Cross-Linking of Mackerel Surimi Actomyosin by Microbial Transglutaminase and Ultraviolet Irradiation†

Shann-Tzong Jiang,* Shou-Zen Leu, and Guo-Jane Tsai

Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan 202, Republic of China

The effect of combining microbial transglutaminase (MTGase) from *Streptoverticillium ladakanum* with ultraviolet (UV) irradiation on the gelation of minced mackerel was investigated. The gel strength of minced mackerel with MTGase alone at a concentration of 0.47 unit/g reached 1789 g·cm, which was 3 times greater than that of control. When MTGase-supplemented minced mackerel was exposed to UV light for the optimal irradiation time of 20 min, the gel strength could be further increased by 25%. SDS−PAGE analysis suggested that UV irradiation accelerated the MTGase to catalyze the cross-linking of myosin heavy chains in mackerel actomyosin.

Keywords: *Microbial transglutaminase; mackerel surimi; actomyosin; Streptoverticillium ladakanum; UV irradiation*

INTRODUCTION

Transglutaminase (TGase) can catalyze the formation of ϵ-(γ-glutamyl) lysyl cross-links, which is considered to be related to the gel strength of minced fish. Seki et al. (1990) isolated TGase from Alaska pollack and found it could induce the gelation of minced fish. Tsukamasa and Shimizu (1990) further reported that the strong gel-forming ability of sardine was due to the formation of the non-disulfide bond, which later was shown to be due to the action of TGase (Tsukamasa et al., 1993). Recently, the Ca^{2+}-independent microbial TGase (MTGase) from *Streptoverticillium mobarense* (Nonaka et al., 1989; Huang et al., 1992; Gerber et al., 1994) or from *Streptoverticillium ladakanum* (Tsai et al., 1995, 1996a,b) has shown potential to increase the gel strength of fish surimi.

Besides TGase's catalysis of covalent cross-linking, the gel strength of fish paste can also be improved by ultraviolet (UV) irradiation (Taguchi et al., 1988, 1989; Ishizaki et al., 1993a,b, 1994). Although the effect of UV irradiation on protein structures is unknown in detail, there is evidence that it can cause the oxidative fragmentation (Curran et al., 1984; Uchida et al., 1990, 1992; Kato et al., 1992) or polymerization of proteins (Fujimori, 1985, 1988; Kano et al., 1987; Ishizaki et al., 1994) and thereby change protein functionality. Taguchi et al. (1989) proved that UV irradiation could activate Mg-ATPase, which is responsible for a stronger binding of myosin to actin. Furthermore, Ishizaki et al. (1994) demonstrated that UV irradiation fragmented flying fish myosin and caused an increase in surface hydrophobicity and the polymerization of myosin heavy chains.

In this study we combine MTGase from *S. ladakanum* with UV irradiation in an attempt to improve the gel strength of mackerel surimi.

* Author to whom correspondence should be addressed (fax +886-2-2462-0684; e-mail sjiang@ind.ntou.edu.tw).

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MATERIALS AND METHODS

Materials. Tris(hydroxymethyl)aminomethane, carbobenzoxyl-L-glutaminylglycine (CBZ-L-Gln-Gly), sodium dodecyl sulfate (SDS), Tween 20, and L-glutamic acid-γ-monohydroxamic acid were purchased from Sigma (St. Louis, MO). β-Mercaptoethanol (β-Me), Coomassie blue G-250, ammonium sulfate, glycerol, and trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany). Blue Sepharose Fast Flow and CM Sepharose CL-6B were from Pharmacia Biotech (Uppsala, Sweden). Beef extract, yeast extract, agar, and soluble starch were ordered from Difco (Detroit, MI). *S. ladakanum* ATCC 27441 was obtained from the Taiwan Culture Collection and Research Center, Hsinchu, Taiwan. All other chemicals were of reagent grade.

Purification of MTGase. The protocol for the purification of MTGase from the culture filtrate of *S. ladakanum* followed that of Tsai et al. (1996a) with some modification. Instead of an ammonium sulfate fractionation step (Tsai et al., 1996a), the culture filtrate was directly loaded onto a CM Sepharose CL-6B column (2.6 × 40 cm, bed volume = 92 mL), which was previously equilibrated with 50 mM sodium acetate buffer, pH 5.5 (buffer I). The absorbed MTGase was then eluted with buffer I containing 0.4 M NaCl at a flow rate of 1 mL/min. Fractions with TGase activity were collected, dialyzed against 50 mM phosphate buffer, pH 7.0 (buffer II), and further applied onto a Blue Sepharose Fast Flow column. These procedures are described in greater detail in Tsai et al. (1996a).

