EFFECT OF TRANSGLUTAMINASE ON THE PROPERTIES OF HEAT-INDUCED GEL OF MIXED ACTOMYOSIN FROM CULLED HEN AND FISH

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Abstract – The effect of transglutaminase (TG) on thermal gelation of actomyosins from culled hen, fishes (scale-eye plaice, plain sculpin, and Arabesque greenling), and the mixture of hen and fish were investigated. The solubilities of individual and mixed actomyosins were measured by adding 8 M Urea, 2% 2-mercaptoethanol, and 2% SDS containing 20 mM Tris-HCl (pH 8.0). The solubility of scale-eye plaice actomyosin was the lowest among the fishes regardless of the addition of TG and mixing with hen actomyosin. In particular, TG-treated scale-eye plaice actomyosin showed about 50% solubility. Force-indentation curves for TG-treated actomyosins indicated high penetration force than that of untreated actomyosins, except scale-eye plaice actomyosin, which did not form a gel, and shrinking of aggregated protein and loss of water were observed. Cross-linking of myosin heavy chain was observed in all TG-treated actomyosin solutions, resulted in the formation of elastic gels. It is concluded that plain sculpin and Arabesque greenling can be used as ingredients of surimi mixture of fish and hen, while scale-eye plaice is inappropriate for mixed surimi.

Key Words – myosin heavy chain, cross-link, surimi

I. INTRODUCTION

Laying hens are slaughtered when their egg productivity starts to decline. The meat of these hens is inferior to chicken meat, so its utilization is restricted. Surimi is popular seafood product in Japan. Surimi is typically made from white-fleshed fish such as pollack or hake, though many unvalued fishes are also caught in a haul, and most of those unvalued fishes are discarded.

In order to facilitate effective utilization of culled hen meat and also unvalued fishes, we attempt to develop novel surimi-like products. In our preliminary study [1], no satisfactory gel was formed by simple mixing of hen and fish actomyosins because of the difference of optimal temperature of gelation among those actomyosins.

Transglutaminase (TG) is an enzyme that catalyzes the formation of a covalent bond between a free amine group of lysine and an amide of glutamine, and it is used in a variety of food production, including processed meat and fish products [2].

In this study, effectiveness of TG on thermal gelation of mixed actomyosin of hen and fish was investigated.

II. MATERIALS AND METHODS

Preparation of actomyosin from culled hen — Actomyosin was extracted from pectoral muscle with Weber-Edsall solution, and purified further.

Preparation of fish actomyosin — Three kinds of fishes, *i.e.*, plain sculpin (PS) (*Myoxocephalus jaok*), scale-eye plaice (SP) (*Acanthopsetta nadeshnyi*), and Arabesque greenling (AG) (*Pleurogrammus azonus*) were used. Minced fish meats were washed with low ionic strength buffer, then extracted with 0.45 M NaCl, 3.38 mM Na_2HPO_4 (pH 7.5) for 24 hr, and purified further.

Transglutaminase treatment — Hen and fish actomyosins were mixed (1:1, w/w) in 0.3 M NaCl and 10 mM Bis-Tris (pH 7) to give a protein concentration of 5 or 10 mg/ml. Transglutaminase (Activa TG-K, Ajinomoto Co.) was added to actomyosin solution at 1 U/g of actomyosin, and incubated for 3–4 hr at 37˚C.

Solubility measurement — Solubilizing solution (8 M urea, 2% SDS, 2% 2-mercaptoethanol, and 20 mM Tris-HCl, pH 8.0) was added to TG treated actomyosin, and heated at 100˚C for 2 minutes, then incubated at room temperature for 20 hours. After centrifugation, the supernatant was collected and the amount of protein was determined.

Protein composition of solubilized fraction —

Solubilized protein was analyzed by SDS-PAGE.

Rheological measurement of heat-induced actomyosin gel — Two kinds of thermal treatments were employed to form a gel. One is one-step heating; that is, heating at 37˚C for 4 hours or 90˚C for 30 min. The other is two-step heating; preheating at 37˚C for 4 hr, then heating at 90˚C for 30 min. Penetration test using a 5 mm spherical plunger was done for actomyosin gel.

