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Functional Properties of Oat Globulin Modified by a Calcium-Independent Microbial Transglutaminase

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Oat globulin was modified by a calcium-independent microbial transglutaminase (TG). The TG-polymerized protein had higher solubility than the control at acidic pH and had improved water- and fat-binding properties. Incubation of 10% (w/v) oat globulin dispersions in the presence of TG at 37 °C led to the formation of a well-developed viscoelastic gel network with a microstructure characterized by thick strands and large clusters. The TG-induced gels had higher modulus values, lower loss tangent values, and lower frequency dependency than the heat-induced gels. The TG-induced gel system has the characteristics of classical polymer gel with permanent "chemical" cross-links, whereas the heat-denatured system has the characteristics of a temporary "physical" gel with breakable cross-links. Fourier transform infrared spectroscopy showed marked shift and intensity changes in several major bands, suggesting pronounced changes in protein conformation during TG-induced gelation. Aggregation of protein molecules was also indicated by the progressive increases in two infrared bands (1679−1682 and 1622−1625 cm^{-1}) associated with the formation of intermolecular β-sheets and strands. Results suggest that new food polymers with unique functionality can be produced from oat globulin treated with TG and that elastic gels can be formed near neutral pH, instead of the alkaline pH required for thermally induced oat globulin gels.

KEYWORDS: Transglutaminase; oat globulin; functional properties; gelation, rheology

INTRODUCTION

Oat protein has been shown to possess good nutritional value (*1*) and functional properties (*2*). Oat protein products including globulin have been chemically modified by various methods to improve their functional properties (*3*−*5*). Most of these modifications have limitations such as the production of toxic derivatives or lowering of nutritional quality (*6*). Enzymatic modifications of food proteins, without these limitations, have been widely used in the food industry (*7*). However, most of the enzymes are used to catalyze the breakdown of proteins to generate soluble polypeptides, sometimes resulting in the production of bitter peptides (*6*). Transglutaminase (TG; EC 2.3.2.13) is the only enzyme used commercially in the food industry that is based on the formation of cross-links between protein and other molecules, including the same or different proteins (*8*).

By definition, TG requires calcium ions to induce conformational changes and to express its activity as a metal−enzyme complex (*9*). Calcium-dependent TGs are widely distributed in plant and animal tissues (*8*, *10*, *11*) but have rarely been used in the food industry due to high cost. The mass production of a calcium-independent microbial TG from *Streptoverticullium* species by fermentation technology makes it possible to apply the enzyme at an acceptable cost for large-scale food processing operations (*12*, *13*). The calcium independence is a useful property of the microbial enzyme in modifying functional properties of food proteins because many protein systems (e.g., milk casein and meat myosin) tend to precipitate at relatively low calcium ion concentrations (*13*).

TG has been used to alter the molecular structure and improve the functional properties of food proteins such as whey protein, soy proteins, gluten, and meat proteins (*14*, *15*). The solubility, emulsifying activity, and hydration properties of polymerized proteins were greatly modified (*16*). TG has also been used to modify the gelation properties of legume and milk proteins (*17*−*21*) and to rebuild the dough structure of bug-damaged wheat flour (*22*). In the present investigation, oat globulin was polymerized by a calcium-independent microbial TG, and some functional properties of the polymers will be evaluated. Oat globulin dispersions (10%) will also be incubated with TG to induce gel formation, and the rheological and microstructural properties of TG-induced gels will be examined by small amplitude oscillatory tests and scanning electron microscopy, respectively. Fourier transform infrared (FTIR) spectroscopy will also be used to follow conformational changes in oat globulin during TG-induced gelation. In a separate investigation, the polymerization of oat globulin by TG was followed by SDS-

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PAGE, and some physicochemical and structural properties of the polymers were examined (*23*).

MATERIALS AND METHODS

Preparation of Oat Globulin. Oat seeds (variety Hinoat), grown at the Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Canada, were dehulled, ground in a pin-mill, and defatted by Soxhlet extraction with hexane. Oat globulin was extracted from the defatted oat groats with 1 M NaCl (*24*). The protein content of oat globulin, determined according to the micro-Kjeldahl method (*25*) using a nitrogen to protein conversion factor of 5.80, was 98.9%, similar to that reported previously (*2*), although a different solvent, 0.5 M $CaCl_2$, was used.

Polymerization of Oat Globulin by TG. Microbial TG, derived from the culture of *Streptovericillium* sp. no. 8112, was supplied by Ajinomoto Co. Inc. Oat globulin dispersions (5 mg/mL), prepared in 0.01 M phosphate buffer (pH 7.5) containing 0.2 M NaCl, were incubated with TG at 0.01 unit/mg of protein in a water bath at 37 °C. At specific time intervals, aliquots of reaction mixture were mixed with an equal volume of sample buffer and heated for 5 min in a boiling water bath to inactivate the enzyme. The reaction mixtures were dialyzed exhaustively against distilled water at 4 °C and freeze-dried. The extent of polymerization was determined by following the formation of high molecular weight polymers and the disappearance of the oat globulin acidic and basic polypeptides by SDS-PAGE.

Functional Properties of Oat Globulin Polymers. Protein solubility was determined according to the procedure described by Morr et al. (*26*). Water hydration capacity was determined according to the method of Quinn and Paton (*27*). Fat-binding capacity was determined according to the method of Sosulski and Jone (*28*). Emulsifying activity index and emulsion stability index were determined by using the turbidimetric method (*29*). Foamability and foam stability were estimated according to the procedure of Yasumatsu et al. (*30*).

