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Modulation of thermal stability and heat-induced gelation of β-lactoglobulin by high glycerol and sorbitol levels

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Abstract

The influence of glycerol and sorbitol on the thermal stability and heat-induced gelation of β-lactoglobulin (β-lg) in aqueous solutions was investigated. The thermal stability of β-lg was characterized by measuring the thermal denaturation temperature (T_m) using differential scanning calorimetry, while its gelation properties were characterized by measuring the gelation temperature (T_{gel}) and final gel rigidity (G^*) using dynamic shear rheology. All experiments were carried out using aqueous solutions containing 10% (w/w) β-lg, glycerol (0–70% w/w) or sorbitol (0–55% w/w), and 5 mM phosphate buffer (pH 7.0). No salt was added to these solutions so that there was a relatively strong electrostatic repulsion between the protein molecules, which usually prevents gelation. When the cosolvent concentration was increased from 0% to 50%, T_m increased from 74 to 86 °C for sorbitol, but only from 74 to 76 °C for glycerol, which indicated that sorbitol was much more effective at stabilizing the native state of the globular protein than glycerol. Protein solutions containing sorbitol (0–55%) did not form a gel after heating, but those containing glycerol formed gels when the cosolvent concentration exceeded about 10%, with G^* increasing with increasing glycerol concentration. We attribute these effects to differences in the preferential interactions of polyols and water with the surfaces of native and heat-denatured proteins, and their influence on the protein–protein collision frequency.

Keywords: β-Lactoglobulin; Heat denaturation; Gelation; Glycerol; Sorbitol

1. Introduction

The globular proteins in milk are a major source of functional ingredients for utilization in foods because of their characteristic physicochemical properties, e.g. surface activity, foaming capacity, emulsion stabilization and gel formation (Elofsson, Dejmek, & Paulsson, 1996; Foegeding, Davis, Doucet, & McGuffey, 2002; Galani & Apenten, 1999; Girardet et al., 2001; Kazmierski & Corredig, 2003; Kim, Decker, & McClements, 2004; Lefevre & Subirade, 1999; Losso & Nakai, 2002). Globular proteins are often used to improve the texture and stability of foods such as bakery products (Kinsella & Whitehead, 1989), processed meats (Vittayanont, Steffe, Flegler, & Smith, 2003), and dairy emulsions (Turgeon, Sanchez, Gauthier, & Paquin, 1996). Their ability to provide desirable functional characteristics in particular food applications depends on their molecular structure and concentration, the composition of the solution surrounding them, and their thermal–mechanical history (Foegeding, Allen, & Dayton, 1986; McClements, 2002; Morr & Foegeding, 1990; Schmidt, 1981; Shimada & Matsushita, 1980; Ziegler & Foegeding, 1990). An improved understanding of the influence of these factors on protein functionality might be used to rationally improve the quality of protein-based food products.

β-Lactoglobulin (β-lg) is a globular protein (radius ≅ 2 nm) isolated from bovine whey proteins (Arnaudov, De Vries, Ippel, & van Mierlo, 2003; Dumay, Kalichevsky, & Cheftel, 1994). β-lg has a molecular weight of ~18,600 Da and an isoelectric point of ~5.2 (Nicolai,

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Urban, & Schurtenberger, 2001). β-lg can be used as a gelling agent in foods because of its ability to form a rigid three-dimensional protein network that traps water and other solution components (Doi, 1993; Gezimati, Singh, & Creamer, 1996; Liu, Relkin, & Launay, 1994; Twomey, Keogh, Mehra, & O'Kennedy, 1997). Native globular proteins dispersed in water at neutral pH do not form gels because their intermolecular repulsive interactions (mainly electrostatic, hydration and configurational entropy) are higher than their attractive interactions (mainly van der Waals force and hydrophobic) (Bryant & McClements, 1998). Nevertheless, they can be made to form gels by heating above the thermal denaturation temperature (T_m), of the proteins under conditions where the electrostatic repulsion between the proteins is not too large (Baier & McClements, 2003a; Baldini, Beretta, & Chirico, 1999; Burova, Grinberg, Visschers, Grinberg, & de Kruif, 2002). The driving force for aggregation is the increased hydrophobic attraction between the denatured proteins, as well as the increased tendency for intermolecular disulfide bonds to form (Gosal & Ross-Murphy, 2000; Hoffmann, van Miltenburg, van der Eerden, van Mil, & de Kruif, 1997; Puyol, Perez, & Calvo, 1999). The microstructure of the gel network formed and the bulk physicochemical properties of the resulting gel are highly dependent on the strength of the electrostatic interactions between the protein molecules, and hence the ionic strength and pH of the solution (Barbut, 1995; Doi, 1993; Langton & Hermansson, 1992; Verheul & Roefs, 1998a, 1998b). When the electrostatic repulsion between the protein molecules is relatively strong (pH far from p*I*, low ionic strength) proteins tend to form filamentous aggregates, which lead to transparent elastic gels with good water holding capacity. On the other hand, when the electrostatic repulsion between the protein molecules is relatively weak (pH near the p*I*, high ionic strength) the proteins tend to form particulate aggregates, which give rubbery opaque gels with poor water holding capacity.

