ヨード酢酸を添加したスケトウダラ坐りゲルの物性

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Physico-chemical Properties of a *Suwari* Gel from Alaska Pollack Surimi with Iodoacetic Acid Added

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Abstract

The physico-chemical properties of a *suwari* gel from Alaska pollack surimi in the presence of iodoacetic acid (IAA), considered as an inhibitor of transglutaminase (TGase), were studied to assess the contribution of TGase to the setting of fish flesh paste. IAA at more than 0.25% completely inhibited the activity of muscle TGase, decreased breaking force and breaking strain of the *suwari* gel by about 35%, increased its expressible water by about 30%, and increased the elastic modulus and viscosity beyond values determined from the stress-relaxation curves by about 20%. Cross-linked myosin heavy chain formation was suppressed. From the result that the gel was also obtained in the presence of IAA, it would appear that TGase may not always be required for the setting of surimi paste. Accordingly, the non-covalent thermal aggregation of protein through hydrogen and hydrophobic bonds may possibly be essential to the setting of Alaska pollack surimi.

Key words: *suwari*. iodoacetic acid. transglutaminase. non-covalent protein aggregation

I. Introduction

The existence of transglutaminase (TGase) in the Alaska pollack surimi paste[8] and a gradual decrease of myosin heavy chain (MHC) with a concomitant increase of cross-linked myosin heavy chain (CMHC) during the setting of the paste[7] suggested that the setting of the paste essentially involves a cross-linking of MHC catalyzed by TGase[2]. In a previous report[6], however, we described that the setting was still induced even in the presence of *p*-chloromercuribenzoic acid (PCMB), one of the SH reagents considered as an inhibitor of TGase, suggesting that the catalytic formation of CMHC from MHC by TGase was not always necessary for the occurrence of the setting of the paste. In this article, we describe the effects of iodoacetic acid (IAA), another powerful SH reagent, on the physico-chemical properties of *suwari* gel.

II. Materials and Methods

1. *Materials*

Unsalted Alaska pollack (*Theragra chalcogramma*) frozen surimi (SA grade) from Golden Alaska Seafoods Inc. was used. IAA (GR-grade) was purchased from E. Merck, Darmstadt. Monodansylcadaverine (MDC) was obtained from Sigma Chem. Co. Other chemicals used in this experiment were reagent grade.

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2. *Setting of the paste*

The thawed surimi was minced and ground together with, per 100 g of the mice, 3 g of NaCl, 1 g of sterilizer (Solmighty, Ueno Fine Chem. Co.), 30 ml of 0.2 M phosphate buffer (pH 7.0), and various amounts of IAA (0, 0.25, 0.5, and 1.0% of the mince) in a mortar for 10 min at 4°C. The resulting paste was stuffed into polyvinylidene chloride casings (2 cm in diameter) and set at 30°C in a water bath to give the *suwari* gel. After setting for the prescribed periods, the gels were cooled in running water, and subjected to the following tests.

3. *Measurement of pH and elasticity of the gel*

The pH of the paste and *suwari* gel was measured by putting them in a hollow chamber of an electrode (Horiba Ltd., Twin compact pH meter B-112).

Puncture tests were done on the gels sliced into 2 cm height by using a rheometer (Fudoh Kogyo Co., NRM 2010J) with a spherical plunger (5 mm in diameter) at a table speed of 6 cm/min.

Stress-relaxation tests were done on the gels of the same shape as above by using the same rheometer with a flat plunger (4×4 cm^2 area) at a table speed of 30 cm/min and a strain of 0.1. Stress-relaxation curves were analysed as reported previously[3], by a four element mechanical model, in which two sets of Maxwell's model were paralleled to each other. An instantaneous elastic modulus of this mechanical model (G_0), the modulus of the Maxwell's model showing longer relaxation time (G_1), the modulus of another Maxwell's model (G_2), the viscosity of the former model (η_1), and that of latter one (η_2) were obtained by the progressive approximate methods[3].

The amount of expressible water of the gel was obtained from its weight loss during a compression at 1 kg/cm^2 for 3 min between two folds of filter paper (Toyo Roshi Co., No. 3).

4. *TGase activity measurement*

The activity of TGase of the paste added with various amounts of IAA was measured as reported previously[5], after setting at 30°C with 2.5 mM MDC.

