

8

9 *3.4.2. Changes in blood glucose levels under A-SCP administration in OGTT*

10 Blood glucose levels increased to 30 min in all the groups (Fig. 6a). The levels of the A-SCP
11 group were slightly higher than those of the SCP group and peaked to 301 mg/dL. The levels
12 of the A-SCP group were significantly lower than those of the control group at 15, 30 and 60
13 min, similarly to those of the SCP group. There was no difference in the blood glucose levels
14 between the A-SCP and SCP groups. The AUC of the A-SCP group was also significantly
15 lower than that of the control group, similarly to that of the SCP group (Fig. 6b).

16

17 **4. Discussion**

18 We obtained approximately 100 g SCP/kg by-products; the skin, fin and bone of Bester
19 sturgeon. The yield of SCP (9.8%) was similar to that of collagen (11.9% on a wet weight
20 basis) prepared from the skin of Bester sturgeon in previous study (Zhang et al., 2014).
21 Moreover, SCP had the physical properties, e.g., white, odorless, and easily soluble in water,
22 and it could be advantage in use SCP as an ingredient for functional foods.

23 First, we examined the hypoglycemic effects of SCP in normal mice. In OGTT, SCP
24 significantly decreased blood glucose levels as compared with those of the control group (Fig.
25 1a). The BSA hydrolysate also decreased blood glucose levels. BSA is one of the major

1 protein contained in whey which has the potential to improve glycemic control (Frid, Nilsson,
2 Holst, & Björck, 2005; Gillespie, Calderwood, Hobson, & Green, 2015; Nilsson, Stenberg,
3 Frid, Holst, & Björck, 2004; Schopen et al., 2017). Besides, long-term intake of BSA
4 improved fasting insulin and glucose in mice even though the mice were fed high fat diet
5 (McManus et al., 2015). Thus, in this study, the BSA hydrolysate might enhance insulin
6 secretion.

7 In previous study, we confirmed that a matter of lipid (0.38% on a dry weight basis
8 of SCP) existed in SCP (data not shown). By thin-layer chromatography (TLC) analysis, the
9 lipid was mainly composed of triglyceride and contained little free fatty acid and sterol.
10 Furthermore, we removed the residual lipid from SCP by supercritical fluid extraction (SFC)
11 using carbon dioxide, and then no triglyceride, free fatty acid and sterol were detected in the
12 SFC-treated SCP by TLC. In OGTT with normal ICR mice, blood glucose levels also
13 decreased in the SFC-treated SCP group as well as in the SCP group. Therefore, we
14 demonstrated that the peptides in SCP affect hyperglycemia, not the residual lipid in SCP.

15 Next, we fractionated SCP with gel filtration to discuss its structure-function
16 relationship. Using gel filtration with Sephadex G-25, we obtained three fractions (Fig. 2b). In
17 OGTT, although all the three fractions showed hypoglycemic effect, the f1 and f2 groups
18 showed higher effect than the f3 group (Fig. 3a and Fig. 3b). However, there was no
19 correlation between the potency and the mean molecular weights of the fractions. Therefore,
20 we considered that the hypoglycemic effect was occurred by not the molecular weight but the
21 structure of the peptides in the fractions. With analysis of amino acid sequence, the f1 and f2
22 fractions possessed the Gly-X-Y repetitive sequences, while the f3 fraction contained both the
23 repetitive and non-repetitive sequences (Table 1). These results suggest that the peptides in the
24 f1 and f2 fractions are derived from the triple helix region of the collagen molecules, and the
25 peptides in the f3 fraction are derived from both the triple helix region and the non-helical