This study is concerned with the influence of neutral cosolvents on the functionality of globular proteins in aqueous solutions. These cosolvents are small non-ionic molecules that form miscible solutions with the solvent molecules (water) surrounding the proteins, e.g., sugars and polyols. Sorbitol and sucrose have been shown to increase the thermal denaturation temperature and alter the final gel properties of bovine serum albumin (BSA) (Baier & McClements, 2003b; Baier, Decker, & McClements, 2004) and whey protein isolate (WPI) (Dierckx & Huyghebaert, 2002). On the other hand, glycerol has been shown to have little impact on the thermal denaturation temperature of BSA (Baier et al., 2004), but it does influence the final properties of globular protein gels (Baier et al., 2004; Chantrapornchai & McClements, 2002).

Neutral cosolvents can alter the functionality of globular proteins in aqueous solutions through a variety of different mechanisms (Record, Zhang, & Anderson, 1998; Saunders, Davis-Searles, Allen, Pielak, & Erie, 2000; Timasheff, 1998; McClements, 2002). First, the thermal stability of proteins is influenced by differences in the preferential interactions between the protein surface and cosolvent-solvent molecules in the surrounding solution (Baier & McClements, 2003b; Dierckx & Huyghebaert, 2002). Second, the strength of protein–protein interactions is influenced by the ability of cosolvents to modulate the attractive and repulsive forces between proteins (Record et al., 1998; Timasheff, 1993; McClements, 2002; Baier et al., 2004). Third, the frequency of protein–protein encounters is reduced by the presence of cosolvents due to their ability to increase the viscosity of the aqueous solution surrounding the proteins (Kulmyrzaev, Bryant, & McClements, 2000; Kulmyrzaev, Cancalliere, & McClements, 2000). In the present study, the influence of glycerol and sorbitol on the thermal stability and rheological properties of β-lg gels was examined to obtain a better understanding of the influence of these neutral cosolvents on the functionality of this commonly used protein.

2. Experimental procedures

2.1. Materials

Analytical grade glycerol (G-6279, >99 wt.% pure) and sorbitol (S-7547, >98 wt.% pure) were purchased from the Sigma Chemical Company (St. Louis, MO). Powdered β-lg was obtained from Davisco Foods International (lot no. JE001-1-922, Le Sueur, MN). As stated by the manufacturer, the β-lg content of the protein fraction of the powder determined by electrophoresis was 98 wt.% (the remainder being mostly globulins). The decrease in mass of the powder upon drying was 2.6 wt.%, and the nitrogen content of the powder was 15.6 wt.%. Distilled and de-ionized water was used for the preparation of all buffer solutions. The pH of the β-lg solutions used in this study was measured using a pH meter to be 7.0 ± 0.1 (pH Meter 320, Corning Inc., Corning, NY).

2.2. Differential scanning calorimetry

The influence of cosolvents on the thermal denaturation of β-lg solutions was measured using a differential scanning calorimeter (Q 100, TA-Instruments, New Castle, DW). Initially, a 10 wt.% β-lg solution was prepared by dispersing powdered protein into an aqueous cosolvent solution (0–70 wt.% glycerol or 0–55 wt.% sorbitol) and stirring for at least two hours. The protein solution was then placed in the sample pan (about 15 mg) of the DSC instrument and cosolvent solution (glycerol or sorbitol) with the same cosolvent concentration was placed in the reference cell. The heat flow required to keep the two cells thermally balanced was then recorded as their temperature was increased from 10 to 110 °C at 90 °C h^{-1}. The cells were then held at 110 °C for 15 min, cooled back to 10 °C and rescanned. The first scan was therefore of native protein, while the second scan was of heat-treated protein. The data was not normalised with respect to protein concentration

since only the position of the maximum in the thermal transition temperatures of the proteins was of interest. Measurements were carried out on two or three separate samples (replicates) and reported as the average. The thermal denaturation temperature (T_m), defined as the temperature at which a maximum occurred in the endothermic peaks, was reproducible to within 0.2 °C.