Morphological observation of gel structure — Scanning electron microscopy was performed to observe microstructure of actomyosin gel.

III. RESULTS AND DISCUSSION

Changes in solubility of TG-treated actomyosin by heating

Solubilizing solution, which contains urea-SDS-mercaptoehanol, was added to TG-treated acto-myosin and solubilized protein was determined. The change in solubility of actomyosin is shown in Figure 1. Individual actomyosin of hen, AG, and SP without TG showed over 90% of solubility during incubation at 37˚C for 3 hours, while the solubility of PS actomyosin decreased to 85%. When TG was added to actomyosins, the solubilities of hen, PS, and AG decreased to 80%, and 50% for SP (Fig. 1a). This indicates that TG forms more cross-linking in SP actomyosin than the others.

In the case of mixed actomyosin of hen and fish without TG, the solubilities of mixed actomyosin of hen + AG and hen + PS remained at almost 100%, while that of hen + SP decreased to 80%. Addition of TG induced decrease of solubility to about 80% in hen + AG and hen + PS actomyosins. There was no effect of TG on solubility of hen + SP actomyosin (Fig. 1b).

It is known that cross-linking of myosin heavy chains takes place in walleye pollack surimi gel [3]. In order to investigate cross-linking formation of the proteins included in actomyosin during incubation at 37˚C with or without TG, the solubilized fraction was analyzed by SDS-PAGE (Figures 2 and 3). There was no additional band above myosin heavy chain (HC) in individual actomyosin without TG. Myosin HCs of PS and AG decreased with time of incubation, and the new bands appeared below HC band (Fig. 2), suggesting degradation of HC by endogenous proteases during incubation. No new bands appeared above HC band in the mixed actomyosin. Furthermore, there was no decrease of HC band (Fig. 3).

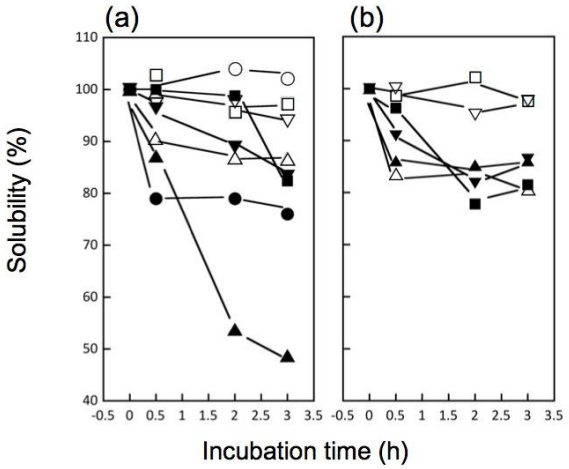

Figure 1. Solubility of actomyosin incubated at 37˚C with or without TG for various time.
(a) individual actomyosin,○, □, △, ▽: hen, AG, SP, and PS. Open symbol represents without TG and closed is with TG. (b) mixed actomyosin of hen and fish. Symbols are the same as in (a), excluding hen actomyosin.

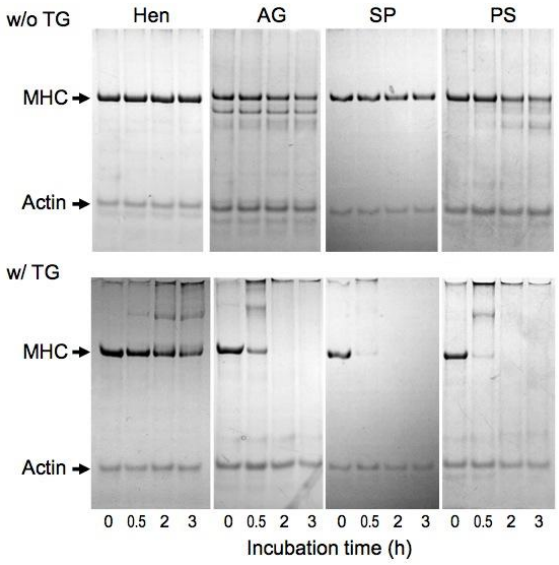

Figure 2. Changes in SDS-PAGE pattern of solubilized fraction from individual actomyosin with or without TG at 37˚C. MHC denotes myosin heavy chain.

