Rheology of Milk Protein Gels and Protein-Stabilized Emulsion Gels Cross-Linked with Transglutaminase

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Oscillatory shear measurements have been used to investigate the rheological properties of enzymically cross-linked milk protein gels at neutral pH with and without emulsion droplets. A Ca^{2+}-independent transglutaminase extracted from microorganisms was used as the enzyme source. Storage and loss moduli are presented for gels formed from enzyme-treated β-lactoglobulin solutions (13 and 14 wt % protein) and β-lactoglobulin-stabilized emulsions (7−9 wt % protein, 32.5 wt % oil). The frequency dependence of the small-deformation elastic moduli of the enzyme-treated gels is weaker than for the equivalent heat-set β-lactoglobulin gels (90 °C for 30 min), and the strain dependence of the elastic moduli of the enzyme-treated gels is of opposite sign to that of the heat-set gels at large deformations. These differences in rheological behavior are consistent with a network consisting of permanent covalent cross-links for the enzyme-induced gels and predominantly physical cross-links for the heat-set gels. Thermal processing after enzyme treatment is very effective in making a strong gel from either a β-lactoglobulin solution or a β-lactoglobulin-stabilized emulsion. Lecithin addition to the β-lactoglobulin-stabilized emulsion gel before enzyme treatment was found to have a weakly positive effect on the gel strength arising from lecithin−protein complexation. When β-lactoglobulin was replaced with sodium caseinate, the rate and extent of enzyme-induced cross-linking was found to increase substantially.

Keywords: *Emulsion gel; transglutaminase; β-lactoglobulin; sodium caseinate; enzymic cross-linking; thermal denaturation; rheology; lecithin−protein interaction*

Milk protein gels are traditionally formed by treating casein with acid or proteolytic enzyme (chymosin) or by thermal denaturation of whey proteins. With the exception of some intermolecular disulfide bond formation in whey protein gels, the resulting network structure in such milk protein gels is typically held together by noncovalent physical cross-links—electrostatic interactions, hydrogen bonding, and hydrophobic bonds (Phillips *et al.*, 1994). An alternative way of making a milk protein gel would be by enzymically cross-linking the protein molecules to produce a network of covalent linkages. Because of the different cross-links, such a protein network might be expected to have quite different rheological properties from a conventional milk protein gel.

The enzyme transglutaminase (glutaminyl-peptide γ-glutamyltransferase, EC 2.3.2.13) catalyzes the acyl transfer reaction between protein-bound glutaminyl residues and primary amines. When the ϵ-amino groups of lysine residues act as acyl acceptors, ϵ-(γ-glutamyl) lysine cross-links are formed (Folk and Finlayson, 1977). Hence, transglutaminase catalyzes the polymerization and gelation of soluble proteins through the formation of intermolecular covalent cross-links (Nio *et al.*, 1985). The cross-linking rate depends on the accessibility of reactive lysine and glutaminyl residues in the protein substrate. While the flexible bovine caseins are known to be good substrates of transglutaminase (Ikura *et al.*, 1980, 1984; Nio *et al.*, 1985, 1986; Nonaka *et al.*, 1989; Traoré and Meunier, 1991, 1992; Sakamoto *et al.*, 1994), the globular whey proteins have been shown to be poor substrates (Ikura *et al.*, 1984; Nonaka *et al.*, 1989; Aboumahmoud and Savello, 1990; Traoré and Meunier, 1992). It has been reported that globular proteins become more susceptible to polymerization by transglutaminase following partial unfolding after chemical modification (Ikura *et al.*, 1984) or coexistence with reducing agents such as dithiothreitol and cysteine (Nonaka *et al.*, 1989; Aboumahmoud and Savello, 1990; Traoré and Meunier, 1992). As the protein located at the oil−water interface in emulsions is partially unfolded, the susceptibility to transglutaminase of globular proteins adsorbed at the surface of emulsion oil droplets might also be expected to increase.

While Ca^{2+}-dependent transglutaminases are widely distributed in various animal tissues and organs (Folk and Finlayson, 1977), and various biological functions have been proposed for them, the use of mammalian transglutaminase for large-scale food-processing operations is recognized to be impractical because of the high cost of extraction and purification. This situation has been potentially transformed by the discovery of microbial transglutaminase produced by *Streptoverticillium* species (Ando *et al.*, 1989). A remarkable characteristic of this enzyme is its Ca^{2+}-independent catalytic property. Its increasing availability offers the opportunity for using this microbial enzyme as a powerful tool in novel minimal processing technology.

In our laboratory we are currently interested in the rheology of particle gels formed from networks of aggregated protein-coated emulsion droplets. Considerable research has already been carried over the past few years on the effect of globular protein-coated oil droplets on the microstructure and rheology of heat-set emulsion gels containing small-molecule surfactants (Jost *et al.*, 1986, 1989; Aguilera and Kessler, 1989; Xiong and Kinsella, 1991; Yost and Kinsella, 1992; Aguilera *et al.*, 1993; Dickinson and Hong, 1995; Dickinson and Yama-

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moto, 1996a,b). On the other hand, relatively little research has been carried out so far on emulsion gels formed by enzymic cross-linking (Nio *et al.*, 1986; Matsumura *et al.*, 1993), and apparently no work has been done on globular protein gels or protein-stabilized emulsion gels formed by a combination of enzymic cross-linking and heat treatment. A systematic investigation of the effect of oil droplets on the mechanical properties of transglutaminase-induced gels is therefore of interest. In this paper we report the oscillatory shear rheological measurements of emulsion gels cross-linked using Ca^{2+}-independent transglutaminase with β-lactoglobulin or sodium caseinate as the protein emulsifier. We also investigate the effects of (i) heat treatment following enzyme-induced gelation of β-lactoglobulin systems and (ii) lecithin addition to the β-lactoglobulin-stabilized emulsion prior to the enzyme treatment.