Determination of TGase Activity and Protein Concentration. The TGase activity was measured by using the colorimetric hydroxamate method (Folk, 1970). One unit of enzyme activity was defined as that which caused the formation of 1 mmol of hydroxamic acid/min. A microprotein assay kit (Bio-Rad) was used to measure the protein concentration. Methods have been described in detail elsewhere (Tsai et al., 1996a).

Effects of MTGase and UV Irradiation on Cross-Linking of Mackerel Actomyosin. Actomyosin (AM) was extracted from dorsal muscle of mackerel according to the method described by Noguchi and Matsumoto (1970). The AM solution (8.5 mg/mL) was prepared with 50 mM Tris-HCl buffer (pH 7.5). A plate with a cooling system was filled with AM solution to a depth of 0.5 cm and irradiated at 10 °C under a photochemical mercury lamp (model UVSL 58, UVP Co., Ltd.) at a wavelength of 365 nm and an intensity of 850 μW/cm^2. To avoid any localized denaturation that might have resulted from shearing force caused by stirring, the AM

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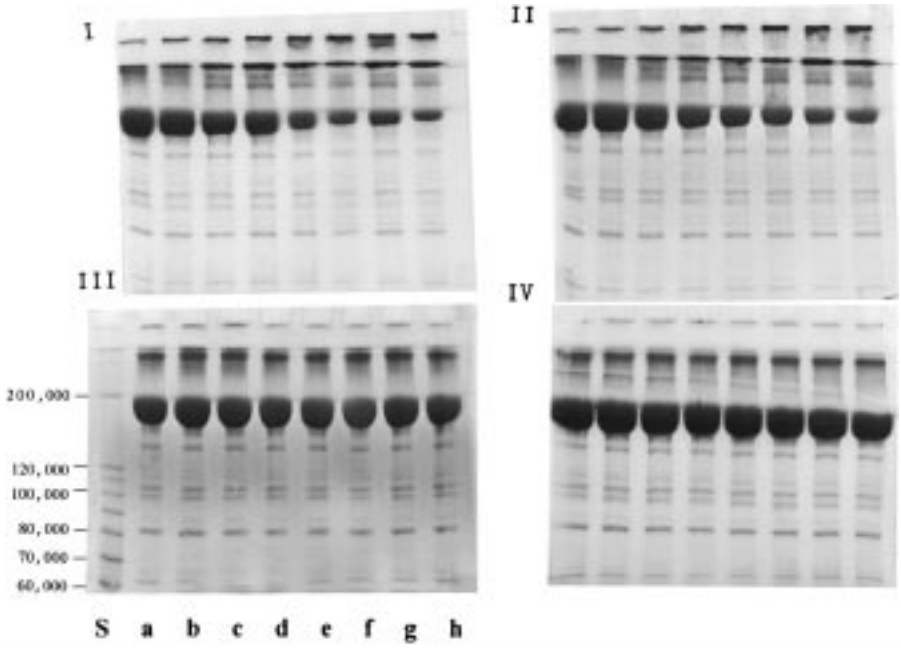

Figure 1. SDS−PAGE profiles of mackerel AM, with/without MTGase (0.1 unit/g) and/or UV irradiation (850 μW/cm^2 at 10 °C for 10 min) followed by incubation at 30 °C for various time periods: (I) MTGase and UV; (II) MTGase only; (III) UV only; (IV) control. Lanes: S, protein markers; a, 0 min; b, 5 min; c, 10 min; d, 15 min; e, 20 min; f, 25 min; g, 30 min; h, 35 min.

solution was gently swirled during UV irradiation. Trials of both the MTGase-supplemented (0.1 unit/g) AM with/without UV irradiation and the AM with/without the addition of MTGase in combination with UV irradiation were performed. The cross-linking of AM was analyzed by SDS−PAGE.

Effect of MTGase on Gelation of Minced Mackerel. Live mackerel (*Scomer japonicus*) was purchased from a fish market in northern Taiwan. Surimi was prepared as described previously by Jiang and Lee (1992). It was packaged in polyethylene bags at 1 kg each and stored at −30 °C until use.

