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Effect of transglutaminase-induced cross-linking on gelation of myofibrillar/soy protein mixtures☆

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Abstract

Microbial transglutaminase (MTGase)-catalyzed interaction and gelation of mixed myofibrillar (MPI)/soy (SPI) protein isolates were investigated at varying ionic strengths and MPI:SPI ratios, with or without SPI being preheated (80 °C). MTGase treatments in deionized water converted myosin heavy chain and actin into lower molecular-weight polypeptides, which gradually diminished as the ionic strength increased up to 0.6 M NaCl. A reduced intensity in the electrophoretic bands of soy proteins (7S and 11S except the basic subunits) was observed in all treatments, suggesting cross-linking with MPI. The enzyme treatment slightly increased the thermal transition (denaturation) temperatures of MPI/SPI but greatly enhanced ($P < 0.05$) the elasticity of the mixed protein gels when compared with untreated samples, independent of incubation time.

Keywords: Transglutaminase; Myofibrillar proteins; Soy proteins; Gelation

1. Introduction

Consumer acceptance of processed meats is determined by the product quality, particularly flavor, texture, and storage stability. One of the common practices in assuring high quality in processed meats is incorporation of protein ingredients in the product formulation. The main purpose is to improve the physical characteristics (especially texture-related properties), flavor, and cooking yield, as well as to reduce the production cost (Xiong, 2000). The increased consumer demand for low-fat products in recent years has drawn further attention to utilizing non-protein ingredients as potential substances to restore the earlier-mentioned product characteristics as fat is removed and water is added (Desmond, Troy, & Buckley, 1998; Keeton, 1996; Mansour & Khalil, 1999).

A variety of isolated proteins have been used as functional ingredients in ground or emulsified muscle foods, of which soy proteins are probably the most widely employed (Pietrasik & Li-Chan, 2002). Soy proteins, in the form of isolates or concentrates, are utilized in processed meats owing to the specific functionalities they are able to impart, for example, as binders to improve the product's yield and texture, as possible gelling agents to enhance the emulsion stability upon heating (Renkema & van Vliet, 2002), and as a meat replacement to reduce the formulation costs (Chin, Keeton, Longnecker, & Lamkey, 1999).

In comminuted muscle foods that are of a continuous, cross-linked structure, functional properties of soy protein additives will depend, to a large extent, on their interaction with muscle proteins. Yet, soy proteins, including the two major globular fractions, glycinin and β-conglycinin, are remarkably resistant to denaturation (Feng & Xiong, 2002; Petruccelli & Añon, 1995). None of the major soy globulins exhibits appreciable structural changes and hence, interaction with muscle proteins, under the normal meat processing conditions (temperature 65–73 °C, pH 5.5–6.0, and ionic strength 0.1–0.6). The lack of interaction is considered one of the major factors that minimize the role of soy proteins as a functional component intended for an improved cohesiveness and physical stability of comminuted and emulsified meats (Feng & Xiong, 2002; McCord, Smyth, & O'Neill, 1998).

Transglutaminase (E.C. 2.3.2.13), an enzyme that promotes polymerization of proteins through intermolecular

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ε-(γ-glutamyl)lysine cross-links, has attracted much attention and long been considered as a potential agent to enhance the interactions and functionality of food proteins (Folk, 1980; Kurt & Rogers, 1984; Motoki & Nio, 1983; Tanimoto & Kinsella, 1988). Yet, it was not until recently that the enzyme was successfully applied in food processing, for example, in the manufacture of restructured meat (Kuraishi, Sakamoto, Yamazaki, Susa, Kuhara, & Soeda, 1997), edible films (Yildirim & Hettiarachchy, 1998), and emulsion products (Liu, 1998).

The purpose of the present study was to examine the effectiveness of a microbial transglutaminase (MTGase) as a catalyst for the interaction of muscle and soy proteins under several processing conditions. Our hypothesis was that MTGase would induce polymerization and therefore, enhance the rheological and gelation properties, of mixed muscle/soy proteins, and that the catalysis would be affected by different meat processing conditions.

2. Materials and methods

2.1. Samples

Myofibrillar protein isolate (MPI) was prepared from trimmed breast meat (36–48 h postmortem, pH 5.9–6.2) of postrigor broiler chickens (three per batch) by washing three times with 4 vol. (v/w) of 0.1 M NaCl, 50 mM Na_2HPO_4 buffer (pH 7.0), followed by washing with 8 vol. (v/w) of 0.1 M NaCl (pH 6.0) (Xiong, 1993). All isolations were conducted at 5 °C. Three batches of the MPI samples were prepared on different days from different chickens to allow for experiment replications. Protein concentration of the final pellet (MPI, ~7% protein) was determined by the Biuret method using bovine serum albumin as standard (Gornall, Bardawill, & David, 1949). Soy protein isolate (SPI) was prepared from low-heat defatted soy flakes with alkaline extraction (pH 8.0) followed by acidic precipitation (pH 4.5) according to Puppo, Lupano and Añon (1995). The final protein suspension was adjusted to pH 7.0, freeze-dried, and then pulverized (mortar-pestle) yielding a protein content of 96.4%. MTGase used in all treatments was Activa TG-TI enzyme preparation (99% maltodextrin and 1% MTGase) donated by Ajinomoto (Ajinomoto USA, Inc., Teaneck, N.J.). All chemicals used were at least reagent grade.