2.3. Rheology measurements

The influence of cosolvents on the dynamic viscoelastic properties of 10 wt.% β-lg solutions was measured using a constant stress rheometer (Bohlin CS10, Bohlin Instruments, Cranbury, NJ). The rheometer applied an oscillating stress of specified frequency to the sample and measured the resulting strain. The magnitude of the complex shear modulus (G^*) and the phase angle (δ) were calculated from the resulting stress–strain relationship. A concentric cylinder (C25) measurement system was used, which had a rotating inner cylinder of 25-mm diameter, and a static outer cylinder of 27.5-mm diameter. Measurements were made at a frequency of 0.1 Hz and at a maximum strain of 0.001, which was within the linear viscoelastic region of the material (as determined by a strain sweep).

β-lg solutions containing 0–70 wt.% glycerol or 0–55 wt.% sorbitol were placed in the measurement cell of the rheometer and allowed to equilibrate to 30 °C for 5 min. Solutions were covered with a thin layer of mineral oil to retard evaporation during the experiments. The solutions were heated from 30 to 90 °C at 90 °C h^{-1}, held for 70 min, cooled from 90 to 30 °C at −90 °C h^{-1}, and then held at 30 °C for 30 min. Measurements were carried out on two or three protein solutions prepared at different times from the same β-lg powder (replicates).

2.4. Statistical analysis

All experiments described above were carried out using at least two or three freshly prepared samples (replicates). The results presented are the means and standard deviations of the experiments.

3. Results and discussion

3.1. Determination of reversibility of β-lg thermal denaturation

The thermographs of native (first scan) and heat-treated (second scan) 10 wt.% β-lg solutions in 5 mM phosphate buffer pH 7.0 were recorded (Fig. 1). The scan of the native protein showed an endothermic transition between 65 and 80 °C, with a single endothermic peak at ~74 °C, whereas the heat-treated scan exhibited no thermal transition (Fig. 1). The endothermic transition observed for the native protein has been attributed to the thermal denaturation of β-lg molecules (Dierckx & Huyghebaert, 2002). Previous studies indicate that the thermal denaturation of proteins leads to substantial changes in protein conformation and aggregation, as well as a redistribution of disulfide bonds and free sulfhydryl groups within and between β-lg molecules (Baldini et al., 1999; Freire, 1995), which is likely to account for the irreversible nature of the thermal transition.

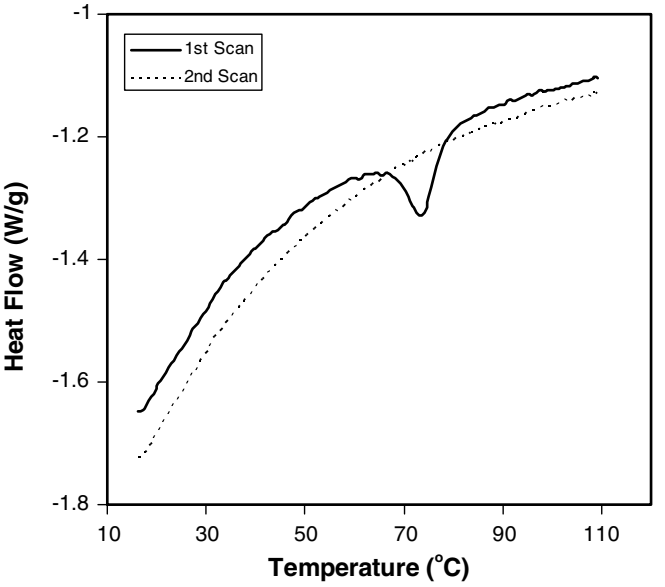

Fig. 1. Heat flow profiles of 10 wt.% β-lg solutions (5 mM phosphate buffer, pH 7.0) scanned at 90 °C h^{-1}. The first scan represents native protein, while the second scan represents heat-treated protein.

3.2. Influence of glycerol and sorbitol on thermal denaturation

The influence of glycerol (0–50 wt.%) and sorbitol (0–50 wt.%) on the heat flow of 10 wt.% β-lg in 5 mM phosphate buffer pH 7.0 was also studied by DSC. For all of the glycerol/sorbitol concentrations used, the heat-treated protein showed no thermal transition, whereas the native protein exhibited an endothermic transition (data not shown). These results indicated that the thermal denaturation of β-lg was irreversible in both the presence and absence of cosolvent molecules. The same phenomenon was also found for whey protein in the presence of sucrose or sorbitol (Dierckx & Huyghebaert, 2002), BSA in the presence of glycerol (Baier et al., 2004) and BSA in the presence of sorbitol (Baier & McClements, 2003b). The heat flow of protein solutions containing no cosolvent, 50 wt.% glycerol, and 50 wt.% sorbitol are compared in Fig. 2. The presence of cosolvents had a pronounced influence on the thermal transition temperature (T_m) of the protein. The T_m value of the β-lg solutions without cosolvent was ~74 °C and shifted to 76 and 86 °C with the presence of 50 wt.% glycerol and 50 wt.% sorbitol, respectively (Fig. 2). The value of T_m progressively shifted to higher temperatures as the cosolvent concentration increased for sorbitol, whereas glycerol had little influence on the ther-

