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Role of transglutaminase-mediated cross-linking of protein in the temperature-dependent setting of Alaska pollack surimi

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Abstract

During the low temperature setting of fish paste, myosin heavy chain (MHC) is polymerized to cross-linked myosin heavy chain (CMHC), which is considered to occur by the action of endogenous transglutaminase (TGase). In this study the contribution of TGase on the setting of Alaska pollack surimi at different temperatures was studied. Alaska pollack surimi was ground with 3 % NaCl, 30 % H_2O and with or without ethylene glycol bis (β-aminoethylether) N, N, N',N'-tetraacetic acid (EGTA), an inhibitor of TGase. Among the pastes without EGTA, highest TGase activity was observed at 25°C but breaking force of the gel set at 25°C was lower than that set at 30°, 35°, and 40°C. Addition of EGTA (5 m mol/kg) to the paste suppressed TGase activity at all setting temperatures from 20° to 40°C. Gelation of the pastes and cross-linking of MHC on addition of EGTA were suppressed completely at 20° and 25°C, partially at 30° and 35°C, and not at all at 40°C. The findings suggested that during the setting of Alaska pollack surimi TGase mediated cross-linking of MHC was strong at around 25°C but the thermal aggregation of MHC by non-covalent bonds was strong at above 35°C. Setting of surimi at 40°C and cross-linking of its MHC did not involve TGase.

Key words : Surimi, Transglutaminase, Myosin heavy chain

Introduction

Mechanically deboned water-washed fish flesh is called surimi. Now-a-days, some very popular analog or fabricated food products like crab-leg, beef, scallop or shrimp analogs are produced from surimi along with Japanese style traditional *kamaboko* products. In the manufacturing process of *kamaboko* or analog

products, the surimi is first ground with salt (2 - 3 %) and water (25 - 50 %) and then heated at a low temperature (0 - 40°C) before further heating at cooking temperature (80-90°C). The process of heating the surimi paste at low temperature is called setting or *suwari* in Japanese. The setting is generally performed to improve the elasticity of the final products, where salt-ground paste turns to an elastic and semi-transparent gel, called *suwari* gel. It was recently found that a myosin heavy chain (MHC) band disappeared in a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of surimi paste from Alaska pollack with the progress of setting; consequently, the bands due to macromolecular cross-linked myosin heavy chain (CMHC) appeared (Itoh *et al.* 1980, Lee *et al.* 1990, Numakura *et al.* 1985, Numakura *et al.* 1990). It was also found that a Ca^{+2} dependent enzyme, transglutaminase (TGase) existed in the surimi paste (Seki *et al.* 1990) and this enzyme mediated e - (g-glutamyl)lysine bond was formed during setting (Kimura *et al.* 1991). From these findings, it has been presumed that an enzymatically catalyzed cross-linking of MHC by covalent bonds plays the major role in the formation of protein network structure in *suwari* gel during setting.

TGase is also found to induce setting in some other easy-setting species, like hoki (Kimura *et al.* 1991) and sardine (Tsukamasa *et al.* 1993) at low temperature around 30°C. However, in our previous investigations, we observed that at 30°C setting proceeded in the actomyosin paste from which TGase was completely removed (Nowsad *et al.* 1994a) and in fish flesh pastes in which TGase was totally inactivated by the addition of SH reagents, such as *P*-chloromercuribenzoic acid (PCMB), iodoacetic acid (IAA) and N-ethylmaleimide (NEM) (Nowsad *et al.* 1994b, 1994c, 1994d). Further more, when CMHC was extracted from the top of the electrophoresed disc in the presence of concentrated urea and electrophoresed again, the band for MHC reappeared (Nowsad *et al.* 1993). These results concluded that CMHC was also formed by the aggregation of MHC through weak bonds such as hydrogen and hydrophobic bonds. The contribution of TGase in the elasticity of *suwari* gel from easy-setting species was found to be around 40 % and that from hard-setting species was almost nil or negligible (Nowsad *et al.* 1995). However, on the other hand, Tsukamasa *et al.* (1993) very recently suggested that the contribution of TGase to the low temperature setting was more important than that of non-covalent protein-protein interactions, from the findings that both the gelation of sardine myofibril sol and e-(g-glutamyl)lysine cross-link formation in it were completely suppressed at 25°C by the addition of EGTA, another inhibitor of Ca^{+2} dependent TGase. It is assumed that such discrepancy in results has arisen from the difference in setting temperatures within the same species in addition to the difference of species as we observed in easy-setting and hard-setting species (Nowsad *et al.* 1995). The TGase-mediated cross-linking of MHC may be temperature-specific and intense at relatively low temperature below 30°C.