2.2. Protein–protein interactions

Three experiments were conducted with 2–3 replications to examine the effects of processing conditions on MTGase (0.1 unit/ml)-catalyzed MPI/SPI cross-linking (1:1 ratio, 0.2% protein concentration each unless indicated otherwise): (1) ionic strengths (0, 0.15, 0.3, 0.45, and 0.6 M NaCl in 50 mM Na_2HPO_4, pH 6.5); (2) MPI:SPI ratio (3:1, 1:1, and 1:3, pH 6.5); and (3) preheating of SPI (80 °C for 5, 15, 30, and 60 min). Two groups of control samples, i.e. SPI-alone and mixed MPI/SPI (no MTGase), were also prepared at the above conditions. All treatments were incubated with 0.1% (w/v) of the MTGase preparation at 5 °C and analyzed after 0, 2, 5, 15, 30, 60, 120, and 240 min. Protein changes with possible cross-linking reactions in all control and enzyme-treated samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

SDS–PAGE was performed accordingly to Laemmli (1970) with some modifications (Ramírez-Suárez, Xiong, & Wang, 2001). A 5–18% acrylamide gradient separating gel (width × height × thickness = 80 × 60 × 1.5 mm), with a 3% acrylamide stacking gel, was prepared. Treated protein samples (0.2% protein concentration) were mixed (at a 1:1 v/v ratio) with SDS–PAGE sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.125 M Tris, pH 6.8) and dissolved by heating in boiling water for 3 min. Aliquots of 30 μg of protein per lane were loaded onto the acrylamide gel.

The major SPI protein bands were tentatively identified by comparing their relative mobility, molecular weights, and staining intensity to those of the same proteins published in the literature (Yagasaki, Takagi, Sakai & Kitamura, 1997; Yaklich, 2001). Molecular weights (MW) of unknown proteins were estimated from the curve generated by plotting the log[MW] vs. migration distance of the protein bands.

2.3. Differential scanning calorimetry (DSC)

Changes in thermal stability of MPI/SPI mixtures (ratio 1:1, total protein concentration 4.5%, pH 6.5) as a result of 0.5% MTGase (preparation) treatments were measured using a model 2920 Modulated DSC machine (TA Instruments, Inc., New Castle, Del.) according to Srinivasan, Xiong, and Blanchard (1997). To demonstrate the changes, samples incubated at 5 °C for 0.5 and 4 h were analyzed. Protein mixtures were accurately weighed (ca. 15 mg) into aluminum capsules and hermetically sealed. An empty capsule was used as reference. Samples were heated from 20 to 130 °C at a constant rate of 10 °C/min. Temperature at maximum heat flow (T_{max}), i.e. temperature at peak of the endotherm, was measured using the Universal Analysis Ver 1.2 N software (TA Instruments, New Castle, Del.) as described in the DSC User's Manual. Analysis was carried out in triplicate for all samples.

2.4. Gelation

MPI and SPI alone (2.25% protein) and their mixture (1:1 ratio, 4.5% total protein) were suspended in distilled

deionized water. After adjusting the pH to 6.5, protein solutions were incubated with 0.5% MTGase preparation for 0, 0.5, and 4 h at 5 °C. The MTGase-treated samples were subjected to dynamic rheological testing using a model VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ) equipped with two parallel plates of 1 mm apart (Xiong, 1993). Samples were loaded in the space between the parallel plates immediately after incubation, and the exposed rim was covered with a thin layer of mineral oil to prevent dehydration. A 3-min incubation time was given before the start of the measurement to allow for temperature equilibrium. Gels were formed by heating the protein mixtures from 20 to 82 °C at a rate of 1 °C/min. The gelling samples were continually sheared in an oscillatory mode at a fixed frequency of 0.1 Hz with a maximum strain of 0.02. Changes in the storage modulus (G', i.e. rigidity due to elastic response of the material) were monitored throughout the gelling process.

