Fisheries Science 63(1), 111-114 (1997)

Relationship between the Myofibrillar Protein Gel Strengthening Effect and the Composition of Sarcoplasmic Proteins from Pacific mackerel

Katsuji Morioka,*1 Tadashi Nishimura,*1
Atsushi Obatake,*1 and Yutaka Shimizu*2

*1Laboratory of Aquatic Product Utilization, Faculty of Agriculture, Kochi University, Nankoku, Kochi 783, Japan
*2Formerly: Laboratory of Fisheries Chemistry, Faculty of Agriculture, Kyoto University, Sakyo, Kyoto 606, Japan

(Received January 18, 1996)

To determine which protein bands appeared in SDS-polyacrylamide gel electrophoresis (PAGE) patterns of sarcoplasmic proteins (Sp-P) are responsible for the gel strengthening effect of Sp-P on the myofibrillar protein (Mf-P) gel, the relations between the jelly strength of Mf-P gel added with each of three different sarcoplasmic fractions and the relative amounts of protein bands in the SDS-PAGE pattern of each sarcoplasmic fraction were examined.

The heat-coagulability of each sarcoplasmic fraction was highly correlated with the jelly strength of each gel. Relative amounts of 94, 64, and 40 kDa components in the SDS-PAGE pattern of each sarcoplasmic fraction also showed a good correlation with the jelly strength of gel. These results suggest that the high jelly strength of Sp-P added Mf-P gel is attributable to a large amount of heat-coagulable proteins, especially the 94, 64, and 40 kDa components of Sp-P.

Key words: sarcoplasmic proteins, gel, jelly strength, Pacific mackerel

In manufacturing fish jelly products such as kamaboko, chikuwa, and fish sausage in Japan, a process of washing the meat is generally carried out.[1,2] By washing the meat, the elasticity of the product is increased. This effect of washing is mainly due to an increase in the concentration of myofibrillar proteins (Mf-P), the essential constituents for the elasticity of the fish jelly product, accompanying the removal of fat, blood, and sarcoplasmic proteins (Sp-P) which are assumed to inhibit the gel-formation of Mf-P.

The inhibitory effect of Sp-P was first demonstrated by Okada.[3] He reported that the strength of Sp-P added Mf-P gel became lower than that of the control gel (water was added instead of Sp-P solution). From this result, he concluded that Sp-P from arrow-tooth flounder inhibited the gel formation of the kamaboko gel (heat-induced gel at 90°C). On the other hand, we recently reported that the jelly strength of Mf-P gel added with native Sp-P from Pacific mackerel was higher than that of Mf-P gel added with heat-denatured Sp-P, which did not interact with Mf-P.[4,5] This result indicates that Sp-P does not interfere with the gel formation of Mf-P, but positively contributes to it. As Sp-P consists of many proteins, this discrepancy may be due to the difference in the composition of Sp-P. However, it remains unclear whether the effect of Sp-P on the gel strength of Mf-P is related to its composition.

The present study explored the relationship between the effect of Sp-P on the gel strength of Mf-P and the composition of Sp-P from Pacific mackerel.

Materials and Methods

Materials

Pacific mackerel *Scomber japonicus* and threadfin bream *Nemipterus virgatus* were purchased from the Kochi wholesale market, and were kept on ice before use. All chemicals were of reagent grade.

Preparation of Sarcoplasmic Fractions

Pacific mackerel was used for preparation of sarcoplasmic fraction. Shimizu *et al.*[6] and Nakagawa and Nagayama[7] reported a difference in extractability among sarcoplasmic components. On the basis of this nature of Sp-P, three different sarcoplasmic solutions were prepared from Pacific mackerel as follows.

The white muscle was excised from the dorsal part of the trunk and minced with a chopper. Minced meat was homogenized with 5 volumes of phosphate buffer (I=0.05, pH 7.0) with a non-bubbling homogenizer (Nihon Seiki, Osaka) at 3,000 rpm for 3 min. The homogenate was then centrifuged at 12,000 × *g* for 15 min. The supernatant obtained was filtrated through absorbent cotton to remove fat and used as a whole sarcoplasmic fraction (W-SF).