In this experiment the role of TGase to the setting of Alaska pollack surimi at various temperatures was investigated. EGTA was added to the surimi paste to offset and compare the influence of TGase on setting and cross-linking of MHC.

Materials and methods

Suwari gel

Alaska pollack *Theragra chalcogramma* frozen surimi (Alaska Ocean Seafood, Anacortes, WA, U.S.A., SA-grade, unsalted) was thawed at 4°C, minced (3-mm-hole) and ground together with 30 % water, 3 % NaCl (in surimi weight), and EGTA {5 m mol/kg of surimi (Tsukamasa *et al.* 1993)} in a hand mortar for 10 min in a cold room at 4°C. The resulting surimi paste was stuffed into polyvinylidene chloride casing (2.8 cm in diameter), set in water baths at variable temperatures (20, 25, 30, 35, 40°C) for 1, 3, and 5 h, and cooled in running tap water. Control gels were prepared without EGTA keeping other conditions constant.

Physico-chemical measurements of suwari gel

pH of *suwari* gel was measured by putting a gel slice (1 mm in thickness) in a hollow chamber of the electrode (Horiba Ltd., Twin Compact pH Meter, B-112).

Suwari gel was sliced into 2 cm in thickness and subjected to a puncture test using a rheometer (Fudoh Rheo Meter, NRM-2010J-CW) with a spherical plunger (5 mm in diameter) at a table speed of 6 cm/min.

TGase activity measurement

Surimi mince with EGTA (5 m mol/kg) and without EGTA were incubated at different temperatures after grinding with 3 % NaCl, 2.5 mM monodansylcadaverine (MDC), and 30 % water and the activity of TGase was measured as described by Wan *et al.* (1992). Protein concentration was measured according to Umemoto (1966) after precipitating with an equal volume of 10 % trichloroacetic acid.

SDS-PAGE of suwari gel

A small piece of *suwari* gel (0.5 g) was solubilized in 9.4 ml of 8 M urea-Tris-HCl buffer (Numakura *et al.* 1985) by heating in boiling water for 1.5 min and stirring overnight at 24°C. After centrifugation at 30,000 x *g* for 30 min, the supernatant was filtered (Toyo Roshi Co., No. 1) and the filtrate was used as SDS-PAGE sample. SDS-PAGE was carried out using a vertical disc gel system as described previously (Nowsad *et al.* 1993). Densitometry of the disc gel and the calculation of the amount of subunit proteins were done as described before (Nowsad *et al.* 1993).

Results and discussion

Alaska pollack surimi showed a peak setting ability at 30°C as the breaking force of the gel without EGTA increased almost linearly in proportion to setting time up to 5 h as presented at the left of Fig. 1. The breaking force increased in proportion to the rise of setting temperature from 20°C, showed its maximum value at 30°C and then gradually decreased at 35°-40°C. During setting at 20°C, however, gelation did not occur till 3 h. In the gel set at 25°C, only a little gel strength was recorded up to 1 h. On the other hand, when EGTA at the rate of 5 m mol / kg was ground with the surimi mince, the paste did not transform into gel during setting at 20° and 25°C as shown at the right of Fig. 1, suggesting that EGTA suppressed the gelation completely at these temperatures. This result is quite consistent with that of Tsukamasa *et al.* (1993). However, when the setting temperature was raised further, gelation of this paste began to occur. The breaking force of the EGTA added gels increased in proportion to the rise of setting temperature. Maximum value was observed in the gel set at 40°C. However, the breaking force was very little at 30°C.

