

# Microbial Transglutaminase Affects Gel Properties of Golden Threadfin-bream and Pollack Surimi

S.-T. JIANG, J.-F. HSIEH, M.-L. HO, AND Y.-C. CHUNG

**ABSTRACT:** The properties of surimi gels from threadfin-bream and pollack surimi set at 30 °C or 45 °C with microbial transglutaminase (MTGase) from *Streptovorticillium ladakanum* were determined. The optimal amounts of MTGase and setting conditions were: 0.3 unit/g surimi either at 30 °C for 90 min or at 45 °C for 20 min for threadfin-bream, and 0.2 unit/g surimi at 30 °C for 60 min for pollack. The strength of golden threadfin-bream surimi gels with 0.35 unit MTGase set at 30 °C for 90 min or 45 °C for 20 min was 3400 g × cm, almost 3-fold of the control. SDS-PAGE analyses indicated that inter- and/or intramolecular cross-linking formed in the myosin heavy chain of MTGase-containing surimi gels.

**Key Words:** MTGase, surimi, gel property, pollack, threadfin-bream

## Introduction

**S**URIMI MADE FROM WATER-WASHED FISH MINCE AND MIXED with cryoprotectants has a unique ability to form elastic gels through the interaction of myosin molecules (Lee and others 1997). Gel-forming of myosin occurs at 2 stages during low-temperature setting (0 to 45 °C) and high-temperature (90 °C) heating (Hashimoto and Arai 1978; Boye and Lanier 1988). The polymerization of the myosin heavy chain (MHC) catalyzed by TGase occurs during low-temperature setting (Nowsad and others 1994).

Transglutaminase (TGase; EC 2.3.2.13) catalyzed the formation of  $\epsilon$ -( $\gamma$ -glutamyl)lysine cross-links, which is covalent and important for gel-formation and viscoelastic properties (Seki and others 1990; Sakamoto and others 1995; Seguro and others 1995). Kimura and others (1991) found the formation of  $\epsilon$ -( $\gamma$ -glutamyl)lysine during the setting process of kamaboko gels and consequently considered TGase to be a setting-promoting enzyme. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that the cross-linked MHC increased during the setting process in TGase-contained pollack surimi (Nowsad and others 1994). Although TGase is widely distributed in animal tissues or organs, plants, and microorganisms (Connellan and others 1971; Chung and Folk 1972; Jiang and Lee 1992; Hanigan and Goldsmith 1978; Brenner and Wold 1978; Takagi and Doolittle 1974; De Backer-Royer and Meunier 1992), the production and purification of TGase from microbial sources have attracted most researchers. Extracellular TGase has been purified from cultural filtrate of *Streptovorticillium mobarense* (Gerber and others 1994; Nonaka and others 1989) and *Streptovorticillium sp.* (Ando and others 1989). Intracellular TGase were also found in *Bacillus subtilis* (Ramanujam and Hageman 1990) and in the spherules of *Physarum polycephalum* (Klein and others 1992). The effects of the microbial transglutaminase (MTGase) from *Streptovorticillium mobarense*, *Streptovorticillium sp.*, and *Streptovorticillium ladakanum* on protein gels have also been extensively studied (Nonaka and others 1989, 1992, 1994; Sakamoto and others 1994, 1995; Seguro and others 1995; Tanaka and others 1990; Tsai and others 1996; Jiang and others 1998).

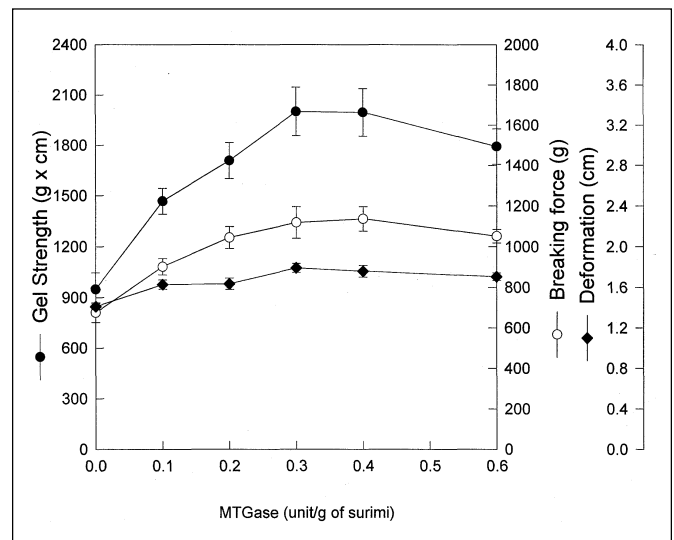
The objectives of this study were to investigate the effects of TGase produced by *Streptovorticillium ladakanum* on the gel-

forming abilities of golden threadfin-bream surimi and pollack surimi and to establish an optimum condition for the production of TGase-containing surimi analog.