Minced meat was homogenized with 10 volumes of deionized water and centrifuged as mentioned above. The supernatant was used as a deionized water soluble sarcoplasmic fraction (DW-SF). The precipitate obtained was then homogenized with 5 volumes of phosphate buffer (I=0.05, pH 7.0) and the homogenate was centrifuged. The supernatant was collected and used as a salt-soluble

sarcoplasmic fraction (SS-SF).

Each sarcoplasmic fraction was concentrated to 85% in water content by dialysis against polyethylene glycol #20,000. After concentration, the sample was dialyzed against phosphate buffer (I=0.05, pH 7.0) for one day, and then centrifuged at 12,000×*g* for 15 min. The supernatant obtained was dialyzed against polyethylene glycol #20,000 to adjust the water content to 85%, and then used for the gel preparation.

Preparation of Myofibrillar Proteins

Threadfin bream was used for preparation of Mf-P. The white muscle was excised from the dorsal part of the trunk and minced. Minced meat was then washed with 5 volumes of 0.09 M KCl-5 mM EDTA-0.039 M borate buffer (pH 7.0) at 3,000 rpm for 5 min. The homogenate was centrifuged at 8,000×*g* for 20 min. The precipitate was similarly washed three more times. The final centrifugation was done at 15,000×*g* for 30 min to dehydrate to 85% water content and used as Mf-P. Eight percent sucrose was added to Mf-P and the mixture was kept at −20°C until use.

Gel Preparation

The concentrate of each sarcoplasmic fraction was added to Mf-P in the ratio of 1 to 3 on a protein basis and mixed for 5 min in a cold room at 5°C. The mixture was then ground with 2.5% NaCl for 10 min at 5°C. The meat paste obtained was stuffed in a glass ring (inner diameter, 13 mm; height, 15 mm), wrapped in polyvinylidene chloride film, and then heated in a water bath at 80°C for 10 min. After heating, gels were immediately cooled in ice water and brought to room temperature on testing.

Instrumental Test

Jelly strength was evaluated with a Yamaden RE-3305 rheometer by using the cylindrical plunger (diameter, 3 mm) at a speed of 1 mm/sec. From the force-deformation curve recorded, puncture force (g) and maximum dent (cm) were obtained. Jelly strength (g·cm) was expressed as the product of puncture force and maximum dent. A mean score of three gels (diameter, 13 mm; height, 15 mm) was determined for each sample.

Analytical Methods

SDS-polyacrylamide gel electrophoresis (PAGE) was used for analyzing the composition of the sarcoplasmic fraction as mentioned previously.[8] SDS-PAGE was performed by the method of Laemmli[9] using 10% gel.

The coagulation test of Sp-P was performed by heating the test tubes containing Sp-P solution in a water bath at 90°C for 10 min. Immediately after heating, the test tubes were cooled in ice water and then centrifuged at 12,000×*g* for 15 min. The supernatants thus obtained were used for determination of protein concentration. Protein concentration was determined by the method of Lowry *et al.*[10] Bovine serum albumin was used as a standard protein. Heat-coagulability (%) was obtained from the following formula: (A−B)/A×100, where A and B are values of the protein concentration before and after heating, respectively.

To estimate the molecular weight of Sp-P components, 5 m*l* of W-SF solution was applied to a Sephadex G-200 column (2.5×85 cm) equilibrated with phosphate buffer (I=0.05, pH 7.0). Flow rate was 10 m*l*/h and 5 m*l* fractions were collected.

Results and Discussion

The effect of proteins remaining in the supernatant after heating the W-SF solution at various temperatures on jelly strength of Mf-P gel was investigated. The W-SF solution (protein concentration 15%) was heated at various temperatures for 15 min. After heating, the W-SF solution was cooled and then centrifuged at 12,000×*g* for 15 min. 3.7 g of supernatant thus obtained from the heated W-SF solution was mixed with 10 g of Mf-P (protein concentration 13.9%), and then the heat-induced gel was prepared from the mixture as mentioned above. As shown in Fig. 1, the jelly strength of Mf-P gel added with each sarcoplasmic fraction decreased with a decrease of the protein concentration remaining in the supernatant after pre-incubation of W-SF. This result indicated that the high jelly strength of Mf-P gel added with Sp-P was attributable to a large amount of heat-coagulable proteins in Sp-P. However, it remains unclear which heat-coagulable components of Sp-P are responsible for this effect of Sp-P. In the next experiment, therefore, the effect of three different sarcoplasmic fractions on the strengths of the Mf-P gel was examined in order to clarify the relationship between the jelly strength of Mf-P gel added with Sp-P and its composition.