The HC bands in all of TG-treated actomyosins decreased and the high molecular weight bands appeared, especially in fish actomyosins (Fig. 2). Cross-linking of myosin HC in SP actomyosin was faster than the others. The HC in mixed actomyosin also decreased and high molecular weight components appeared with TG, though no complete disappearance of the HC band within 3 hours was observed (Fig. 3). HC decrease in SP actomyosin was the fastest among the fishes, while the decrease of HC in hen-SP mixed actomyosin was slower compare to hen-PS and hen-AG.

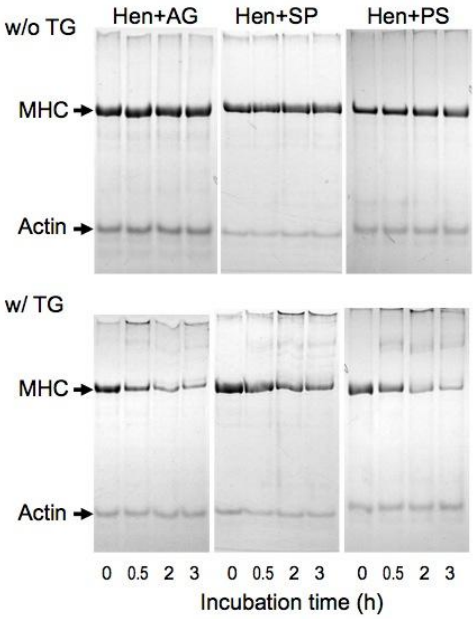

Figure 3. Changes in SDS-PAGE pattern of solubilized fraction from mixed actomyosin with or without TG at 37˚C.

Rheological measurement was done for thermally treated actomyosin with or without TG (Figures 4 and 5). When individual actomyosin or mixed actomyosin was incubated at 37˚C for 4 hours without TG, no steady gel was formed; while, they formed gel in the presence of TG.

Regardless of the presence or absence of TG, fish actomyosins did not form gel after heating at 90˚C for 30 minutes, whereas hen actomyosin formed steady gel and the gel strength increased by addition of TG. Mixed actomyosins of hen and fish also did not form steady gel by heating

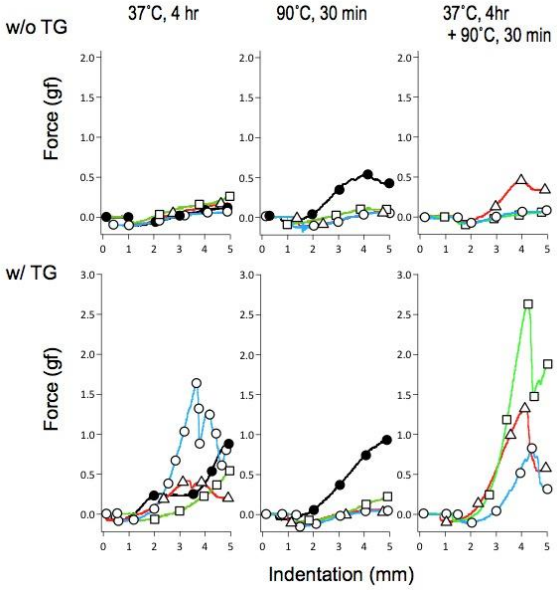

Figure 4. Force *vs.* indentation curve of thermally treated individual actomyosin. ●; hen, △; AG, □; SP, and ○; PS.

