Formation and properties of glycinin-rich and β-conglycinin-rich soy protein isolate gels induced by microbial transglutaminase

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Abstract

The gelation and gel properties of glycinin-rich and β-conglycinin-rich soy protein isolates (SPIs) induced by microbial transglutaminase (MTGase) were investigated. At the same enzyme and protein substrate concentrations, the on-set of gelation of native SPI and the viscoelasticity development of correspondingly formed gels depended upon the relative ratio of glycinin to β-conglycinin. The turbidity analysis showed that the glycinin components also contributed to the increase in the turbidity of SPI solutions incubated with MTGase (at 37 °C). Textural profile analysis indicated that the glycinin components of SPIs principally contributed to the hardness, fracturability, gumminess and chewiness values of corresponding gels, while the cohesiveness and springness were mainly associated with the β-conglycinin components. The strength of MTGase-induced gels of various kinds of SPIs could be significantly improved by the thermal treatment. The protein solubility analyses of MTGase-induced gels, indicated that besides the covalent cross-links, hydrophobic and H-bondings and disulfide bonds were involved in the formation and maintenance of the glycinin-rich SPI gels, while in β-conglycinin-rich SPI case, the hydrophobic and H-bondings were the principal forces responsible for the maintenance of the gel structure. The results suggested that various kinds of SPI gels with different properties could be induced by MTGase, through controlling the glycinin to β-conglycinin ratio.
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1. Introduction

Soy proteins have been widely applied in food products, due to their nutritive value and ability to improve texture. In usual, soy protein isolates (SPI) consist of about 90% protein; their major components are glycinin, or 11S, and β-conglycinin, or 7S, which represent 34% and 27%, respectively, of the proteins occurring in the isolate (Iwabuchi & Yamauchi, 1987a, 1987b). The native glycinin is an oligomeric protein having a molecular weight (MW) of approximately 350 ku and consisting of six subunits (AB)$_6$. The acidic ($M_r = 37$–42 ku) (A) and basic subunits ($M_r = 17$–20 ku) (B) are linked by disulfide bridges. The β-conglycinin is a kind of trimers composed of three major subunits (α′, α and β) associated in various combinations by non-covalent interactions (Thanh & Shibasaki, 1976, 1977, 1979). To date, the ability of gel formation of soy proteins or their individual constituents, induced by heat treatment, has been widely

investigated (Kang, Matsumura, & Mori, 1991; van Kleef, 1986; Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992; Nagano, Akasaka, & Nishinari, 1994; Nakamura, Utsumi, & Mori, 1984; Nakamura, Utsumi, & Mori, 1986a; Nakamura, Utsumi, & Mori, 1986b; Renkema, Lakemond, de Jongh, Gruppen, & van Vliet, 2000; Renkema, Knabben, & van Vliet, 2001, Renkema, Renkema, Gruppen, & van Vliet, 2002a; Renkema et al., 2002b; Renkema, 2004; Renkema & van Vliet, 2004). Besides this treatment, many other treatments have also been tried to induce the gel formation of soy proteins, including the acidification by addition of glucono-δ-lactone, hydrolyzing by protease, and cross-linking by transglutaminase (TGase) (Alting, Hamer, de Jongh, Visschers, & Simons, 2002; Chanyongvorakul, Matsumura, Sakamoto, Motoki, & Mori, 1994; Chanyongvorakul, Matsumura, Nonaka, Motoki, & Mori, 1995; Kang et al., 1994; Nio, Motoki, & Takinami, 1985; Nonaka et al., 1989; Tay et al., 2005). Of all these treatments, the gelation of proteins by means of TGase seems to be most potential and attractive.

There have been several reports on the gelation of globulins or glycinin of soy proteins induced by TGase (Chanyongvorakul et al., 1994, 1995; Nio et al., 1985). As for the underlying mechanism of gelation, Nio et al. (1985) pointed out that TGase and a higher concentration of a substrate protein were indispensable for firm gel formation. In another study, they demonstrated that the gelation mechanism of α_{s1}-casein by TGase was through the formation of covalent or ε-(γ-glutamyl)lysyl cross-links (Nio, Motoki, & Takinami, 1986). However, these reports have rarely proposed the gelation mechanism in detail, and no report has established the relative importance of individual components of soy proteins for the gel formation by TGase. Furthermore, few reports investigated and compared the thermal and other properties of TGase-induced gels from various kinds of soy proteins. Therefore, there is a necessity to compare the gelation of various kinds of soy proteins and their gel properties, and investigate the underlying mechanism of gelation.

In a previous paper (Tang, Wu, Yu, Chen, & Yang, 2005), we investigated the coagulation and gelation of native soy protein isolates (SPI) induced by microbial transglutaminase (MTGase) by measuring the turbidity or the dynamic viscoelasticities of the reaction solutions, and demonstrated that the coagula or aggregates were mainly composed of most of basic subunits of glycinin and some new formed high MW of biopolymers. And the gelation mechanism of SPI induced by MTGase was supposed to be the result of both the cross-linking and non-covalent interactions (especially hydrophobic interaction). In this paper, we also suggested that the glycinin mainly contribute to the coagulation or gelation of native SPI. To prove this point, in the present study, we

aimed to investigate the gelation process of glycinin-rich or β-conglycinin-rich SPIs induced by MTGase, and reveal the relative importance of individual components of SPI for the formation of corresponding gels. In addition, the thermal properties and protein solubility of gels of various SPIs induced by MTGase were also investigated. This evidence could help to further understand the gelation mechanism of SPI induced by MTGase.