MATERIALS AND METHODS

Materials. Transglutaminase (protein-glutamin:amino γ-glutamyltransferase, EC 2.3.2.13) derived from the microorganism *Streptoverticillium* (sp. no. 8112) with a nominal specific activity of 3.0 units/mg was supplied by Ajinomoto Co. Inc. (Japan). Spray-dried sodium caseinate (dry protein content 94.5 wt %) was obtained from De Melkindustrie Veghel (Netherlands). Bovine β-lactoglobulin (L0130, lot 91H7005), *n*-tetradecane (>99 wt %), *N*-α-carbobenzoxy-L-glutaminylglycine (C6154), hydroxylamine−HCl (H9876), and soybean oil (S7381) were obtained from Sigma Chemical Co. Two kinds of technical grade lecithin, "crude egg lecithin" (P9671, ∼60% L-α-phosphatidylcholine) and "crude soybean lecithin" (P3644, ∼40% L-α-phosphatidylcholine), were also obtained from Sigma. Buffer solutions were prepared from analytical grade reagents and double-distilled water.

Emulsion Preparation. Oil-in-water emulsions (38 wt % oil) were prepared at room temperature from various concentrations of protein solution (β-lactoglobulin or sodium caseinate, or a mixture of the two) in 0.02 M bis-tris buffer (pH 7.0) as previously described (Dickinson and Yamamoto, 1996a). Mainly the oil phase was *n*-tetradecane, but in a few experiments it was soybean oil. Freshly prepared protein-stabilized emulsion was mixed with transglutaminase solution in 0.02 M bis-tris buffer (pH 7.0) to give emulsions containing a constant proportion of oil (32.5 wt %) and various concentrations of protein.

Lecithin was added to some of the β-lactoglobulin-stabilized emulsions prior to the addition of enzyme. The lecithin sample was dissolved in 0.02 M bis-tris buffer (pH 7.0) and added to the emulsion to give a lecithin−protein molar ratio $R = 8$, calculated by assuming molecular masses of 716 and 18 360 Da for phosphatidylcholine and β-lactoglobulin, respectively.

Transglutaminase Reaction. Time-dependent transglutaminase activity in 0.02 M bis-tris buffer (pH 7.0) at 40 or 55 °C was determined by measuring peptide-bound γ-glutamyl hydroxamate formed from *N*-carbobenzoxy-L-glutaminylglycine and hydroxylamine (Folk, 1971).

Immediately prior to determination of viscoelastic properties, a sample of protein solution or protein-stabilized emulsion was mixed with a transglutaminase solution at pH 7.0 to give an enzyme to protein weight ratio of 1:25. The enzyme-catalyzed cross-linking reaction was conducted at 40 °C in most of the experiments.

Droplet Size Determination. The droplet size distribution and average droplet diameter d_{43} of the freshly prepared emulsions and the emulsions treated with transglutaminase at 40 °C were measured using a Malvern Mastersizer S2.01. For the determination of the effect on protein emulsifying capacity of enzyme treatment prior to emulsification, a solution of β-lactoglobulin or sodium caseinate at pH 7.0 was mixed with transglutaminase at 55 °C for 2 h, and this was used to prepare an emulsion (32.5 wt % *n*-tetradecane; 6.0 wt % β-lactoglobulin or 3.0 wt % sodium caseinate), which was then examined for its droplet size distribution.

Table 1. Average Droplet Diameter d_{43} of Protein-Stabilized Emulsions (6 wt % β-Lactoglobulin or 3 wt % Sodium Caseinate, 32.5 wt % *n*-Tetradecane) Subjected to Transglutaminase Treatment at 40 °C for Various Lengths of Time

protein emulsifier	time (h)	d_{43}[a] (μm)
β-lactoglobulin	0	0.71
	0.25	0.88
	0.5	1.02
	1.0	1.01
	2.0	1.30
	5.0	1.23
	2.0[b]	2.28
sodium caseinate	0	1.12
	0.25	2.60
	0.5	2.70
	1.0	3.53
	3.0	5.48
	2.0[b]	0.98

[a] Defined by $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is number of droplets of diameter d_i. [b] Protein treated with transglutaminase at 55 °C *before* emulsification.