To elucidate the dynamic, progressive protein changes in the MPI/SPI mixtures during the heat-induced gel network formation, a separate electrophoresis experiment was carried out. MPI/SPI mixtures (1:1 ratio, 4.5% total protein, pH 6.5) and the individual proteins (2.25% protein, pH 6.5) treated and incubated with 0.5% MTGase preparation (0, 0.5 and 4 h), all in distilled deionized water, were heated from 25 to 90 °C at a rate of 1 °C/min. Samples were taken at 25, 30, 40, 50, 60, 70, 80 and 90 °C; and aliquots of 2 ml (pre-gel) or 2 g (gel) were blended with 18 ml of dissolving solution (5% SDS, 0.1% β-mercaptoethanol) using a Polytron homogenizer (Brinkman Instruments Inc., Westbury, N.Y.). The homogenates were incubated at 80 °C for 1 h to allow for maximal protein solubilization and extraction, and subsequently centrifuged at 3000×g for 15 min. The supernatants, after measuring protein concentration by the Biuret method, were diluted to a 0.2% protein concentration with water and then mixed (at a 1:1 ratio) with SDS–PAGE sample buffer before electrophoresis as described above. Aliquots of 30 μl of protein per lane were loaded onto the gel.

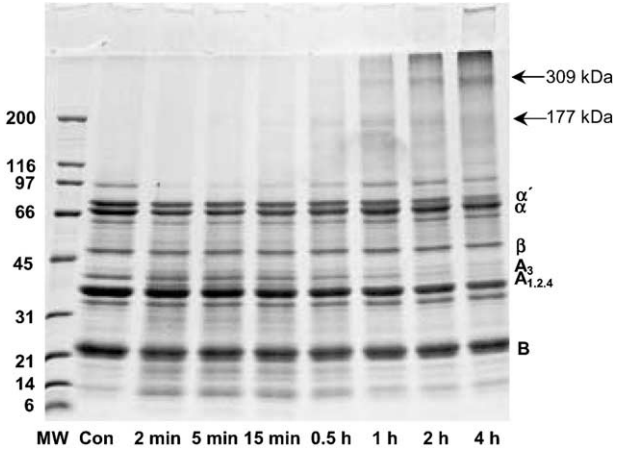

Fig. 1. Electrophoretic patterns of soy protein isolate (SPI, 0.2%) incubated at 5 °C with 0.1% transglutaminase preparation for various times in distilled deionized water. Lane MW = molecular weight standard (in kDa); and lane Con = control without MTGase. α′, α, β = subunits of β-conglycinin; $A_{1,2,3,4}$ and B = acidic and basic subunits of glycinin.

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) was performed on quantitative DSC (T_{max}) and gelation (G') parameters to test significance between treatments. The T_{max} and G' (60–82 °C) values from different replicate measurements were entered into the analysis using the SAS program (SAS, 1995) with a general linear model procedure. When significant main effects were found, their means were separated by the LSD method.

3. Results and discussion

3.1. SDS–PAGE of enzyme-treated protein solutions

3.1.1. Effect of ionic strength

The MTGase treatment of SPI alone in distilled deionized water ('zero' ionic strength) produced the following detectable electrophoretic changes: (1) an occurrence of two high-molecular-weight (HMW) bands at ~310 and ~180 kDa, along with a gradually intensified smear around the region, (2) an initial reduction, followed by a reappearance, of the 97 kDa protein band, and (3) a progressive disappearance of the A_3 component of glycinin and, to a lesser extent, of the α′ subunit of β-conglycinin (Fig. 1). The intensity of the ~310 kDa band increased with incubation time; and by 4 h an appreciable amount of cross-linked products was produced that did not enter the gel. These HMW polypeptides were derived ostensibly from the acidic (A_3) as well as the α′ subunits. Although the formation of cross-links by MTGase for soy proteins has been reported by Mizuno, Mitsuiki, and Motoki (2000) and by Nonaka, Toiguchi, Sakamoto, Kawajiri, Soeda, and Motoki (1994), not much effect was observed under the conditions used in the present experiment, i.e. a low temperature (5 °C, suitable for meat).

Incubation of mixed MPI/SPI with MTGase in distilled deionized water (0 M NaCl) resulted in an almost instant (in 2 min) reduction of myosin heavy chain (MHC, 200 kDa) and disappearance of actin (45 kDa; Fig. 2). The reaction was accompanied by a simultaneous occurrence of a major band immediately below MHC, along with numerous polypeptides (smears) beneath the respective bands, and of an emergence of a distinctive new protein band at about ~31 kDa. When MPI alone was incubated with MTGase, a similar result showing changes in myofibrillar proteins was observed

except that the actin band exhibited a marked attenuation but not a total disappearance and that the ~31 kDa polypeptide was salient from the beginning of incubation (Ramírez-Suárez & Xiong, 2002). The formation of the giant polymers (at the top of the gel) was visible only towards the end of the incubation (Fig. 2). Furthermore, the mixed MPI/SPI gel showed only a faint ~310 kDa polymer and was devoid of the smear above 200 kDa seen in the SPI-alone gel (Fig. 1), probably due to cross-linking with muscle proteins. In the mixed protein system, the disappearance of the A_3 component also seemed to be instantaneous. The discrepant electrophoretic patterns of samples with or without SPI suggested the occurrence of muscle–soy protein interactions.