1 region known as the telopeptide region which does not contain the Gly-X-Y repetitive
2 sequence. For instance, f3-6 in Table 1 comes from the N-terminal telopeptide of type 1
3 sturgeon collagen α 1-chain (DNA Data Bank of Japan accession number EU241879,
4 LC008481-8485). Therefore, the f3 fraction most likely contains the Gly-X-Y repetitive
5 sequences relatively less than the f1 and f2 fractions per unit weight. Because it seemed that
6 the existence of the Gly-X-Y repetitive sequences related to the potency of the fractions (Fig.
7 3a and 3b), we hypothesized that the Gly-X-Y repetitive sequences which were derived from
8 the triple helix region bring the hypoglycemic effect. To verify the hypothesis, we prepared
9 A-SCP from the atelocollagen, which only consist of the Gly-X-Y sequences and form the
10 triple helical structure, and evaluated its hypoglycemic effect. As shown in Fig. 6a and Fig. 6b,
11 A-SCP indicated equivalent hypoglycemic ability to that of SCP. This result clearly shows
12 that the effective peptides derived from the Gly-X-Y repetitive sequences, namely the triple
13 helix region of the collagen molecule has the hypoglycemic effect. Li-Chan et al. (2012)
14 reported that two kinds of peptides including Gly-X-Y sequences showed hypoglycemic
15 effect (Li-Chan, Hunag, Jao, Ho, & Hsu, 2012). Our finding is consistent with the report of
16 Li-Chan et al. (2012). The peptides in the f1 and f2 fractions formed oligo-peptides which
17 differs from the peptides reported by Li-Chan et al. (2012). Several studies reported that
18 collagen peptides administrated orally digested into di- and/or tri-peptides and absorbed into
19 the blood (Iwai et al., 2005; Ohara, Matsumoto, Ito, Iwai, & Sato, 2007; Shigemura,
20 Kubomura, Sato, & Sato, 2014). For this reason, SCP is more likely to affect after digestion
21 into di-/tri-peptides in gastrointestinal tract.

22 In hypoglycemic studies, DPP-IV is well known as an enzyme inactivating incretin
23 hormone which stimulate insulin secretion; glucose-dependent insulintropic polypeptide
24 (GIP) and glucagon-like peptide-1 (GLP-1). Several food-derived peptides are reported to
25 exert hypoglycemic effect via DPP-IV inhibition and GLP-1 secretion (Higuchi, Hira, Yamada,

1 & Hara, 2013; Ishikawa et al., 2015; Mochida et al., 2010). DPP-IV inhibition is also
2 observed in collagen peptides, and it is expected that the inhibition effect is attributed to Pro
3 rich structure of collagen peptides because DPP-IV degrades peptides with Pro and Ala in the
4 penultimate N-terminal residues (Hsieh et al., 2015; Iba et al., 2016; Li-Chan et al., 2012;
5 Mentlein, Gallwitz, & Schmidt, 1993). There are many peptides containing Pro and Ala
6 residues in SCP, and thus SCP might show DPP-IV inhibitory activity. Now, we are studying
7 the detail mechanisms of the hypoglycemic effect by SCP.

8

9 **5. Conclusion**

10 Collagen peptides (SCP), which were white, odorless and water-soluble, were prepared from
11 Bester sturgeon by-products: skin, fin and bone. In OGTT with normal mice, SCP
12 significantly improved glucose tolerance. The result implies that SCP has a potential as a
13 beneficial ingredient for functional foods preventing type 2 diabetes mellitus. Moreover, it is
14 suggested that the hypoglycemic effect of SCP might be caused by the peptides which possess
15 structural properties of the Gly-X-Y repetitive sequence and/or an abundance of Pro and Ala
16 residues derived from the triple helical region of the collagen molecule.

17

18 **Conflict of interest**

19 The authors have declared no conflicts of interest.

20

21 **Acknowledgment**

22 The authors thank Dr. Hiroki Saeki, Faculty of Fisheries Sciences, Hokkaido University, for
23 helpful discussion. This work was supported in part by the Regional Innovation Cluster
24 Program (Global Type), Ministry of Education, Culture, Sports, Science and Technology,
25 Japan.