Rheological Measurement. Viscoelasticity was investigated by dynamic oscillatory rheometry using a controlled stress Bohlin CS-50 rheometer. A 2 mL sample of protein solution or protein-stabilized emulsion containing transglutaminase was poured into the Couette-type cylindrical cell (2.5 cm i.d., 2.75 cm o.d.) of the rheometer and covered with a thin layer of low-viscosity silicone oil to prevent evaporation. For samples with enzyme present, gelation was induced *in situ* by incubation at 40 °C. For β-lactoglobulin samples without enzyme, gelation was induced by subjecting the sample to the following thermal treatment process: temperature increased at constant rate of 2 K min^{-1} from 40 to 90 °C, kept at 90 °C for 30 min, cooled at 1 K min^{-1} from 90 to 30 °C, and kept at 30 °C for 15 min. Some samples were subjected to this thermal treatment after the enzyme treatment.

Small deformation shear rheological properties were mostly determined in the linear viscoelastic regime (maximum strain amplitude 0.5%) with storage and loss moduli (G' and G'') measured at a constant frequency of 1 Hz. In addition, some small deformation measurements were made as a function of frequency (2×10^{-3} to 2 Hz), and some large deformation measurements were carried out at strains up to nearly 100%. For the enzyme cross-linked gel (formed by the enzyme reaction at 55 °C for 2 h), the frequency dependency experiment was performed at 55 °C.

RESULTS AND DISCUSSION

Emulsion Droplet Size Distributions. The effect of transglutaminase treatment *after* emulsification on the aggregation of protein-coated emulsion droplets was studied by monitoring time-dependent changes in the droplet size distribution following addition of the enzyme. Table 1 shows values of the average diameter d_{43} for emulsions stabilized by β-lactoglobulin or sodium caseinate subjected to various treatment times. We can see that after just 15 min there is a significant increase in the effective average droplet size for both types of emulsion. This can be attributed to emulsion flocculation caused by the linking together of adsorbed protein molecules attached to different droplets. Figure 1 shows that just 15 min of enzyme treatment leads to a very pronounced broadening of the cumulative size distribution for the caseinate-stabilized droplets. It is clear that the rate of cross-linking of the casein-coated droplets is much faster than that for the β-lactoglobulin-coated droplets; this is consistent with the much greater susceptibility of casein to the enzyme reported previously (Ikura *et al.*, 1980, 1984; Nio *et al.*, 1985, 1986; Nonaka *et al.*, 1989; Traoré and Meunier, 1991, 1992; Sakamoto *et al.*, 1994).

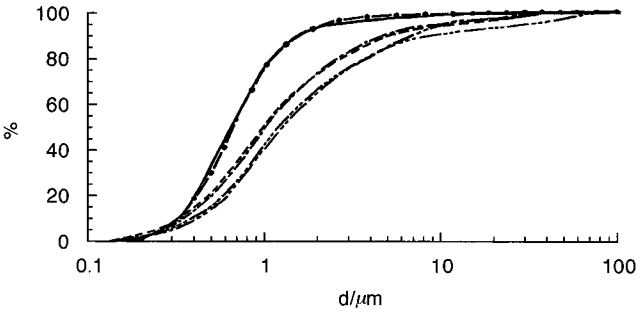

Figure 1. Influence of transglutaminase treatment at 40 °C on the cumulative droplet size distribution of a caseinate-stabilized emulsion (3 wt % protein, 32.5 wt % *n*-tetradecane, pH 7). The plot shows the volume percentage of droplets with diameter less than *d*: (—), untreated emulsion; emulsion treated for (- - -) 15 min, (— · —) 30 min, (— · · —) 1 h, and (— ··· —) 3 h; and (— ● —) emulsion made with enzyme-treated caseinate (55 °C for 2 h).

Some experiments were also carried out on emulsions made with milk protein that had been treated with transglutaminase (at 55 °C for 2 h) *before* emulsification. Here we observed an extremely large difference in behavior between β-lactoglobulin and sodium caseinate. The emulsion made with the enzyme-treated β-lactoglobulin (6 wt %) was found to be very coarse, with a d_{43} value more than 3 times larger than that made with the untreated whey protein (Table 1). In contrast, the emulsion made with enzyme-treated caseinate (3 wt %) had the same cumulative droplet size distribution as that made with untreated caseinate (Figure 1) and the same d_{43} value within experimental error (Table 1). What this means is that, although the casein molecules are presumably extensively polymerized by the enzyme treatment, the polymerization has apparently no significant influence on casein adsorption properties during emulsification or the ability of casein molecules to establish an effective steric stabilizing layer around freshly formed fine emulsion droplets. On the other hand, the globular β-lactoglobulin, which reacts much more slowly in the presence of transglutaminase, is greatly reduced in emulsifying effectiveness by the treatment. This may be because cross-linking and polymerization of β-lactoglobulin reduces further the molecular flexibility of the globular protein and, hence, its effectiveness in packing at the newly formed oil—water interface to form a coherent stabilizing layer.

Stability of Transglutaminase Activity. We investigated the activity of our sample of enzyme at 40 and 55 °C in 0.02 M bis-tris buffer (pH 7.0) over a reaction time scale similar to that employed in the rheological measurements. The results are illustrated in Figure 2. We have found that the transglutaminase activity is very stable at 40 °C, with about 90% of the original activity remaining after 4 h. At 55 °C, however, the activity decreases rapidly with incubation time, with only 13% of the original activity remaining after 30 min and about 1—2% after 3 h. Our results are broadly consistent with literature data on the thermal stability of transglutaminase under similar reaction conditions: in the work of Ando *et al.* (1989), it was reported that, after 10 min of incubation, there was ~100% activity of microbial transglutaminase remaining at 40 °C, but only 74% at 50 °C.