5. *SDS-polyacrylamide gel electrophoresis (SDS-PAGE)*

The *suwari* gels were subjected to SDS-PAGE (5% acrylamide gel) as reported previously[4], after being dissolved in 8 M urea containing 2% SDS, 2% 2-mercaptoethanol, and 20 mM Tris-HCl (pH 8.0). Densitometry of the stained disc was carried out using a chromatoscanner (Shimadzu Corporation, CS-910) at the wavelengths of 640 and 700 nm[4]. The relative amount of each subunit was calculated from the ratio of the area of its absorption band to the sum total of the area of all the bands in the densitogram pattern.

III. Results

Changes in the activity of TGase in the surimi pastes with 0-1.0% IAA added during setting is presented in Table 1. The activity was 1.4-6.9 n mol/mg protein in the control paste where IAA was not added. However, the addition of IAA completely inhibited the activity in the pastes. It was 0.0 n mol/mg protein in all the gels with 0.25, 0.5, and 1.0% IAA added, respectively.

The pH of the pastes and *suwari* gels are shown in parentheses in Table 1. In spite of the addition of phosphate buffer to the pastes, their pH decreased with both the increasing amount of IAA added and the

Table 1 Changes in the activity of TGase in the surimi pastes with IAA added. The activity is expressed as the amount of MDC incorporated into the muscle proteins (n mol/mg protein). Numbers in parentheses represent the pH of the pastes without MDC.

Amount of IAA (%)	TGase activity*						
	Setting time (h)						
	0	1	2	5	10	20	30
0.0	— (6.9)	1.4 (6.9)	2.5 (6.9)	3.5 (6.9)	5.3 (6.7)	6.4 (6.4)	6.9 (6.3)
0.25	— (6.7)	0.0 (6.7)	0.0 (6.6)	0.0 (6.6)	— (6.3)	— (6.2)	— (6.1)
0.5	— (6.6)	0.0 (6.6)	0.0 (6.4)	0.0 (6.4)	— (6.1)	— (6.0)	— (6.0)
1.0	— (6.6)	0.0 (6.4)	— (6.2)	0.0 (6.1)	— (5.9)	— (5.7)	— (5.7)

* Mean from three replicates
—: not measured

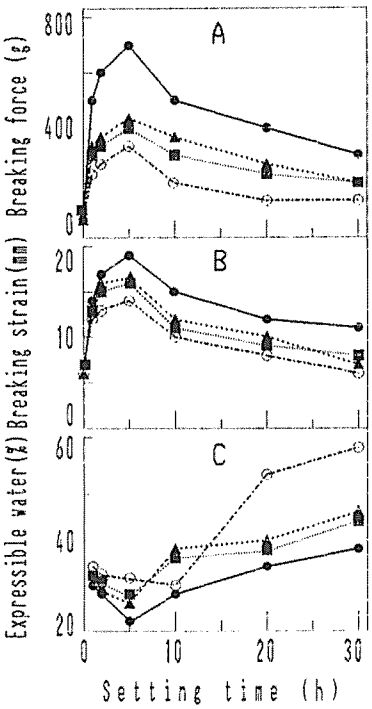

Fig. 1. Effects of IAA on the breaking force (A), breaking strain (B), and expressible water (C) of the *suwari* gels. Amount of IAA added to the surimi: —●—: 0%; —▲—: 0.25%; —■—: 0.5%; —○—: 1.0%.

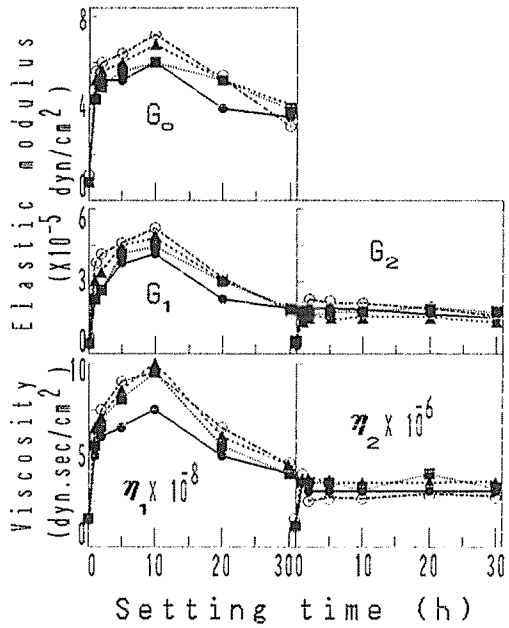

Fig. 2. Effects of IAA on the elastic modulus and viscosity of the *suwari* gels calculated from their stress-relaxation curves. Symbols are the same as those described in Fig. 1.

elongation of setting period. In the control gel to which IAA was not added, however, the pH did not change till 5 h of setting.