Frozen surimi was thawed in a 5 °C refrigerator until the central temperature reached 0 °C, then sliced, and ground for 10 min. After the addition of 2.5% NaCl, grinding was resumed for another 20 min at 5 °C. Finally, 5% potato starch and various amounts of purified MTGase (0−0.52 unit/g of meat) were mixed uniformly for 5 min at 5 °C. The resulting samples were stuffed into poly(vinyl chloride) tubes (diameter = 1.5 cm), sealed, and then allowed to set at 30 °C for 60 min. Samples were finally heated at 90 °C for 30 min to fix the protein gel. After storage at 4 °C for 12 h, the gel strength was measured using a rheometer (model CR-200D, Sun Scientific Co., Ltd.; plunger, 5 mm; speed, 60 mm/min). The gel strength was expressed as the product of breaking load and breaking strain (g·cm).

Effects of MTGase and UV Irradiation on Gelation of Minced Mackerel. *(1) Effect of Varying UV Irradiation Time.* The MTGase-supplemented (0.47 unit/g) mackerel mince was spread to a depth of 0.5 cm on a plate with a cooling system and irradiated at 10 °C and an intensity of 1100 μW/cm^2 for various time periods (0−40 min). After irradiation, the minces were then collected and stuffed into poly(vinyl chloride) tubes. The resulting samples were set at 30 °C for 60 min and followed by heating and gel strength measurement as described above.

(2) Effect of Varying Setting Time. The same protocol as described above in (1) was used, except that the mackerel paste was irradiated for a fixed time of 20 min and various setting time periods of 0, 20, 40, 60, and 120 min were investigated.

SDS−PAGE. For the SDS−PAGE analysis, 10 mL of the AM sol was pretreated with 10 mL of sample buffer (0.5 M Tris-HCl, pH 6.8, 10% SDS, 5% β-Me, and 20% glycerol). After 3 min of boiling, 10 mL of 0.1% bromophenol blue was added. For minced mackerel, 0.1 g of mince was added to 1 mL of 20 mM Tris-HCl buffer (pH 8.0) containing 2% SDS, 8 M urea, and 2% β-Me and then boiled for 3 min. Ten milliliters of 0.1% bromophenol blue was then added to the mixture. After pretreatment, 10 mL of the dissociated samples was subjected to SDS−PAGE analysis using a slab gel (stacking gel 3.75% and resolving gel 7.5%) (Hames, 1990). The gel was then stained with Coomassie brilliant blue G-250 (Neuhoff et al., 1988).

RESULTS AND DISCUSSION

Effects of MTGase and UV Irradiation on Cross-Linking of Mackerel Actomyosin. The effects of MTGase and UV irradiation on cross-linking of mackerel AM are demonstrated in Figure 1. The intensity of the myosin heavy chains (MHC) noticeably decreased when both MTGase and UV irradiation were used in combination, compared with those with MTGase alone during 15−35 min of incubation at 30 °C (Figure 1-I,-II). Decreases were apparently observed after 10 min, and intensity continued to fall through 30 min of incubation (Figure 1-I). MTGase alone also caused a noticeable decrease (Figure 1-II), whereas there was very little change under UV irradiation alone or in the control (Figure 1-III,IV). In another similar trial, in which mackerel AM was supplemented with MTGase and then irradiated for various times, after 40 min, the MHC was barely visible (Figure 2-II), whereas the MHC band of the sample with MTGase alone was still clear even after 40 min of irradiation (Figure 2-I). The disappearance of the MHC band is taken as evidence of MHC cross-linking, because it would be more difficult for cross-linked MHC to dissociate in sample buffer during SDS−PAGE analysis. The UV irradiation made the polymerization of MHC ∼2-fold faster than that of sample with MTGase alone (Figure 2).

McLaren and Hidalgo-Salvatierra (1964) showed that cysteine, peptide bonds, and the aromatic amino acids present in protein molecules are very sensitive to UV irradiation and hypothesized that UV light could therefore cause conformational change in muscle proteins. Ishizaki et al. (1993b) found that the surface hydrophobicity of AM from coalleye pollack, sardine, flying fish,

