Effect of Genetic Polymorphism on the Gelation of β-Lactoglobulin

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SUMMARY: The rheological properties of heat-induced gels made from β-lactoglobulin variants A, B and C were compared. The relative G' values (elastic moduli) for gels formed in 90 mM NaCl solutions were A = B > C. Conversely, in 30 mM $CaCl_2$ the relative G' values were C > A = B. The differences in rheological properties were due to A and B variants forming less rigid gels in $CaCl_2$ (~ 7 kPa) than NaCl (~ 20 kPa), and variant C forming gels of similar rigidity in both salt solutions. It was concluded that genetic variation in β-lactoglobulin changes the effect of salts on gelation but does not cause a universal increase or decrease in gel forming ability.

Introduction

Genetic polymorphs are naturally occurring variants of proteins with minor changes in amino acid sequence. They provide a useful probe of structure-function relationships, and may be a future source of differentiated functionality of food proteins. β-Lactoglobulin (β-LG) is the predominant protein in most whey protein products. It is a globular protein with 162 amino acids and a molecular weight of about 18,000. It exists commonly as the A and B polymorphic forms or variants. There is also a C variant, which occurs in the Jersey breed at a low frequency. The B variant can be considered as the parent form, and the A variant differs by replacement of a Gly side chain at position 64 with an Asp, and replacement of an Ala side chain with a Val at position 118. The C variant differs in the substitution of a His side chain for a Gln at position 59.

One of the main functional properties of whey proteins is their ability to form viscoelastic gels when heated [1)]. Heat-induced gelation of proteins is due to the combined reactions of denaturation and aggregation. Heating causes denaturation by altering tertiary and secondary structures in proteins. Denatured proteins will form aggregates when structural changes have progressed to a stage that favors intermolecular interactions. Aggregates are linked by hydrophobic interactions, hydrogen bonds and disulfide bonds. The aggregation process converts a whey protein solution (fluid) to a gel (solid) if the protein concentration is above a critical minimum (C_o) to form a continuous matrix.

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Denaturation and aggregation reactions of proteins are altered by solution pH, ionic strength and types of ions present[2]. At protein concentrations > C_o, a critical balance of pH, ionic strength and ion type is required for the aggregation process to produce a gel matrix. Insufficient aggregation will produce a dispersion of protein particles, whereas excessive aggregation will cause precipitation of the proteins in the solution. Gels formed under conditions which minimize aggregation are call "fine-stranded" because the gel network is composed of strands with diameters representing one to several molecular diameters[2]. When solution conditions favor random aggregation, a "particulate" gel network is formed. At a fixed protein concentration, the rheological properties will depend on the type of network formed[2].

Rheological properties of β-LG gels have been shown to be different between A and B variants. Gels made from variant A are more rigid and have different viscoelastic properties than those made from variant B [3,4]. These differences in rheological properties could be due to sequence-specific chemical properties of β-LG, variation in solution conditions or a combination of both factors. The studies of Huang *et al.* [3] and McSwiney *et al.* [4] used different solution conditions, which would suggest that the rheological differences are sequence specific. However, in those studies, the solutions were not equilibrated to the same chemical potential so one cannot rule out ionic effects which would alter the type of network formed (i.e., fine-stranded vs. particulate). The goal of this investigation was to determine if β-LG variants A, B and C form similar gels when gelation occurs in solutions which were equilibrated to the same chemical potential and under conditions which favored fine-stranded or particulate matrices.

β-LG Solutions

Single variant β-LG was prepared from AA, BB or CC milk according to the procedure of Mailliart and Ribideau-Dumas[5]. Protein solutions were prepared at 15% protein (w/v), 0.01%(w/v) sodium azide, and either 90 mM NaCl or 30 mM $CaCl_2$ (i.e., equal ionic strength). Solutions were adjusted to pH 7.0 at 22 ± 2°C and exhaustively dialyzed against either 90 mM NaCl or 30 mM $CaCl_2$. All three β-LG variants were exhaustively dialyzed in one container, for each salt type, to assure similar chemical potential. Solutions were adjusted to 10% protein prior to gelation with the appropriate salt solution (90 mM NaCl or 30 mM $CaCl_2$).

Rheological Analysis

A Bohlin CVO controlled stress rheometer and C25 cup and bob sample cell were used in all experiments. The 10% protein dispersions were gelled *in situ* by the following heating regime: (i) heating from 20 to 80°C at 1°C/min, (ii) holding at 80°C for 1 h, (iii) cooling at 1°C/min from 80 to 20°C, (iv) hold at 20°C for 15 min followed by a frequency sweep, (v) heating from 20 to 80°C at 1°C/min and (vi) hold at 80°C for 15 min followed by a frequency sweep. The frequency sweeps at 20 and 80°C were down-up sweeps of 10 - 0.001 Hz, followed by 0.001 - 10 Hz. Rheological properties of G' (storage or elastic modulus), G" (loss or viscous modulus) and δ (phase angle) were determined from oscillation (fixed frequency of 0.1 Hz) and frequency sweep measurements. Gel point was determined as the temperature where the phase angle was 45°. All experiments were replicated two or three times.