Rheological Properties of TG-Induced Gels. The viscoelastic properties of TG-induced gels of oat globulin were evaluated in situ by small amplitude dynamic oscillatory measurements using a Stresstech controlled stress rheometer (Rheologica Instruments AB). Dispersions (10% w/v) of oat globulin were prepared in 0.01 M phosphate buffer (pH 7.5) with 0.2 M NaCl. Enzyme was added at a level of 0.01 unit/mg of protein. The reaction mixture was vacuum-evacuated to remove dissolved air and pipetted to the stainless steel platform. A stainless steel flat plate (30 mm diameter) was lowered to form a gap of 1.000 mm. The temperature was controlled by a programmable temperature-controlled water bath (Cole-Parmer, Veron Hills, IL) set at 37 °C. Light paraffin oil was applied to the edge of the protein dispersion to prevent evaporation. Oscillatory measurements were performed at fixed time intervals over an incubation period of 12 h at a fixed frequency of 1 Hz and a strain amplitude of 0.05, which was within the linear viscoelastic region by previous tests. The storage modulus (G'), loss modulus (G''), and loss tangent (tan δ) were computed from raw oscillatory data using Stresstech software, version 3.0 (Rheologica Instruments AB). The effect of oscillatory frequency on the dynamic rheological properties of the gel network formed at selected time intervals was evaluated at 23 °C. Frequencies were selected from 0.01 to 10 Hz, and the strain amplitude used was the same as above. Preliminary experiments (data not shown) showed that the relative activity of TG at 23 °C was <20% of the maximum (at ~40 °C), so the residual enzyme activity during rheological measurements can be regarded as minimal.

The rheological properties of heat-induced gels were also evaluated according to the procedures described previously (*31*). Oat globulin dispersions (10% w/v) prepared in 0.01 M phosphate buffer (pH 9.0), containing 0.2 M NaCl, were introduced to the rheometer according to the procedure described above. The protein sample was heated at 100 °C for 20 min by connection to a recirculating boiling water bath, followed by rapid cooling to room temperature by connection to an ice−water bath. Oscillatory measurements were performed on the final gels after equilibration for at least 30 min.

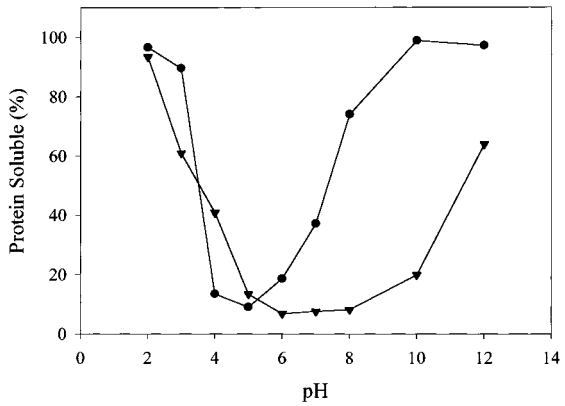

Figure 1. Solubility curves of oat globulin: (●) native oat globulin; (▼) transglutaminase-polymerized oat globulin (4 h of incubation). Error bars represent standard deviations of the means.

Microstructure of TG-Induced and Heat-Induced Gels. The microstructure of oat globulin gels formed by TG and heat treatments was examined by scanning electron microscopy (SEM). TG-induced and heat-induced gels were prepared according to procedures described above, using stainless steel molds (3 mm i.d., 2 mm height) sealed with a bottom steel plate and a top glass plate, similar to that described by Chanyongvorakul et al. (*19*). Gel samples were fixed and dehydrated according to the method of Chanyongvorakul et al. (*19*), and the microstructure was examined with a Cambridge SEM 360 (Leica Cambridge Ltd.) at a voltage of 10 kV.

FTIR spectroscopy was used to follow structural changes in oat globulin during TG-induced gelation. Oat globulin dispersions (10% w/v) were prepared in D_2O instead of H_2O because D_2O has greater transparency in the infrared region of interest (1600−1700 cm^{-1}). To ensure complete H/D exchange, samples were prepared 1 day before and kept at 4 °C prior to infrared measurements. TG was added to the protein dispersion at the same level as described above just prior to FTIR measurements.

Infrared spectra of the TG−oat globulin mixture were recorded in a Bio-Rad Excalibur FTIR spectrometer (Bio-Rad Laboratories, Cambridge, MA) equipped with a deuterated triglycine sulfate (DTGS) detector. Samples were held in an IR cell with a 25 μm path length CaF_2 window. An Omega temperature controller (Omega Engineering, Stanford, CA) was used to hold the temperature at 37 °C, with an accuracy of ±0.5 °C, and the IR spectra were recorded at fixed time periods. The scans, performed at 4 cm^{-1} resolution, were averaged. Preliminary data (not shown) showed that increasing the number of scans from 32 to 64, 128, 256, and 512 did not significantly improve the resolution of the IR spectra. Hence, 32 scans were used for most experiments to save time, which was particularly important for monitoring changes during TG treatment at selected time intervals. Deconvolution of the infrared spectra was performed using Bio-Rad software (Merlin version 1) and according to the method of Kauppinen et al. (*32*). The half width used for deconvolution was 10 cm^{-1}, and the enhancement factor, γ, was 2.0. Band assignment of oat globulin in the amide I region (1600−1700 cm^{-1}) followed the system of Susi and Byler (*33*).

Statistical Analysis. Data were analyzed using a SAS program (*34*). Pairwise comparison of all treatment means was performed using the least significant differences (LSD) procedure at $P \leq 0.05$.