## Results and Discussion

### Effect of MTGase on the gel-forming ability of golden threadfin-bream and pollack surimi

The breaking force, deformation, and gel strength of the golden threadfin-bream gels reached a maximum when 0.3 unit/g surimi of MTGase was added (Fig. 1). The gel strength of the sample with 0.3 unit/g surimi of MTGase was 2000 g × cm, which was about 2-fold of the control. When added MTGase was higher than 0.4 unit/g gel, the decreases ( $p < 0.05$ ) in gel strength, deformation, and breaking force became rigid and brittle. Therefore,



**Fig. 1**—Effect of MTGase on the gel-forming ability of golden threadfin-bream surimi. Gels set at 30 °C for 120 min. Vertical bars represent standard deviation.

0.3 unit/g surimi appears to be an optimum amount of MTGase for the production of golden threadfin-bream surimi-based products.

The effect on gel-forming ability of pollack surimi was similar to that of golden threadfin-bream. However, the highest breaking force and gel strength were obtained with the addition of 0.2 unit MTGase/g (Fig. 2). Again, excess amounts of MTGase caused the significant decrease ( $p < 0.05$ ) in the gel-forming ability of pollack surimi.

The gel-forming ability of golden threadfin-bream or pollack surimi increased with the addition of MTGase. However, it increased up to a certain level, and further increase in MTGase decreased the gel strength. This phenomenon was also observed in several previous studies (Asagami and others 1995; Sakamoto and others 1995; Tsai and others 1996; Jiang and others 1998). The highest gel strength of pollack surimi was obtained from 1 to 2 units MTGase/g (Sakamoto and others 1995), however, the optimal MTGase for minced mackerel was 0.34 unit/g (Tsai and others 1996). According to Jiang and others (1998), the gel strength of minced mackerel increased with the increase of MTGase up to

0.47 unit/g. Asagami and others (1995) studied 5 different kinds of frozen surimi and concluded that the amount of MTGase added highly depended on fish species and also some other factors such as freshness, protein quality, and harvesting season.

### Effect of setting condition on gel-forming ability of MTGase-containing golden threadfin-bream and pollack surimi

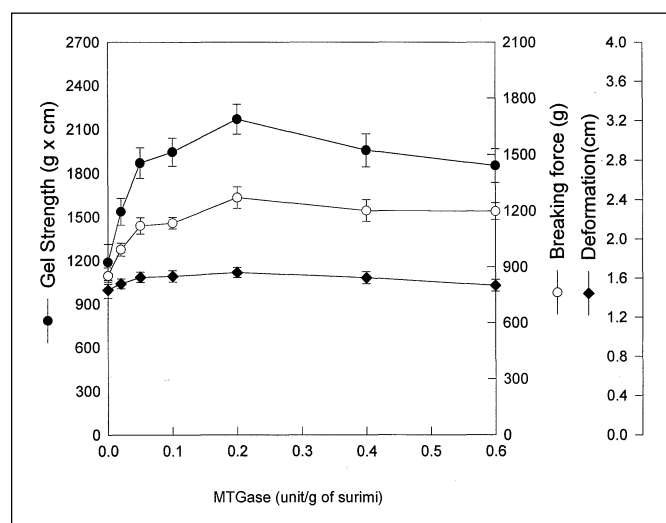
Kumazawa and others (1995) reported that TGase participated in the setting process of high-grade surimi gels and mainly accelerated the cross-linking of the myosin heavy chain (MHC). When MTGase-containing golden threadfin-bream gels were set at 45 °C for 2 h, the breaking force and gel strength sharply increased within 20-min setting and began to level off afterwards (Fig. 3). The effect of setting on the gel-forming ability of samples without MTGase was not as pronounced as that with MTGase. The gel strength of golden threadfin-bream gels with 0.35 unit/g MTGase set at 30 °C for 90 min or 45 °C for 20 min was 3400 g × cm, which was about 3-fold of the control. Based on the gel-strength data, the optimal setting condition for MTGase-containing threadfin-bream surimi gels was 30 °C, 90 min or 45 °C, 20 min.