Results

Gelation was determined during the initial heating (20°C - 80°C at 1°C/min) as the temperature where G' = G". Gelation occurred in 30 mM $CaCl_2$ at a lower temperature than in 90 mM NaCl (Table 1). The relative decrease in gel point temperature was greater for the A and B variants than the C variant. There were no major trends among β-LG variants.

Tab. 1. Gel Point Temperature

	Temperature Where G' = G" [a)]	
β-LG Variant	90 mM NaCl	30 mM $CaCl_2$
A	76.8	67.8
B	75.6	71.4
C	74.5	73.0

[a)]Average values for two replications.

Frequency sweep data were fit to a power law model as described by the following equation,

$$G' = k\,(Hz)^n \qquad \text{eq.1}$$

where "k" is G' at a frequency of 1 Hz and "n" reflects the frequency dependence of G'. The r^2 values (coefficient of determination) for the 20°C frequency sweeps varied from 0.92 - 0.99 and those run at 80°C had a range of 0.77 - 0.99. In both cases, the data with a less precise fit to the model showed a slight hysteresis between the up and down sweeps, which was mainly observed in the 80°C data. The second sweeps had greater values, suggesting a slow curing of the gel network during the frequency sweep.

Tab. 2. Power Law Model Constants for β-Lactoglobulin Gels[a)]

	Gel Temperature During Frequency Sweep			
Salt Type and	20°C		80°C	
β-LG Variant	n	$G'_{1\ Hz}$ (kPa)	n	$G'_{1\ Hz}$ (kPa)
90 mM NaCl				
A	0.057	19.4	0.060	7.7
B	0.062	21.3	0.072	8.1
C	0.066	16.4	0.075	5.4
30 mM $CaCl_2$				
A	0.072	6.8	0.101	1.8
B	0.070	7.4	0.094	1.9
C	0.069	15.9	0.098	3.9

[a)]Average values for 2-3 replications.

The elastic rigidity ($G'_{1\ Hz}$) of all gels was greater at 20°C than at 80°C (Tab. 2.). An indication of overall elasticity is a lack of frequency dependence for G' values, which is indicated by low "n" values. All gels were more elastic at 20°C and there were no variant-specific trends. Gels formed in 30 mM $CaCl_2$ were the most frequency dependent. The main difference among variants was in $G'_{1\ Hz}$. The magnitude of $G'_{1\ Hz}$ for gels formed in 90 mM NaCl was variant A = B > C, whereas for gels formed in 30 mM $CaCl_2$ it was C > A = B. This was due to how the variants responded to the different salts. In going from NaCl to $CaCl_2$, gels with significantly lower $G'_{1\ Hz}$ values were formed with variants A and B, while variant C formed gels which only had slightly decreased $G'_{1\ Hz}$ values.

Changes in phase angle with frequency indicate the contribution of viscous and elastic elements of a gel. The frequency dependence of the phase angle for all gels at 20°C was quite similar (Fig. 1). At frequencies • 0.1 Hz, the phase angle remained constant at values <10°.

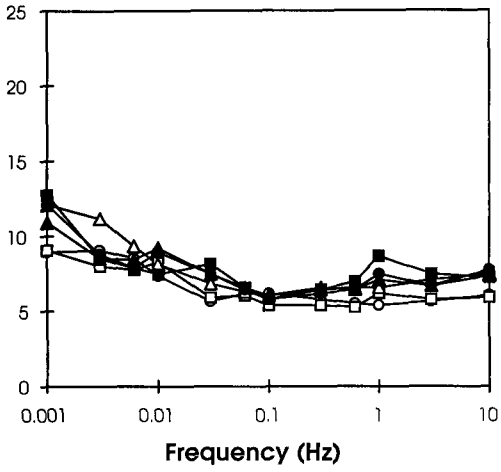

Fig. 1. Frequency dependence of phase angle at 20°C. β-Lactoglobulin gels from variant A (circles) B, (squares) and C (triangles) were formed from solutions containing either 90 mM NaCl (open symbols) or 30 mM $CaCl_2$ (closed symbols).