RESULTS AND DISCUSSION

Functional Properties. Figure 1 shows the pH-solubility curves of the native and TG-treated oat globulin. The native protein shows a typical bell-shaped solubility curve with minimum solubility near pH 5, corresponding to the isoelectric point of oat globulin (*24*). TG treatment led to a broadening of the solubility curve and a shift of the pH minimum to higher pH value. The polymerized oat globulin has lower solubility

Table 1. Some Functional Properties of Unmodified and Transglutaminase-Polymerized Oat Globulin[a]

property[b]	unmodified protein	TG-polymerized protein
bulk density (g/mL)	0.52 ± 0.01	0.20 ± 0.01
FBC (mL/g)	1.6 ± 0.11	4.5 ± 0.04
WHC (mL/g)	1.0 ± 0.03	1.5 ± 0.10
EAI (m^2/g)	21.4 ± 0.47	7.51 ± 0.24
ESI (min)	6.90 ± 0.10	4.29 ± 0.63
foamability (%)	64 ± 2.0	140 ± 2.2
foam stability (%)	55 ± 0.10	32 ± 0.11

[a] Means ± standard deviations. [b] FBC, fat-binding capacity; WHC, water hydration capacity; EAI, emulsifying activity index; ESI, emulsion stability index; foam stability was determined at 30 min after foam formation.

than the control over a wide range of pH. However, the solubility was slightly increased at pH 5 and markedly increased at pH 4 (**Figure 1**), probably due to the shift of the solubility minimum. The decreased solubility could be attributed to increase in molecular size or the elimination of cationic ammonium groups of lysyl residues in the polymerized protein. Cross-linking of cationic lysyl residues with glutamyl residues could lower the overall charge density and could decrease protein solubility. Lowered solubility was also observed in the TG-induced soy glycinin homologous polymers (*16*) and whey protein/soy glycinin heterologous polymers (*35*).

The increased solubility at pH 4 and 5 could be due to altered intra- and intermolecular charge repulsion in the polymerized oat globulin. This suggests that hydrogen bonds and ionic and dipole−dipole interactions between the polymerized protein and water were effective at specific pH (*35*).

Table 1 shows the effects of TG treatment on some functional properties of oat globulin. Results indicate that the water hydration capacity (WHC), fat-binding capacity (FBC), and foamability were significantly ($P < 0.5$) increased by TG treatment, whereas the emulsifying activity index (EAI), emulsion stability index (ESI), and foam stability were decreased ($P < 0.5$). The increase in WHC (the ability to swell and take up water) in the polymerized oat globulin could be due to the formation of large clusters of protein molecules. The increase in FBC can be partly attributed to marked decrease in bulk density because fat absorption depends on the physical entrapment of oil (*36*).

The decreases in EAI and ESI may be due to low solubility of the polymerized protein. Solubility is an important factor in determining the emulsifying properties of proteins (*37, 38*). As solubility decreases, the ability of the protein to form and stabilize emulsions is reduced. The TG-catalyzed cross-linking of oat globulin may also lead to loss of flexibility and the ability to unfold at the oil−water interface, properties essential for emulsion formation (*39*). The results are in agreement with those reported in TG-treated soy glycinin (*16*) and cowpea globulin (*40*).

The increase in foamability in the polymerized oat globulin could be attributed to increased hydrodynamic size (*41*), molecular weight (*42*), and elimination of lysyl charged amino groups. Although the combined effect of these factors could also improve foam stability (*43, 44*), a decrease in foam stability was observed in the present study. Improvement in foaming properties was observed in TG-treated soy glycinin (*35*).

Rheological Properties of TG-Induced Gels. Results showed that TG treatment of oat globulin dispersions at a concentration of 10% or above led to the formation of a semitransparent, self-supporting gel network, quite different from the heat-set gels

Table 2. Dynamic Viscoelastic Properties of Transglutaminase-Induced and Heat-Induced Gels of Oat Globulin[a]

incubation time (h)	G'[b] (dyn·cm^{-2})	G''[c] (dyn·cm^{-2})	tan δ[d]
1	43.9	70.8	1.47
2	326	205	0.63
4	2030	672	0.33
8	5740	2070	0.36
12	5110	1650	0.32
heat-induced gel[e]	340	118	0.35

[a] Averages of duplicate determinations. [b] Storage modulus. [c] Loss modulus. [d] Loss tangent. [e] Heat-induced gel was formed by heating the oat globulin dispersion (10%, pH 9.0) at 100 °C for 20 min.

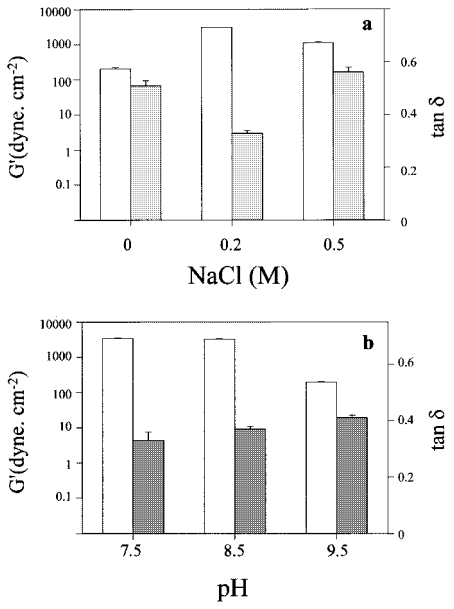

Figure 2. Effect of salt concentration (a) and pH (b) on storage modulus (G', open bars) and loss tangent (tan δ, solid bars) of oat globulin gels induced by transglutaminase treatment. Error bars represent standard deviations of the means.

at the same concentrations (*31*), which were off-white and opaque. Heat-set gels of oat globulin can be formed only at alkaline pH and in the presence of added salt (*31*). Added salt was also required for the formation of enzyme-induced oat globulin gels.

Table 2 shows the changes in rheological properties of oat globulin gel during TG incubation. Both G' and G'' values increased progressively with increase in incubation time, reaching a maximum at 8 h. The tan δ value decreased rapidly, leveling off after 4 h. The thermal-induced oat globulin gel, formed in situ by heating a 10% protein dispersion at 100 °C for 20 min, had much lower storage and loss modulus and slightly higher loss tangent value when compared with TG-induced gels after 4 h of incubation (**Table 2**).

Figure 2 shows the effects of salt and pH on the rheological properties of TG-induced oat globulin gels. Highest G' with lowest tan δ values were observed at 0.2 M NaCl (**Figure 2a**) and pH 7.5−8.5 (**Figure 2b**). The optimal conditions were then selected for preparing gels for microscopic examination.