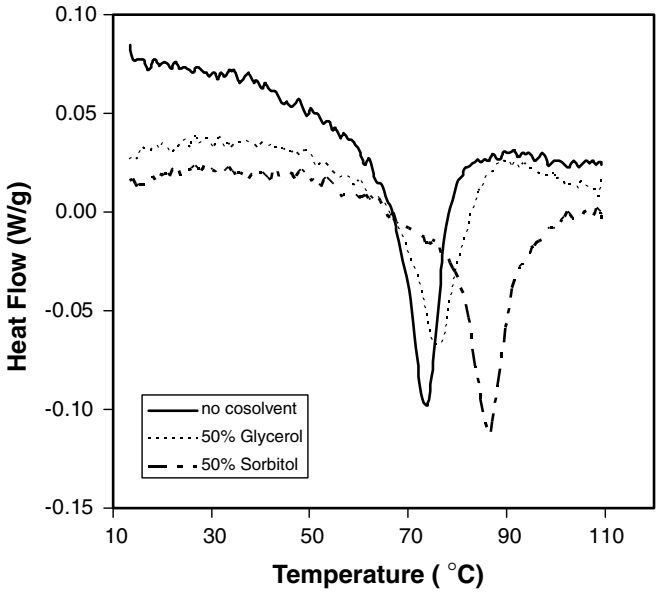

Fig. 2. Heat flow profiles of 10 wt.% β-lg solutions (5 mM phosphate buffer, pH 7.0) containing 0 wt.% cosolvent, 50 wt.% sorbitol and 50 wt.% glycerol scanned at 90 °C h^{-1}.

mal stability of the protein (Fig. 3). The ability of cosolvents to alter the thermal stability of proteins can be attributed to the preferential accumulation or exclusion of cosolvent molecules around the protein surface compared to solvent molecules (Arakawa, Bhat, & Timasheff, 1990; Xie & Timasheff, 1997). Two different physicochemical phenomena contribute to the preferential accumulation or exclusion of cosolvent and solvent molecules around proteins: steric exclusion and differential interactions (McClements, 2002). The steric exclusion contribution depends on the relative size of the cosolvent and solvent molecules, whereas the differential interaction contribution depends on differences in the strength of the molecular interactions between the cosolvent and solvent molecules and the protein surface.

Our results indicated that adding glycerol to the solution caused little difference in the free energy difference between the native and unfolded states of the protein at the thermal denaturation temperature (Timasheff, 1993, 1998). On the other hand, adding sorbitol increased the free energy difference between the unfolded state and folded states of the protein appreciably, thereby increasing the thermal stability of the protein (Freire, 1995; Leharne & Chowddhry, 1998). Sorbitol is a considerably larger molecule than glycerol, so that it seems likely that steric exclusion effects may play an important role in stabilizing the protein, i.e., the zone where the cosolvent molecules are excluded from the protein surface is larger for sorbitol. Nevertheless, there may also be a differential interaction contribution from differences in the molecular interactions of the two polyols with the protein surface groups.

3.3. Rheological properties of gels

The dependence of the dynamic shear modulus (G^*) and phase angle (δ) of 10 wt.% β-lg solutions containing different glycerol or sorbitol concentrations on temperature was recorded when they were heated from 30 to 90 °C, held at 90 °C for 70 min, cooled back to 30 °C, and then held at 30 °C for 30 min. The time-dependence of the temperature, G^* and δ for protein solutions containing 0% and 50 wt.% cosolvent are shown in Figs. 4–7.