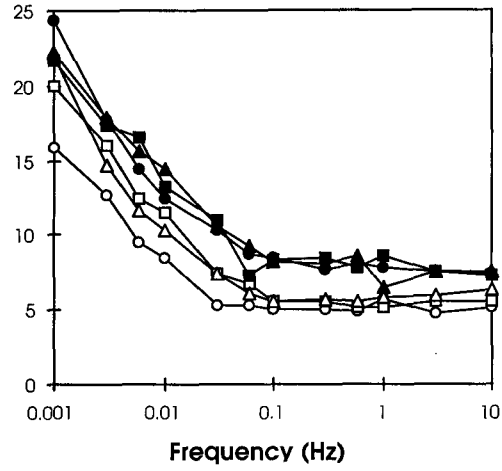

Fig. 2. Frequency dependence of phase angle at 80°C. β-Lactoglobulin gels from variant A (circles) B, (squares) and C (triangles) were formed from solutions containing either 90 mM NaCl (open symbols) or 30 mM $CaCl_2$ (closed symbols).

Gels at 80°C show salt-specific trends, with those formed in $CaCl_2$ having an overall greater phase angle (Fig. 2). This suggests a more viscous nature. Changes in phase angle at 20 and 80°C did not show variant-specific trends.

Discussion

The above results showed that the rheological properties of heat-induced β-LG variant A, B and C gels depend on solution conditions. The conditions of pH 7.0 and either 90 mM NaCl or 30 mM $CaCl_2$ were selected because whey protein isolate (>70% β-LG) respectively forms fine-stranded and particulate gels in these salt solutions at pH 7.0[2,6]. The lower gelation temperature (Tab. 1) and $G'_{1\ Hz}$ values (Tab. 2) are similar to results for gelation of a β-LG A, B mixture in 100 mM NaCl or 20 mM $CaCl_2$[7]. The phase angle at 80°C also showed ion-specific differences (Fig. 2.). These results suggest that salt-associated differences can be explained by formation of primarily fine-stranded (90 mM NaCl) or particulate (30 mM $CaCl_2$) networks.

In 90 mM NaCl the A and B variants formed gels with similar rigidity and the C variant gels were less rigid. This finding differs from previous studies which showed that gels made from variant A are more rigid and have different viscoelastic properties than those made from variant B [3,4]. While it is not possible to precisely determine the cause of variation among investigations, the most plausible explanation is differences in ionic conditions. Moreover, the results from this investigation suggest that no single variant has an inherently greater gelling ability. Sequence-specific differences appear to be important to how ions affect gelation, such that the variant forming the most rigid gel will depend on the ionic conditions.

In 30 mM $CaCl_2$ the A and B variants formed gels with similar rigidities which were lower than the C variant gels (Tab. 2). In contrasting the gels formed in the different salt solutions, the C variant was less sensitive to ionic conditions than the A and B variants (Tab. 2). One plausible explanation is that $CaCl_2$ is less effective in shifting the C variant into a particulate structure. This is supported by only a 1.5°C lowering of the gel point temperature for the C variant, as compared to 9°C and 4.2°C decreases for variants A and B respectively. This hypothesis is not supported by changes in G' ("n" values in Tab. 2) or phase angle (Fig. 2)

with frequency. These two parameters showed general salt-associated differences, suggesting similar network types among variants in one salt solution.

The effect of $CaCl_2$ on whey protein denaturation and gelation has been investigated using whey protein isolate[8], β-LG A,B mixture[7] and β-LG B[9]. Calcium chloride either causes or stabilizes a structural transition at 40 - 45°C which allows for aggregation that would not normally occur until a temperature of > 60°C[8,9]. The aggregation reaction which starts at 40 - 45°C produces a particulate gel structure when gels are formed by heating at 80°C. From this investigation on β-LG variants, it is clear that $CaCl_2$ has a different effect on the C variant as compared to the A and B variants. However, the molecular mechanism is not apparent and will require future investigation.

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References

[1] J. N. de Wit, *J. Dairy Sci.* **81**, 597 (1998)

[2] E. A. Foegeding, E. L. Bowland, C. C. Harden, *Food Hydrocolloids*, **9**, 237 (1995).

[3] X. L. Huang, G. L. Catignani, E. A. Foegeding, H. E. Swaisgood, *J. Agric. Food Chem.* **42**, 1064 (1994)

[4] M. McSwiney, H. Singh, O. Campanella, L. K. Creamer, *J. Dairy Res.* **61**, 221 (1994)

[5] P. Mailliart, B. Ribadeau-Dumas, *J Food Sci.* **53**, 743 (1988)

[6] E. L. Bowland, E. A. Foegeding, *Food Hydrocolloids*, **9**, 47 (1995)

[7] E. A. Foegeding, P. R. Kuhn, C. C. Harden, *J. Agric. Food Chem.* **40**, 2092 (1992)

[8] S. Barbut, E. A. Foegeding, *J. Food Sci.* **58**, 867 (1993)

[9] H. Li, C. C. Harden, E. A. Foegeding, *J. Agric. Food Chem.* **42**, 2411 (1994)