The effect of oscillatory frequency on the rheological properties of the two types of gel is demonstrated in **Figure 3**. Pronounced frequency dependency for both G' (**Figure 3a**) and G'' (**Figure 3b**) was observed in TG-induced gels after 1 h of incubation. Less frequency dependency was observed after 2 h of incubation, and a flat plateau over a wide range of frequencies

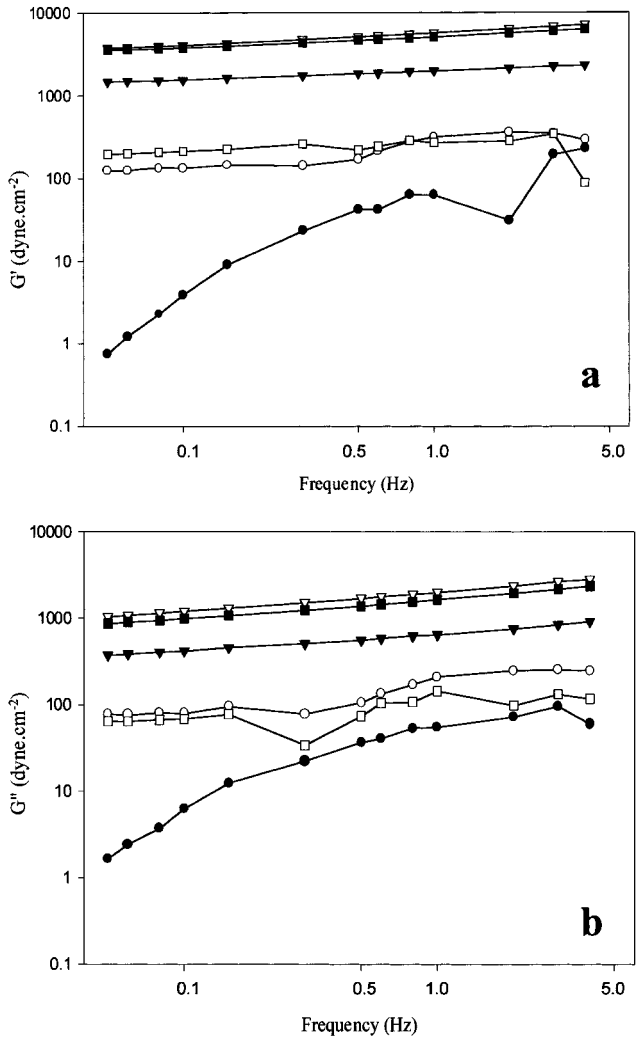

Figure 3. Frequency dependence of storage (a) and loss (b) modulii for oat globulin gels induced by transglutaminase (TG) treatment or heat treatment (100 °C for 20 min): (●) 1 h of TG treatment; (○) 2 h of TG treatment; (▼) 4 h of TG treatment; (▽) 8 h of TG treatment; (■) 12 h of TG treatment; (□) heat-induced gels.

was observed in gel samples after 4 h or longer of enzyme treatment. Some frequency dependency was observed in the heat-induced gel.

The increasing modulus values and decreasing loss tangent value during TG incubation suggest the formation of a viscoelastic network structure. In TG-induced gelation, cross-linking points in the networks are constituted mainly of covalent ϵ-(γ-glutamyl) lysyl bonds (*18*). The association of protein molecules by isopeptide bonds may occur more regularly than heat-induced gels, and the formation of the irreversible bonds may contribute to reinforced protein−protein interactions, leading to a well-developed network structure (*18*). The lower G' and G'' and higher tan δ values of the heat-set gels may be attributed to non-covalent forces including hydrogen bonds and electrostatic and hydrophobic interactions involved in gel network formation (*31*), similar to other heat-induced protein gels (*45*, *46*).

The minimum concentration required to form a self-supporting gel of 11S soybean and broad bean globulins catalyzed by TG was lower than the minimum concentration for thermal gelation (*18*), indicating that the covalent bonding by TG was more effective in forming a gel network. Large deformation tests showed that TG-induced 11S globulin gels were more rigid and elastic than thermally induced gels, and creep compliance experiments showed higher elastic moduli and viscosities (except complex viscosity) in the TG-induced gels (*19*).

The frequency dependence of the shear (or storage) modulus for a gel is characteristic of the gel type (*45*). G' shows a plateau over a wide range of frequencies when the system behaves like a gel (particularly when the network is stabilized by covalent cross-links) but decreases with decreasing frequency when the system behaves like a sol or weak gel (*47*). The low frequency dependence observed in TG-induced oat globulin gels after prolonged incubation indicates the formation of an elastic network. Similar observations were reported in β-lactoglobulin in which heat-set gels were strongly frequency dependent, whereas the TG-induced gels were nearly independent of frequency (*21*). Such behavior is consistent with the TG-induced gel systems having the characteristics of classical polymer gels with permanent "chemical" cross-links, whereas the heat-denatured systems have characteristics typical of a temporary "physical" gel with breakable or deformable cross-links (*48*, *49*).

Despite the difference in chemical forces involved, similar effects of ionic strength on gel strength were observed in both TG-induced (**Figure 2a**) and heat-induced oat globulin gels (*31*). Weak gel network formation in the absence of added salt may be attributed to the predominance of electrostatic forces at low ionic strength (*50*). Suppression of ionic repulsion at appropriate NaCl concentration (0.2 M for TG-induced gels and 0.4 M for heat-induced gels) would enhance protein−protein interactions and the formation of a stable gel network (*51*). At higher salt concentration, however, extensive cross-linking between protein molecules may lead to compaction and collapse of the gel matrices, as indicated by lower G' and tan δ values. In the case of TG-induced gels, ionic strength may also affect the number of isopeptide bonds formed, in relation to enhanced solubility, accessibility, and interactions between enzyme and protein. This will have a direct influence on the observed gel modulus values.