A previous study with a peptide mapping showed that the MTGase-induced band immediately below 200 kDa originated from MHC, while the smear underneath the intact actin band (45 kDa) was derived from actin (Ramírez-Suárez & Xiong, 2002). Since MTGase is known to catalyze both intra and intermolecular associations (Ohtsuka, Seguro, & Motoki, 1996), it is highly possible that formation of intramolecular ε-(γ-glutamyl)lysine isopeptide bonds led to an increased structural compactness in myosin and actin, and therefore, a faster electrophoretic mobility. This type of intramolecular cross-linking has been observed between Gln-41 and Lys-50 in intact actin (Eli-Berchoer, Hegyi, Patthy, Reisler, & Muhlrad 2000). A similar electrophoretic behavior has also been reported by Matsumura, Lee, and Mori (2000) on MTGase-treated whey proteins.

The protein changes in the salt-free solution reached a maximum in about 5–15 min. Thereafter, most were gradually reversed; after 2 h, essentially all the MPI components, but not those of SPI, were recovered (Fig. 2). The result suggested that the enzyme-catalyzed 'intramolecular association' in the MPI/SPI mixture was reversible, although it seemed to follow a different kinetics than MPI alone (Ramírez-Suárez & Xiong, 2002), i.e. the presence of soy proteins accelerated the reaction. The apparent reversibility by transglutaminase was previously noted by Folk (1969), and Fink, Chung, and Folk (1980). Ramírez-Suárez et al. (2001) suggested that the reversibility phenomenon probably stemmed from certain proteolytic/plastein (reversible cross-linking) activity which could be present in the MTGase preparation. As indicated by Makarova, Aravind, and Koonin (1999), transglutaminases have evolved from ancient proteases; their 'hidden proteolytic activity' (under the condition of the present study) would probably be counteracted or greatly diminished by its opposing effect, namely, cross-linking activity that prevails upon the application of heat. To test this hypothesis, further research is required.

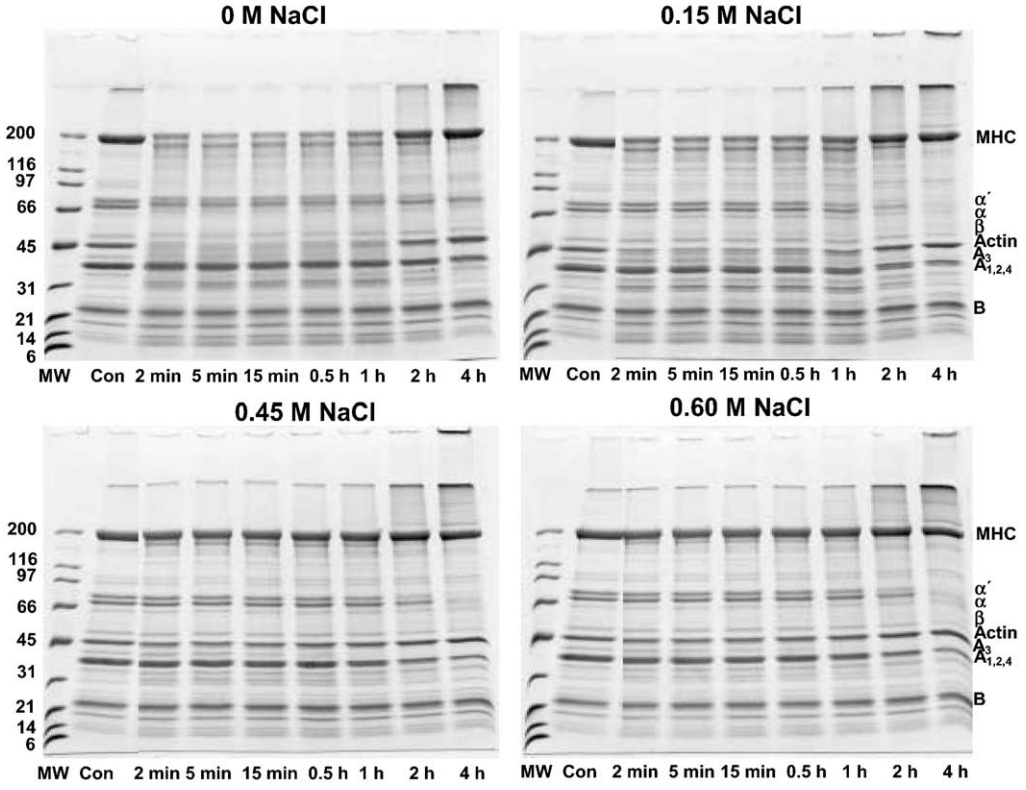

Fig. 2. Effect of ionic strength (NaCl concentration) on cross-linking of mixed MPI/SPI (1:1 ratio, 0.2% total protein) after incubation at 5 °C with 0.1% transglutaminase preparation for various times. Lane MW = molecular weight standard (in kDa); and lane Con = control without MTGase. MHC = myosin heavy chain; α′, α, β = subunits of β-conglycinin; $A_{1,2,3,4}$ and B = acidic and basic subunits of glycinin.