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22

1 **TABLE 1.**2 **AMINO ACID SEQUENCES OF PEPTIDES IN THE f1, f2 AND f3 FRACTIONS**

Fraction	Peak No.	Amino acid sequence	Molecular weight
f1	f1-1	A-HP-G-P-A-G-P-T-G-K	868.09
	f1-2	A-HP-G-P-V-G-P-A-G-P	835.06
	f1-3	E-HP-G-P-A-G-P-HP-G-P	907.08
	f1-4	E-HP-G-P-A-G-P-HP-G-P	907.08
	f1-5	T-G-G-I-G-G-HP-G-G-S	774.93
	f1-6	A-V-G-P-V-G-P-I-G-P	863.16
f2	f2-1	A-A-G-P-HP-G	484.58
	f2-2	A-HP-G-P-A-G	484.58
	f2-3	G-P-G-G-P-A	454.56
	f2-4	L-HP-G-P-T-G	556.69
	f2-5	G-P-L-G-P-A	510.66
f3	f3-1	A-P-A-G	314.38
	f3-2	G-Y-G-P-G-P-A	617.75
	f3-3	G-G-Y-E	424.46
	f3-4	G-E-Y-G-P	521.59
	f3-5	F-HP-G-G-HP-G-A-K-G	819.01
	f3-6	F-V-A	335.43
	f3-7	A-P-N-P-F-R-H-Y	1001.20

3

Fig. 1. (a) Changes in blood glucose levels under the administration of SCP or BSA hydrolysate in the oral glucose tolerance test (OGTT). The open circles are the control group (○), the closed circles are the SCP group (●) and the open squares are the BSA hydrolysate group (□). (b) AUC