In the absence of cosolvent, G^* was close to zero (<0.3 Pa) (Fig. 4) and δ was high and erratic (>70°) throughout the heating, holding and cooling periods (Fig. 6), indicating fluid-like behavior of the solutions. Nevertheless, there did appear to be an appreciable increase in G^* during the cooling period (Fig. 4), which sug-

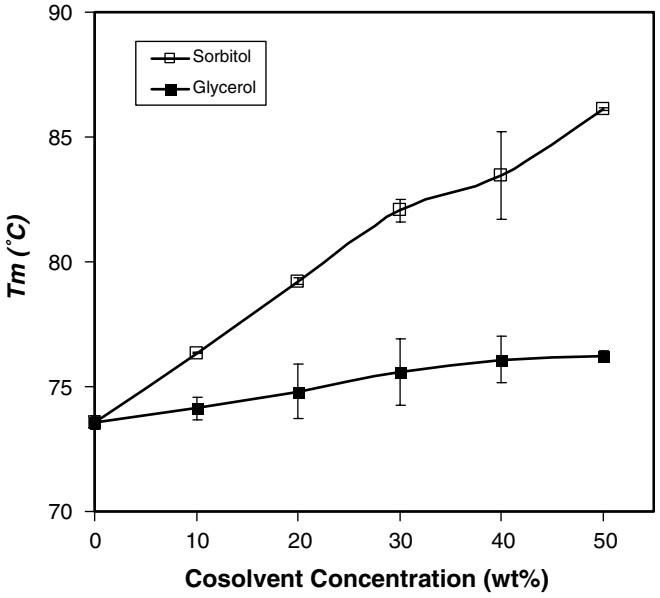

Fig. 3. Dependence of thermal denaturation temperature on cosolvent concentration for 10 wt.% β-lg solutions (5 mM phosphate buffer, pH 7.0) containing sorbitol or glycerol.

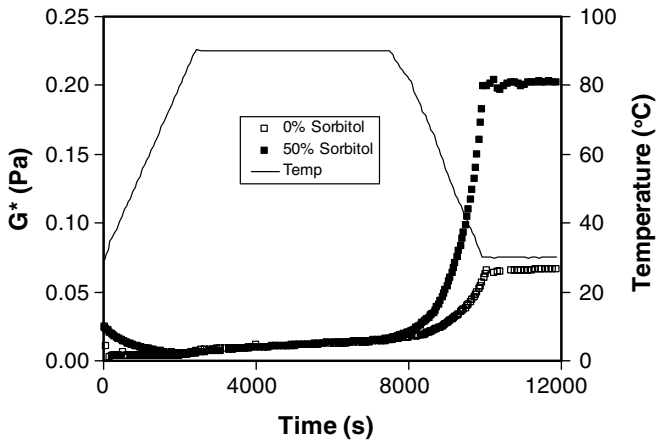

Fig. 4. Time-dependence of sample temperature and complex shear modulus of 10 wt.% β-lg solutions containing 0 or 50 wt.% sorbitol (pH 7.0) measured by controlled stress rheometery.

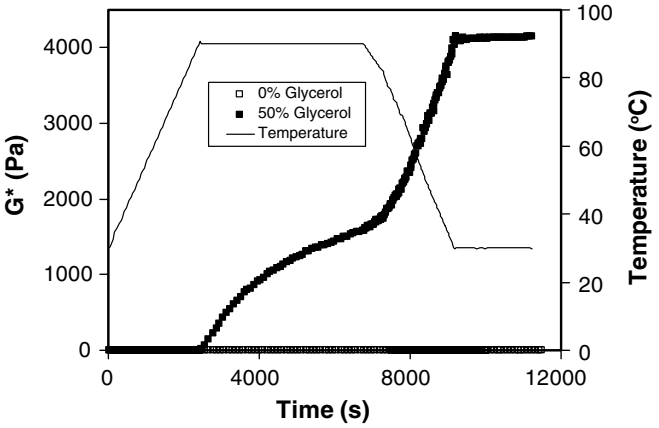

Fig. 5. Time-dependence of sample temperature and complex shear modulus of 10 wt.% β-lg solutions containing 0 or 50 wt.% glycerol (pH 7.0) measured by controlled stress rheometery.

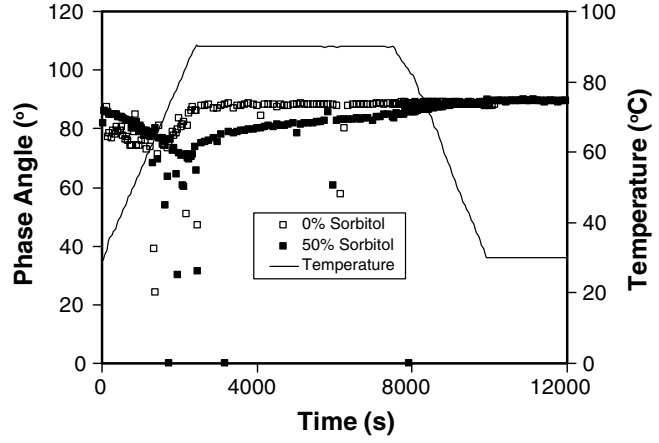

Fig. 6. Time-dependence of sample temperature and phase angle of 10 wt.% β-lg solutions containing 0 or 50 wt.% sorbitol (pH 7.0) measured by controlled stress rheometery.