2. Materials and methods

2.1. Materials

Throughout the experiment, 0.05 M Tris–HCl buffer (pH 7.5, 25 °C) containing 0.05% sodium azide was used as the standard buffer. The whole soybean seed powder was provided by Henan Hebi Co. (China). Commercial microbial transglutaminase (MTGase) was obtained from Chanshou Biological Co. Ltd. (China). N_α-CBZ-GLN-GLY and L-glutamic acid γ-monohydroxamate were purchased from Sigma Chemical Co. The purification of MTGase and its activity's determination were according to Tang, Yang, Chen, Wu, and Peng (2005). All other chemicals were of analytical reagent or better grade. Silicone oil was a gift from the Guangzhou office of Pharmacia Co.

2.2. Preparation of crude glycinin and β-conglycinin

The crude glycinin and β-conglycinin were prepared according to Iwabuchi and Yamauchi (1987), with a minor modification. A sample (100 g) of defatted meal was extracted once with 2 L of 0.03 Tris–HCl buffer containing 10 mM β-mercaptoethanol (2-ME), at pH 8.0 at room temperature. After centrifugation (4000g, 15 min), the supernatant was acidified to pH 6.4. After placing at 4 °C for 2–4 h and then centrifugation, the precipitate (crude glycinin) was obtained, and resolubilized in a, pH 7.6, phosphate buffer (2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, 0.4 M NaCl, 10 mM 2-ME, pH 7.6; ionic strength 0.5). The corresponding supernatant was further separated into the crude β-conglycinin and whey fractions by acidification to pH 4.8. This resulting precipitate was centrifuged, completely resolubilized in 0.03 M Tris–HCl buffer by adjusting to pH 7.6. The above-mentioned crude glycinin and β-conglycinin solutions were dialyzed completely against desalted water at 4 °C, and lyophilized to yield the glycinin-rich and β-conglycinin-rich SPIs.

2.3. Enzymatic polymerization experiments

All the enzymatic reactions were carried out at 37 °C in standard buffer (pH 7.5) containing 2% (w/v) various soy proteins and an enzyme level of 20 units per gram of

protein substrate. The reaction mixtures were incubated for various times (0, 15, 30, 60, 90, 120, 240 and 360 min, respectively), then stopped by directly mixing with the sample buffer (2×) of electrophoresis. The reaction mixtures were analyzed by SDS–PAGE.

2.4. Turbidity measurements

The changes in the turbidity of the SPI solutions (after the addition of a certain amount of MTGase) were evaluated by measuring the optical density at 660 nm (OD_{660}) with a spectrophotometer (Spectrumlab 52, LengHuang Technol., China). Two milliliters of the SPI solution (7%, w/v) was placed in a cell and incubated at 37 °C for 5 min. Then, 20 units per gram of protein substrate of enzyme were added, and a change in OD_{660} was measured as a function of the reaction time. The same SPI solution without addition of enzyme was used as the blank.

The effect of heating on the turbidity of the gel was also evaluated by the change of OD_{660} value. The gel was obtained in the same cell at 37 °C for 6 h. The change in the OD_{660} was measured as a function of the heating time at 95 °C. All the turbidity measurements were carried out twice, and the mean values were given.

2.5. SDS–PAGE

SDS–PAGE electrophoresis was performed on a discontinuous buffered system according to the method of Laemmli (1970) using 12% separating gel and 4% stacking gel. The samples [the enzyme mixture directly mixed with sample buffer (2×), 1:1 (v/v)] or [the moderate samples dissolved with sample buffer (1×)] were heated for 5 min in boiling water before electrophoresis. Every sample (10 μL) was applied to each lane. Before the sample entering the separating gel, electrophoresis was performed at 10 mA, and the other was at 20 mA. The gel was stained with 0.25% Coomassie brilliant blue (R-250) in 50% trichloroacetic acid, and destained in 7% acetic acid [methanol:acetic:water, 227:37:236 (v/v/v)].

2.6. Textural properties

The textural characteristic of gels was analyzed according to texture profile analysis (TPA; Bourne, 1978) using a TA.XT2 Texture Analyzer (Stable Micro Systems Ltd., England), in the compression mode.

The gel samples (5 mL) were directly formed from the mixtures of protein solutions and enzyme in sealed cylinders (20 × 25 mm), by incubation at 37 °C for 4 h. Before the measurement, the gel samples were pre-cooled in ice bath to room temperature. The samples were compressed twice by a cylinder probe to 50% of their original height at a constant cross-head speed of 1 mm/s. From the TPA curves, the following texture parameters were

obtained: hardness at 50% of deformation, springness, cohesiveness, adhesiveness, gumminess and chewiness (Pons, 1996). Hardness was defined by peak force (g) during the first compression cycle. Fracturability was defined as the force (g) required to produce the first fracture during the first compression. Springness was defined as a ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve (dimensionless). Gumminess was obtained by multiplying hardness and cohesiveness. Chewiness was obtained by multiplying hardness, cohesiveness and springness.

2.7. Determination of the protein solubility of gels

The protein solubility of the gels was determined according to Lupano, Renzi, and Romera (1996), Lupano (2000), and Yamul and Lupano (2003). Samples were dispersed either in distilled water (DW), in a pH 8.0 buffer (0.086 M Tris, 0.09 M glycine, and 4 mM Na_2EDTA) (B), or in the same buffer containing 0.5% sodium dodecyl sulfate (SDS) and 8 M urea (BSU), or in the BSU plus 1% (v/v) 2-ME (BSUM). Samples (0.1% protein, w/v) were homogenized at room temperature with FJ-200 High-Speed Homogenizer (Shanghai Specimen Model Co., China) at 10,000 rpm for 1 min, and then centrifuged at 15,000g for 20 min. The UV absorbance (at 280 nm) of these supernatants was measured while the corresponding solvent was used as the blank. In each a solvent, the absorbency of 0.1% (w/v) native protein was defined as 100% protein solubility in this solvent. Thus, the protein constituent solubility of gels in a solvent was expressed as 100× the absorbance (at 280 nm) in the supernatant/the absorbance of 0.1% (w/v) native protein. Three independent extractions were carried out with each solvent.