Fig. 1 shows the effects of IAA on the breaking force, breaking strain, and expressible water of the *suwari* gels. The breaking force and breaking strain rapidly increased till 5 h of setting and gradually decreased thereafter in all the gels. Both the breaking force and breaking strain were the highest in the control gel and the value gradually lowered in other gels with the increasing amount of IAA added. The amount of expressible water of the gels decreased till 5 h but increased thereafter. The amount was the lowest in the control get and the highest in the gel with 1.0% IAA added.

Fig. 2 shows the effects of IAA on the elastic modulus and viscosity of the *suwari* gels calculated from their stress-relaxation curves. In all the gels, G_0, G_1, and η_1 rapidly increased at the initial stage of setting and decreased after 10 h, but G_2 and η_2 reached a constant after showing a rapid increase up to 2 h. Differing from the results of breaking force, breaking strain, and expressible water, G_0, G_1, and η_1 were higher in the gels with IAA added than in the control gel. However, G_2 and η_2 were almost similar in both control and IAA added gels.

Fig. 3 shows the SDS-PAGE patterns for the gels without IAA and with 0.25% IAA added. In the former gel, a rapid weakening of a MHC band was observed with a concomitant strengthening of a CMHC band with the progress of setting. In the latter gel, however, only a little weakening of the MHC band was noticed and the CMHC band was somewhat weakened. A band due to 170 kDa (170 k) protein was strengthened with the setting time in both the gels. Fig. 4 shows the relative amount of protein subunits calculated from the densitogram patterns of the discs in Fig. 3. The decrement of MHC was intense in the control gel but not so in the gels with IAA added. The CMHC intensely increased till 30 h in the former, but slightly increased up to 2 h and decreased thereafter in the latter. The amount of 170 k protein gradually increased, and this tendency was

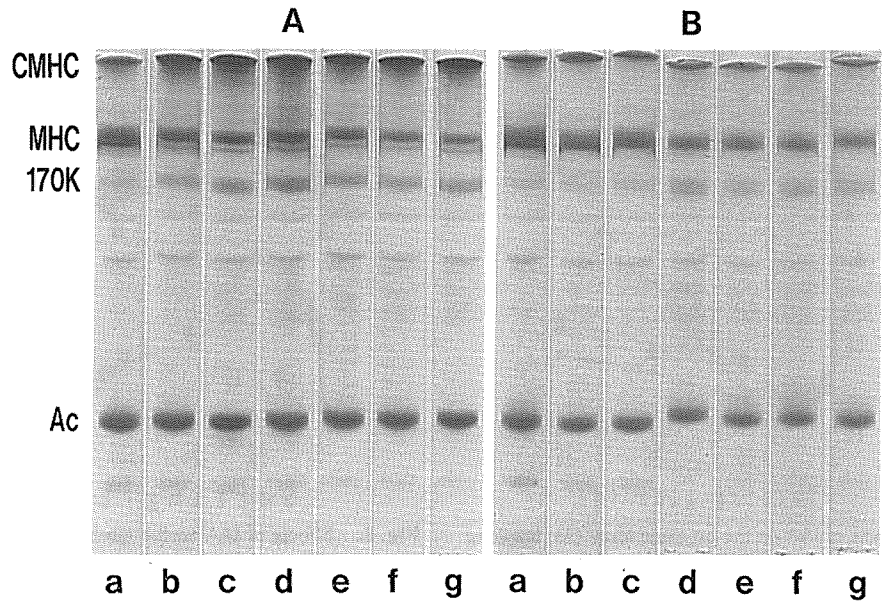

Fig. 3. SDS-PAGE discs of the *suwari* gels without (A) and with 0.25% (B) IAA added.
a: surimi paste; b: *suwari* gel set at 30°C for 1 h; c: gel set for 2 h; d: gel set for 5 h; e: gel set for 10 h; f: gel set for 20 h; and g: gel set for 30 h.