Figure 2 shows the SDS-PAGE patterns of three different sarcoplasmic fractions extracted from the white meat of Pacific mackerel. The 55, 43, and 40 kDa components were major in DW-SF. On the other hand, the 94, 64, and 40 kDa components were major in SS-SF.

The heat-coagulabilities of W-, DW-, and SS-SF and the strengths of each sarcoplasmic fraction added gels are

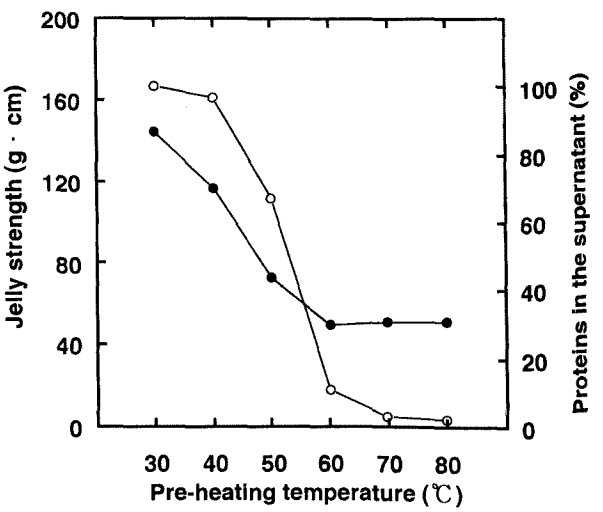

Fig. 1. The percentage of proteins remaining in the supernatant and the jelly strength of the myofibril gels added with the supernatant after heating the Sp-P solution from Pacific mackerel at various temperatures.

●, jelly strength (g·cm); ○, proteins in the supernatant (%).

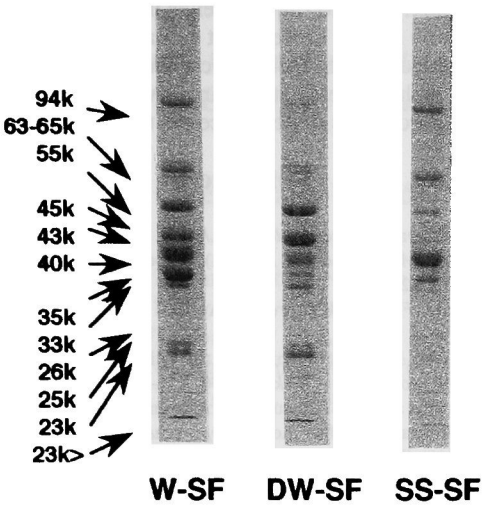

Fig. 2. SDS-polyacrylamide gel electrophoretic patterns of three kinds of sarcoplasmic fractions extracted with various buffers from Pacific mackerel.

Preparations of each fraction were as follows: W-SF was extracted with phosphate buffer (I=0.05, pH 7.0); DW-SF was extracted with deionized water (I=0); SS-SF was extracted with phosphate buffer (I=0.05, pH 7.0) from the residues of DW-SF.

Arrows indicate components of Sp-P in Table 2.

Table 1. Effect of various Sp-P fractions from Pacific mackerel on gel formation of threadfin bream myofibrillar proteins

Protein fraction added	Puncture force (g)	Maximum dent (cm)	Jelly strength (g·cm)	Heat coagulability (%)
W-SF*1	142±6	0.72±0.03	103±7	95.8
	(113±5	0.68±0.02	76±6)*5	
DW-SF*2	133±8	0.66±0.01	88±7	90.9
	(112±6	0.68±0.03	77±7)*5	
SS-SF*3	185±18	0.81±0.01	150±17	98.3
	(118±10	0.70±0.05	83±13)*5	
C.C*4	0.78	0.89	0.84	