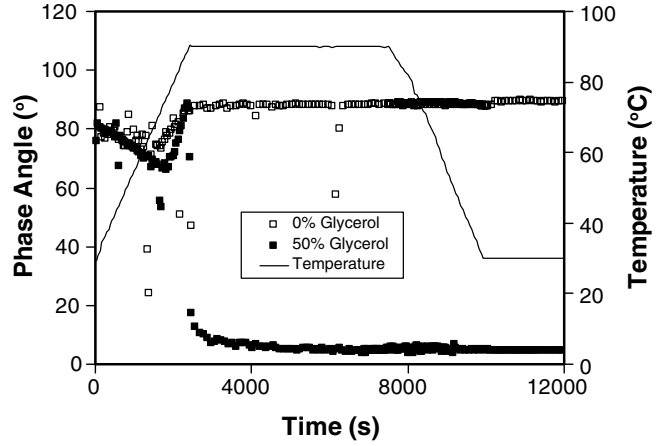

Fig. 7. Time-dependence of sample temperature and phase angle of 10 wt.% β-lg solutions containing 0 or 50 wt.% glycerol (pH 7.0) measured by controlled stress rheometery.

gested that some structure formation occurred in the solutions. The DSC results showed that the proteins were thermally denatured around 74 °C, however extensive protein aggregation and gelation probably did not occur because of the relatively strong electrostatic repulsion between the protein molecules in the absence of added salt. The slight increase in structure in the solutions may have been due to the formation of some linear filamentous structures as reported by other workers (Bryant & McClements, 2000).

In the presence of 50 wt.% sorbitol, G^* was also low and δ was high and erratic throughout the heating, holding and cooling periods (Figs. 4 and 6), indicating that the solutions remained fluid-like. It is postulated that the protein solutions containing sorbitol did not gel because the sorbitol increased the thermal denaturation temperature of the proteins above 90 °C (Fig. 3), so that there were few denatured proteins available to participate in gel formation. Having said this, there was an appreciable increase in G^* upon cooling the protein solution containing 50 wt.% sorbitol (Fig. 4), which suggests that there may have been some protein unfolding and structure formation in the solutions during heat treatment, but not enough to cause gelation. The fact that the final G^* was higher for the system containing sorbitol than for the system containing no cosolvent (even though gelation did not occur), suggests that either larger linear filamentous protein aggregates were formed during heating or that the strength of the attraction between protein filaments was higher in the presence of sorbitol. Previous studies suggest that cosolvents that increase the thermal denaturation of proteins should also increase the attraction between protein molecules since both processes decrease the contact area between the protein surface and the surrounding cosolvent-solvent system (McClements, 2002). Hence, sorbitol would have been expected to promote the aggregation of any thermally denatured protein molecules or protein filaments formed during the heating process.

In the presence of 50 wt.% glycerol, G^* remained close to zero and δ was high and erratic when the sample was heated from 30 to 90 °C (Figs. 5 and 7), indicating that the sample remained fluid-like. The DSC data showed that the proteins unfolded around 78 °C (Fig. 3) and yet the systems remained fluid-like until the temperature was appreciably higher than this (Fig. 5). This suggests that the aggregation of the unfolded proteins may have been delayed because the high viscosity of the glycerol solution slowed down protein–protein encounters. During the isothermal holding period at 90 °C, G^* increased appreciably and δ decreased to a relatively low value (<5°), indicating that gel formation occurred. G^* Continued to increase throughout the isothermal holding period suggesting that gelation was not completed even after holding the protein solutions for 70 min at this elevated temperature. During the cooling cycle from 90 to 30 °C, the rigidity of the gels increased steeply while the phase angle remained low and constant. These results may be attributed to the further incorporation of proteins into the gel network and/or the

increasing of the bond strength between the proteins previously trapped within the gel network. It is postulated that the increase in gel strength is due to bond strengthening rather than further incorporation of proteins into the network, because this latter phenomenon should occur more rapidly at higher temperatures. The shear modulus and phase angle of the gels remained relatively constant throughout the isothermal holding period at 30 °C, indicating that there was no further gel strengthening during this period. When the gels containing glycerol were examined at the end of the heat-cool cycle they appeared to be transparent and there was no evidence of free water on their surface. This suggests that the protein network formed involved the association of filamentous protein aggregates, rather than particulate protein aggregates.