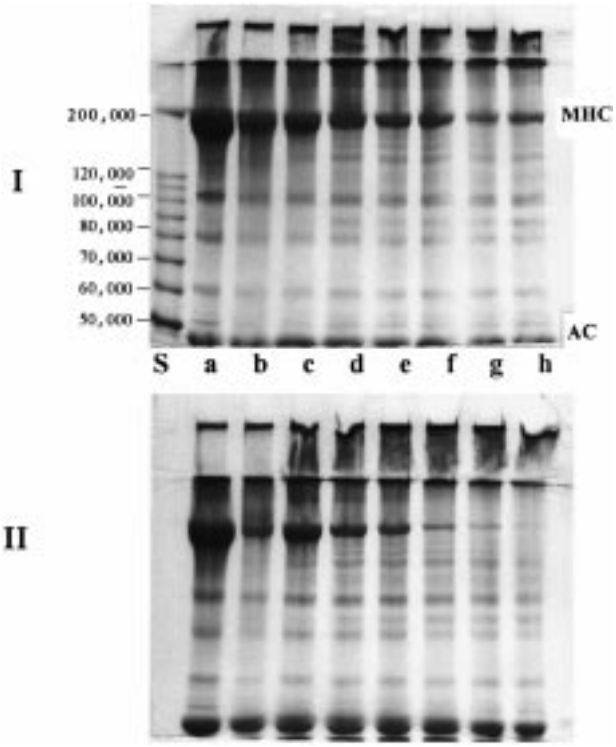

Figure 2. Changes in SDS−PAGE profiles of MTGase-supplemented (0.1 unit/g) mackerel AM with UV irradiation (wavelength, 365 nm; intensity, 850 μW/cm^2) at 10 °C for various time periods: (I) without UV irradiation; (II) with UV irradiation. Lanes: S, protein marker; a, 0 min; b, 5 min; c, 10 min; d, 15 min; e, 20 min; f, 25 min; g, 30 min; h, 40 min.

and pork was significantly increased, while the surface SH content ultimately decreased after an initial increment during UV irradiation. On the basis of these results and data from differential scanning calorimetry analyses, these authors suggested that UV irradiation prompted the unfolding of AM. Ishizaki et al. (1994) further pointed out that this AM unfolding could cause the formation of myosin polymers; evidence that this polymerization does in fact occur was provided by the reduced intensity of the MHC band on SDS−PAGE profiles.

Although TGase is known to catalyze the cross-linking between glutamine and lysine residues in proteins, the glutamine and lysine residues must be located in a flexible region (Berbert et al., 1983) or in regions that are predicted to be reverse turns (Wold, 1985). The compact globular structures of the 11S and 7S soybean proteins, for instance, make them rather poor substrates for TGase, despite their relatively high glutamine contents (Larre et al., 1992). Chemical modifications, however, such as succinylation (Lorand et al., 1971) or citraconylation (Larre et al., 1992) have been shown to open up globular proteins to TGase. Likewise, whereas the covalent cross-linking of rabbit AM by MTGase from *Streptoverticillium* S-8112 (Huang et al., 1992) and that of mackerel AM by MTGase from *S. ladakanum* (Tsai et al., 1995) have already been demonstrated, the MTGase activity should be further increased in the presence of UV light, which exposes glutaminyl groups to MTGase. This accounts for the more rapid disappearance of the MHC band on the SDS−PAGE profiles in the presence of both MTGase and UV light than when either treatment is used alone (see Figures 1 and 2).

Effect of MTGase and UV Irradiation on Gelation of Minced Mackerel. In an earlier paper (Tsai et al., 1996a), the highest gel strength of minced mackerel was obtained when 0.34 unit/g MTGase was added, whereas the gel strength became markedly decreased if the amount of MTGase was further increased to 0.99 unit/g. In the present study we repeated this experiment with smaller intervals to find more precisely the optimal amount of MTGase for application in minced mackerel. As shown in Figure 3, the gel strength increased with increasing amounts of MTGase until it reached a maximum (~3 times greater than the control) at an MTGase concentration of 0.47 unit/g of meat. Further increases in MTGase concentration decrease the gel strength. This optimal MTGase concentration of 0.47 unit/g was used in the following parts of this study.

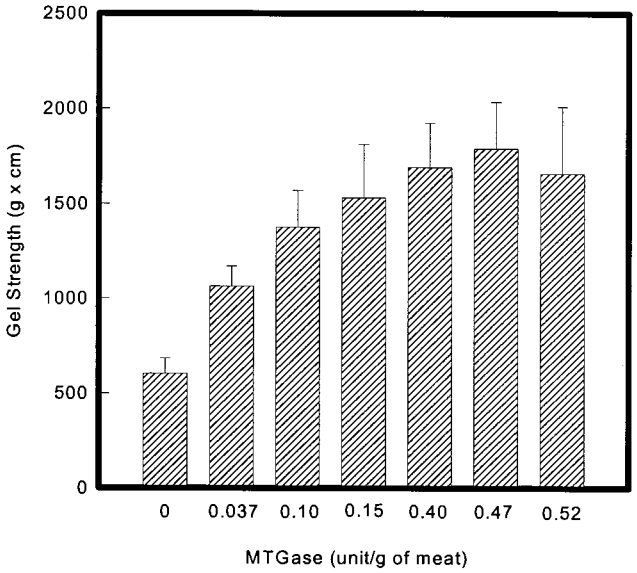

Figure 3. Gel strength of minced mackerel gels supplemented with MTGase (0−0.52 unit/g of meat) after a 30 min setting at 30 °C.