Rheology of β-Lactoglobulin Gels with or without Oil Droplets. The effect of the transglutaminase treatment on the time-dependent storage modulus G' at 1 Hz of β-lactoglobulin gels in the presence and absence of protein-coated oil droplets is shown in Figure 3. We see that both β-lactoglobulin solutions and

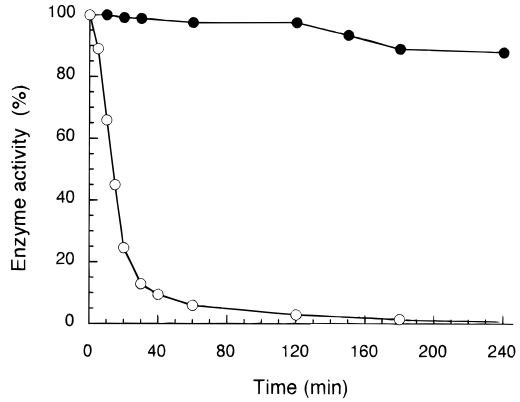

Figure 2. Time-dependent loss of activity of transglutaminase in 0.02 M bis-tris buffer solution at pH 7.0: (●) 40 °C; (○) 55 °C.

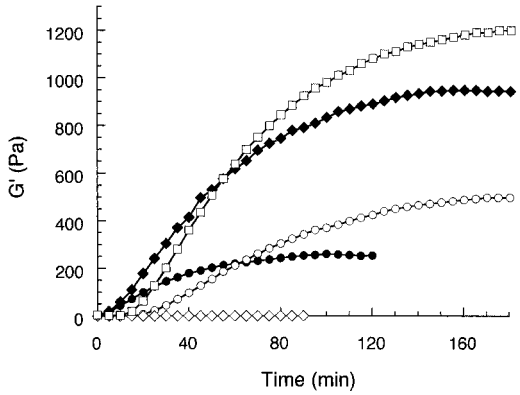

Figure 3. Development of elasticity at 40 °C of transglutaminase-treated β-lactoglobulin gels (13 or 14 wt % protein) and transglutaminase-treated β-lactoglobulin-stabilized emulsion gels (8 or 9 wt % protein, 32.5 wt % *n*-tetradecane). Storage modulus G' at 1 Hz is plotted against time: (○) 13 wt % protein gel; (□) 14 wt % protein gel; (●) 8 wt % protein emulsion gel; (◆) 9 wt % emulsion gel. Also plotted is the continuously low modulus of a 14 wt % β-lactoglobulin solution incubated at 55 °C without transglutaminase.

β-lactoglobulin-stabilized emulsions are gelled by the enzyme treatment (40 °C, pH 7.0) but that the protein concentration in the emulsions (8 or 9 wt %) is considerably lower than that in the solutions (13 or 14 wt %) for gels of similar strength. [The 14 wt % protein emulsion without enzyme present does not form a gel ($G' < G''$) when kept at a temperature (55 °C) below the normal denaturation temperature.] This strong reinforcing effect of protein-coated oil droplets on the strength of the enzyme cross-linked globular protein network is qualitatively similar to the previously established reinforcing effect of emulsion droplets on the viscoelasticity of heat-set gels formed from whey proteins (Jost *et al.*, 1986, 1989; Aguilera and Kessler, 1989; Xiong and Kinsella, 1991; Yost and Kinsella, 1992; Aguilera *et al.*, 1993; Dickinson and Hong, 1995; Dickinson and Yamamoto, 1996a).

Previous work had suggested that transglutaminase has little activity with native β-lactoglobulin (Coussons *et al.*, 1992), and indeed many of the literature studies on the cross-linking of globular proteins by transglutaminase have usually involved the reducing agent dithiothreitol (Tanimoto and Kinsella, 1988; Aboumahmoud and Savello, 1990; Traoré and Meunier, 1992). Nevertheless, we have found here that a concentrated solution of β-lactoglobulin at pH 7 can be used to make a gel with this transglutaminase sample without any previous protein treatment or the addition of a reducing agent. However, the rate of increase in G' with time in

Table 2. Comparison of Storage and Loss Moduli, G' and G'', for β-Lactoglobulin Gels and β-Lactoglobulin-Stabilized Emulsion Gels (32.5 wt % *n*-Tetradecane) Made by Transglutaminase Treatment and Thermal Treatment

system type	protein concn (wt %)	enzyme gel[a] G' (Pa)	enzyme gel[a] G'' (Pa)	heat-set gel[b] G' (Pa)	heat-set gel[b] G'' (Pa)
emulsion gel	6.0	not gelled		not gelled	
	7.0	13	3	58	15
	8.0	250	20	410	87
	9.0	890	78	3500	560
protein gel	12.0	not gelled		not gelled	
	13.0	420	35	not gelled	
	14.0	1080	72	104	70
	15.0			1590	390

[a] Treated with transglutaminase at 40 °C for 2 h (protein:enzyme 25:1 w/w); measured at 40 °C. [b] Thermal treatment: 40−90 °C (2 °C min^{-1}), 90 °C for 30 min, 90−30 °C (−1 °C min^{-1}), 30 °C for 15 min; measured at 30 °C.

the early stages (see Figure 3) is much slower for the β-lactoglobulin solution than for the β-lactoglobulin-stabilized emulsion. This is probably because far fewer intermolecular protein cross-links (*i.e.* only a relatively small number of linkages between protein molecules adsorbed to different droplet surfaces) are likely to be required to produce a macroscopic network structure from the concentrated emulsion, as compared with the concentrated protein solution without filler oil droplets. A further contributory factor is that the partly unfolded adsorbed β-lactoglobulin molecules may be more susceptible to attack than the solubilized native β-lactoglobulin molecules.