When MTGase-containing pollack gels were set at 30 °C, the peak values for breaking force, deformation, and gel strength were obtained after 60-min setting, while at 45 °C the peak values were obtained within shorter setting for 30, 15, and 30 min, respectively (Fig. 4). Although the gel-forming ability of pollack surimi without MTGase was improved greatly by setting at 30 °C ( $p < 0.05$ ), it was still inferior to those with MTGase. The optimal setting conditions for pollack surimi were found to be 60 and 30 min at 30 °C and 45 °C, respectively.

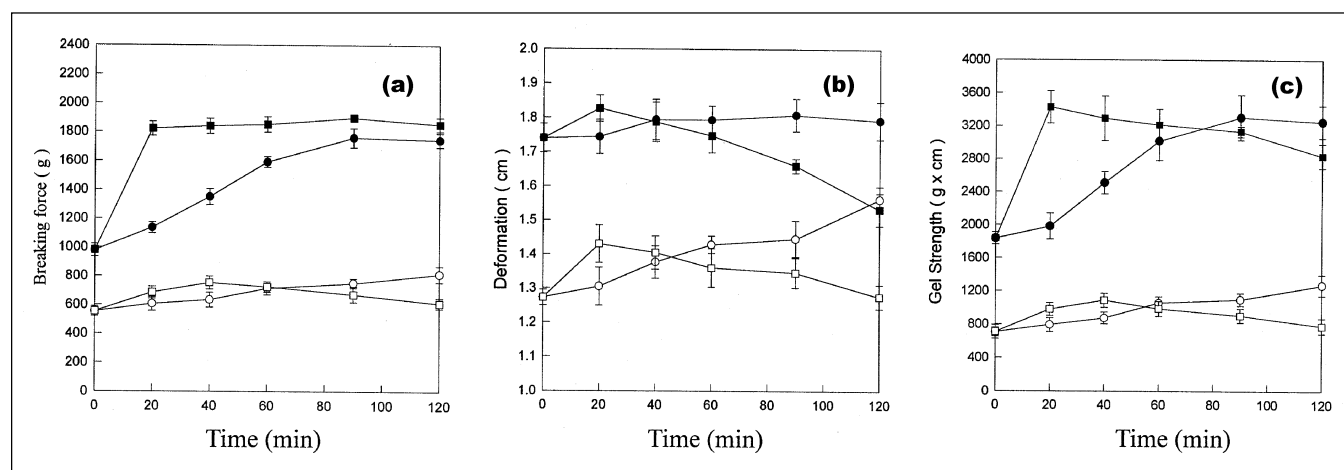
The decreases in gel-forming ability of both golden threadfin-bream and pollack surimi when set at 45 °C beyond optimal time are believed to be because of the endogenous protease activity at neutral and around 45 °C (Boye and Lanier 1988).