of blood glucose levels. Data are expressed as mean ± SEM (n=6-7). (a) Values at the same time point with the different letter indicate statistically significant differences ($P < .05$; Steel-Dwass's test).

Fig. 2. (a) Gel filtration of SCP on Sephadex G-50. (b) Gel filtration of the F1 fraction on Sephadex G-25. Each 6.0 mL fraction was collected. The closed circles indicate absorbance at 230 nm.

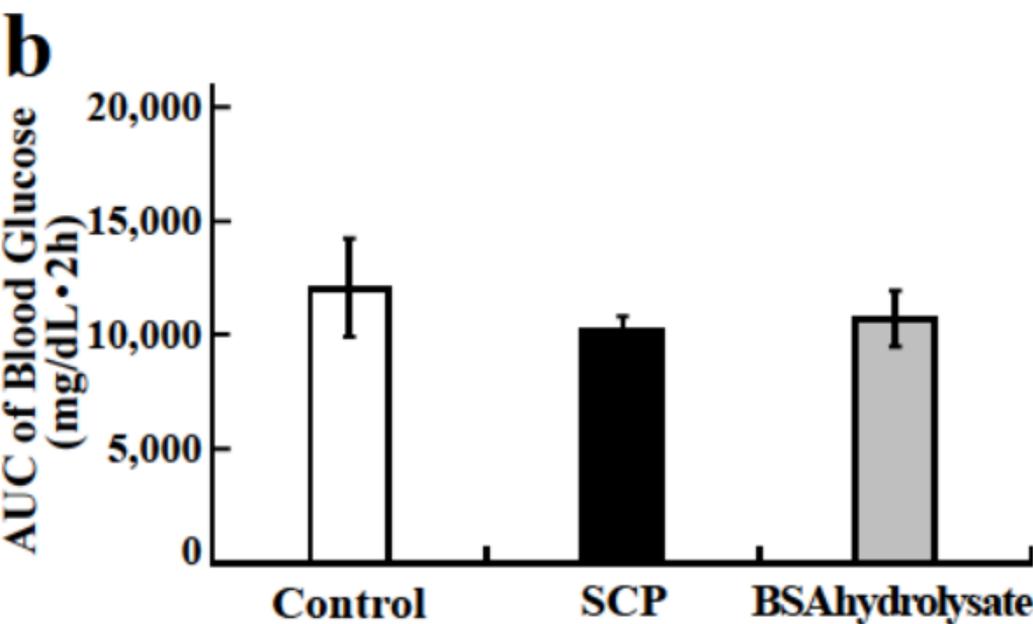
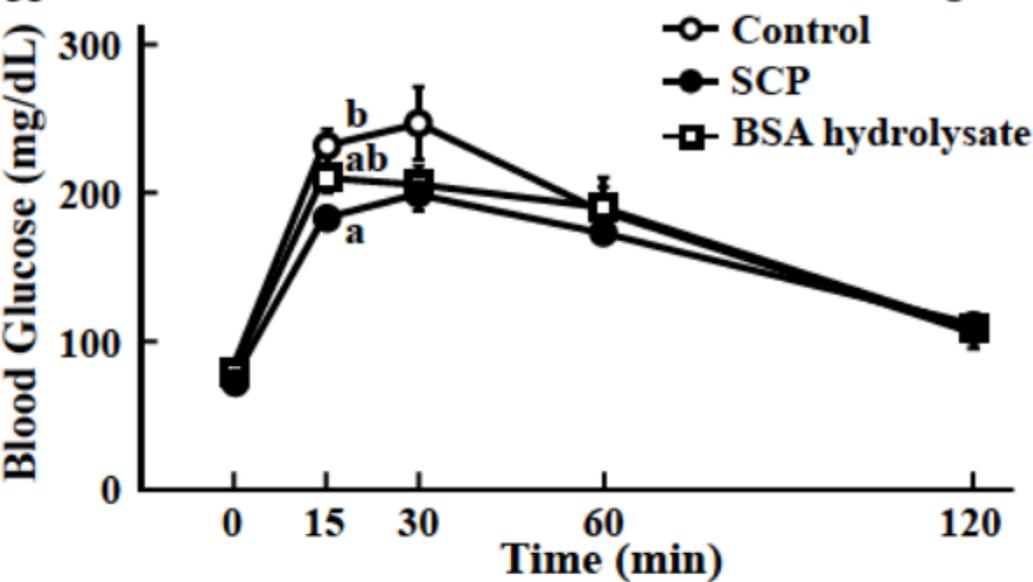
Fig. 3. (a) Changes in blood glucose levels under the administration of the f1, f2 or f3 fractions in the OGTT. The open circles are the control group (○), the closed circles are the f1 group (●), the open squares are the f2 group (□) and the closed squares are the f3 group (■). (b) AUC of blood glucose levels. Data are expressed as means ± SEM (n=7). Values at the same time point (a) or bars (b) with the different letter indicate statistically significant differences ($P < .05$; Steel-Dwass's test).