2.8. Low amplitude dynamic oscillatory measurements

Rheological measurements using parallel plates ($d = 27.83$ mm) were carried out in a Haake RS600 Rheometer (HAAKE Co., Germany). The sample dispersions were placed between parallel plates and the gap between two plates was set to 1.0 mm. Excess sample was trimmed off and a thin layer of silicone oil applied to the exposed free edges of the sample to prevent moisture loss. The lower plate was held at 37 °C. The equipment was driven through the RheoWin 3 Data Manager (HAAKE Co., Germany). Storage modulus (G'), loss modulus (G'') and phase angle (δ) were recorded as a function of time. In order to ensure all measurements were carried out within the linear viscoelastic range, first stress amplitude sweep was performed at a shear oscillation of 0.1 Hz or other values.

In the gelation experiments, the changes of rheological behaviors (G', G'' and δ or $\tan\delta$) of SPI dispersions during incubation with MTGase were recorded and analyzed, immediately after the sample (preheated to 37 °C) was mixed with MTGase and loaded onto the lower plate of the rheometer. The time of last cross-over point of the G' and G'' during the incubation was defined as the on-set time of gelation (T_{gel}). And in the experiments to investigate the effect of heating–cooling treatment on the viscoelasticities of gels, the formed gels were heated from 25 to 90 °C at a heating rate of 1 K/min, kept at 90 °C for 20 min, and cooled down to 25 °C at a cooling rate of 1 K/min.

2.9. Statistics

An analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

3.1. Composition of glycinin-rich and β-conglycinin-rich SPIs

The composition and purity of glycinin-rich and β-conglycinin-rich SPIs was evaluated by SDS–PAGE (Fig. 1). The relative content of glycinin in glycinin-rich SPI was close to 80% (vs total protein), and that of β-conglycinin in β-conglycinin-rich SPI was also near 80%, as evaluated by comparing the optical density. The total protein contents of those two SPIs were 92.5% and 93.0% (dry weight), as determined by the Kjeldahl method ($N \times 6.25$). In the β-conglycinin-rich SPI, the relative content of α'- and α-subunits was much higher than that of β-subunit (Fig. 1, lane a).

3.2. SDS–PAGE analysis of cross-linking

In order to testify the importance of cross-linking of various protein constituents of SPI for the formation of MTGase-induced gels, we investigated the coagulation of 2% (w/v) glycinin-rich and β-conglycinin-rich SPIs induced by 20 units per gram of protein substrate of MTGase, by SDS–PAGE in the presence of 2-ME (Fig. 2). A majority of protein constituents of β-conglycinin and acidic subunits (AS) of glycinin declined continuously with increasing the incubation time from 0 to 360 min, and correspondingly, new high MW biopolymers increased on the top of separating and stacking gel, while the basic subunits (BS) of glycinin were almost intact during the whole incubation (Figs. 2(a) and (b)). In the subunits of β-conglycinin, the susceptibility of β-subunit to MTGase was lower than that of α'- and
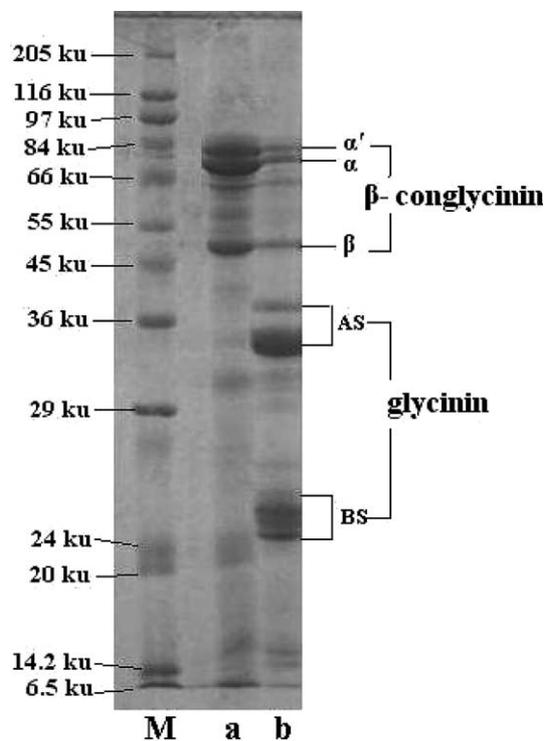

Fig. 1. The SDS–PAGE patterns of glycinin-rich and β-conglycinin-rich SPIs. The lanes a and b indicate the β-conglycinin-rich and glycinin-rich SPIs, respectively. Lane M indicates the standard protein markers.

α-subunits. Similar results have been reported in TGase or MTGase-induced cross-linking reactions of soy globulins and pea legumin (Kang et al., 1994; Larré, Kedzior, Chenu, Viroben, & Gueguen, 1992; Nonaka et al., 1989; Zhang, Matsumura, Matsumoto, Hayshi, & Mori, 2003). The reactivity difference of protein constituents of SPI to MTGase could be considered to relate with the native structure of glycinin and β-conglycinin. Usually, the BS of glycinin is buried in the interior of hexamers of glycinin (Nielsen, 1985), and the relative hydrophobicity of β-subunit of β-conglycinin is higher than that of α'- and α-subunits (Thanh & Shibasaki, 1976, 1977). Therefore, the active site of this enzyme (MTGase) is not easily accessible for BS of glycinin and β-subunit of β-conglycinin.