### Effect of MTGase on the cross-linking of golden threadfin-bream and pollack actomyosin

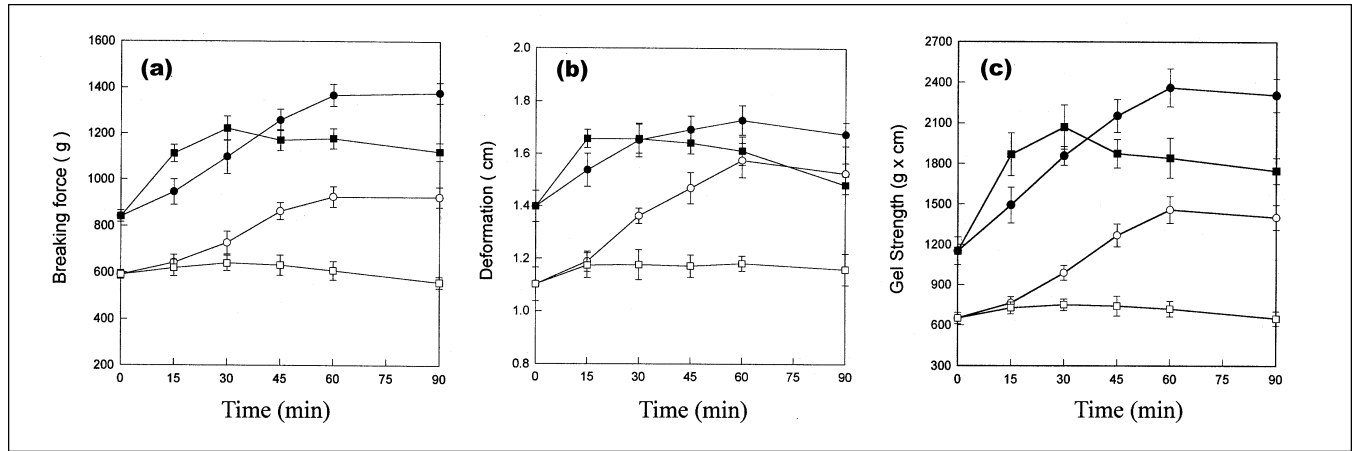
SDS-PAGE shows that the intensity of MHC decreased with an increase in the amount of MTGase (Fig. 5). Components with higher MW were observed, and their intensity got thicker with an increase in the amount of MTGase. These components were considered to result from the MHC cross-linking (Seguro and others 1995; Jiang and others 1998). The disappearance of MHC evi-



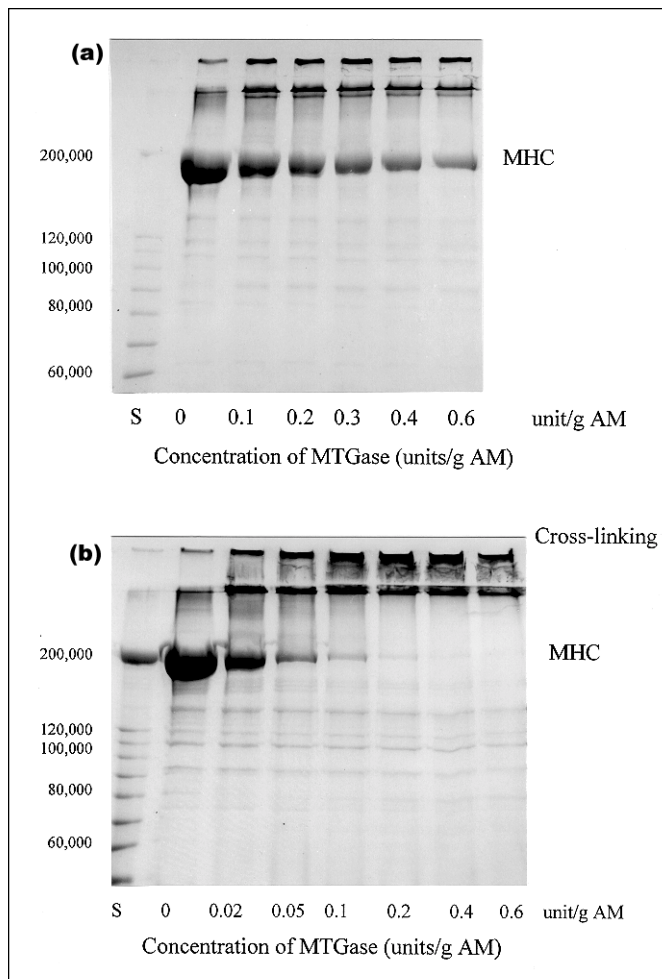
**Fig. 2—Effect of MTGase on the gel-forming ability of pollack surimi. Gels set at 30 °C for 120 min. Vertical bars represent standard deviation.**