The addition of NaCl gradually diminished the protein changes: the smearing became less noticeable, and the occurrence of neo-polypeptides below the main muscle proteins became less pronounced (Fig. 2). At 0.6 M NaCl, minimum intramolecular MPI interactions were observed although more cross-linked protein polymers (>200 kDa) were produced, suggesting that small conformational changes due to ionic strength probably occurred either to MTGase or to the MPI protein substrates which altered the enzyme reaction. In contrast, changes in SPI were more remarkable in the presence of salt, since band intensity of β-conglycinin (α′, α) and the acidic subunits ($A_{1,2,3,4}$) of glycinin in the MPI/SPI mixture decreased appreciably at the end of MTGase incubation. On the other hand, the basic subunits (B) of glycinin remained resistant to the enzyme probably because these polypeptides were present in the interior of the glycinin (11S) molecule as suggested by Lakemond, de Jongh, Hessing, Gruppen, and Voragen (2000a, 2000b). The authors found that at ionic strengths of 0.03–0.5, the acidic subunits were predominantly facing the outside of the glycinin complex (model proposed by Marcone, Kakuda, & Yada, 1998), and therefore, they were more accessible to the enzyme.

High concentrations of salt have been shown to modify soy globulin complex (Lakemond et al., 2000b), and this appeared to increase the susceptibility of the reactive side chain groups (Lys-NH_2 and Gln-$CONH_2$) to MTGase attack. Because none of the SPI subunits produced a 'compact' structure ('intramolecularly' associated, with a reduced MW), disappearances of the individual SPI components can be ascribed to cross-linking into mega-polymers unable to enter the gel or aggregates insoluble in the SDS–PAGE buffer. The lack of corresponding, appreciable changes in MPI seems to indicate that there were minimal MPI/SPI interactions at high-ionic-strength conditions.

3.1.2. Effect of MPI:SPI ratio

Electrophoretic analysis of the MPI/SPI mixtures (in distilled deionized water) at varying ratios of the constituting proteins essentially yielded the same result as presented in Fig. 2 (0 M NaCl), i.e. myosin and actin exhibited major changes and α′ subunit of α-conglycinin and acidic subunits of glycinin were cross-linked only at the end of treatment (data not shown). Hence, MTGase catalysis of MPI/SPI cross-linking or intramolecular association was independent of the preponderance of one group of protein over the other.

3.1.3. Effect of preheating SPI

Heat pretreatment of SPI was conducted to test the hypothesis that a brief exposure to denaturation temperatures, which unfolds the protein structure and hence, increases the accessibility of the reactive side chain groups, would render the protein substrate more susceptible to MTGase. Heating of SPI before MTGase treatment for 5–15 min (Fig. 3b) delayed the reoccurrence of MHC and actin (from 2 to 4 h) although it did not change the overall electrophoretic pattern when compared with native SPI (Fig. 3a). This suggests that the presence of partially denatured soy proteins caused the already modified myosin less susceptible to MTGase during the reversing protein cross-linking reaction. Interestingly, longer heating (30–60 min, Fig. 3c) did not have much effect on the enzyme catalysis, when compared to the control (Fig. 3a). This may be explained because a more extensive protein denaturation resulted in an aggregation and precipitation of soy proteins (Feng & Xiong, 2002), thereby diminishing their participation in the MTGase-mediated myosin cross-linking reaction.

