Chapter 15
Beta-Lactoglobulin Fibril Dispersions: Structural and Rheological Characteristics

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15.1 Introduction

Processed foods are created as a result of mostly physical responses of proteins, mono- and polysaccharides, and lipids in aqueous media to different processing methods. Measured rheological responses occur at the macroscopic level. However, these responses are affected by changes and properties at the molecular and microscopic levels. One major challenge is to establish links between macroscopic rheological properties with changes at the molecular and microscopic levels (Rao 2007). Rheological data on a food together with data on its composition and structure or microstructure should lead to understanding the inter relationships between them (Genovese et al. 2007).

Particles with a wide range of sizes are found in foods. The size of colloidal (Brownian) particles may be considered to be between 1 nm and 10 µm (Russel et al. 1989). Examples of foods that contain colloidal particles are milk, cloudy fruit juices, and mayonnaise (an emulsion). Brownian motion promotes collisions between pairs of colloidal particles, while interparticle forces determine whether two colliding particles aggregate. Particle shape, size and size distribution, deformability, and liquid polarity may affect the structure/rheology (Tsai and Zammouri 1988).

Many foods are dispersions of solids in a liquid medium, usually an aqueous solution, or of liquid droplets in another liquid, which is called an emulsion. It is well known that the apparent viscosity of dispersions depends on the volume

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fraction of the solids. Three kinds of forces coexist to various degrees in flowing dispersions: hydrodynamic, Brownian, and colloidal forces. Hydrodynamic forces arise from the relative motion of particles to the surrounding fluid. The Brownian force is the ever-present thermal randomizing force. Colloidal forces are potential forces and are elastic in nature (Zhou et al. 2001). The relative magnitude of these forces and, therefore, the bulk rheological behavior are influenced by the particle size. For sub-nanometer-size dispersions, Brownian motion and interparticle forces quickly equilibrate. Colloidal dispersions can be defined as polyphasic or heterogeneous systems where the dispersed phase is subdivided into discrete units (particles/droplets) that are large compared to simple molecules, but small enough so that interfacial and inertial forces are significant in governing system properties (Sennet and Olivier 1965).

Here, we briefly cover recent results on the structure and rheology of fibrils made from whey protein β-lactoglobulin (β-lg). A more extensive review of the subject can be found elsewhere (Loveday et al. 2012a). When heated at a low pH ~2–3, and ~85 °C, β-lg will self-assemble into amyloid-like fibrils that are 4–10 nm wide and up to 10 μm long, and consist of β-sheets whose strands run perpendicular to the fibril axis. These long fibrils have the potential to enhance viscosity and form gels at low protein concentration due to their extreme aspect ratio. For practical application, whey protein isolate (WPI) is often used to make β-lg fibrils, as it contains up to 80 % w/w β-lg and is available at much lower cost than highly purified β-lg.

Salts are ubiquitous in food and biological systems, and we have investigated the effect of $CaCl_2$ on fibril network assembly during heating. $CaCl_2$ was chosen because it accelerates self-assembly much more effectively than NaCl (Loveday et al. 2010), and significant amounts of Ca^{2+} are present in many dairy-based food products.

WPI fibrils formed without $CaCl_2$ were long and semiflexible, and associated in large entangled networks more than 10 μm across on the TEM grid (Fig. 15.1a). Fibrils formed in the presence of 100 mM $CaCl_2$ were shorter, and were bent and twisted (Fig. 15.1b), and these are termed "wormlike" fibrils. At $CaCl_2$ concentrations between 0 and 100 mM, "long semiflexible" and "wormlike" fibril types coexist (Loveday et al. 2010). The morphology of fibrils is characterized by two length scales: persistence length, l_p, and contour length L_c. Persistence length, defined as $l_p = k/(k_B T)$, is the typical length at which thermal fluctuations begin to bend the polymer in different directions; it characterizes the flexibility or rigidity of a filament (MacKintosh 1998). The persistence length of food protein nanofibrils has been obtained from many experimental techniques (Loveday et al. 2012a), including dynamic light scattering, microscopic observation of thermal fluctuations, and transmission electron microscopy (TEM) data. The contour length, L_c, of a filament is its length at maximum extension. A filament is considered flexible when $l_p \ll L_c$, and rigid when the opposite holds ($l_p \gg L_c$); many biological filaments are in a third intermediate category; semiflexible filaments with l_p and L_c are of comparable magnitude (Storm et al. 2005). Typical values of the persistence and contour lengths of β-lg fibrils are given in Table 15.1.