*1 W-SF was extracted with phosphate buffer (I=0.05, pH 7.0).
*2 DW-SF was extracted with deionized water (I=0).
*3 SS-SF was extracted with phpsphate buffer (I=0.05, pH 7.0) from the residues of DW-SF.
*4 C.C shows a correlation coefficient of puncture force, maximum dent, and jelly strength to heat-coagulability.
*5 Each Sp-P solution was heated at 90°C for 10 min before adding to the myofibrillar protein.

shown in Table 1. The jelly strength of SS-SF added gel was the highest of the three. The jelly strength of W-SF added gel was a little higher than that of DW-SF added gel. When a heat-denatured sarcoplasmic fraction was added to Mf-P gel, the strength of each pre-heated sarcoplasmic fraction added gel became lower than that of an unheated sarcoplasmic fraction added gel. Sp-P lost its ability to interact with Mf-P by the heat-treatment.[5] This decline in jelly strength of Mf-P added with heat-denatured Sp-P might be due to the lack of heat-induced interaction between Mf-P and heat-denatured Sp-P. From these results, it was confirmed that a native Sp-P contributed to the strength of the heat-induced Mf-P gel.

The heat-coagulability of the sarcoplasmic fraction was then compared in relation to puncture force, maximum dent, and jelly strength. The heat-coagulability of SS-SF showed the highest value of the three sarcoplasmic solutions. The heat-coagulability of the sarcoplasmic fraction was highly correlated with the puncture force (r=0.78) and maximum dent (r=0.89). The heat-coagulability was also highly correlated with the jelly strength (r=0.84). These results suggest that a high strength of Sp-P added Mf-P gel is due to a large amount of the heat-coagulable proteins in Sp-P.

In order to explore the relationship between the jelly strength of sarcoplasmic fraction added gel and the composition of Sp-P, the relative amount of each protein component in SDS-PAGE patterns of sarcoplasmic fractions was calculated by using a densitometer (Table 2). The relative amount of 40 kDa component, which was a major one of SS-SF, showed a good correlation (r=0.93) with the jelly strength of the gel. The relative amounts of 94 and 64 kDa components were also highly correlated (r=0.92, r=0.93) with the jelly strength. The amount of 63 kDa component, which was a minor one, also showed a good correlation (r=0.84) with the jelly strength. On the other hand, the relative amounts of other protein components except for 35 kDa component showed inverse correlations with the jelly strength of the gel. These components may reduce the strength of the gel. However, considering that the strength of native DW-SF added gel was higher than that of heat-denatured DW-SF added gel, these components may not strongly inhibit gel formation. These results suggest that a high jelly strength of Mf-P gel added with Sp-P is due to the large amounts of 94, 64, and 40 kDa components in

Table 2. Relationship between the jelly strength of Sp-P added gel and the composition of Sp-P from Pacific mackerel

Protein fraction	Jelly strength (g·cm)	Relative amount of each component (%)										
		94k*	65k	64k	63k	55k	43k	40k	35k	33k	26k	25k	others
W-SF	103±7	7.9	1.0	5.6	1.5	13.1	10.6	23.1	19.1	3.4	3.0	3.5	8.2
DW-SF	88±7	3.1	2.8	1.5	1.8	21.0	20.0	13.4	4.9	6.3	2.4	6.2	16.6
SS-SF	150±17	14.0	0	11.7	2.7	6.6	4.1	39.5	9.8	3.6	0	0	8.0
Correlation coefficient to jelly strength		0.92	−0.85	0.93	0.84	−0.89	−0.87	0.93	0.58	−0.61	−0.86	−0.92	—

* The value shows the molecular weight (Da) estimated by SDS-PAGE.

the SDS-PAGE pattern of Sp-P.