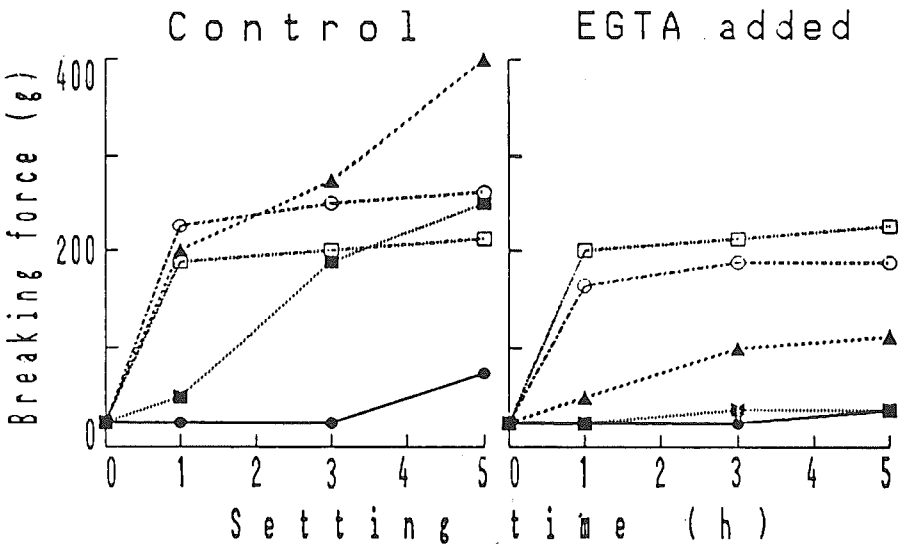

Fig. 1. Changes of breaking force in the control and EGTA added gels set at various temperatures. Setting temperatures (°C) -O-: 20, - -: 25, -Δ-: 30, -O-: 35, - -: 40

The pH of both the pastes with or without EGTA was more or less constant (range: 6.8-6.9, data not shown), suggested that the muscle pH was not affected by the addition of EGTA.

TGase has been considered essential in this setting (Seki *et al.* 1990, Kimura *et al.* 1991, Tsukamasa *et al.* 1993). Therefore, the activity of TGase in the gels

without EGTA and with EGTA was examined. The results are presented in Table 1. Highest TGase activity was observed in the gel without EGTA at 25°C, followed by that at 30°, 20°, 35°, and 40°C. Although the activity was high at 25°C, the breaking force was lower in this gel compared to the gels set at 30°, 35°, and 40°C. During setting at 35° and 40°C, TGase activity reduced to 0.32 - 0.35 n mol MDC / mg protein, a 25 % of the activity showed at 25°C after 5 h. But the *suwari* gels at these temperatures were stronger than that at 25°C. On the other hand, no TGase activity was observed in the gels with EGTA added at any setting temperature, suggesting that the action of TGase was completely inhibited by the addition of EGTA, and according to Tsukamasa *et al.* (1993), formation of e-(g-glutamyl)lysine cross-links was also inhibited in such gels. However, the surimi paste lost its stickiness and transformed into gel at 30°, 35°, and 40°C. This gelation did not occur due to TGase-mediated cross-linking of MHC. Non-covalent protein-protein interactions as we described before (Nowsad *et al.* 1994a, Nowsad *et al.* 1994b, Nowsad *et al.* 1994c, Nowsad et al. 1994d) might be important for this gelation.

Table 1. TGase activity of the paste with or without EGTA added*

Temperature	Without EGTA		With EGTA	
(°C)	Incubation time (h)			
	1	5	1	5
20	0.35	1.14	0.0	0.0
25	0.70	1.56	0.0	0.0
30	0.70	1.25	0.0	0.0
35	0.32	0.35	0.0	0.0
40	0.16	0.32	0.0	0.0

*TGase activity was expressed in terms of nmol MDC imcorporated into 1 mg surimi protein

The changes of protein subunits during the gelation of the pastes without and with EGTA at different temperatures were investigated electrophoretically, the SDS-PAGE patterns have been shown in Fig. 2. In the gels without EGTA at all temperatures (top row), as setting progressed, the intensity of MHC decreased and that of CMHC increased. That meant, MHC gradually cross-linked to CMHC in proportion to setting time at all setting temperatures examined. However, the degree of such cross-linking was very slow and the lowest at 20°C but fast and the highest at 25°C, followed by 30°, 35° and 40°C.

On the other hand, when EGTA was added to the pastes, the intensity of MHC was unchanged in the gels set at 20° and 25°C but gradually decreased in the gels set at 30°, 35°, and 40°C. However, a gradual concomitant increment of CMHC was observed in the latter three gels. This meant that the cross-linking of MHC was stopped in the EGTA added gels set at 20° and 25°C, but again proceeded at the temperatures above 30°C.