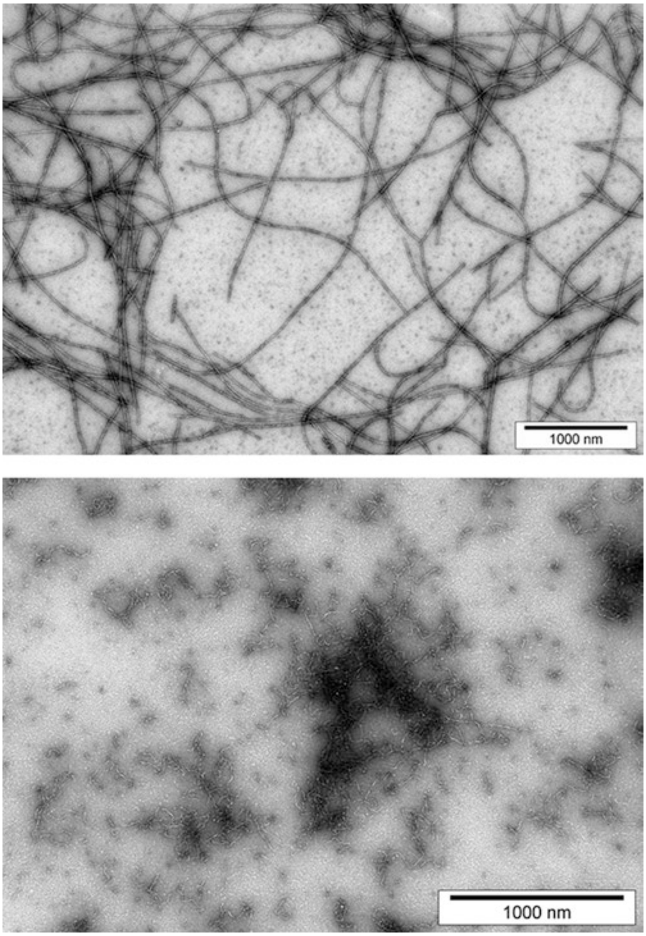

Fig. 15.1 β-lactoglobulin nanofibrils made by heating 1 % w/v protein at pH 2 and 80 °C with either (**a**) no added salts or (**b**) with 100 mM $CaCl_2$

15.2 Rheological Properties of Whey Protein Isolate Fibril Gels

On heating dispersions of β-lg at low pH and a constant temperature, the magnitude of the storage modulus (G') increases with time as the proteins self-assemble into fibrils. From the data of such a gel-cure experiment, the magnitudes of the equilibrium modulus and gel time, which reflect the morphology of the fibrils, can be determined by using the relationship (Kavanagh et al. 2000):

$$G' \approx G'_{\mathrm{inf}} \exp\left(-B/t\right) \quad (15.1)$$

Table 15.1 Contour length (L_c) and persistence length (l_p) data for β-lactoglobulin fibrils

Study and method for measuring l_p	Concentration (% w/v)	Ionic strength	pH	Heating temperature (°C)	Heating time (h)	L_c (nm)	l_p (nm)
Aymard et al. (1999)	4	0.013	2	80	?[a]	?	600
X-ray and neutron	4	0.03	2	80	?	?	300
scattering	4	0.1	2	80	?	?	38
Mudgal et al. (2009)	1.5	<0.05	2	80	10	2500	788
TEM image analysis	4	<0.05	3.35	85	3	130	36
	8	<0.05	3.35	85	3	300	34
Loveday et al. (2010)	1	0	2	80	6	>2523	2607 ± 511
TEM image analysis	1	0.1 (NaCl)	2	80	6	>1569 (LS)[b]	4307 ± 747 (LS)
						>494 (WL)	80 ± 15 (WL)
	1	0.3 ($CaCl_2$)	2	80	6	>1170 (LS)	1846 ± 140 (LS)
						>514 (WL)	67 ± 4 (WL)

[a] Not specified

[b] Both long semi-flexible (LS) and worm-like (WL) fibrils co-existed under these conditions

where t is the time in seconds, G'_{inf} is the value of G' at infinite time, and B is the time taken for G' to reach G'_{inf}. Equation (15.1) satisfactorily reproduced both the asymptotic limit as $t \rightarrow \infty$ and the logarithmic singularity as $t \rightarrow t_{\mathrm{gel}}$, the gelation time (Kavanagh et al. 2000). Values of both parameters, G'_{inf} and t_{gel}, are required for testing of the data against physical models, such as a percolation-based kinetic gelation model. G'_{inf} can also be obtained by extrapolating the linear portion of a G' versus 1/time plot to find the G' intercept (Gosal et al. 2004a).