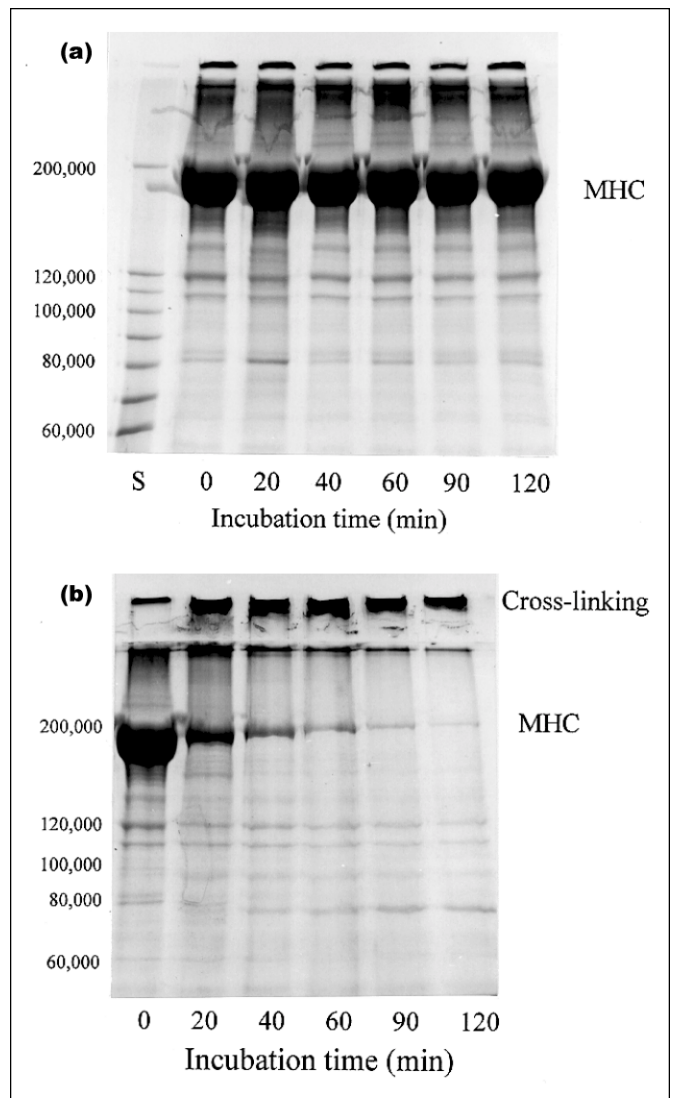
**Fig. 3—Effect of setting temperature on the gel-forming ability of golden threadfin-bream surimi with or without MTGase. Gels set at 30 °C = ○●; 45 °C = □■. Gels with 0.35 unit/g MTGase: solid symbols; gels without MTGase: open symbols. Vertical bars show standard deviation.**



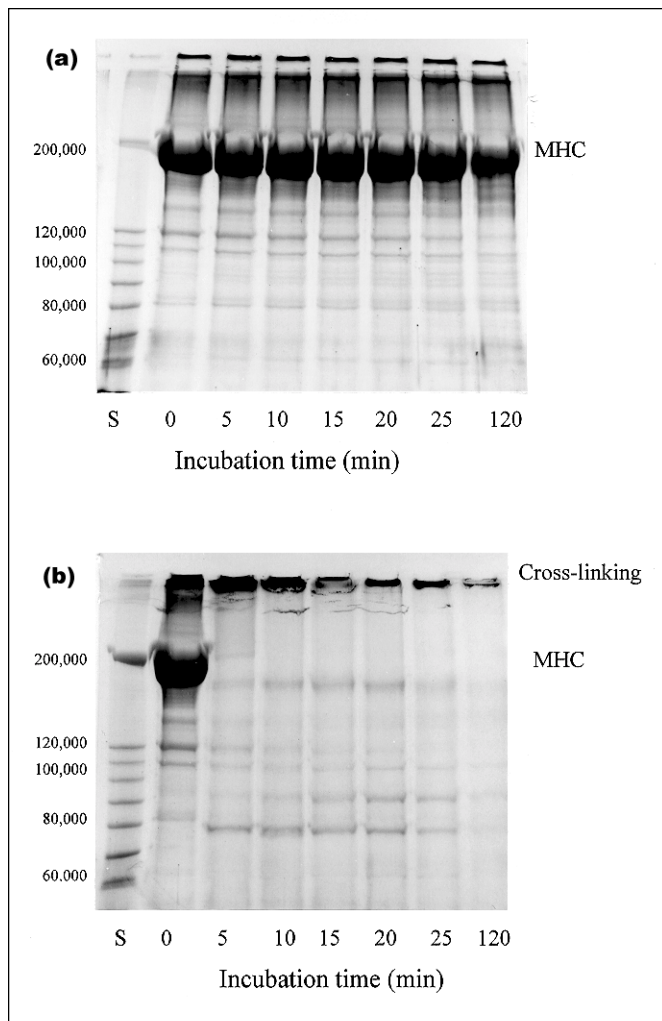
**Fig. 4—**Effect of setting temperature on the gel-forming ability of pollack surimi with or without MTGase. Gels set at 30 °C = ○●; 45 °C = □■. Gels with 0.2 unit/g MTGase: solid symbols; gels without MTGase: open symbols. Vertical bars represent standard deviation.