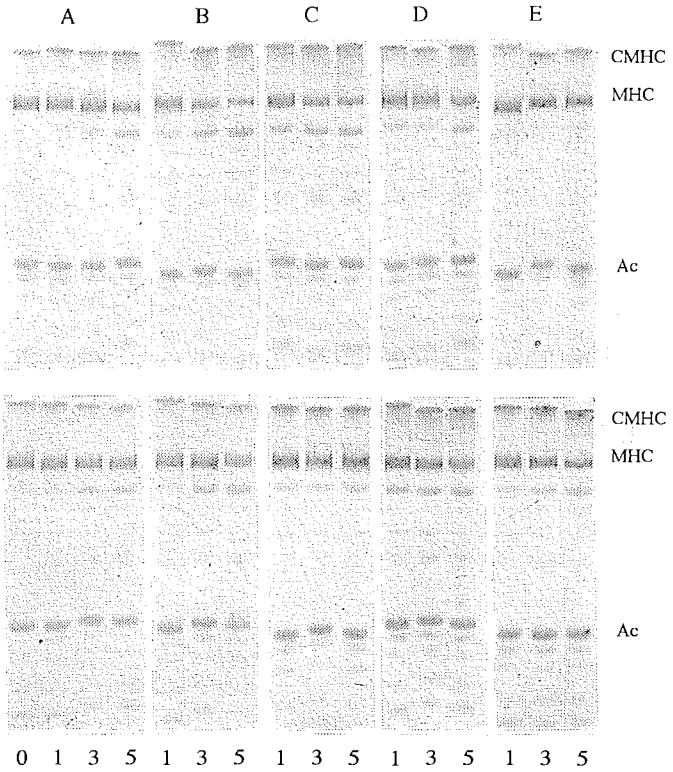

Fig. 2. SDS-PAGE patterns for control (top) and EGTA added (bottom) gels. Numbers in X axis indicate setting time in hour. Letters at the top indicate setting temperature (°C): A, 20; B, 25; C, 30; D, 35; E, 40

The electrophoresed discs were scanned densitometrically to calculate the changes in the amount of various subunit proteins. The per cent decrease of MHC and increase of CMHC have been shown in Fig. 3. As can be seen at the top-left, the amount of remaining MHC was high in the gel set at 20°C, followed by that at 40°, 35°, 30°, and 25°C, suggesting that higher amount of MHC was cross-linked in the gels in reversed order. At the bottom-left, the percent increment of CMHC in these gels also followed the similar reverse order. In case of EGTA added gels (right-top and -bottom), although the amounts of MHC and CMHC were virtually constant at 20° and 25°C, they began to change at higher setting temperatures. Much decrement of MHC and greater formation of CMHC were found at 35°C. However, the formation of CMHC was very little at

30°C which corresponded well with its low breaking force. The effect of EGTA in the gels set at 20° and 25°C was in good agreement with that of Tsukamasa *et al.* (1993), because gelation and MHC cross-linking were completely suppressed here with the inhibition of TGase activity. But, it is interesting that when setting temperature of such paste was raised from 25°C to 40°C, MHC began to cross-link again in proportion to both temperature and time. The results suggest that this cross-linking of MHC was occurred not by the action of TGase but by non-covalent bonds.

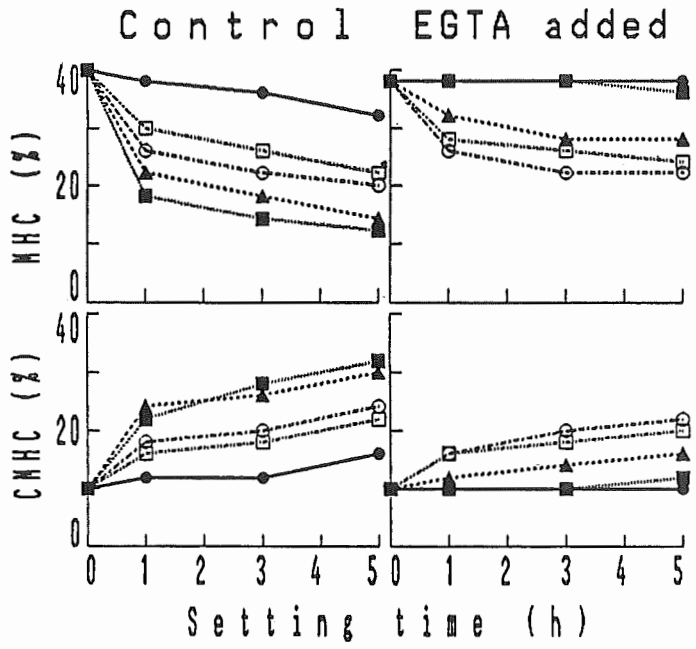

Fig. 3. Changes of MHC and CMHC in the control and EGTA added gels at various temperatures.