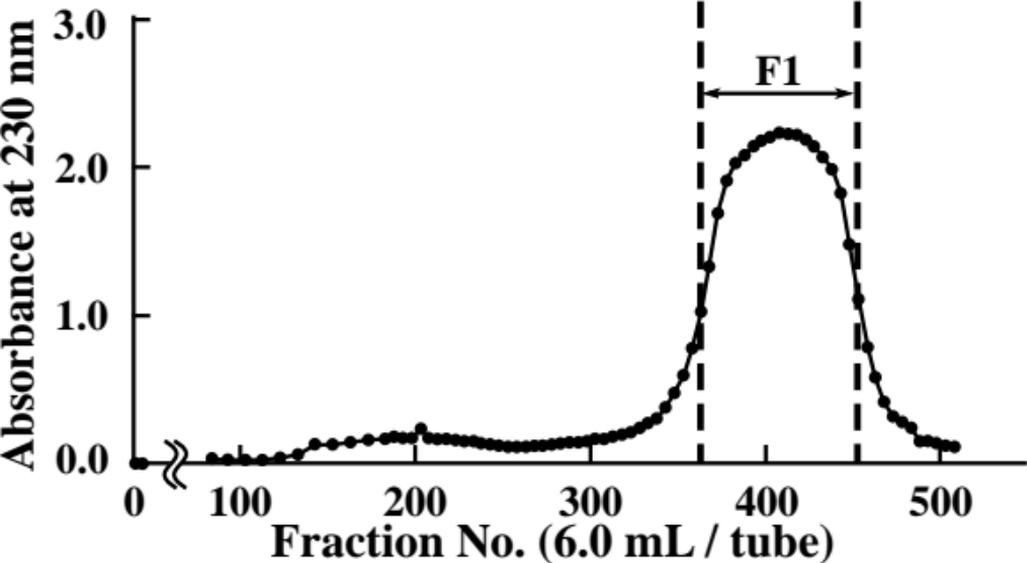
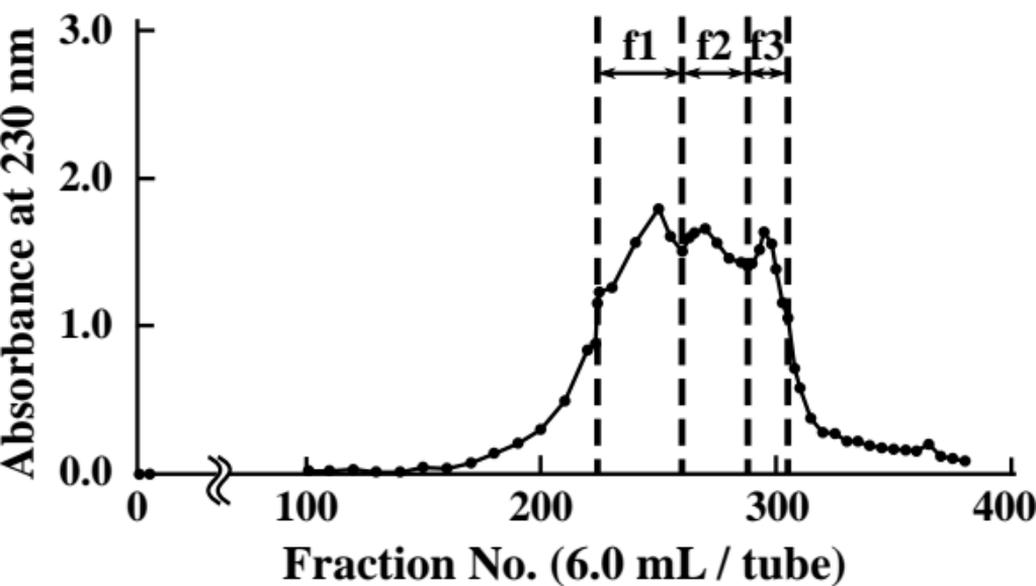
Fig. 4. RP-HPLC chromatograms of the f1 (a), f2 (b) and f3 (c) fractions. The signal intensity at 220 nm was monitored. Each number on the peak corresponds to the Peak No. of Table 1.