The influence of pH on TG-induced gelation of oat globulin may also be related to charge effect. From the pH-solubility curve (**Figure 1**), the p*I* of the oat globulin polymers is around pH 6−8, and protein−protein interactions are generally optimal near the p*I*, where charge repulsion is balanced by charge attraction. Oat globulin has low solubility near neutral pH, preventing the formation of a self-supporting gel network even at high protein concentration and after extensive heat treatments (*31*). Enhanced solubility by pH adjustment (*31*) or chemical modifications (*4*) is thus required to enhance heat-induced gelation. The present data show that TG treatment can improve gel-forming ability of oat globulin near neutral pH.

Microstructure of TG-Induced and Heat-Induced Gels. **Figure 4** shows the SEM micrographs of both the TG-induced and heat-induced oat globulin gels. Large differences in the organization of gel network structure were observed between the two gel types. Thick strands and large clusters were found in TG-induced gels (**Figure 4a**), whereas thin strands and smaller clusters were observed in the heat-set gels (**Figure 4b**). The results suggest a better developed network structure in the TG-induced gels. Similar results were observed in legume proteins (*19*) where TG-induced gels were composed of larger unit particles forming more developed strands and clusters than heat-induced gels. The differences were again attributed to the involvement of stronger covalent isopeptide cross-links in the TG-induced gels, as compared to weaker non-covalent chemical forces in heat-induced gelation (*19*).

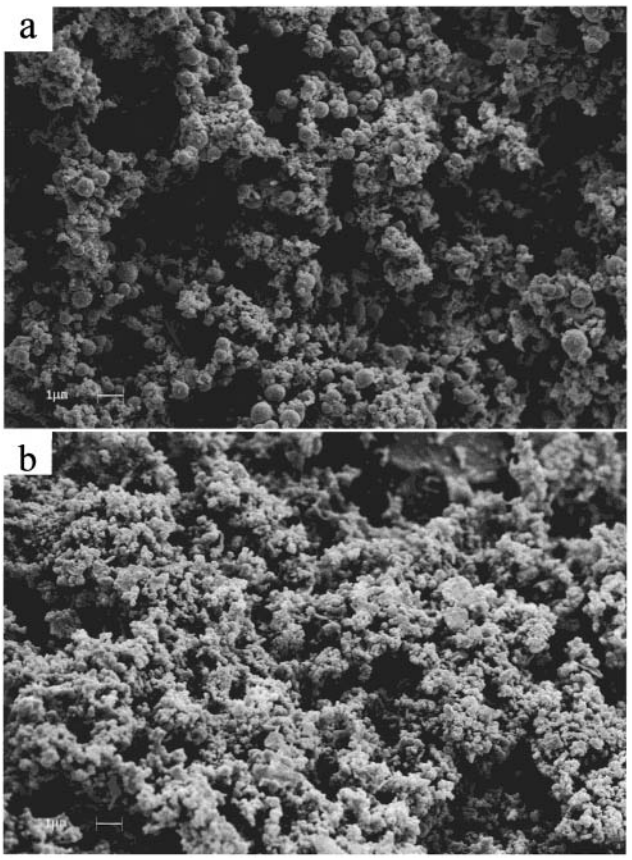

Figure 4. Scanning electron micrographs of oat globulin gels: (a) transglutaminase-induced gel; (b) heat-induced gel.

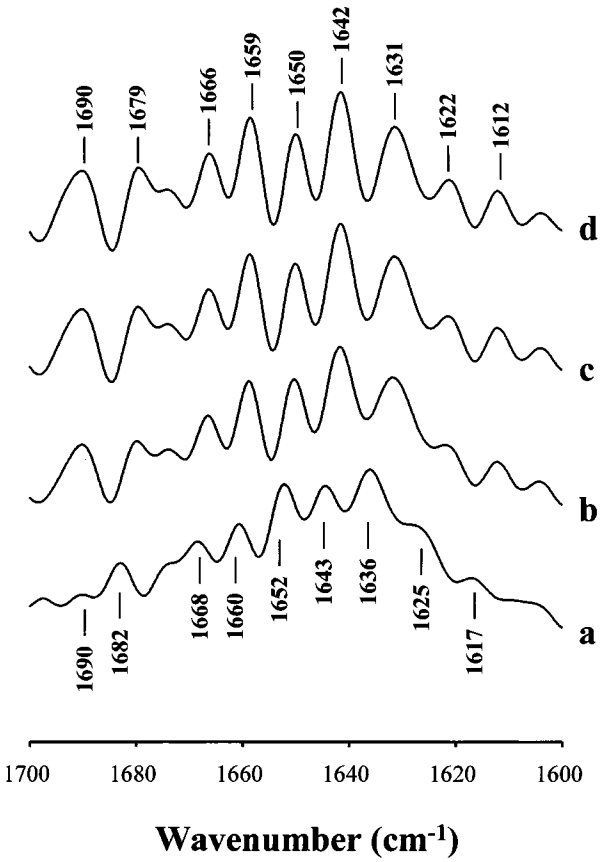

Figure 5. Stacked plot of deconvoluted infrared spectra of oat globulin during transglutaminase-induced gelation: (a) control (0 min); (b) 10 min of TG treatment; (c) 30 min of TG treatment; (d) 240 min of TG treatment.

FTIR Spectroscopy of Oat Globulin during TG-Induced Gelation. Figure 5 shows the deconvoluted FTIR spectra of 10% oat globulin dispersion incubated with TG for different time periods at 37 °C. The control sample, at 0 min of incubation, shows several bands in the amide I region (**Figure 5a**), corresponding to the major secondary structure components: α-helices (1652 cm^{-1}), β-sheets (1636 and 1682 cm^{-1}), β-turns (1660 and 1668 cm^{-1}), and random coils (1643 cm^{-1}). The spectrum is slightly different from that published (*52*), perhaps due to the use of a buffer containing 0.2 M NaCl (versus D_2O) in the present study. Marked shifts in the band positions were observed even with a short incubation time (10 min; **Figure 5b**) with the enzyme, and further shifts were shown upon longer incubation periods (**Figure 5c**,**d**). There were pronounced increases in the intensity of several major bands (1622−1625, 1631−1636, 1642−1643, 1659−1660, 1679−1682, and 1690 cm^{-1}). There were also dramatic increases in two minor bands corresponding to side-chain vibration (1612 cm^{-1}) and β-type (1690 cm^{-1}) structures, respectively (**Figure 5**).