Table 2 compares the viscoelastic parameters (G' and G'') of cross-linked β-lactoglobulin gels and emulsion gels induced by enzyme treatment (40 °C for 2 h) with those of heat-set β-lactoglobulin gels and emulsion gels induced by thermal treatment (90 °C for 30 min, cooled to 30 °C) for the same protein concentrations. We see that, whereas the transglutaminase treatment produces a stronger protein gel (no oil droplets) than the thermal treatment, the opposite is the case for the emulsion gel. This may be because, once substantial growth of droplet aggregates has occurred in the enzyme-treated emulsion gel through formation of some permanent covalent cross-links, many of the adsorbed β-lactoglobulin molecules become unavailable for cross-linking with other adsorbed β-lactoglobulin molecules due to topological constraints imposed by the permanent nature of the network. In contrast, the weaker reversible physical cross-links induced by the thermal treatment allow rearrangements and hence reinforcement of the network structure through further subsequent (mainly noncovalent) cross-linking.

Figure 4 shows the frequency dependence of the storage modulus for enzyme-treated and heat-treated emulsion gels containing 7, 8, and 9 wt % protein. We see that the G' values for the transglutaminase-induced emulsion gels are less frequency dependent than those for the heat-set emulsion gels. The difference in frequency dependence of G' for the enzyme-cross-linked network and the heat-set network is even more pronounced for the protein gels in the absence of emulsion droplets (Figure 5). The behavior is consistent with the enzyme-treated systems having the characteristics of classical polymer gels with permanent "chemical" cross-links, whereas the heat-treated systems have characteristics typical of a "physical" gel with breakable or deformable cross-links (Ferry, 1980; Ross-Murphy, 1984).

Thermal Gelation of β-Lactoglobulin following Enzyme Treatment. The application of heat treatment after enzyme-induced gelation is useful for (i) inactivating the transglutaminase to inhibit further covalent cross-linking and (ii) generating novel protein gel structures with a mixture of covalent and physical cross-links. What we have found here is that the combination of the two treatments is very effective in making gels of high elastic modulus from either a β-lactoglobulin solution or a β-lactoglobulin-stabilized emulsion.

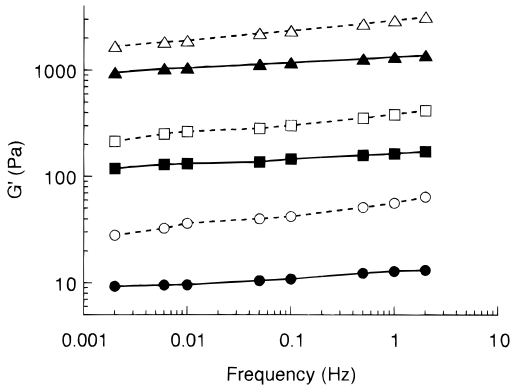

Figure 4. Frequency dependence of storage modulus G' for emulsion gels (7−9 wt % β-lactoglobulin, 32.5 wt % *n*-tetradecane) induced by transglutaminase treatment (55 °C for 2 h) or heat treatment (90 °C for 30 min) and measured at 55 or 30 °C, respectively. Solid symbols denote enzyme treatment, and open symbols denote heat treatment: (●, ○) 7 wt % protein; (■, □) 8 wt % protein; (▲, △) 9 wt % protein.

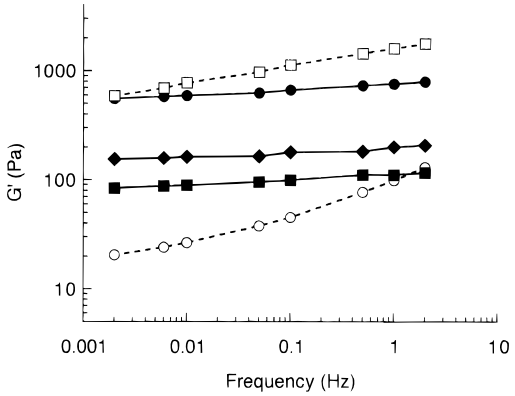

Figure 5. Frequency dependence of storage modulus G' for protein gels (13−15 wt % β-lactoglobulin) induced by transglutaminase treatment (55 °C for 2 h) or heat treatment (90 °C for 30 min): (■) enzyme gel, 13 wt % (measured at 55 °C); (◆) enzyme gel, 13 wt % (measured at 30 °C); (●) enzyme gel, 14 wt % (measured at 55 °C); (○) heat-set gel, 14 wt % (measured at 30 °C); (□) heat-set gel, 15 wt % (measured at 30 °C).