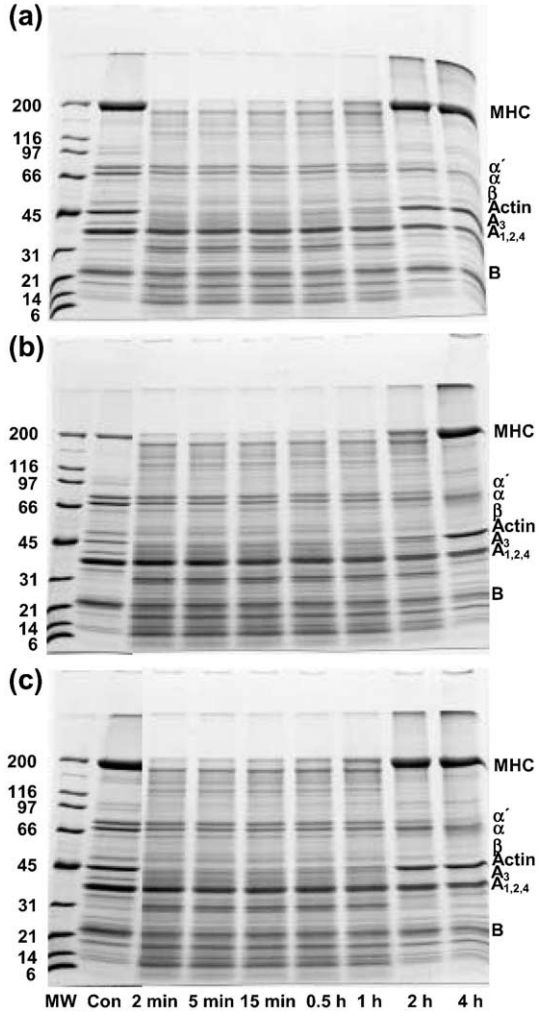

Fig. 3. Electrophoretic patterns of the mixtures (1:1 ratio, 0.2% total protein) of MPI with native SPI (a), or with SPI preheated at 80 °C for 15 min (b) or 30 min (c). The mixtures were incubated at 5 °C with 0.1% transglutaminase preparation for various times. Lane MW = molecular weight standard (in kDa); and lane Con = control without MTGase. MHC = myosin heavy chain; α′, α, β = subunits of β-conglycinin; $A_{1,2,3,4}$ and B = acidic and basic subunits of glycinin.

3.2. Differential scanning calorimetry

In order to distinguish the effect of different stages of the MTGase reactions, the control (0 time), 0.5 h incubation (showing maximum protein changes), and 4 h incubation (showing a complete reversion of the reaction) of mixed MPI/SPI samples in distilled deionized water (from Fig. 2, 0 M NaCl) were selected for DSC analysis. The samples displayed similar thermal curves, all exhibiting three endothermic transitions (shown by arrows) corresponding to the three major proteins in the mixture, i.e., myosin (T_{max} = 60–62 °C) (Smyth, Smith & O'Neill, 1998; Smyth, Smith, Vega-Warner, & O'Neill, 1996; Vittayanont, Vega-Warner, Steffe, & Smith, 2001) from muscle proteins, and β-conglycinin (75–78 °C) and glycinin (90–94 °C) from soybean proteins (Scilingo & Añon, 1996) (Fig. 4). The first endotherm (60–62 °C) was unaffected ($P > 0.05$) by the enzyme treatment; however, the second and third endotherms increased ($P < 0.05$) by 1–2 and 2–3 °C, respectively, after the enzyme treatment, irrespective of incubation time. The shifts in the transition temperatures of the MPI/SPI mixture presumably resulted from MTGase-induced conformational changes in the major proteins and the formation of more stable heterogeneous polymers due to modification of intra and intermolecular interactions (as can be seen by SDS–PAGE), which was in general agreement with Tanimoto and Kinsella (1988).

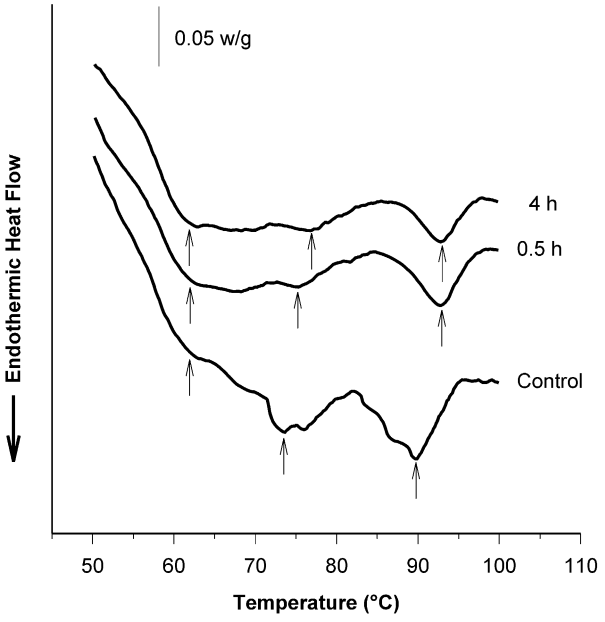

Fig. 4. Representative DSC thermal curves of mixed MPI/SPI (1:1 ratio, 4.5% total protein) after incubation at 5 °C with 0.5% transglutaminase preparation for 0.5 and 4 h. Control = no enzyme.