Under the same conditions, high MW biopolymers (not entering the stacking and separating gels) seemed to be more easily formed from glycinin-rich SPI than from β-conglycinin-rich SPI. For instance, new formed biopolymers (not entering the separating gel) distinctly appeared after glycinin-rich SPI was incubated with MTGase for 30 min, while it required about 60 min for β-conglycinin-rich SPI to be catalyzed to form the same amount of biopolymers (Fig. 2). This suggested that the AS of glycinin be more effectively polymerized or cross-linked by MTGase than those constituents of β-conglycinin.
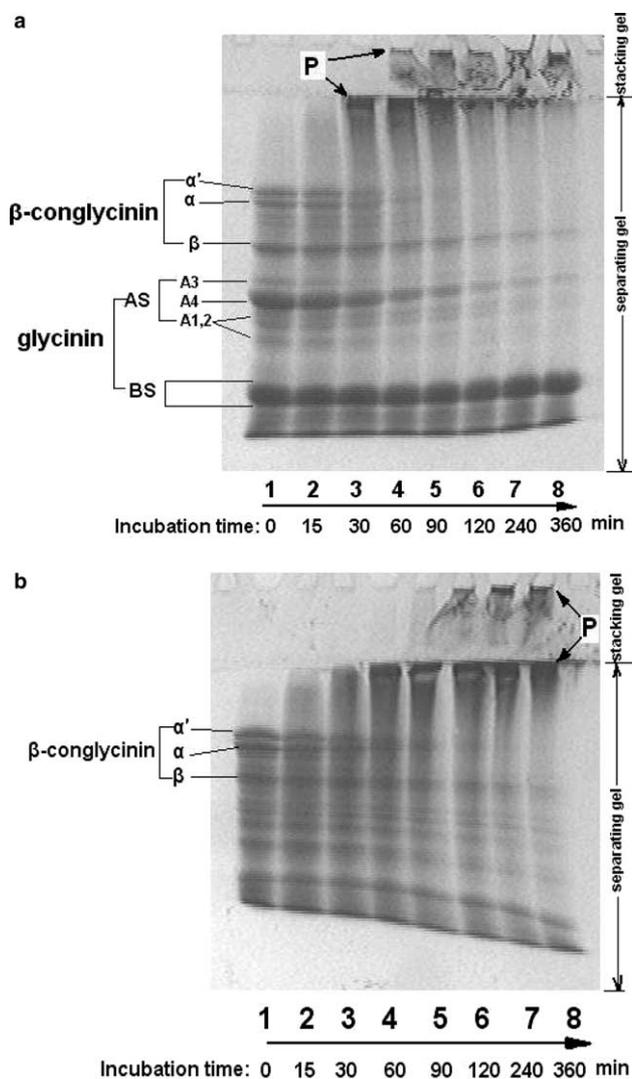
Fig. 2. SDS–PAGE analysis of glycinin-rich SPI (a) and β-conglycinin-rich SPI (b) incubated with MTGase at 37 °C for various times. The concentration of total protein is 2% (w/v), and the enzyme amount applied is 20 units per gram of protein substrate. The numbers 1–8 indicate the soy proteins incubated with MTGase at 37 °C for 0, 15, 30, 60, 90, 120, 240 and 360 min, respectively. The P indicates the biopolymers formed from the protein constituents of glycinin or β-conglycinin, which cannot enter the separating and stacking gels.

3.3. Dynamic gelation process

In our previous experiments, we studied the coagulation and gelation of native SPI induced by MTGase, and pointed out that this coagulation or gelation of native SPI could be observed by the changes in the turbidity and/or dynamic viscoelasticities, and a high concentration (>3%) of native SPI solutions could be induced by MTGase to form gels (Tang, Wu et al., 2005). Nio et al. (1985) also reported that a high concentration (above 5%) of soy 11S and 7S globulins were indispensable for gel formation, induced by TGase from guinea pig livers.

Fig. 3 shows the changes in the mechanical moduli (including the storage modulus, G' and loss modulus, G'') and tangent of phase angle or $\tan(\delta)$ of 7% (w/v) glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixture thereof, incubated with MTGase at 37 °C, as a function of incubation time. In all cases, the G' (and G'' to a lesser extent) increased pronouncedly after an initial incubation time (e.g., 3 min for glycinin-rich SPI), and a plateau was reached (after a certain incubation time) whose final value was dependent upon the ratio of glycinin to β-conglycinin (Fig. 3). This indicated a rapidly increasing degree of covalent cross-linking of the components forming the three-dimensional network, and that a more elastic gel matrix was formed. Similar change patterns of the mechanical moduli were observed in the MTGase-catalyzed gelation of soybean glycinin (Kang et al., 1994) and casein micelles (Schorsch, Carrie, Clark, & Norton, 2000). The on-set of gelation of SPI
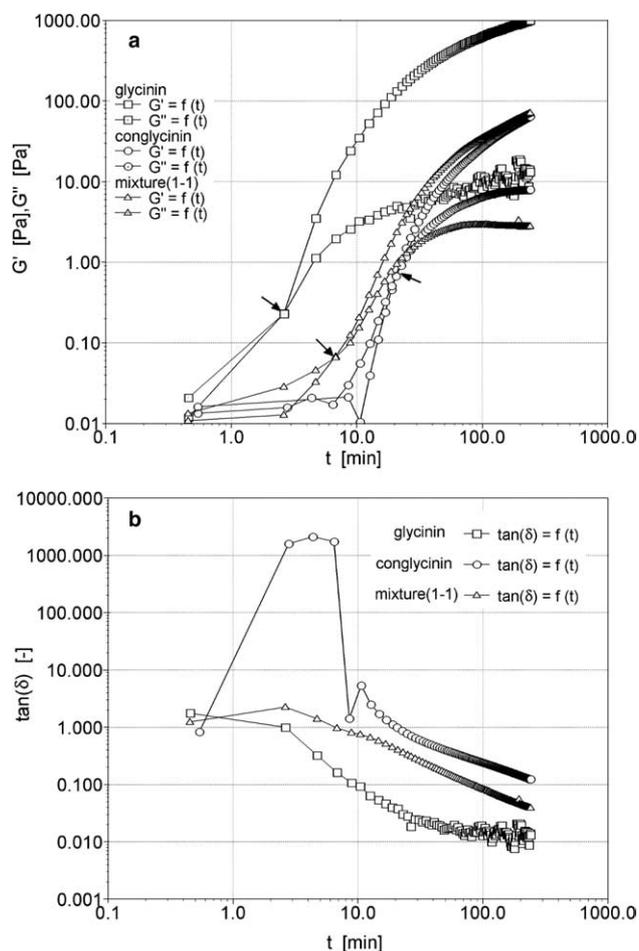