Figure 6 shows plots of G' against time for systems subjected to enzyme treatment at 40 °C for 2 h, then heated at constant rate to 90 °C for 25 min, held at 90 °C for 30 min, cooled at constant rate to 30 °C for 60 min, and held at 30 °C for 15 min. We see that the heat treatment produces a remarkably large increase in the elastic moduli of the enzyme-treated emulsion gels (32.5 wt % *n*-tetradecane, 7−9 wt % protein). Figure 6 also shows a substantial, but proportionately much smaller, increase in the moduli of the β-lactoglobulin gels without oil droplets (13 or 14 wt % protein). Table 3 lists some final G' and G'' values for the protein gels and emulsion gels formed by the combined enzyme/heat treatment. It is noteworthy that, while neither the 12 wt % β-lactoglobulin solution nor the 6 wt % β-lactoglobulin-stabilized emulsion forms a gel with enzyme treatment or heat treatment alone, both systems do form gels when subjected to the combined enzyme/heat treatment. This

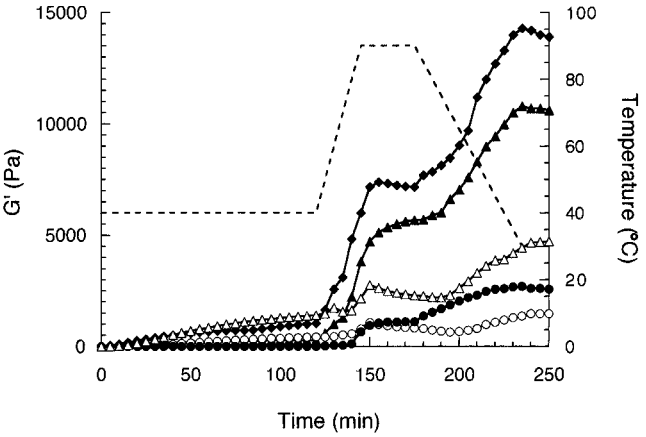

Figure 6. Effect of heat treatment following enzyme-induced cross-linking on the developing elasticity of emulsion gels (7−9 wt % β-lactoglobulin, 32.5 wt % *n*-tetradecane) and protein gels (13 or 14 wt % β-lactoglobulin). The storage modulus G' at 1 Hz is plotted against time: (●) emulsion gel, 7 wt % protein; (▲) emulsion gel, 8 wt % protein; (◆) emulsion gel, 9 wt % protein; (○) 13 wt % protein gel; (△) 14 wt % protein gel. The dashed line shows temperature as a function of time.

Table 3. Storage and Loss Moduli, G' and G'', for β-Lactoglobulin Gels and β-Lactoglobulin-Stabilized Emulsion Gels (32.5 wt % *n*-Tetradecane) Made by a Combination of Transglutaminase Treatment and Thermal Treatment (Experimental Conditions As Indicated in Table 2)

system type	protein concn (wt %)	G' (Pa)	G'' (Pa)
emulsion gel	6.0	58	9
	7.0	2500	196
	8.0	10700	710
	9.0	13800	1050
protein gel	12.0	158	17
	13.0	1470	155
	14.0	4700	430

suggests a synergistic effect between the covalent cross-links produced by the enzyme action and the predominantly physical cross-links (hydrogen bonding, hydrophobic interactions) arising from subsequent β-lactoglobulin thermal denaturation/aggregation.

Large Deformation Rheological Behavior. All of the rheological data quoted above relate to experiments carried out at very low strains in the linear viscoelastic regime. The significance of such a small deformation experiment lies in the fact that the stress applied in making the rheological measurement does not itself modify the gel structure and hence the gel rheology. Hence, the viscoelastic parameters determined in such an experiment give useful direct information about the strengths and lifetimes of intermolecular and interparticle interactions, as well as indirect information about the gel microstructure (Clark and Lee-Tuffnell, 1986; Clark and Ross-Murphy, 1987). The danger of relying wholly on such measurements, however, is that they may give quite misleading information about mechanical gel properties relevant to food processing or eating characteristics, especially for particle gels having a small linear range (van Vliet *et al.*, 1991; van Vliet, 1995).

Figure 7 shows the complex shear modulus $G^* = (G'^2 + G''^2)^{1/2}$ as a function of strain amplitude in the range 10^{-3}−1 (*i.e.* 0.1% to 100%) for various β-lactoglobulin gels and emulsion gels. To facilitate comparison between the different systems, all of the data values are normalized here with respect to the limiting low-strain modulus G_0^*. A first general point to note is the wide