**Fig. 5—**Changes in SDS-PAGE profiles of actomyosin (AM) with various amounts of MTGase incubated at 30 °C for 30 min. A: golden threadfin-bream AM; B: pollack AM. (S: protein marker)



**Fig. 6—**Changes in SDS-PAGE profiles of gold threadfin-bream actomyosin (AM) with or without MTGase incubated at 30 °C for various time periods. A: without MTGase added; B: with 0.35 unit MTGase/g surimi. (S: protein marker)



**Fig. 7—Changes in SDS-PAGE profiles of gold threadfin-bream AM with or without MTGase incubated at 45 °C for various time periods. A: without MTGase; B: with 0.35 unit MTGase/g surimi. (S: protein marker)**

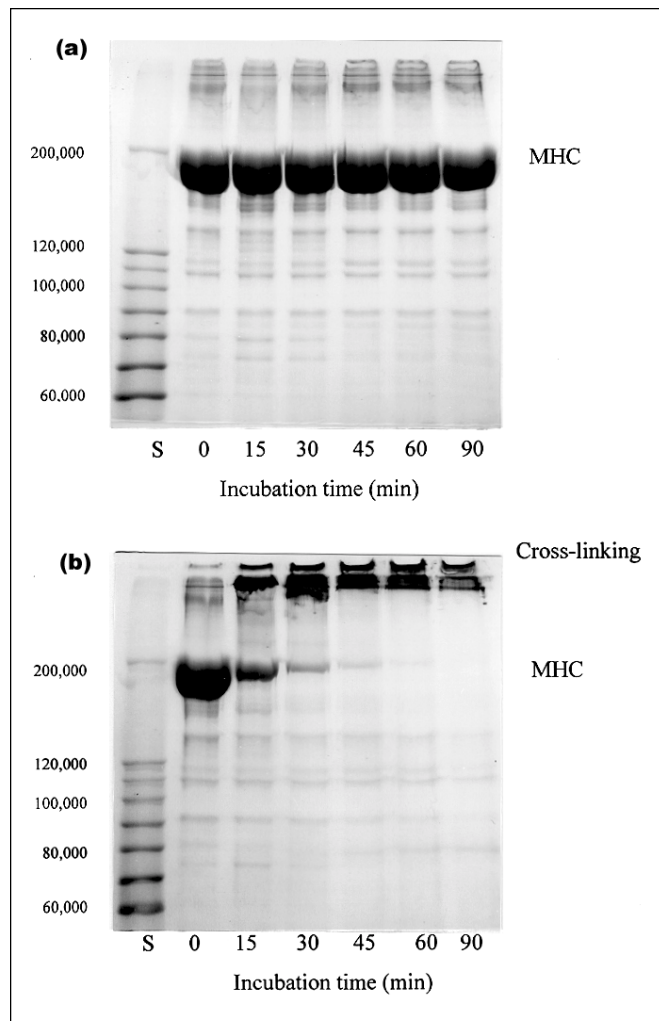
denced the occurrence of MHC cross-linking because the  $\epsilon$ -( $\gamma$ -glutamyl)lysine isopeptide bonds formed in the cross-linked MHC by MTGase, which is difficult to dissociate by the mixture of SDS and mercaptoethanol during SDS-PAGE analysis (Jiang and others 1998).

When MTGase-containing golden threadfin-bream AM was incubated at 30 °C or 45 °C, the MHC decreased gradually, and cross-linking of MHC became prominent with incubation time, being more rapid at 45 °C than at 30 °C (Figs. 6 and 7). However, no cross-linked MHC was observed on golden threadfin-bream without MTGase during 120-min incubation at 30 °C or 45 °C (Figs. 6 and 7).