Cytosolic TGase showed its optimum activity at 25°C. The breaking force of the *suwari* gels set at the temperatures above 25°C was higher than that at 25°C. Therefore, TGase was not only factor that promoted setting. When the paste with added EGTA was incubated at 25°C, TGase activity was inhibited. This inhibition brought about a complete suppression of surimi gelation and cross-linking of MHC at this temperature. But with the same paste at the elevated setting temperatures, although TGase was inactive, gelation of the paste and the cross-linking of MHC occurred. At 40°C, the rate and extent of gelation and MHC cross-linking were almost similar in both control and EGTA added gels. The fact suggests that addition of EGTA could not suppress setting and cross-linking at 40°C. Therefore, by the addition of EGTA gelation of surimi paste and the cross-linking of MHC were suppressed completely at 20° and 25°C, partially at 30° and 35°C but not at all at 40°C. The results indicate that setting at 40°C was not involved with a TGase-mediated cross-linking at all, although the control gel showed a little TGase activity at this temperature. On the other hand, the highest increment of breaking force was recorded in the gels without EGTA at 30°C, a transitional temperature where both TGase and non-covalent protein

interactions might work together. However, this non-covalent protein aggregation phenomenon may be inactive in Alaska pollack below 30°C where TGase-mediated cross-linking is strong, but well active at elevated temperatures around 35°C where TGase-mediated cross-linking is weak. On the other hand, the extent of non-covalent aggregation of MHC varies significantly with the variation of species as reported before (Nowsad *et al.* 1995).

References

Itoh, Y., R. Yoshinaka and S. Ikeda, 1980. Formation of polymeric molecules of protein resulting from intermolecular SS bonds formed during the gel formation of carp actomyosin by heating. *Nippon Suisan Gakkaishi,* **46 :** 621-624.

Kimura, I., M. Sugimoto, K. Toyoda, N. Seki, K. Arai and T. Fujita, 1991. A studyon the cross-linking reaction of myosin in kamaboko "*suwari*" gels. *Nippon Suisan Gakkaishi,* **57**: 1389-1396.

Lee, N., N. Seki, N. Kato, N. Nakagawa, S. Terui and K. Arai, 1990. Gel forming ability of myosin heavy chain in salted meat paste from threadfin bream. *Nippon Suisan Gakkaishi,* **56**: 329-336.

Nowsad, AKM. A., S. Kanoh and E. Niwa, 1993. Electrophoretic behavior of cross-linked myosin heavy chain in *suwari* gel. *Nippon Suisan Gakkaishi,* **59**: 667-671.

Nowsad, AKM. A., S. Kanoh and E. Niwa, 1994a. Setting of transglutaminase-free actomyosin paste prepared from Alaska pollack surimi. *Fisheries Science,* **60**: 295-297.

Nowsad, AKM. A., S. Kanoh and E. Niwa, 1994b. Setting of surimi paste in which transglutaminase is inactivated by *p*-chloromercuribenzoate. *Fisheries Science,* **60** : 185-188.

Nowsad, AKM. A., S. Kanoh and E. Niwa. 1994c. Physico-chemical properties of a *suwari* gel from Alaska pollack surimi with iodoacetic acid added. *Bull. Fac. Bioresources, Mie Univ.,* **13**: 25-31.

Nowsad, AKM. A., S. Kanoh and E. Niwa, 1994d. Setting of surimi paste in which transglutaminase is inactivated by *N*-ethylmaleimide. *Fisheries Science,* **60**: 189-191.

Nowsad, AKM. A., E. Niwa and S. Kanoh, 1995. Contribution of transglutaminase to the setting of various actomyosin pastes. *Fisheries Science,* **61**: 79-81.

Numakura, T., N. Seki, I. Kimura, K. Toyoda, T. Fujita, K. Takama and K. Arai, 1985. Cross-linking reaction of myosin in the fish paste during setting (*SUWARI*). *Nippon Suisan Gakkaishi,* **51**: 1559-1565.

Numakura, T., I. Kimura, K. Toyoda and T. Fujita, 1990. Temperature-dependent changes in gel strength and myosin heavy chain of salt-ground meat from walleye pollack during setting. *Nippon Suisan Gakkaishi,* **56:** 2035-2043.

Seki, N., H. Uno, N. Lee, I. Kimura, K. Toyoda, K. Arai and T. Fujita, 1990. Transglutaminase activity of Alaska pollack muscle and surimi and its reaction with myosin B. *Nippon Suisan Gakkaishi,* **56:** 125-132.

Tsukamasa, Y., K. Sato, Y. Shimizu, C. Imai, M. Sugiyama, Y. Minegishi and M. Kawabata, 1993. e-(g-Glutamyl)lysine crosslink formation in sardine myofibril sol during setting at 25°C. *J. Food Sci.,* **58**: 785-787.

Umemoto, S., 1966. A modified method for estimation of fish muscle protein by Biuret method. *Nippon Suisan Gakkaishi,* **32**: 427-435.

Wan, J., J. Miura and N. Seki, 1992. Effect of monovalent cations on cross-linking of myosin heavy chain in *suwari* gel from walleye pollack. *Nippon Suisan Gakkaishi,* **58***:* 583-590.