Figure 6 shows that with an increase in TG treatment time, there was a progressive decrease in the intensity of the α-helix band (**Figure 6a**) with a concomitant increase in the random coil band (**Figure 6b**), both leveling off after 30−60 min of incubation. The results suggest marked conformational changes in the protein. There were also pronounced increases in the intensity of two bands (1679−1682 and 1622−1625 cm^{-1}) (**Figure 6c**,**d**), which were attributed to antiparallel β-sheets and strands and were associated with the aggregation process (*33*).

The present data demonstrate that TG treatment of oat globulin under conditions that induce gelation could lead to marked alterations in FTIR spectral characteristics, indicating pronounced conformational changes in the protein. Our previous studies (*52*, *53*) also showed marked changes in FTIR and Raman spectra of oat globulin heated under aggregation conditions. There were both similarities and differences in the FTIR spectra and in the changes in band intensity when the heat-induced oat globulin aggregates (*52*) were compared with the TG-induced oat globulin gels. In both systems, marked shift in band positions was observed, with rapid decreases in the intensity of the α-helix band. Although decreases in a β-sheet band (1634 cm^{-1}) were observed only in the heat-induced aggregates, increases in the random coil band were observed only in the TG-treated protein. All of the changes in band intensity occurred rapidly and leveled off after 30−60 min, indicating changes in protein conformation at the early stages of heat or TG treatment. Increases in "aggregation" peaks were also observed in both systems, although in the case of thermal aggregation, only small increases in the 1682 cm^{-1} band were detected (*52*). The data are in agreement with the classical two-stage process of protein aggregation/gelation (*54*). The first stage involves partial protein denaturation, with association of the unfolded polypeptides into aggregates or gel networks occurring during the second stage.

Nagano et al. (*55*) studied the effects of heating on gel properties and conformation of soybean glycinin and β-conglycinin using rheological and FTIR methods. They observed that the increase in storage modulus during gel formation corresponded to the increase in absorbance at 1618 cm^{-1}, suggesting that heat-induced gels of glycinin and β-conglycinin are formed by cross-links with intermolecular β-sheet structures. Our data also show the concomitant increases in both G' and two FTIR bands associated with the formation of antiparallel β-sheets and strands, suggesting that the formation of TG-

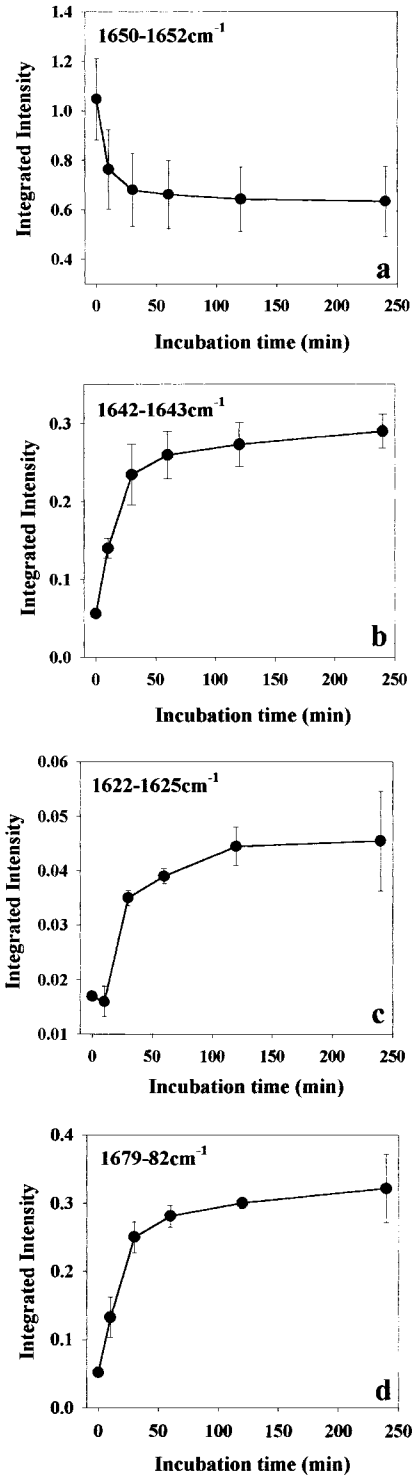

Figure 6. Plot of integrated intensity of (a) 1650–1652 cm^{-1} band, (b) 1642–1643 cm^{-1} band, (c) 1622–1625 cm^{-1} band, and (d) 1679–1682 cm^{-1} band in the infrared spectra of 10% oat globulin treated with transglutaminase for different time periods. Error bars represent standard deviations of the means.

induced oat globulin gels may be partially attributed to cross-links of intermolecular β-sheets. Contrary to the linear relationship between percent change in absorbance to G' in the soybean protein gels (*55*), our data show that the intensity of the two "aggregation" bands increased more rapidly than the increases in G' and leveled off after 1 h of TG incubation, but G' continued to increase after 4 h of enzyme treatment. The discrepancy is likely due to the dependence of TG-induced gelation on the formation of other covalent bonds, that is, the ϵ-(γ-glutamyl) lysyl bonds, particularly during the later part of the gelation process. The data suggest that the formation of the initial several isopeptide bonds in the first few minutes would have a major effect on the conformation of the polypeptides. However, the gel network could be quickly fixed with a few covalent bonds and would not be modified structurally thereafter, but only be reinforced by additional covalent bonds and other connectivity.