Fig. 3. Changes of the storage modulus (G'), loss modulus (G'') (a) and tangent of phase angle or $\tan(\delta)$ (b) of 7% (w/v) glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixtures, incubated with MTGase at 37 °C, with the incubation time. The amount enzyme applied is 20 units per gram of protein substrate. The arrows indicate the last cross-over point of G' and G'', which is defined as the on-set point of gelation.

and the development pattern of correspondingly formed gels, induced by MTGase, obviously depended on the relative ratio of glycinin and β-conglycinin of SPI. The on-set time of gelation of various kinds of SPIs was in the order: glycinin-rich SPI (only 2.6 min) < its 1:1 mixture with β-conglycinin-rich SPI (7.0 min) < β-conglycinin-rich SPI (25.0 min) (Fig. 3(a)), suggesting that the protein constituents of glycinin be more important for the initial network formation of gels than that of β-conglycinin, in the MTGase-induced gels of soy proteins. This result is in accordance with that of SDS–PAGE (Fig. 1). The G' increased in the order glycinin-rich SPI > 1:1 mixture of glycinin-rich and β-conglycinin-rich SPIs > β-conglycinin-rich SPI, and the final G' value of glycinin-rich SPI after 240 min was almost 100-fold higher than that of β-conglycinin-rich SPI (980 Pa vs ~10 Pa) (Fig. 3(a)), indicating that glycinin also accounted for the development of mechanical strength of formed gels. The differences of on-set of gelation and the mechanical properties between glycinin-rich and β-conglycinin-rich SPIs may be explained by the changes of interactive force pattern of protein substrate (Tang, Wu et al., 2005).

The development pattern of G'' was not similar with that of G'. Although the G'' of glycinin-rich SPI increased most remarkably during the initial period, the increasing velocity of the G'' of β-conglycinin-rich SPI was fastest after the on-set of gelation (Fig. 3(a)). After a time of about 25 min or more, the G'' value of β-conglycinin-rich SPI exceeded that of the 1:1 mixture. The G'' usually reflects the viscous property of a gel. Therefore, it can be affirmed that β-conglycinin mostly help to strengthen the viscous property of a gel from soy proteins. A perfectly elastic material will show $\tan \delta = 0$, and $\tan \delta$ approaches infinity for a purely viscous material (Nagano, Fukuda, & Akasaka, 1996). The development pattern of $\tan \delta$ (Fig. 3(b)) also illuminated the formation of more elastic gels with the incubation time. Interestingly, the on-set of gelation of the 1:1 mixture of glycinin-rich SPI and β-conglycinin-rich SPI occurred at lowest G' value (Fig. 3(a)). This seemed to suggest that there be an interaction between the protein constituents of glycinin and β-conglycinin, in the initial network formation of gels.

During the whole incubation, the turbidity of the glycinin-rich SPI solution increased most evidently, followed by its 1:1 mixture with β-conglycinin-rich SPI, and that of β-conglycinin-rich SPI on the contrary decreased in the initial incubation time and then slowly increased (Fig. 4). During the initial 60 min, the exponential increase in the turbidity of glycinin-rich SPI and its 1:1 mixture with β-conglycinin-rich SPI was observed (Fig. 4). These results revealed that glycinin components contributed to the increase of turbidity of soy protein solutions incubated with MTGase, and also reflected the importance of MTGase-induced coagulation or aggre-
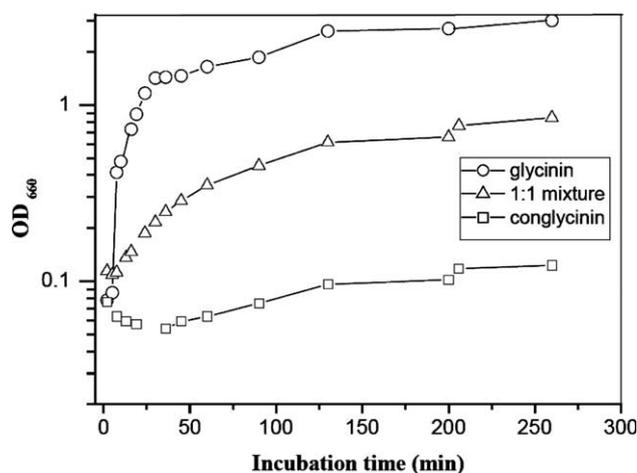

Fig. 4. The turbidity of 7% (w/v) glycinin-rich and β-conglycinin-rich SPIs and its 1:1 mixtures thereof, incubated with 20 units per gram of protein substrate of MTGase at 37 °C, as a function of incubation time.

gation of glycinin for the gel formation of soy proteins. The cross-linking of glycinin components of SPI by MTGase (Fig. 2(a)) seemed to account for the increase of turbidity. The turbidity differences of formed gels of various SPIs implied that the mechanism of gel-formation of various SPIs by means of MTGase might be a bit different. The dissimilarity of changing pattern of inter-active forces among various SPIs during the incubation with MTGase may be responsible for this difference. In all, those glycinin-rich SPIs seem to form strong and turbid gels, and those rich in β-conglycinin to form weak but transparent gels, when MTGase is used as the only coagulating agent to induce the gelation.