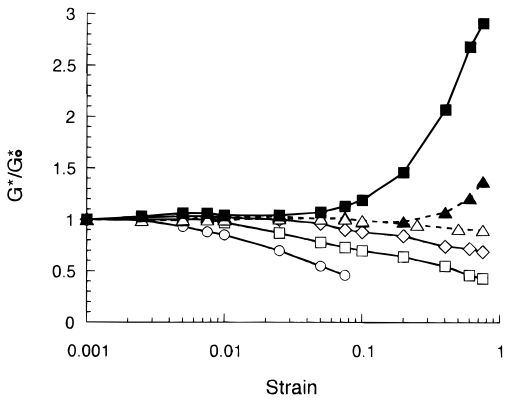

Figure 7. Dependence on extent of deformation of complex modulus G^* for protein-stabilized emulsion gels (7 or 8 wt % β-lactoglobulin, 32.5 wt % *n*-tetradecane) and protein gels (13 or 14 wt % β-lactoglobulin) made by enzyme treatment (55 °C for 2 h) or heat treatment (90 °C for 30 min) or a combination of the two. The reduced modulus G^*/G_0^* is plotted against the shear strain: (■) enzyme-induced emulsion gel, 8 wt % protein; (▲) enzyme-induced protein gel, 13 wt % protein; (○) heat-set emulsion gel, 7 wt % protein; (□) heat-set emulsion gel, 8 wt % protein; (△) heat-set protein gel, 14 wt % protein; (◇) emulsion gel induced by heating following enzyme treatment, 8 wt % protein.

variation in large deformation rheological behavior exhibited by the different systems. A second more specific point is that, irrespective of whether the gelation is triggered by enzyme action or thermal denaturation, the extent of the linear viscoelastic region (*i.e.* the range of constant G^*) is much shorter for the emulsion gels (7−8 wt % protein, 32.5 wt % oil) than for the pure β-lactoglobulin gels (13−14 wt % protein), *i.e.* up to 0.4−2% strain for the former as against up to 10−20% for the latter. This reflects the predominantly "particle gel" character of a protein-stabilized emulsion gel as opposed to the predominantly "polymer gel" character of a pure protein gel. A third point to note is that the modulus of the enzyme-treated protein gel, and especially of the enzyme-treated emulsion gel, *increases* under conditions of large shear deformation, whereas the opposite is the case for the corresponding heat-set systems. This behavior is again consistent with the "chemical" gel characteristics of the enzyme-treated systems, in which the combination of permanent cross-links and additional stresses induced by unrelaxed entanglements produces a highly strained network with a proportionately larger restoring force. On the other hand, in the heat-set systems at high strains, we can infer that there is a breakdown or rearrangement of some of the "physical" cross-links, which has the effect of *reducing* the effective shear modulus. Interestingly, when the enzyme-treated emulsion gel is itself subjected to thermal processing, the resulting large deformation behavior then lies between that for the enzyme-treated system and that for the heat-set system, but much closer to the latter than the former. We conclude that, in principle, it may be possible, by a judicious combination of enzymic and thermal treatments, to produce a globular protein-stabilized emulsion gel with rheological behavior essentially independent of strain over a very wide range of deformations.

Effect of Lecithin on Rheology of β-Lactoglobulin Emulsion Gels. Small molecule emulsifiers can have a substantial effect on the rheology of heat-set protein-stabilized emulsion gels. For instance, in a previous paper (Dickinson and Yamamoto, 1996b), we found that there was a considerable positive influence of pure or crude egg lecithin (but *not* soybean lecithin)

Table 4. Effect of Addition of Egg or Soybean Lecithin (Lecithin:Protein Molar Ratio 8:1) after Emulsification on the Storage and Loss Moduli, G' and G'', of β-Lactoglobulin-Stabilized Emulsion Gels (7 wt % Protein, 32.5 wt % *n*-Tetradecane or Soybean Oil) Prepared by Enzyme Treatment and/or Heat Treatment (Experimental Conditions As Indicated in Table 2)

lecithin type	treatment type	*n*-tetradecane		soybean oil	
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)
not added	enzyme	13	3	26	4
	heat	59	15	54	19
	enzyme + heat	2500	196		
egg	enzyme	149	15	380	33
	heat	610	99	2700	340
	enzyme + heat	3500	270	4100	310
soybean	enzyme	33	6	340	26
	heat	191	49	490	95
	enzyme + heat	740	63	2200	170

at molar ratio $R = 16$ on the modulus of heat-set whey protein concentrate emulsion gels and that the addition of any of these lecithins (pure or crude, from eggs or soybeans) led to an increase in the strength of β-lactoglobulin-stabilized emulsion gels.

In the present work, we study the effect of crude lecithin from egg or soybean ($R = 8$) on the enzyme-induced gelation of a β-lactoglobulin-stabilized emulsion with either *n*-tetradecane or soybean oil as the oil phase. Table 4 compares the G' and G'' values for enzyme-treated systems with those for the equivalent heat-treated systems and combined enzyme/heat-treated systems. We see that the effect of added lecithin on the enzyme-treated systems is rather modest compared with its effect on the systems subjected to heat or enzyme/heat treatments. While similar trends are obtained with both the hydrocarbon and triglyceride oils, the modulus values determined for the soybean oil emulsions are consistently higher. The results in Table 4 suggest that thermal denaturation of the protein is a necessary precondition for forming a rheologically important complex between the protein and the emulsifier. The relatively nonreinforcing effect of soybean lecithin can be attributed to displacement of protein from the oil−water interface (Dickinson and Yamamoto, 1996b).