Fig. 5. (a) SDS-PAGE of atelocollagen from the skin of Bester sturgeon. M: High molecular weight marker; Lane 1: Prepared atelocollagen. The high molecular marker contained Myosin (MW 220 kDa), α -2-Macroglobulin (MW 170 kDa), β -Galactosidase (MW 116 kDa), Transferrin (MW 76 kDa) and Glutamate dehydrogenase (MW 53 kDa). (b) SDS-PAGE of digested atelocollagen by pepsin at different temperatures. Lane 1: undigested atelocollagen; Lane 2: digested atelocollagen at 15 °C; Lane 3: digested atelocollagen at 35 °C. α (I): Type I collagen α -chain, α 2(I): Type I collagen α 2-chain, β : β -chain.

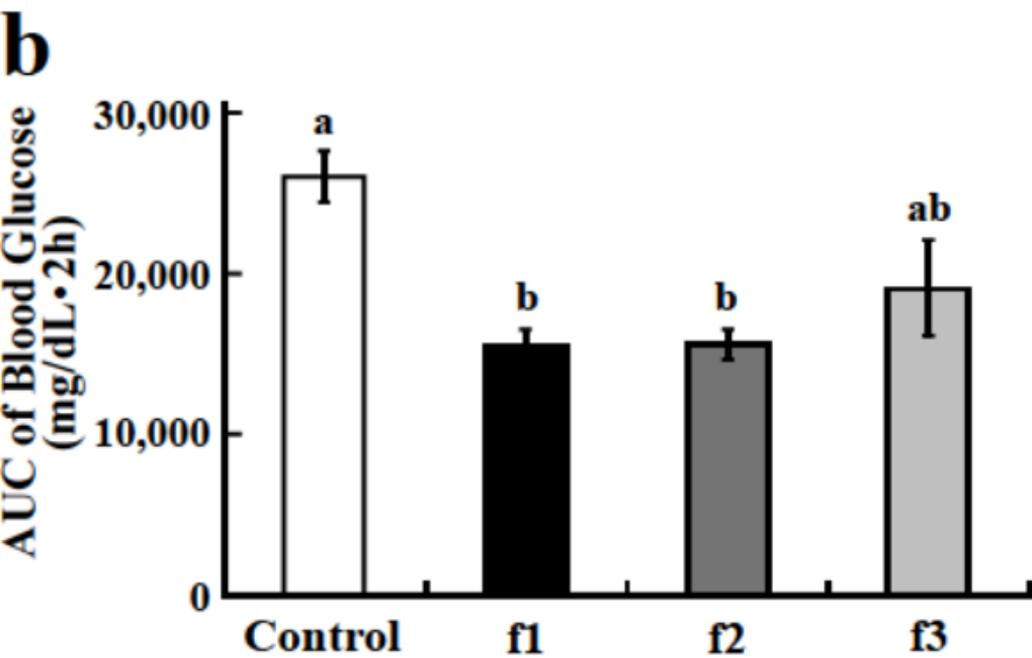
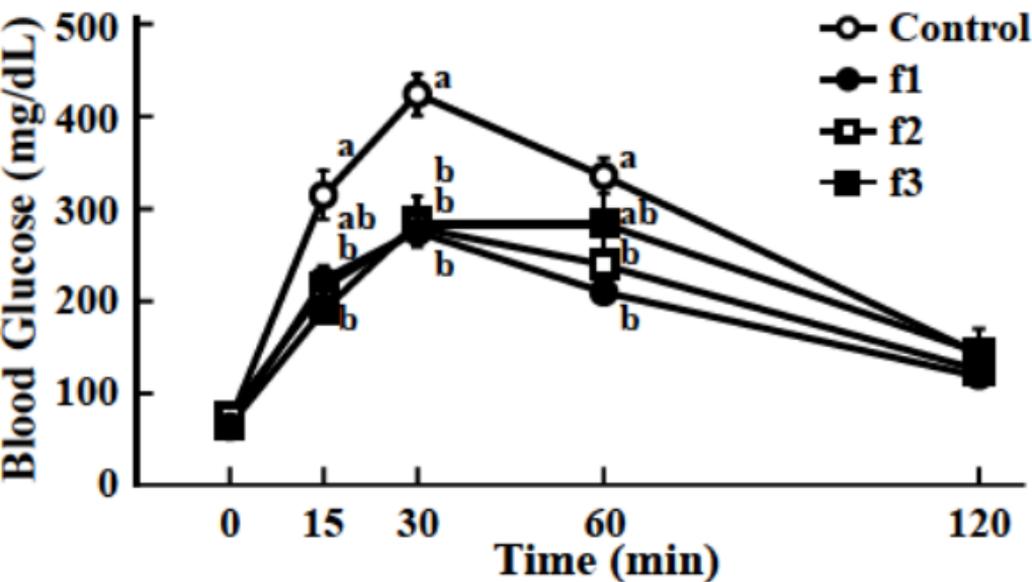
Fig. 6. (a) Changes in blood glucose levels under the administration of SCP or A-SCP in the OGTT. The open circles are the control group (○), the closed circles are the SCP group (●) and the open squares are the A-SCP group (□). (b) AUC of blood glucose levels. Data are expressed as means ± SEM (n=7). Values at the same time point (a) or bars (b) with the different letter indicate statistically significant differences ((a) $P < .05$;