3.4. Textural profile analysis of finally formed gels

The TPA results of finally formed gels of glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixture thereof, are shown in Table 1. Six parameters were obtained: hardness, fracturability, springness, cohesiveness, gumminess and chewiness. The hardness is related with the strength of the gel structure under compression. The hardness of glycinin-rich SPI gels was highest (81 g), and that of β-conglycinin-rich SPI gels lowest (8.1 g) (Table 1), suggesting that glycinin constituents basically account for the gel formation and strength development of MTGase-induced gels. This result is consistent with the G' data in Fig. 3. The fracturability (originally called brittleness) of formed gels was also tightly related with the glycinin constituents of SPI (Table 1). The fracturability of glycinin-rich SPI gels was significantly higher ($P < 0.05$) (compared to that of other gels), suggesting that this kind of gels have obvious and firm surfaces and be not brittle. Chanyongvorakul et al. (1995) also pointed out that MTGase-induced soy glycinin gels were more rigid and elastic than thermally induced gels. The

Table 1
TPA parameters of finally formed gels of 7% (w/v) glycinin-rich SPI, β-conglycinin-rich SPI, and their 1:1 mixture thereof

Substrate protein	Hardness (g)	Fracturability (g)	Springiness (–)	Cohesiveness (–)	Gumminess (g)	Chewiness (g)
Glycinin-rich SPI	81.2 ± 6.0a	41.8 ± 7.0a	1.03 ± 0.01b	0.34 ± 0.05c	22.9 ± 2.5a	23.6 ± 3.5a
1:1 Mixture of glycinin and β-conglycinin-rich SPI	20.8 ± 2.6b	1.3 ± 0.3b	1.04 ± 0.02b	0.52 ± 0.02a	10.8 ± 1.3b	11.2 ± 1.0b
β-Conglycinin-rich SPI	8.1 ± 0.4c	0.7 ± 0.2c	1.13 ± 0.06a	0.46 ± 0.05ab	3.70 ± 0.40c	4.18 ± 0.52c

Means with different letters in the same column are significantly different ($P < 0.05$).

chewiness and guminess of formed gels exhibited the same profiles, and thus are complementary parameters of hardness.

Springiness (sometimes also called as "elasticity") is a measure of how much the gel structure is broken down by the initial compression. High springiness will result when the gel structure is broken into few large pieces during the first TPA compression whereas low springiness results from the gel breaking into many small pieces (Lau, Tang, & Paulson, 2000). This parameter of β-conglycinin-rich SPI gels was significantly higher than that of gels containing glycinin ($P < 0.05$) (Table 1). The cohesiveness is a measure of the degree of difficulty in breaking down in the gel's internal structure (Lau et al., 2000). There was a dramatic decrease in cohesiveness of the glycinin-rich SPI gels (Table 1), indicating the cohesiveness also depended upon the relative ratio of glycinin and β-conglycinin. The cohesiveness value of β-conglycinin-rich gels was a bit lower than that of its mixture with glycinin-rich SPI ($P > 0.05$), also suggesting that there may be an interaction between glycinin and β-conglycinin components, during the gelation of SPI induced by MTGase. These results of springiness and cohesiveness of formed gels agree with that of the G'' in Fig. 3.

3.5. Effect of thermal treatment on the dynamic viscoelasticities and turbidity of MTGase-induced gels

Fig. 5 shows the normal heating and cooling cycle (heating: $25 \rightarrow 95\,°C$; holding: $90\,°C$, 20 min; cooling: $90 \rightarrow 25\,°C$) on the dynamic viscoelasticities of finally formed gels of various SPIs (7%, w/v), including glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixture. In all cases, there were two respective "endothermal peaks" in the G' curves during the thermal treatment, with the temperature of the peaks corresponding to ~78 and ~90 °C, respectively (Fig. 5). These two temperatures are coincident with the denaturation temperatures of β-conglycinin and glycinin at pH 7.6 (Renkema et al., 2001). In the heat-induced gelation of soy 7S globulins, Nagano et al. (1992) also confirmed the close relationship between the rheological behavior of 7S globulin gel and its DSC curve (or thermal properties). The G' values of glycinin-rich and β-conglycinin-rich SPI gels were nearly unchanged when heated up to near 70 °C, and after that, obviously declined to a minimum at 78–80 °C (Fig. 5).
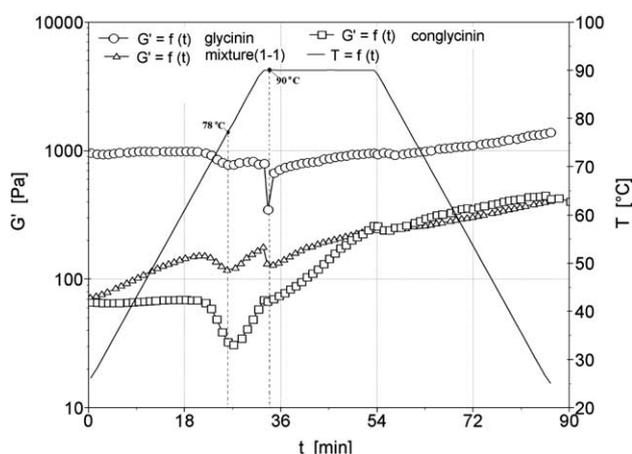

Fig. 5. Changes of the G' of finally formed gels of 7% (w/v) various SPIs (including glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixtures thereof) during the heating–cooling treatment cycle. The gels were induced by 20 units per gram of protein substrate of MTGase, at 37 °C for 240 min. The heating–cooling cycle: heating from 25 to 90 °C at 2 °C/min, then holding at 90 °C for 20 min, last cooling from 90 to 25 °C.