Conclusions. The present study showed that polymerization of oat globulin by a microbial transglutaminase can lead to significant improvement in some functional properties such as water- and fat-binding capacities. This could enhance the utilization of the protein in specific food systems such as comminuted meats, similar to acylated oat protein isolates with which an improvement in performance in a model weiner system was observed (*5*). Increased solubility of the polymerized oat globulin at pH 4−5 suggests that the product may be used in acidic beverages. Transglutaminase treatment also improved the gelation properties of oat globulin, particularly near neutral pH. This could eliminate the need for the use of alkaline pH or chemical modifications to improve solubility, a prerequisite for enhancing the heat-induced gelation properties of the protein.

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LITERATURE CITED

(1) Hischke, H. H., Jr.; Potter, G. C.; Graham, W. R., Jr. Nutritive value of oat proteins. I. Varietal differences as measured by amino acid analysis and rat growth responses. *Cereal Chem.* **1968**, *45*, 374−378.

(2) Ma, C.-Y. Preparation, composition and functional properties of oat protein isolates. *Can. Inst. Food Sci. Technol. J.* **1983**, *16*, 201−205.

(3) Ma, C.-Y. Functional properties of acylated oat protein. *J. Food Sci.* **1984**, *49*, 1128−1131.

(4) Ma, C.-Y.; Khanzada, G. Functional properties of deamidated oat protein isolates. *J. Food Sci.* **1987**, *52*, 1583−1587.

(5) Ma, C.-Y.; Wood, D. F. Functional properties of oat proteins modified by acylation, trypsin hydrolysis or linoleate treatment. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1726−1731.

(6) Panyam, D.; Kilara, A. Enhancing the functionality of food proteins by enzymatic modification. *Trends Food Sci. Technol.* **1996**, *7*, 120−125.

(7) Adler-Nissen, J. Some fundamental aspects of food protein hydrolysis. In *Enzymic Hydrolysis of Food Proteins*; Adler-Nissen, J., Ed.; Elsevier Applied Science: London, U.K., 1986; pp 9−24.

(8) Folk, J. E. Transglutaminases. *Annu. Rev. Biochem.* **1980**, *49*, 517−531.

(9) Folk, J. E.; Finlayson, J. S. The ϵ-(γ-glutamyl) lysine cross-link and the catalytic role of transglutaminase. *Adv. Protein Chem.* **1977**, *31*, 1−133.

(10) Icekson, I.; Apelbaum, A. Evidence for transglutaminase activity in plant tissue. *Plant Physiol.* **1987**, *84*, 972−974.

(11) Araki, H.; Seki, N. Comparison of reactivity of tranglutaminase to various fish actomyosin. *Bull. Jpn. Soc. Sci. Fish.* **1993**, *59*, 711−716.

(12) Ando, H.; Adachi, M.; Umeda, K.; Matsuura, A.; Nonaka, M.; Uchio, R.; Tanaka, H.; Motoki, M. Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agric. Biol. Chem.* **1989**, *53*, 2613−2617.

(13) Motoki, M.; Seguro, K. Transglutaminase and its use for food processing. *Trends Food Sci. Technol.* **1998**, *9*, 204−210.