## Materials and Methods

### Chemicals

Tris(hydroxymethyl)aminomethane,  $\gamma$ -globulin, carbobenzoxy-L-glutamyl-glycine (CBZ-L-Gln-Gly), sodium dodecyl sulfate (SDS), Tween 20, L-glutamic acid- $\gamma$ -monohydroxamic acid, cysteine, glutathione, sucrose, and dephosphorylated  $\beta$ -



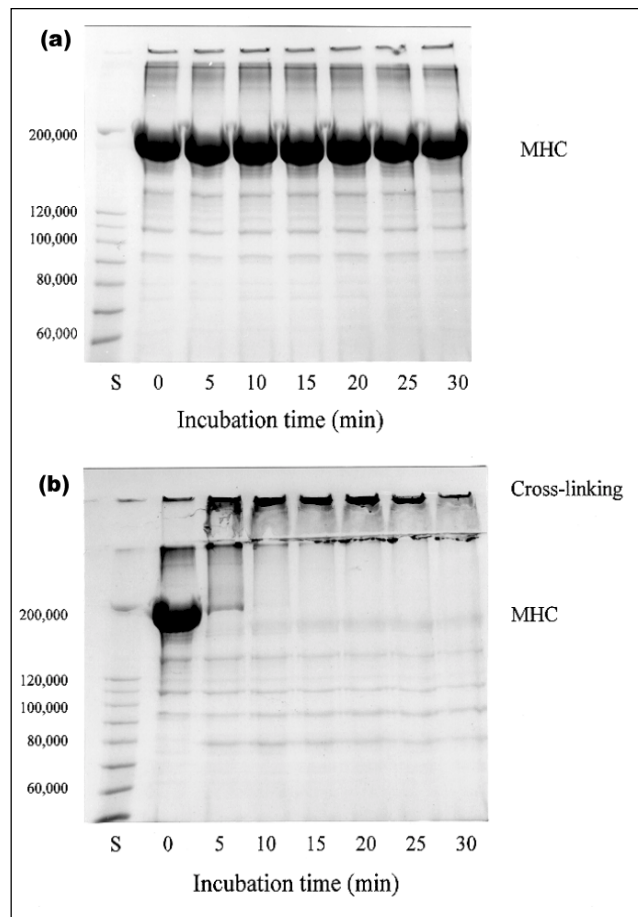
**Fig. 8—Changes in SDS-PAGE profiles of pollack AM with or without MTGase incubated at 30 °C for various time periods. A: without MTGase; B: with 0.2 unit MTGase/g surimi. (S: protein marker)**

Similar SDS-PAGE profiles were obtained from pollack AM with or without MTGase incubated at 30 °C or 45 °C (Figs. 8 and 9). Polymerization of MHC caused by MTGase was more obvious and much faster in pollack than in golden threadfin-bream surimi.

## Conclusions

**M**TGASE CATALYZED THE MHC CROSS-LINKING OF BOTH POLLACK and golden threadfin-bream surimi and increased the gel-forming ability of surimi. Results suggested that 0.3 and 0.2 unit/g MTGase were adequate to improve gel strength of frozen golden threadfin-bream and pollack surimi, respectively.

casein (from bovine milk) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).  $\beta$ -Mercaptoethanol ( $\beta$ -Me), dithiothreitol (DTT), Coomassie blue G-250, ammonium sulfate, glycerol, and trichloroacetic acid (TCA) were from Merck (Darmstadt, Germany). Beef extract, yeast extract, Bacto-agar and soluble starch were purchased from Difco (Detroit, Mich., U.S.A.). *Streptovorticillium ladakanum* ATCC 27441 was ob-



**Fig. 9**—Changes in SDS-PAGE profiles of pollack AM with or without MTGase incubated at 45 °C for various time periods. **A:** without MTGase; **B:** with 0.2 unit MTGase/g surimi. (S: protein marker)

tained from Taiwan Culture Collection and Research Center (Hsinchu, Taiwan). All other chemicals were reagent grade.