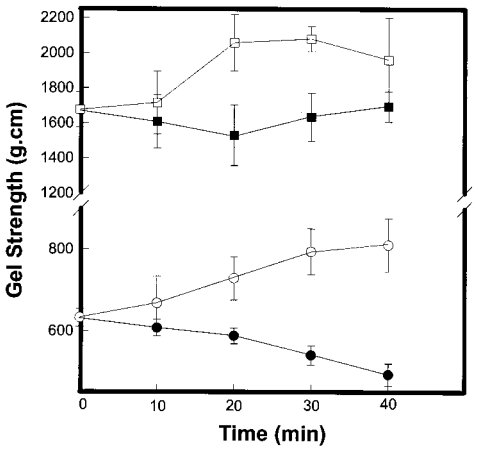

Figure 4. Effect of UV irradiation time at 10 °C (1100 μW/cm^2) on the gel strength of minced mackerel with/without addition of MTGase (0.47 unit/g): □, both MTGase and UV irradiation; ■, MTGase only; ○, UV irradiation only; ●, control.

Although UV irradiation alone could increase the gel strength of minced mackerel, this increase was only about half as great as in samples to which MTGase had been added (Figure 4). After 20 min of UV irradiation of MTGase-supplemented samples, the gel strength was increased by 25%, compared to MTGase alone. Taguchi et al. (1989) also found that the gel strength of the meat pastes of sardine, beef, and pork was increased with UV

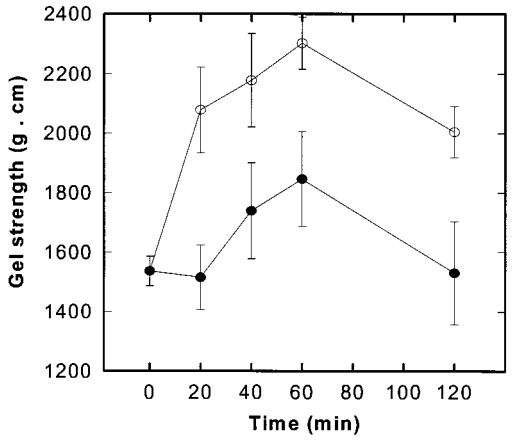

Figure 5. Effect of incubation time on the gel strength of MTGase-supplemented minced mackerel with/without 20 min of UV irradiation: ○, UV irradiation; ●, control.

irradiation time. Ishizaki et al. (1993a) demonstrated that UV-irradiated thermal gel had a denser structure than the control gel, and they also showed that this was due to the hydrophobic coagulation between the myosin molecules that had been unfolded by UV irradiation (Ishizaki et al., 1994). However, in the present study, we found that although gel strength continued to increase with increasing irradiation time in samples which had not been treated with MTGase, it slightly decreased or almost reached a constant level when the UV irradiation was >20 min in the presence of MTGase (Figure 4). It therefore seems that although UV irradiation increases the MTGase activity by increasing substrate availability, prolonged UV irradiation might in fact denature MTGase itself. This might be because the active site of MTGase contains a cysteine residue (Tsai et al., 1996a), which is sensitive to UV light. Another possible reason is that cross-linking of surimi proteins may occur during prolonged irradiation; however, the resulting protein network may be destroyed mechanically during stuffing into the tube, which consequently decreases the gel strength.

During setting, the inter-/intramolecular linkages of muscle proteins are gradually constructed, and accordingly the gel strength is increased. However, at the same time the muscle proteins are also gradually hydrolyzed by the endogenous proteases. Therefore, the optimal setting time needs to be determined for each individual meat paste. As shown in Figure 5, the highest gel strength of MTGase-supplemented minced mackerel was obtained after setting at 30 °C for 60 min, regardless of whether it had been treated with UV irradiation or not. Extending the setting time to 120 min decreased the gel strength.

In conclusion, although MTGase alone causes the cross-linking of MHC of mackerel AM, UV irradiation enhances this polymerization of MHC by MTGase. Accordingly, the gel strength of minced mackerel increased significantly when MTGase and UV irradiation were used in combination. Optimal values for MTGase and UV irradiation time were 0.47 unit/g and 20 min, respectively.

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