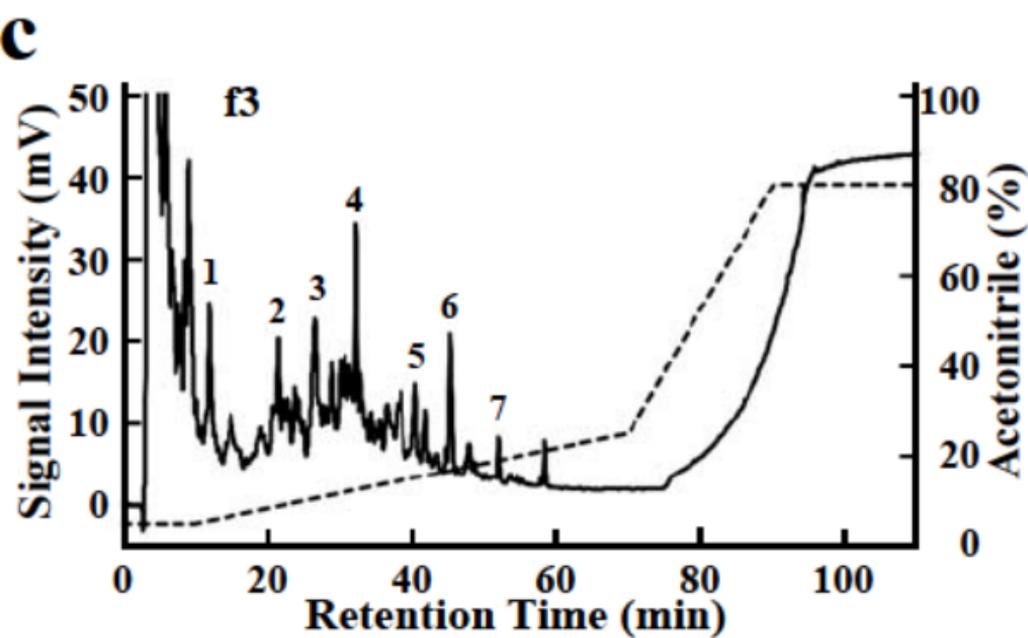
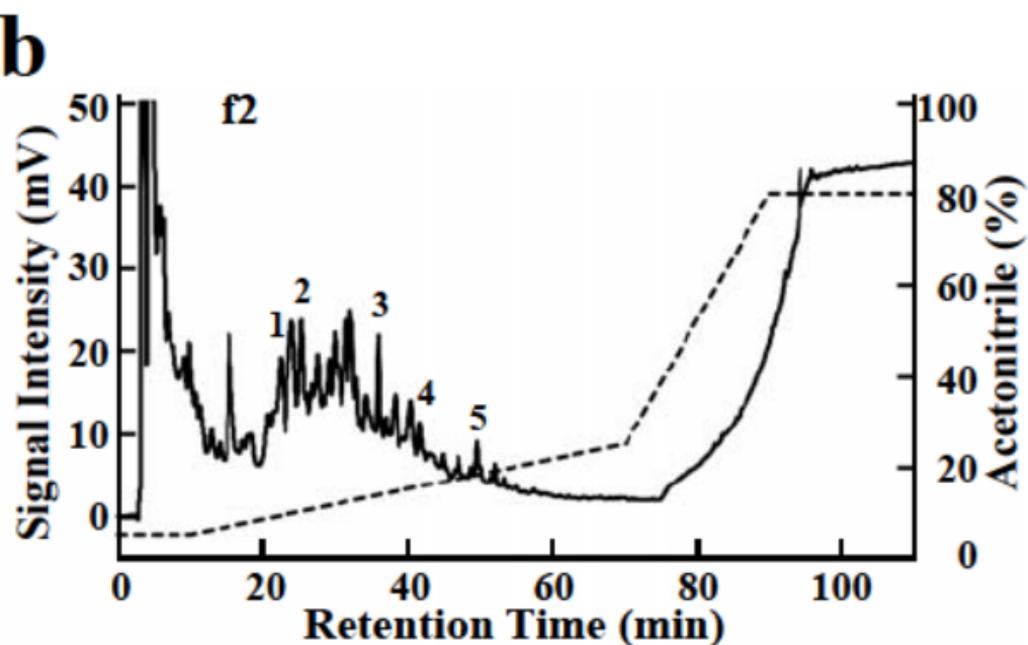
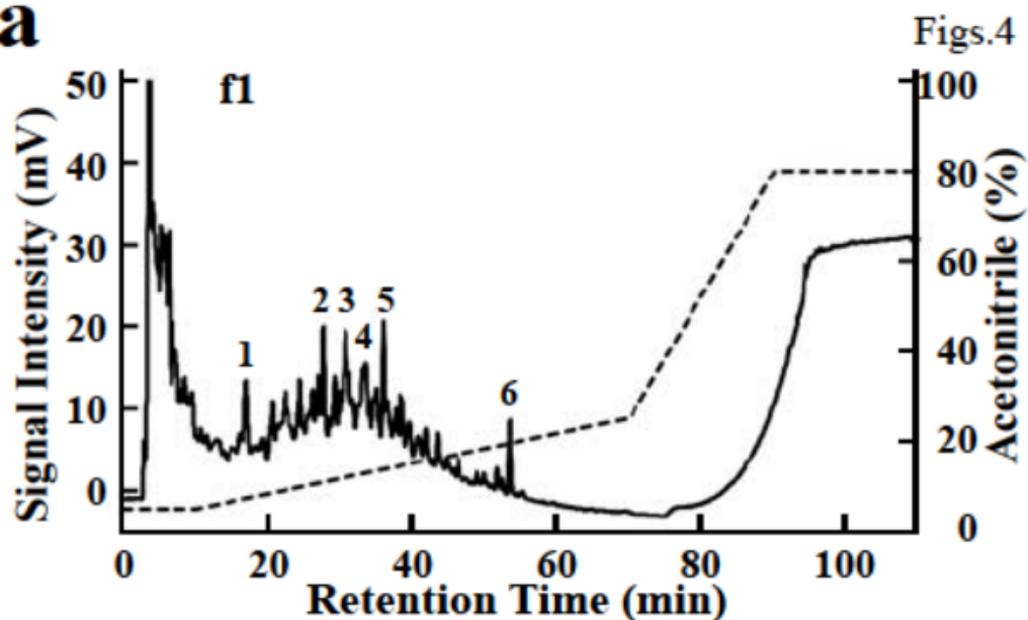
Steel-Dwass's test, (b) $P < .01$; Tukey-Kramer's test).