The β-lg gel-cure data (Fig. 15.2) had an initial lag phase, during which G' was below 10 Pa, followed by a very rapid increase in G', then a further increase in G', but at a slowing rate. $CaCl_2$ had a marked impact on the duration of the lag phase, shortening it in a concentration-dependent manner. For 80 and 120 mM datasets there was dip in G' after long heating times, similar to that seen by Gosal et al. (2004b). They suggested that the dip could be caused by slippage due to gel shrinkage and syneresis after extended heating, or the infiltration of silicone oil between the cone and the sample.

The model in Eq. (15.1) was an excellent fit for 0 and 120 mM data, which had clear linear regions on "log(G') vs. 1/t" axes (Fig. 15.2 inset). The linear regions were less well defined with 40 and 80 mM data, and in choosing which points to

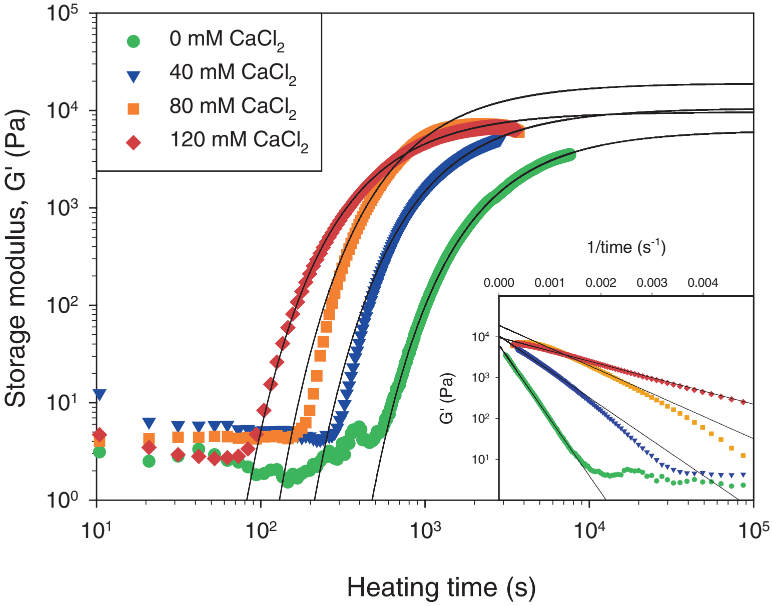

Fig. 15.2 Time course data for 10 % w/w WPI with 0–120 mM $CaCl_2$ heated at 80 °C on the rheometer. Lines show the fit of Eq. 15.1 to data; the *inset* shows the linear fitting (Eq. 15.1)

Table 15.2 Effect of $CaCl_2$ on gel times, t_{gel} (in seconds) for 10 % WPI heated at 80 °C on the rheometer

$CaCl_2$	0 mM	40 mM	80 mM	120 mM
t_{gel} when $G' > 10$ Pa	637	334	209	115
t_{gel} from manual selection	491	271	156	73
t_{gel} from Eq. (15.1)	476	212	129	82

Three different methods were used for estimating t_{gel}, as explained in the text

include in the regression, preference was given to the later points, for which G' was higher and could therefore be measured more accurately. Three different approaches were used to calculate t_{gel}: (a) choosing the time at which G' first exceeded 10 Pa, (b) manually selecting the beginning of the asymptotic increase in G', or (c) extrapolating Eq. (15.1) to the time at which it predicted $G' = 1$ Pa.

Table 15.2 shows the effect of $CaCl_2$ on t_{gel}, as calculated with these three methods. All three showed the same trend: added calcium produced a logarithmic decrease in t_{gel} with increasing $CaCl_2$ concentration. This approach has also been successfully applied to describe the effects of other mono- and divalent cations on β-lg fibril gelation (Loveday et al. 2012b).

15.3 Conclusion

Recent results on the structure and rheology of fibrils made from whey protein β-lactoglobulin (β-lg) are discussed here in brief. A more extensive review of the subject can be found elsewhere (Loveday et al. 2012a). It is shown that protein self-assembly leads to large and useful changes in rheological properties. In situ small amplitude oscillatory measurements are a useful tool that can provide information for both rheological modeling and practical purposes.

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