So far, the protein bands in the SDS-PAGE pattern of Sp-P were tentatively called by the molecular weight estimated from the relative mobility. Nakagawa *et al.*[11] reported that 43, 40, and 35 kDa components in the SDS-PAGE pattern of Sp-P from Pacific mackerel were identified as creatine kinase (intact molecular weight=86k), aldolase (160k) and glyceraldehyde-3-phosphate dehydrogenase (140k), respectively. However, the intact molecular weights of other components of Sp-P from Pacific mackerel remain unclear. To estimate the intact molecular weights of other protein components, W-SF solution was then applied to Sephadex G-200 gel filtration. Sarcoplasmic fractions eluted from the column were applied to the SDS-PAGE. From the position of elution and the relative mobility in the SDS-PAGE pattern, intact molecular weights of the protein bands in the SDS-PAGE pattern of Sp-P were estimated as follows; 200 kDa for the 94 kDa component in SDS-PAGE, 65 kDa for 65 kDa, 180 kDa for 64 kDa, 150 kDa for 63 kDa, 100 kDa for 55 kDa, 87 kDa for 43 kDa, 180 kDa for 40 kDa, 170 kDa for 35 kDa, 55 kDa for 26 kDa, 50 kDa for 25 kDa, and 25 kDa for 23 kDa. Judging from intact molecular weight and subunit molecular weight, the 94 and 40 kDa components were assumed to be phosphorylase b by reference to the data of purified enzyme from sarcoplasmic extract of rabbit muscle[12] and aldolase.[11]

From the results thus obtained, it was concluded that a high jelly strength of Mf-P gel added with Sp-P is attributable to a large amount of heat-coagulable proteins, especially those of the 94, 64, and 40 kDa components in the SDS-PAGE pattern of Sp-P. Judging from intact molecular weight and subunit molecular weight, the 94 and 40 kDa components were assumed to be phosphorylase b and aldolase.

We showed that the jelly strength of Mf-P gel added with Sp-P is related to the composition of Sp-P. The composition and content of Sp-P differ among fish species.[6,8,13] The strengths of the gels formed at 90°C from fish meat pastes also differ among fish species.[14] Our results suggest that this difference in the strengths of the heat-induced gels could be partly due to the difference in the composition of Sp-P. In this study, however, it remains unclear whether the additive content of Sp-P affects the strengthening effect of Sp-P. Further investigations are now in progress to clarify the Sp-P contents dependence on the gel strength.

References

1) E. Tanikawa: *Marine products in Japan*, rev. ed. (rev. and enl. by T. MOTOHIRO and M. AKIBA), Koseisha Koseikaku, Tokyo, 1985, pp. 329–367.
2) T. Suzuki: *Fish and krill protein*, Applied Sci. Pub., London, 1981, pp. 62–114.
3) M. Okada: Effect of washing on the jelly forming ability of fish meat. *Nippon Suisan Gakkaishi*, **30**, 255–261 (1962) (in Japanese).
4) K. Morioka and Y. Shimizu: Contribution of sarcoplasmic proteins to gel formation of fish meat. *Nippon Suisan Gakkaishi*, **56**, 929–933 (1990) (in Japanese).
5) K. Morioka and Y. Shimizu: Heat-coagulation property of fish sarcoplasmic proteins. *Nippon Suisan Gakkaishi*, **58**, 1529–1533 (1992) (in Japanese).
6) Y. Shimizu, S. Karata, and F. Nishioka: Extractability of proteins from fish skeletal muscle at low ionic strengths. *Nippon Suisan Gakkaishi*, **42**, 1025–1031 (1976) (in Japanese).
7) T. Nakagawa and F. Nagayama: Extractability of glycolytic enzymes from fish muscle. *Nippon Suisan Gakkaishi*, **54**, 1971–1974 (1988).
8) K. Morioka and Y. Shimizu: Relationship between the heat-gelling property and composition of fish sarcoplasmic proteins. *Nippon Suisan Gakkaishi*, **59**, 1631 (1993).
9) U. K. Laemmli: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
10) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **191**, 265–275 (1951).
11) T. Nakagawa, S. Watabe, and K. Hashimoto: Identification of three major components in fish sarcoplasmic proteins. *Nippon Suisan Gakkaishi*, **54**, 999–1004 (1988).
12) R. K. Scopes and I. F. Penny: Subunit sizes of muscle proteins, as determined by sodium dodecyl sulphate gel electrophoresis. *Biochim. Biophys. Acta*, **236**, 409–415 (1971).
13) T. Nakagawa, S. Watabe, and K. Hashimoto: Electrophoretic analysis of sarcoplasmic proteins from fish muscle on polyacrylamide gels. *Nippon Suisan Gakkaishi*, **54**, 993–998 (1988).
14) Y. Shimizu: Species variations in gel-forming ability of fish meat paste. *Nippon Suisan Gakkaishi*, **40**, 175–179 (1974) (in Japanese).