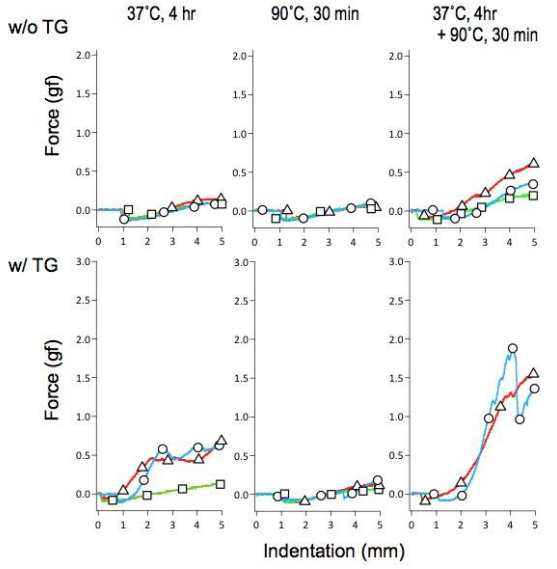

Figure 5. Force *vs.* indentation curve of thermally treated mixed actomyosin. △; hen + AG, □; hen + SP, and ○; hen + PS.

at 90˚C. The results indicate that fish acto-myosin fails to form a gel by rapid high temperature heating, and cross-linking by TG is not formed. Fish actomyosins formed firm gel by two-step heating (37˚C for 4 hours, then 90˚C for

30 minutes) in the presence of TG. Mixed actomyosin of hen and AG or PS showed same tendency, though the mixed actomyosin of hen and SP released water by heating and no steady gel was formed (Fig. 5).

Cross-linking of myosin heavy chain is known to relate gel strength [4], and the present result is in accordance with it.

Actomyosin gel formed by two-step heating with or without TG was observed by SEM (Figures 6 and 7). In general, the gels formed with TG showed finer network structure compare to those without TG. These fine network structure is possibly responsible for high gel strength in TG-treated actomyosin gel (Figs. 4 and 5).

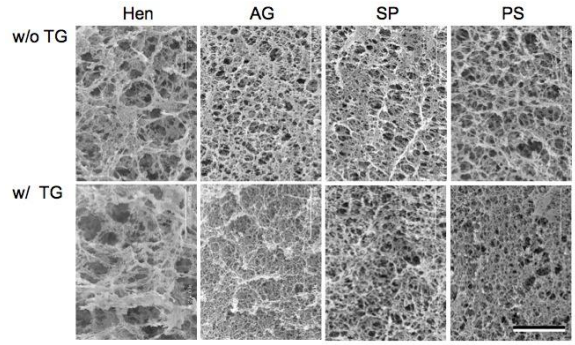

Figure 6. Scanning electron micrographs of gels formed from individual actomyosin by two-step heating.
Scale bar indicates 20 µm.

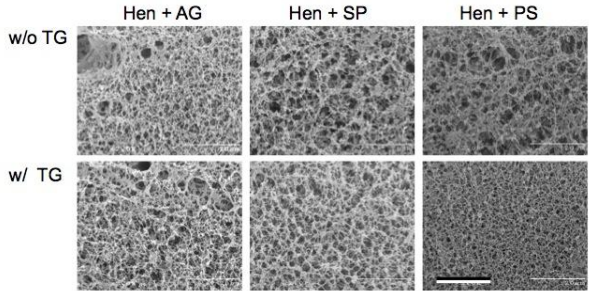

Figure 7. Scanning electron micrographs of gels formed from mixed actomyosin by two-step heating.
Scale bar indicates 20 µm.

SP actomyosin formed coherent gel when treated with TG, while the gel of mixed actomyosins of SP and hen released water on heating. Therefore, SP actomyosin is not a suitable material for mixing with hen actomyosin; instead it is desirable for single usage. On the other hand, mixtures of hen and PS or AG actomyosin formed a stable gel on addition of TG. The present results suggest that PS and AG are promising fish materials for development of novel surimi-like products based on culled hen meat.

ACKNOWLEDGEMENTS

This research was partly supported by a fund from Hokkaido Research Organization, for the Scientific Research on Priority Areas during fiscal years 2010–2012.

The authors thank Dr. H. Takeda and Mr. K. Ebitani of the Hokkaido Research Organization for helpful discussion.

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IV. CONCLUSION