In summary, the final gel strength was strongly dependent on the amount and type of cosolvent present: $G^* = 6.76 \pm 0.02$ mPa for 0% cosolvent, 190 ± 14 mPa for 50% sorbitol, and $4.15 \pm 0.00 \times 10^6$ mPa for 50% glycerol. It is postulated that the cosolvent-free sample did not gel because of the strong electrostatic repulsion between the protein molecules. The sorbitol sample did not gel because of the strong electrostatic repulsion between the protein molecules and the fact that the thermal denaturation temperature of the proteins was increased so that there were few active proteins available to participate in network formation. The glycerol sample gelled because glycerol did not appreciably increase the thermal denaturation temperature so that the majority of proteins were in an active state, and because glycerol increased the strength of the attraction between protein molecules thereby overcoming the electrostatic repulsion.

3.4. Influence of cosolvent concentration on gelation

The final rigidity of all the gels containing sorbitol (0–55 wt.%) was low and the phase angle was high, indicating a fluid-like behavior (Table 1). On the other hand, the final rigidity of the gels containing glycerol increased significantly with increasing glycerol concentration while the phase angle decreased (Table 1, Fig. 8), indicating that glycerol promoted gelation in these samples above a certain level (~20–30 wt.% glycerol). This study shows that it is possible to make protein gels with low salt concentrations by having a sufficiently high amount of glycerol present in the system. This type of gel system may be useful for food applications where low moisture contents are desirable (e.g., in dried food products such as cookies, crackers or other baked goods) or where low salt contents are desirable (e.g., food products specifically designed for those with high blood pressure).

The increase in gel strength with increasing glycerol concentration can be attributed to the fact that glycerol increased the strength of protein–protein interactions through the steric exclusion and differential interaction effects mentioned earlier (Cioci, 1996; Gekko & Timasheff, 1981; Timasheff, 1998). It is possible to use knowledge of the various molecules involved to speculate about the molecular origin of this thermodynamic effect. Glycerol is a larger molecule than water and therefore there is a region surrounding the protein molecules from which glycerol molecules are excluded, but water molecules can enter (Timasheff, 1993, 1998; Record et al., 1998). Hence, there is a concentration gradient between the glycerol-depleted region immediately surrounding the protein and the glycerol-rich bulk solution, which is thermodynamically unfavorable (Parsegian, Rand, & Rau, 1995). This steric exclusion effect tends to favor molecular arrangements that lower the surface area of the protein exposed to the solution, e.g. aggregated over non-aggregated state. It has been proposed that glycerol molecules interact more unfavorably with hydrophobic groups than water molecules (Timasheff, 1993, 1998). Consequently, the exposure of non-polar protein surface groups to the surrounding solution would be less favorable in the presence of glycerol than in its absence. These differential interaction effects would therefore tend to favor molecular arrangements that lower the exposure of protein hydrophobic groups to the aqueous solution, e.g. aggregated over non-aggregated state. Any increase in the attractive force between protein molecules due to preferential interaction effects will tend to increase the elastic modulus of the gel network (Narine & Marag-

Table 1
Dependence of shear modulus and phase angle on sorbitol and glycerol concentrations for 10 wt.% β-lg in aqueous solution (pH 7.0) after a heat–cool scan

Cosolvent concentration (wt.%)	G^* (Pa) (mean ± SD)		Phase angle (°) (Mean ± SD)	
	Sorbitol	Glycerol	Sorbitol	Glycerol
0	0.07 ± 0.00	0.07 ± 0.00	86.0 ± 0.8	86.0 ± 0.8
5	0.09 ± 0.00	–	87.5 ± 0.7	–
10	0.16 ± 0.05	7.29 ± 0.04	87.1 ± 0.1	37 ± 3
20	0.04 ± 0.00	205 ± 8	86.5 ± 1.9	17.9 ± 0.1
30	0.10 ± 0.00	1430 ± 40	88.1 ± 0.1	7.3 ± 0.3
35	0.07 ± 0.00	–	87.8 ± 1.1	–
40	0.05 ± 0.01	2600 ± 200	88.4 ± 0.7	5.5 ± 0.6
50	0.19 ± 0.01	4154 ± 2	89.3 ± 0.4	4.8 ± 0.1
55	0.35 ± 0.01	–	89.4 ± 0.4	–
60	–	8208 ± 96	–	4.8 ± 0.1
70	–	10508 ± 160	–	5.1 ± 0.2