### Production of TGase

One loop of spore suspensions of *S. ladakanum* frozen stock culture was activated in a 125-mL Erlenmeyer flask containing 50 mL medium (1% glycerol, 1.5% yeast extract, 0.2%  $K_2HPO_4$ , 0.1%  $MgSO_4$ , pH 7) and cultured with shaking (150 rpm) at 28 °C for 48 h. One mL of culture suspension was then transferred to 150 mL of fresh medium (pH 7.0) and incubated with shaking at 28 °C. After 4-d incubation, the culture fluid was filtered initially through a Whatman No. 1 filter paper and subsequently a 0.45- $\mu$ m filter membrane. The filtrate was used as crude enzyme of MTGase and stored at -30 °C until analysis or further application.

### Determination of enzyme activity

MTGase activity was measured by the method described previously by Folk (1970). Reaction mixture contained 50  $\mu$ L enzyme, 350  $\mu$ L 0.1 M Tris-acetate buffer (pH 6.0), 25 mL 2.0 M hydroxylamine, and 75  $\mu$ L 0.1 M carbobenzyloxy-L-glutaminyl-glycine. The reaction mixture was incubated at 37 °C for 10 min and then stopped by adding equal volume (50  $\mu$ L) of 15% TCA solution containing 5%  $FeCl_3$ . After 15 min centrifugation

at 4000 g, the supernatant was collected, and the absorbance was measured at 525 nm. The calibration curve was drawn with L-glutamic acid- $\gamma$ -monohydroxamic acid as standard. One unit of MTGase activity was defined as the amount of enzyme that catalyzed the formation of 1  $\mu$ mole of hydroxamic acid within 1 min reaction at 37 °C.

### Preparation of surimi gels

Frozen surimi of golden threadfin-bream (*Nemipterus virgatus*, SA grade) and pollack (*Theragra chalcogramma*, A grade) were purchased from Kasei Co. (Keelung, Taiwan). Surimi (1 block; 10 kg) was thawed at 4 °C until the central temperature reached 0 °C and then was sliced and ground using a mechanical mortar and pestle (capacity: 20 kg/batch) for 5 min. A cooling jacket was equipped outside the mortar; the temperature of surimi could consequently be maintained below 2 °C during grinding. After adding 2.5% NaCl, grinding was resumed for another 20 min. Various amounts of MTGase (0, 0.1, 0.2, 0.3, 0.4, and 0.6 unit/g surimi) were added and ground for another 5 min. The amounts of salt, water, and MTGase added into the surimi were based on the initial weight of surimi. After grinding, the surimi sol was stuffed into polyvinylchloride tubes (1.5 cm dia) and set in a 30 °C water bath for 120 min. The resulting samples were then heated in a 90 °C water bath for 30 min to fix the protein gel (Jiang and others 1998). The optimal levels of MTGase for golden threadfin-bream and pollack surimi were employed for the following experiment. For investigating the effect of setting temperature on gel-forming ability, the MTGase-containing golden threadfin-bream (0.35 unit/g) and pollack (0.2 unit/g) surimi gels were prepared. The sols were set at either 30 °C or 45 °C for 120 min and heated at 90 °C for 30 min. The resulting gels were chilled in ice water for 30 min and kept at 4 °C overnight prior to gel-properties assessment.

### Assessment of gel properties

The strength of surimi gels was determined following the procedure designed by Seki and others (1990). Surimi gels were equilibrated to room temperature for 30 min and cut into 30-mm thick pieces for the puncture test. The breaking force and deformation of samples were measured at a compression speed of 60 mm/min using a Rheometer (Model CR-200D, Sun Scientific Co., Ltd., Japan) equipped with a 5-mm spherical plunger. The value of gel strength ( $g \times cm$ ) was the product of breaking force and deformation. For each treatment, 12 measurements were made.

### SDS-polyacrylamide gel electrophoresis analysis (SDS-PAGE)

Actomyosin (AM) was extracted from golden threadfin-bream and pollack surimi according to the method described by Noguchi and Matsumoto (1970). AM with or without MTGase was incubated at either 30 °C or 45 °C. After 2-h incubation, 0.1 mL sample was mixed with 0.4 mL buffer (2% SDS, 5%  $\beta$ -Me, 62.5 mM Tris-HCl, pH 6.8) and heated in a water bath (95 °C) for 3 min to terminate the reaction. The resulting samples were analyzed by SDS-PAGE according to Hames (1990) using 7.5% polyacrylamide. The gels were stained with Coomassie brilliant blue G-250 (Neuhoff and others 1988). Changes in electrophoretic profile were used to evaluate the cross-linking caused by MTGase.

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