3.3. Gelation

3.3.1. Dynamic rheological properties

Heat-induced rheological changes in MPI, SPI, and their mixtures with or without MTGase treatment are displayed in Fig. 5. The rheograms obtained from different measurements (replications) were essentially identical, and hence, only one set of data is presented. Control MPI (no MTGase) started to develop rigidity (soft gel) after being heated to about 50 °C and attained a maximum storage modulus (G' = 187 Pa) at about 72 °C. On the other hand, control MPI/SPI (no MTGase), showing a G' peak at 60 °C, had a substantially reduced gel strength. The result indicated that under the present experimental condition, non-modified

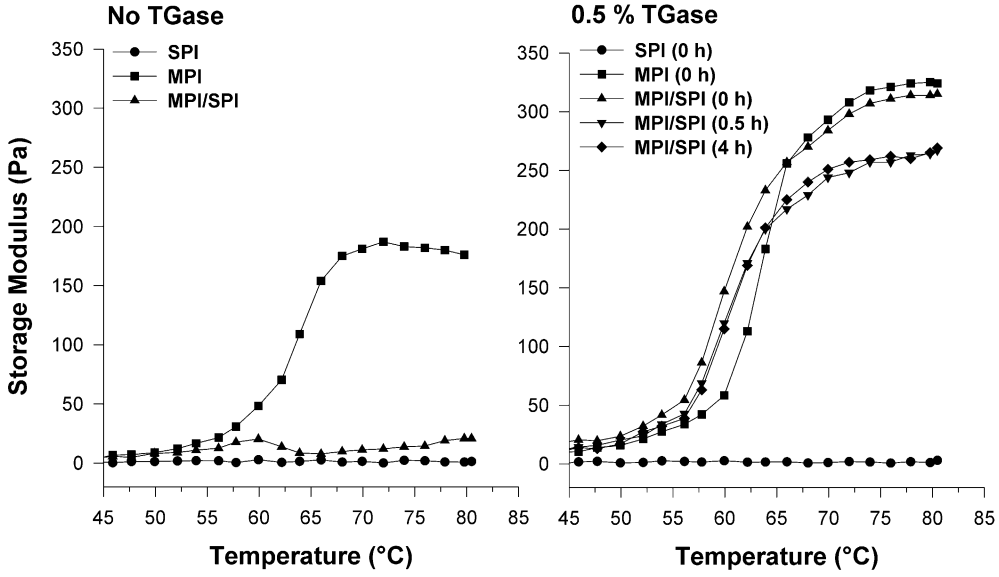

Fig. 5. Representative rheograms of heat-induced gels of MPI, SPI, and their mixtures (1:1 ratio, 4.5% total protein) with or without transglutaminase. The treated samples (right graph) were incubated with the enzyme preparation (0.5%) for 0, 0.5 or 4 h.

soy proteins had a detrimental effect on muscle protein gelation probably by hindering the type of protein–protein interaction responsible for a high elasticity.

Treatment of MPI and its SPI mixture with MTGase greatly improved their gelling ability (Fig. 5). All treatments (except SPI-alone that did not gel) started to form a gel at about 45 °C. Between 55 and 65 °C, the storage modulus rose sharply as a result of Gln-Lys cross-linking among mixed proteins (evidenced by SDS–PAGE later), reaching a maximum value of 325 Pa for MPI. The earlier onset of gelation for MTGase-treated MPI or its SPI mixture, when compared to those without the enzyme, suggests that cross-linked proteins had a lower temperature requirement for producing an elastic structure. It is of interest that MTGase-treated MPI and the MPI/SPI mixture without pre-incubation seemed to have a different gelation kinetics in the 55–65 °C temperature range, although their final G' values were nondistinguishable ($P > 0.05$). The result was a manifestation of shifts in the rate of gel network formation induced by the interaction of muscle and soy proteins.

Incubation time did affect the gelation of mixed MPI/SPI proteins by slightly decreasing ($P < 0.05$) the magnitude of G', i.e. 315 Pa for '0 h' vs. 269 Pa for 0.5 or 4 h at the end of the treatment. A possible explanation would be that MTGase produced more intramolecular cross-links during incubation so that when subjected to heating, the less opened protein structure had fewer available reactive sites for network formation (Oakenfull, Pearce, & Burley, 1997). Therefore, pre-incubation would be unnecessary for producing a viscoelastic, cohesive gel from MTGase-treated protein mixtures. Based on the rate of gel elasticity/rigidity development (Fig. 5), the optimum temperature range for the gelation of enzyme-reacted MPI and MPI/SPI mixtures was 60–65 °C, which was slightly higher than the optimum temperature for the catalysis of MTGase (Ajinomoto, 1998).

3.3.2. SDS–PAGE

In order to identify the proteins that contributed to the gel network structure and to further explain the role of soy proteins in the MTGase-mediated MPI gelation process, gel samples heated to various temperatures were subjected to electrophoresis. There was no visible change in heated SPI in the absence of MTGase (result not shown). However, with MTGase, a substantial amount of cross-linking of soy proteins occurred at ⩾50 °C. Essentially all the SPI constituents, except the basic subunits (B) of glycinin, were linked covalently forming a streak of polymers most of which were too large to enter the separating gel (Fig. 6a). These were likely random aggregates because they produced no elastic characteristics (Fig. 5) seen in a typical protein gel. Similarly, as shown previously (Ramírez-Suárez & Xiong, 2002), all the MPI components, except actin, were cross-linked by MTGase at about 50–55 °C, but the products (gel) were most likely of an ordered structure because they exhibited a high elasticity.