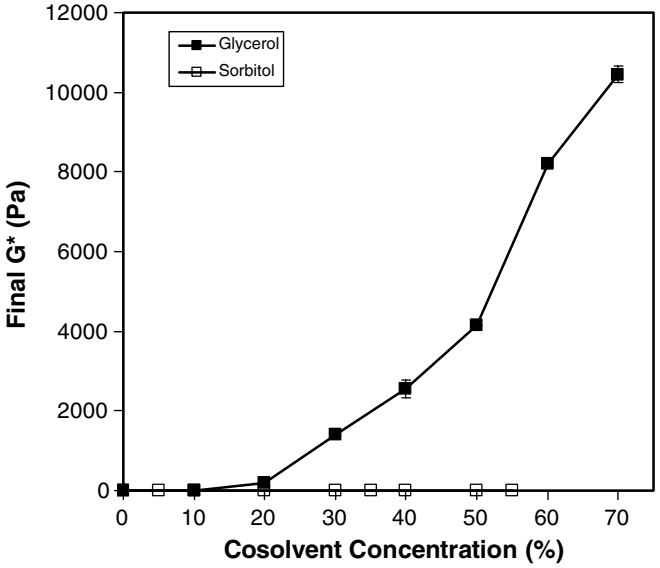

Fig. 8. Dependence of final gel strength (G^*) on cosolvent concentration for 10 wt.% β-lg solutions (5 mM phosphate buffer, pH 7.0) containing sorbitol or glycerol.

oni, 1999). The presence of glycerol in the aqueous phase of the β-lg solutions will impact both the fraction of protein molecules incorporated into the gel structure and the strength of protein–protein interactions through a preferential interaction effect (McClements, 2002). The rigidity of a gel depends on the concentration of particles incorporated into the gel network and the strength of the interaction between the particles (Narine & Maragoni, 1999). As mentioned earlier, it is postulated that the gels containing sorbitol did not gel because this cosolvent increased the thermal denaturation temperature of the protein so much that there were not enough active protein molecules to participate in gel formation. The different behavior of the two cosolvents is highlighted schematically in Fig. 9.

4. Conclusions

The presence of neutral cosolvents in aqueous solutions may alter the thermal stability of globular proteins, the strength of protein–protein interactions, and the kinetics of protein–protein encounters. The results suggest that these physicochemical mechanisms are affected differently by different types of cosolvents, thereby impacting the ability of globular proteins to form gels. For example, glycerol had little influence on the thermal denaturation temperature of β-lg, but it promoted protein–protein interactions and gel formation, whereas sorbitol increased T_m but did not promote gel formation. A better understanding of the influence of protein–cosolvent–solvent interactions on the functionality of globular proteins may help food scientists to design desirable physicochemical characteristics into protein-based foods.

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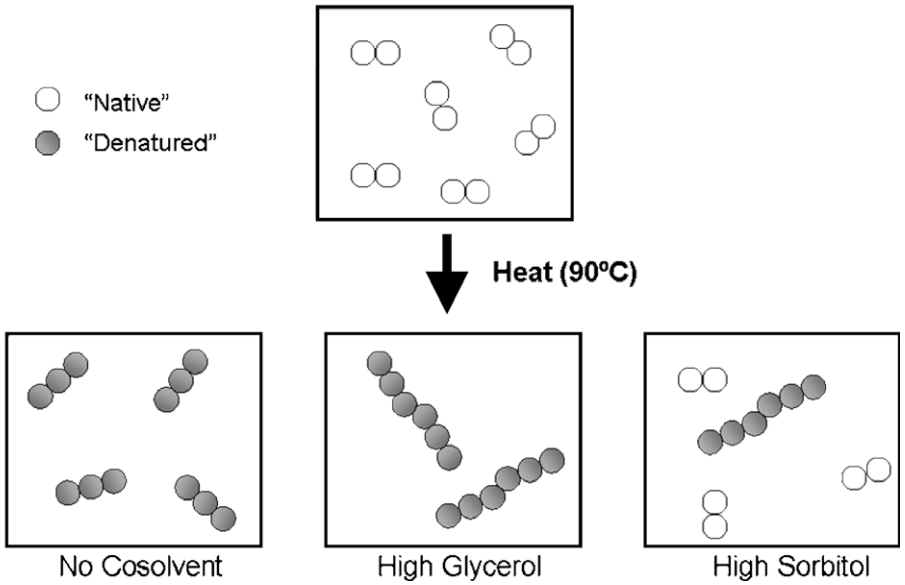

Fig. 9. Proposed mechanism for the influence of sorbitol and glycerol on the thermal gelation behavior of β-lg. Sorbitol substantially increases T_m so that there are few active protein molecules to participate in gel formation, but it strengthens protein–protein interactions thereby promoting aggregation of any denatured proteins. On the other hand, glycerol only causes a slight increase in T_m and also increases the strength of protein–protein interactions, thereby increasing the number and/or size of the protein aggregates formed.

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