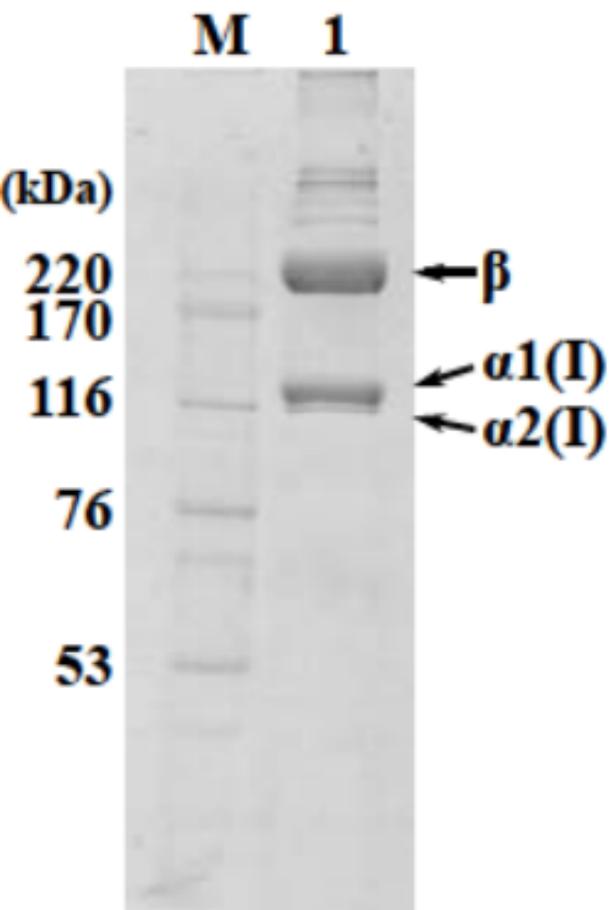


a**b**

Figs.3





a**b**