Rheology of Caseinate Gels and Caseinate-Stabilized Emulsion Gels. The time-dependent rheology of transglutaminase-treated caseinate systems with and without oil droplets has been investigated as a function of protein concentration. Figure 8 shows a plot of G' against time for protein gels containing 8−10 wt % caseinate and protein-stabilized emulsion gels (32.5 wt % *n*-tetradecane) containing 4−6 wt % caseinate. Table 5 gives values of G' and G'' for various caseinate gels and emulsion gels after enzyme treatment for 2 h at 40 °C. As noted above for the β-lactoglobulin systems (Figures 4 and 5), the storage moduli of the enzyme-cross-linked caseinate systems (not shown) are only very weakly dependent on frequency. Comparison with the data in Figure 3 and Table 2 indicates that an enzyme-induced caseinate gel or caseinate-stabilized emulsion gel of elastic modulus ~1 kPa can be produced at a substantially lower overall protein concentration than the equivalent β-lactoglobulin system. This is not surprising in view of the established higher susceptibility of the disordered caseins to transglutaminase—as compared with the globular whey proteins (Ikura *et al.*, 1984; Nonaka *et al.*, 1989; Traoré and Meunier, 1992).

Shown also in Table 5 are a few results for systems where the protein component is a mixture of sodium caseinate plus β-lactoglobulin. The emulsion containing 6.0 wt % β-lactoglobulin does not form a gel when subjected to enzyme treatment (Table 2), but a gel is formed when 1.0 or 2.0 wt % of caseinate is added, and it is stronger than the equivalent pure β-lactoglobulin emulsion gel of the same total protein content. In a similar way, although a 10 wt % β-lactoglobulin solution does not itself gel, the addition of 4 wt % caseinate leads to a strong gel with a much higher modulus than the gel formed from 14 wt % β-lactoglobulin. These results show the potential for enhancing the strength of transglutaminase-induced whey protein gels, and whey protein emulsion gels, through the addition of small amounts of sodium caseinate or skim milk powder.

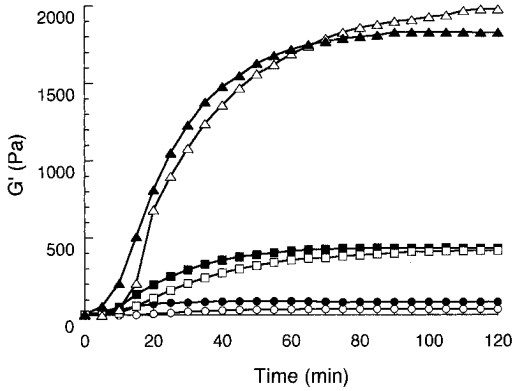

Figure 8. Development of elasticity at 40 °C of transglutaminase-treated caseinate gels (8−10 wt % protein) and transglutaminase-treated caseinate-stabilized emulsion gels (4−6 wt % protein, 32.5 wt % *n*-tetradecane). Storage modulus G' at 1 Hz is plotted against time: (●) emulsion gel, 4 wt % protein; (■) emulsion gel, 5 wt % protein; (▲) emulsion gel, 6 wt % protein; (○) 8 wt % protein gel; (□) 9 wt % protein gel; (△) 10 wt % protein gel.

Table 5. Storage and Loss Moduli, G' and G'', of Protein-Stabilized Emulsion Gels (32.5 wt % *n*-Tetradecane) and Protein Gels Made by Transglutaminase Treatment (40 °C for 2 h; Protein:Enzyme 25:1 w/w) Containing Sodium Caseinate (or Caseinate + β-Lactoglobulin) as the Protein Source

system type	protein concn (wt %)		G' (Pa)	G'' (Pa)
	caseinate	β-lactoglobulin		
emulsion gel	1.0	6.0	122	10
	2.0	6.0	1140	74
	3.0		not gelled	
	4.0		85	12
	5.0		440	32
protein gel	4.0	10.0	3200	191
	7.0		not gelled	
	8.0		38	4
	9.0		400	29
	10.0		1980	90

Conclusions. This work has demonstrated the potential use of transglutaminase-induced cross-linking in the preparation of milk protein gels and milk protein emulsion gels. While the extent and rate of gelation is certainly greater for sodium caseinate systems, we have found that solutions and emulsions containing β-lactoglobulin can also be readily cross-linked by transglutaminase. Compared with the equivalent heat-set gels or heat-set emulsion gels, the enzyme-treated networks are characterized by substantially different rheological behavior in terms of (i) the frequency dependence and (ii) the strain dependence. These differences arise from the fact that the enzyme-induced β-lactoglobulin system consists of a network with "permanent" covalent connections, whereas the thermally denatured β-lactoglobulin network is held together

predominantly by "temporary" physical connections. Thermal processing of a weak enzyme-treated β-lactoglobulin emulsion gel produces a strong gel with a combination of permanent bonds and reversible cross-links. The generation of such networks of connected spherical particles interacting with a combination of flexible bonds and attractive/repulsive nonbonded interactions is the subject of ongoing computer simulations (Dickinson, 1994; Bijsterbosch *et al.*, 1996).

While the results described in this paper refer to laboratory experiments on model emulsion systems prepared with β-lactoglobulin or sodium caseinate as the emulsifier, we expect the broad findings to be relevant also in commercial practice to dairy products prepared with, say, whey protein concentrate or skim milk powder as emulsifier. On the basis of the work reported here, it would seem that the use of transglutaminase during the processing of emulsion gel products offers new oppportunities for developing improved textures in protein-based spreads, desserts, and dressings.

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