(14) Zhu, Y.; Rinzema, A.; Tramper, J.; Bol, J. Microbial transglutaminase—a review of its production and application in food processing. *Appl. Microbiol. Biotechnol.* **1995**, *44*, 277−282.
(15) Yildirim, M.; Hettiarachchy, N. S. Biopolymers produced by cross-linking soybean 11S globulin with whey proteins using transglutaminase. *J. Food Sci.* **1997**, *62*, 270−275.
(16) Motoki, M.; Nio, N.; Takinami, K. Functional properties of food proteins polymerized by transglutaminase. *Agric. Biol. Chem.* **1984**, *48*, 1257−1261.
(17) Nonaka, M.; Sakamoto, H.; Toiguchi, S.; Kawajiri, H.; Soeda, T.; Motoki, M. Sodium caseinate and skim milk gels formed by incubation with microbial transglutaminase. *J. Food Sci.* **1992**, *57*, 1214−1219.
(18) Chanyongvorakul, Y.; Matsumura, Y.; Ikura, K.; Motoki, M.; Sakamoto, H.; Mori, T. Gelation of bean 11S globulins by Ca^{2+}-independent transglutaminase. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 864−869.
(19) Chanyongvorakul, Y.; Matsumura, Y.; Nonaka, M.; Motoki, M.; Mori, T. Physical properties of soy bean and broad bean 11S globulin gels formed by transglutaminase reaction. *J. Food Sci.* **1995**, *60*, 483−488.
(20) Kang, J.; Matsumura, Y.; Ikura, K.; Motoki, M.; Sakamoto, H.; Mori, T. Gelation and gel properties of soybean glycinin in a transglutaminase-catalyzed system. *J. Agric. Food Chem.* **1994**, *42*, 159−165.
(21) Dickinson, E.; Yamamoto, Y. Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *J. Agric. Food Chem.* **1996**, *44*, 1371−1377.
(22) Koksel, H.; Sivri, D.; Ng, P. K. W.; Steffe, J. F. Effects of transglutaminase enzyme on fundamental rheological properties of sound and bug-damaged wheat flour doughs. *Cereal Chem.* **2001**, *78*, 26−30.
(23) Siu, N.-C.; Ma, C.-Y.; Mine, Y. Physicochemical and structural properties of oat globulin polymers formed by a microbial transglutaminase. *J. Agric. Food Chem.* **2002**, *50*, 2660−2665.
(24) Ma, C.-Y.; Harwalkar, V. R. Chemical characterization and functionality assessment of oat protein fractions. *J. Agric. Food Chem.* **1984**, *32*, 144−149.
(25) Concon, J. M.; Soltess, D. Rapid micro-Kjeldahl digestion of cereal grains and other biological materials. *Anal. Biochem.* **1973**, *53*, 35−41.
(26) Morr, C. V.; German, B.; Kinsella, J. E.; Regenstein, J. M.; Van Buren, J. P.; Kilara, A.; Lewis, B. A.; Mangino, M. E. A collaborative study to develop a standardized food protein solubility procedure. *J. Food Sci.* **1985**, *50*, 1715−1718.
(27) Quinn, J. R.; Paton, D. A practical measurement of water hydration capacity of protein materials. *Cereal Chem.* **1979**, *56*, 38−40.
(28) Sosulski, F.; Jones, J. D. Functional properties of rapeseed flours, concentrates and isolates. *J. Food Sci.* **1976**, *41*, 1346−1352.
(29) Pearce, K. N.; Kinsella, J. E. Emulsifying properties of protein: evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716−723.
(30) Yasumatsu, K.; Sawada, K.; Moritaka, S.; Misaki, M.; Toda, J.; Wada, T.; Ishii, K. Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem.* **1972**, *36*, 719−727.
(31) Ma, C.-Y.; Khanzada, G.; Harwalkar, V. R. Thermal gelation of oat globulin. *J. Agric. Food Chem.* **1988**, *36*, 275−280.
(32) Kauppinen, J. K.; Moffat, D. J.; Mantsch, H. H.; Cameron, D. G. Fourier transforms in the computation of self-deconvoluted and first-order derivative spectra of overlapped band contours. *Anal. Biochem.* **1981**, *53*, 1454−1457.
(33) Susi, H.; Byler, D. M. Fourier transform infrared spectroscopy in protein conformation studies. In *Methods for Protein Analysis*; Cherry, J. P., Barford, R. A., Eds.; American Oil Chemists' Society: Champaign, IL, 1988; pp 235−250.
(34) SAS Institute, Inc. *SAS User's Guide, Statistics*, version 6.12; SAS Institute Inc.: Cary, NC, 1997.
(35) Yildirim, M.; Hettiarachchy, N. S.; Kalapathy, U. Properties of biopolymers from cross-linking whey protein isolate and soybean 11S globulin. *J. Food Sci.* **1996**, *61*, 1129−1131.
(36) Kinsella, J. E. Functional properties of food proteins: A review. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *7*, 219−280.
(37) Mangino, M. E. Protein interactions in emulsions: Protein−lipid interactions. In *Protein Functionality in Food Systems*; Hettiarachchy, N. S., Ziegler, G. R., Eds.; Dekker: New York, 1994; pp 147−180.
(38) Zayas, J. F. *Functionality of Proteins in Food*; Springer-Verlag: Berlin, Germany, 1997.
(39) McClements, D. J. *Food Emulsions—Principles, Practice and Techniques*; CRC Press: Boca Raton, FL, 1999.
(40) Aluko, R. E.; Yada, R. Y. Effect of a microbial calcium-independent transglutaminase on functional properties of a partially purified cowpea (*Vigna unguiculata*) globulin. *J. Sci. Food Agric.* **1999**, *79*, 286−290.
(41) Damodaran, S. Structure−function relationship of food proteins. In *Protein Functionality in Food Systems*; Hettiarachchy, N. S., Ziegler, G. R., Eds.; Dekker: New York; 1994; pp 1−37.
(42) Mita, T.; Ishida, E.; Matsumoto, H. Physicochemical studies on wheat protein foams II. Relationship between bubble size and stability of foams prepared with gluten and gluten components. *Colloid Interface Sci.* **1978**, *64*, 143−146.
(43) Kinsella, J. E. Functional properties of proteins: possible relationships between structure and function in foams. *Food Chem.* **1981**, *7*, 273−288.
(44) Damodaran, S. Interfaces, protein films, and foams. *Adv. Food Nutr. Res.* **1990**, *34*, 1−79.
(45) Clark, A.; Ross-Murphy, S. Structural and mechanical properties in biopolymer gels. *Adv. Polym. Sci.* **1987**, *83*, 57−192.
(46) Oakenfull, D.; Pearce, J.; Burley, R. W. Protein gelation. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Dekker: New York, 1997; pp 111−142.
(47) Matsumura, Y.; Mori, T. Gelation. In *Method of Testing Protein Functionality*; Hall, G. M., Ed.; Blackie Academic and Professional: London, U.K., 1996; pp 76−109.
(48) Ferry, J. D. *Viscoelastic Properties of Polymers*, 3rd ed.; Wiley: New York, 1980.
(49) Ross-Murphy, S. B. Rheological methods. In *Biophysical Methods in Food Research*; Chan, H. W.-S., Ed.; Blackwell: Oxford, U.K., 1984; pp 138−199.
(50) Kinsella, J. E. Relationship between structure and functional properties of food proteins. In *Food Proteins*; Fox, P. F., Ed.; Applied Science: London, U.K., 1982; pp 51−103.
(51) Damodaran, S.; Kinsella, J. E. Effects of ions on protein conformation and functionality. In *Food Protein Deterioration, Mechanisms and Functionality*; ACS Symposium Series 206; Cherry, J. P., Ed.; American Chemical Society: Washington, DC, 1982; pp 327−357.
(52) Ma, C.-Y.; Rout, M. K.; Mock, W.-Y. Study of oat globulin conformation by Fourier transform infrared spectroscopy. *J. Agric. Food Chem.* **2001**, *49*, 3328−3334.
(53) Ma, C.-Y.; Rout, M. K.; Chan, W.-M.; Phillips, D. L. Raman spectroscopic study of oat globulin conformation. *J. Agric. Food Chem.* **2000**, *48*, 1542−1547.
(54) Ferry, J. Protein gels. *Adv. Protein Chem.* **1948**, *4*, 1−78.
(55) Nagano, T.; Akasaka, T.; Nishinari, K. Dynamic viscoelastic properties of glycinin and β-conglycinin gels from soybeans. *Biopolymers* **1994**, *34*, 1303−1309.

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