Heated MPI/SPI mixtures without MTGase, which produced a rather weak gel (Fig. 5), were readily dissolved in the SDS/β-mercaptoethanol solution; and essentially an identical electrophoretic pattern was generated by samples heated to different temperatures (Fig. 6b). The addition of MTGase resulted in a pronounced production of highly cross-linked polymers at ⩾50 °C that were too large to even enter the stacking gel (3% acrylamide; Fig. 6c), or became undissolvable

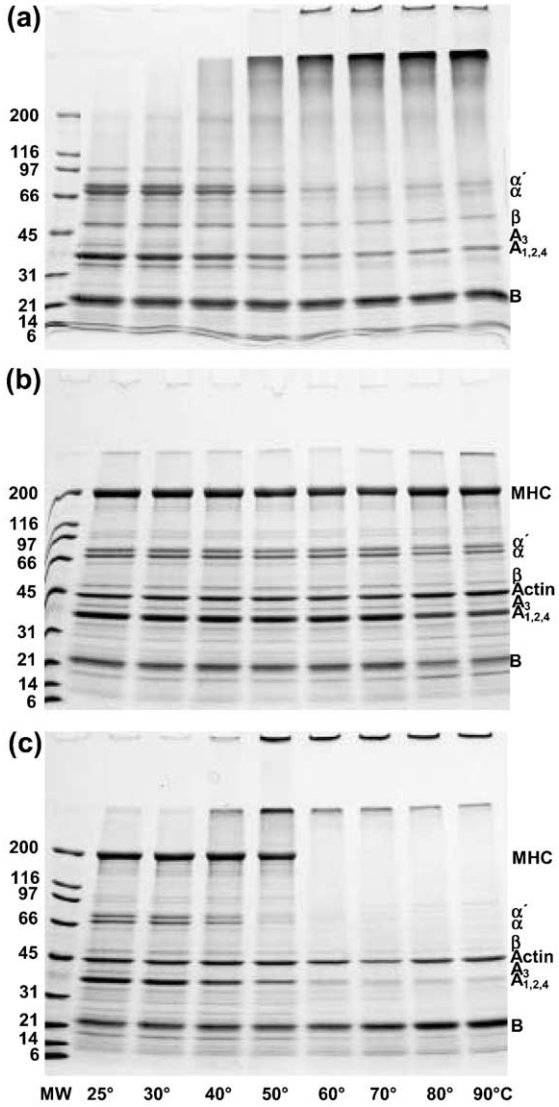

Fig. 6. Electrophoretic patterns of extracted proteins from SPI and MPI/SPI mixtures (1:1 ratio, 4.5% total protein) with or without 0.5% transglutaminase preparation, which were heated to different temperatures during gelation. Lane MW = molecular weight standard (in kDa). MHC = myosin heavy chain; α′, α, β = subunits of β-conglycinin; $A_{1,2,3,4}$ and B = acidic and basic subunits of glycinin. Shown are patterns of SPI alone with transglutaminase, no pre-incubation (a), MPI/SPI mixture without transglutaminase (b), and MPI/SPI mixture with the enzyme, no pre-incubation (c).

by the SDS/β-mercaptoethanol extraction buffer (unpublished observation). As was in the SPI-alone sample, all the soy protein components, except the basic subunits, were gradually diminished and ultimately vanished, and the process was accompanied by a total disappearance of MHC (Fig. 6c). Furthermore, the smear above 200 kDa, seen in the SPI-alone samples, was missing from the heated protein mixture. Moreover, the presumable 'intramolecular' association of myosin and of actin at low temperature MTGase incubation (Fig. 2; 0 M NaCl at 5 °C) was absent at the gelation temperatures (25–90 °C). The results were strong evidence of muscle/soy protein interactions, which can be ascribed to Gln-Lys cross-linking, and such molecular interactions led to ordered heteropolymers or aggregates that produced the gels with great storage modulus (Fig. 5).

4. Conclusions

Pre-incubation of myofibrillar protein isolate with microbial transglutaminase at low-temperature (ca. 5 °C) low-ionic-strength conditions produced 'compact' myosin molecules, but the biochemical process had no effect on subsequent heat-induced gelation and rheological characteristics of the protein isolate. The presence of transglutaminase, which cross-linked soy and muscle proteins, eliminated the adverse effect of soy proteins on muscle protein gelation, and produced a rigid mixed protein gel at a reduced myofibrillar protein concentration. Hence, in comminuted and restructured low-salt muscle foods (e.g. chicken nuggets, restructured meats) that contain low-cost functional ingredient such as soy proteins, transglutaminase may serve as an excellent agent for producing an adhesive mixed protein gel structure with a reduced concentration requirement for extracted myofibrillar proteins.

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