When the gel was heated up to near 90 °C, there was an alike decrease in the G' curve. These results confirmed the postulated mechanism of gelation of native SPI induced by MTGase that covalent cross-links and hydrophobic and H- bondings were involved in the gel formation (Tang, Wu et al., 2005). The occurrence of the "peak" in the G' curve during the heating indicated the incomplete rupture of initially formed gel network. From Fig. 5, it was presumed that in the gel network of MTGase-induced gels, there be some partially intact or "native" glycinin and β-conglycinin whose molecular structure was not completely destroyed by MTGase. Mizuno, Mitsuiki, Motoki, Ebisawa, and Suzuki (2000) have demonstrated the changes in molecular structure of soy proteins as a result of the MTGase treatment.

The pattern of changes in the G' of 1:1 mixture of glycinin-rich and β-conglycinin-rich SPIs was a bit different (Fig. 5). In this case, the G' value persistently increased with increasing temperature in the range from 25 to 90 °C, suggesting that there be an interaction between the glycinin and β-conglycinin components, e.g., those hydrophobic interactions between the β-subunits of β-conglycinin and BS of glycinin (which could

be reinforced by the heating treatment). This result agreed with the results of dynamic gelation process and TPA analyses (Sections 3.3 and 3.4). In a heat-induced aggregation, German, Damodaran, and Kinsella (1982) also suggested the complex formation between dissociated β-conglycinin and BS of glycinin.

The thermal properties of MTGase-induced gels of soy proteins (including glycinin-rich and β-conglycinin-rich SPIs) were evidently different from that of heat-induced gels. In the heat-induced gelation of soy proteins, the protein denaturation induced by heat treatment is often a prerequisite for gel formation of soy proteins (van Kleef, 1986; Nakamura et al., 1986a, 1986b; Puppo and Añón, 1998; Renkema & Vliet, 2002a,b; Utsumi and Kinesella, 1985a, 1985b), since the exposed hydrophobic areas of denatured proteins can interact and aggregate to form gel-network upon cooling. In this case, the soy protein gels were completely thermoreversible at pH 3.8 or 5.2, and the gel strength declined after reheating-recooling cycle at pH 7.6 (Renkema et al., 2002b). Furthermore, Nagano et al. (1996) pointed out that β-conglycinin played an important role in the heat-induced gel formation of commercial or preheated SPI. However, in the present study, after the incomplete rupture by heating treatment (from 70 to 90 °C), the gel network of corresponding gels re-arranged, then recovered or even exceeded the initial gel strength during the holding process (at 90 °C for 20 min). After that, the G' persistently and slowly increased upon cooling from 90 to 25 °C (Fig. 5). These results showed that the strength of MTGase-induced gels of various kinds of SPIs could be improved by additional thermal treatments. The influence of this heating–cooling cycle on the G' of various kinds of SPI gels was different. This also reflected the importance of glycinin to the gel formation of soy proteins by means of MTGase, since glycinin could be more effectively cross-linked by MTGase to be embedded in the gel network than β-conglycinin.

We also investigated the heating treatment (at 95 °C) on the turbidity (OD_{660}) of MTGase-induced gels of various kinds of SPIs (7%, w/v) (Fig. 6). The initial OD_{660} value of the final gel of glycinin-rich SPI was beyond the scope of measurement, and the OD_{660} value of its 1:1 mixture with β-conglycinin-rich SPI increased from initial 1.2 to the measurement limit (3.0), when heated at 95 °C up to 20 min (Fig. 6). The OD_{660} value of β-conglycinin-rich SPI gel slowly increased (from 0.2 to 0.5), and still kept transparent after heated at 95 °C for 120 min. This results indicated that glycinin chiefly contribute to the increase in the turbidity of soy protein gels. From the results of Figs. 5 and 6, it was suggested that the induction of MTGase combined with the thermal treatment be the effective technique of producing transparent and strong gels of soy proteins (especially those rich in β-conglycinin).
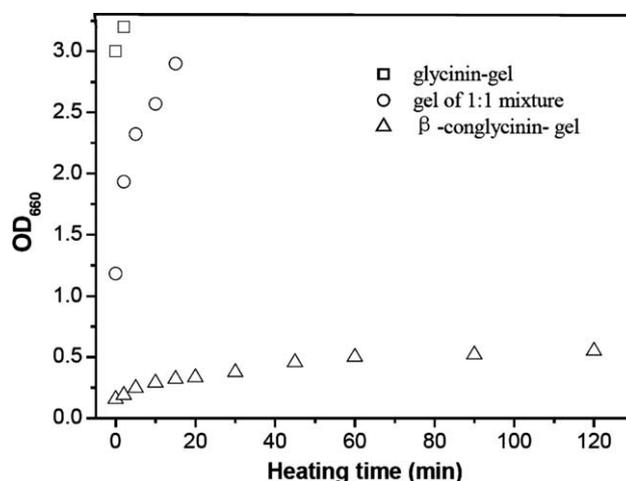

Fig. 6. Changes of the turbidity (OD_{660}) of MTGase-induced gels of 7% (w/v) glycinin-rich and β-conglycinin-rich SPIs and their 1:1 mixtures during the heating process (at 95 °C). The initial gels of various SPIs are induced by 20 units per gram of protein substrate of MTGase at 37 °C for 6 h.

3.6. Solubility of finally formed gels

In the present study, the enzymatic cross-linking by MTGase was used to induce the gelation of SPI instead of the normal heat treatment. In MTGase-induced coagulation of SPI, covalent cross-links and disulfide bonds and hydrophobic and H-bondings were involved in the formation of aggregates of SPI (Tang, Wu et al., 2005). In the present study, we investigated the interactive patterns among MTGase-induced gels of various kinds of SPIs, according to the method of solubility analysis adopted by Lupano et al. (1996), Lupano (2000) and Yamul and Lupano (2003).