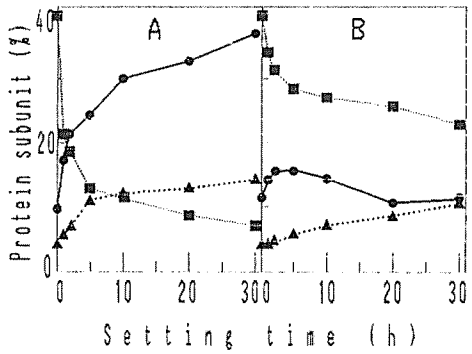

Fig. 4. Relative amount of protein subunits calculated from the densitogram patterns of SDS-PAGE discs in Fig. 3.
A and B: same as in Fig. 3.
—●—: CMHC, —■—: MHC, —▲—: 170 k protein.

more intense in the former than in the latter.

IV. Discussion

No TGase activity was observed in the pastes with IAA added, suggesting that the catalysis of TGase was completely inhibited by the addition of more than 0.25% IAA to the paste.

It is seen in Fig. 1 that the elastic gels were formed even from the pastes with IAA added where the catalytic activity of TGase was completely inhibited. Due to the acidification by IAA, the pH of the pastes and *suwari* gels sharply decreased. In spite of their pH decrease, both breaking force and breaking strain increased with the elongation of setting time. However, their values for the gels with 0.25% IAA added decreased to 60 —70% of that for the control gel without IAA. On the other hand, the expressible water, as an index of setting, also decreased corresponding to increased setting time in the pastes with IAA added, though its value was about 30% more in the gel with 1.0% IAA added than in the control one.

Differing from the breaking force and breaking strain mentioned above, both the G_1 and η_1 were higher by about 20% in the gel with 1.0% IAA added than in the control gel. Therefore, from the results, it was observed that the setting was induced even in the pastes with IAA added, although the quality of the resulting gel was changed.

On the other hand, as mentioned before[7], CMHC has been considered to be formed from MHC during the progress of setting. The protein subunits in the gel with IAA added were compared with those in the control gel in Fig. 3 and 4, in order to confirm whether the CMHC was formed in the presence of IAA. From the results that the MHC decreased throughout the setting of the IAA added paste with a concomitant increase of CMHC at the initial stage, it seems that the MHC is still polymerized to CMHC even in the paste where the activity of TGase is absolutely nil. In this gel, the CMHC might also be formed from MHC throughout setting which were dissolved out in SDS of SDS-PAGE system. It has been reported that the demonstration of non-covalent protein interactions in SDS-PAGE was precluded because SDS readily broke hydrogen bonds, electrostatic bonds, and hydrophobic associations[1]. As to the decrease of MHC, however, there is another possibility that the MHC underwent proteolysis during setting, from the fact that the 170 k protein increased gradually.

Similar to the results from the gels with PCMB added[6], it was also found in the present study that the gel was formed by the setting of the paste, where the TGase activity was completely inhibited by IAA, which also suggests that the catalytic formation of CMHC by TGase is not always necessary for the occurrence of setting of the paste. In this case, setting might be induced by the non-covalent protein aggregation through hydrogen and hydrophobic bonds, the possibility of which was reported earlier[4]. However, the gels with IAA added were inferior to the control gel in the breaking force, breaking strain, and expressible water. This inferiority may be attributed to the depression of pH of the paste by the addition of IAA, the suppression of the formation of SS bonds among the muscle proteins (IAA, being a SH reagent, is known to suppress intermolecular SS bonds of protein), and the inhibition of the activity of TGase. In a sense that the elasticity of the *suwari* gel is strengthened by the formation of CMHC, TGase may also involve in the setting.

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ヨード酢酸を添加したスケトウダラ坐りゲルの物性

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魚肉塩すり身の坐りに及ぼすトランスグルタミナーゼ (TGase) の影響を明らかにするために，その阻害剤として知られるヨード酢酸 (IAA) を加えたスケトウダラ塩すり身を坐らせ，得られる坐りゲルの物性を調べた。ミンチ重量に対して0.25%以上の IAA を加えると，筋肉中の TGase は失活し，坐りゲルの破断強度，破断歪みの35%程度の低下，圧出水分量の30%程度の増加が認められたが，応力緩和では弾性率，粘性率が20%程度増加した。さらにこのとき，TGase によって触媒されるミオシン重鎖多量体の形成も抑制された。これらの結果から，坐りにおける TGase の意義は非共有結合によって生ずる筋肉タンパク質の網状構造を強化することにあると察せられる。