The solubility of the protein constituents of MTGase-induced gels of various SPIs in various solvents was variable. In any solvent, the solubility of the protein constituents of SPI gels was in the order: β-conglycinin-rich SPI > its 1:1 mixture with glycinin-rich SPI > glycinin-rich SPI ($P < 0.05$) (Table 2). This was consistent with gel strength or hardness differences of various SPI gels (Fig. 3 and Table 1), and also reflected the importance of covalent cross-linkages of glycinin for the formation of gel network, not easily disrupted by various solvents. In distilled water (DW), the high solubility (66.6%) of protein constituents of the gels from β-conglycinin-rich SPI indicated that, although a majority of subunits of β-conglycinin could be polymerized by MTGase to form higher MW biopolymers (Fig. 2), the cross-linking extent seemed to be limited and the protein aggregation caused by the covalent crosslinkage was also limited, because most of those resultants could be still solubilized in the DW.

Table 2 also shows that the protein constituents were similarly soluble in B and DW, except in their 1:1

Table 2
Solubility of the protein constituents of MTGase-induced gels from various SPIs, including glycinin-rich SPI, β-conglycinin-rich SPI and their 1:1 mixtures thereof[a]

Various solvents[b]	Solubility of finally formed gels (%)[c]		
	Glycinin-rich SPI	1:1 Mixture of glycinin-rich and β-conglycinin-rich SPIs	β-Conglycinin-rich SPI
DW	$22.7 \pm 1.5^{f,j}$	$27.7 \pm 1.9^{g,i}$	$66.6 \pm 2.0^{e,h}$
B	$20.0 \pm 2.0^{f,j}$	$40.1 \pm 1.6^{f,i}$	$68.2 \pm 2.5^{e,h}$
BSU	$36.3 \pm 2.2^{e,j}$	$59.1 \pm 2.4^{e,i}$	$104.3 \pm 5.3^{d,h}$
BSUM	$77.1 \pm 3.5^{d,j}$	$86.0 \pm 4.0^{d,i}$	$107.1 \pm 4.8^{d,h}$

All the gels were induced by 20 units per gram of protein substrate of MTGase at 37 °C for 4 h. Protein content of gels: 7%, w/v. Protein concentration of all solubilization assays: 0.1%, w/v.

[a] All data were the mean values ± SD ($n = 3$).

[b] DW, distilled water; B, a pH 8.0 buffer (0.086 M Tris, 0.09 M glycine, and 4 mM Na_2EDTA); BSU, the same buffer containing 0.5% SDS and 8 M urea; BSUM, BSU plus 1% (v/v) 2-ME.

[c] The protein solubility of various gels in various solvents was evaluated by the absorbance at 280 nm.

[d,e,f,g] Indicate significant ($P < 0.05$) difference between the solubility within a same column.

[h,i,j] Indicate significant ($P < 0.05$) difference between the solubility in a same row.

mixtures case, in which the solubility (40.1%) in B was significantly higher than that in DW (27.7%) ($P < 0.05$). The differences of solubility between in DW and in B reflect the contribution of electrostatic forces to the formation of gel structure (Lupano et al., 1996; Lupano, 2000). Therefore, in glycinin-rich or β-conglycinin-rich SPIs cases, the formation or maintenance of gel structure did not involve the electrostatic forces, and in their 1:1 mixture case, the contribution of the electrostatic forces was obvious. Although the underlying reason of this phenomenon is not known in the present, it may be associated with the occurrence of interactions between the subunits of glycinin and that of β-conglycinin. In addition, SDS and urea, which could cause the disruption of hydrophobic and H- bondings, practically increased the protein solubility in all cases (Table 2). Particularly in β-conglycinin-rich SPI case, the protein constituents of formed gels were completely solubilized by BSU. Thus, the hydrophobic and H-bondings (not covalent cross-links) would be the principal forces responsible for the maintenance of the gel structure of β-conglycinin-rich SPI. Interestingly, the interactive forces to maintain the structure of MTGase-induced SPI gels were similar with that in thermally induced gels. In the latter cases, the hydrophobic interactions for the gel formation of SPI was very prominent (Renkema & Vliet, 2002a, 2002b).

In glycinin-rich or its mixture with β-conglycinin cases, the solubility of the protein constituents of gels was improved further by the presence of 1% (v/v) 2-ME (Table 2). This fact indicated that in these cases, the disulfide bonds be also involved in the maintenance of the gel structure, probably because the disulfide bonds between the acidic subunits and basic subunits of glycinin were broken during the cross-linking reactions catalyzed by MTGase, and came into being again, during the formation of gel networks. That the increasing extent (2.1-fold) of solubility in the BSUM (compared to that in the BSU) in glycinin-rich SPI case was much higher than that in its 1:1 mixture with β-conglycinin case (1.45-fold), also illuminated the importance of the disulfide bonds among glycinin to the formation of gel structure for those soy proteins containing glycinin.

4. Conclusions

The glycinin-rich SPI was induced by MTGase to form more strong and turbid gels, and the β-conglycinin-rich SPI to form more weak and transparent gels. Higher hardness, fracturability, gumminess and chewiness values were obtained for the MTGase-induced gels of glycinin-rich SPI, while higher springiness and cohesiveness values were mainly related with the β-conglycinin. The strength of MTGase-induced gels of various kinds of SPIs (especially those containing β-conglycinin) could be improved by the thermal treatment. Besides the covalent cross-linking, hydrophobic and H-bondings and disulfide bonds were involved in the formation and maintenance of glycinin-rich SPI gels. In the β-conglycinin-rich SPI cases, hydrophobic and H-bondings were the primary forces responsible for the maintenance of corresponding gels. Therefore, many kinds of SPI gels with different properties could be prepared by means of MTGase, by controlling the glycinin to β-conglycinin ratio, and additional thermal treatment.

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