Kinetics of microstructure formation of high-pressure induced gel from a whey protein isolate

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Abstract. The kinetic process of pressure-induced gelation of whey protein isolate (WPI) solutions was studied using in situ light scattering. The relationship of the logarithm of scattered light intensity ($I$) versus time ($t$) was linear after the induced time and could be described by the Cahn-Hilliard linear theory. With increasing time, the scattered intensity deviated from the exponential relationship, and the time evolution of the scattered light intensity maximum $I_m$ and the corresponding wavenumber $q_m$ could be described in terms of the power-law relationship as $I_m \sim t^\beta$ and $q_m \sim t^{-\alpha}$, respectively. These results indicated that phase separation occurred during the gelation of WPI solutions under high pressure.

1. Introduction

Milk whey proteins are a by-product of cheese manufacturing, and are widely used as functional ingredients in many food products. An important functional property of whey proteins is gelation. Hydrostatic pressure has recently been shown to induce gelation in whey proteins under appropriate conditions (Dumay, Kalichevsky, & Cheftel, 1998; He & Kanno, 2006a; He, Azuma, & Kanno, 2006b; Kanno, Mu, Hagiwara, Ametani, & Azuma, 1998; Kanno, He, Ametani, & Azuma, 2002; Van Camp & Huyghebaert, 1995a). The contribution of intermolecular S-S bonds to the gelation of whey proteins has been demonstrated (He et al., 2006b; Kanno et al., 1998).

The protein gel is a three-dimensional network of unfolded protein in which free water is dispersed in interstices of the network. Gel properties, such as its rheological properties, water-holding capacity, appearance, and swelling behavior, depend on the morphological characteristics of the network (Ziegler & Foegeding, 1990). Therefore, kinetic studies on network formation during gelation are of great interest.

However, there is limited information on network formation during pressure-induced gelation of whey proteins. Detailed study of network formation during gelation is important for understanding the mechanism of gelation and designing new functional gels. In this study, the kinetics of pressure-induced gelation of a WPI solution was investigated using light scattering and morphological analysis.

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2. Materials and Methods

2.1 Materials

WPI powder from bovine milk was purchased from Davisco Food International, Inc. (Saitama, France). This product contains approximately 1% moisture, 95% protein, 3% ash, 1% lactose, and 1% lipids. WPI solutions (20%~28%, w/v) were prepared in distilled water, and their initial pH values (6.8 to 6.9) were adjusted to 7.0 by the addition of 0.5 mol/L NaOH.

2.2 High-pressure measurements

For the Kinetics study under high-pressure, we used thermo-jacketed high-pressure sample houses with flat quartz as the window material and water as the pressure-transmitting medium. The sample houses have a maximum operating pressure of 300 MPa. Each WPI solution was put into a quartz cell (1.5 ml) sealed with a polymeric film, then the quartz cell was set in a high-pressure house. The pressure of the sample house was measured with a precision strain gauge pressure transducer. Temperature was controlled using a water bath and a thermocouple that was inserted into a hole drilled into the high-pressure houses. All pressure measurements were performed at 25°C.

2.3 Light scattering

Light scattering was carried out in a SLM 48000 fluorescence-spectrophotometer (SLM Co.) in which the sample house was modified, as described above, to allow measurement of the scattered light intensity using incident light at an angle of 90° under high pressure.

Since the intensity of the incident light is altered when the incident light wavelength changes, correction of scattering light intensity is essential for quantitative application of the scattering data obtained by fluorescence-spectrophotometry. In the empirical method, the correction factor \( F(\lambda) \) can be written in the form

\[
F(\lambda)I_{\text{app}}^\text{water} \equiv 1
\]

where \( I_{\text{app}}^\text{water} \) is the apparent scattered intensity of water. The scattered intensity \( I \) can then be written as

\[
I = F(\lambda)I_{\text{app}}
\]

where \( I_{\text{app}} \) is the apparent scattered intensity of the sample.

2.4 Scanning electron microscopy (SEM).

Each gel was cut into small pieces with a razor blade and fixed in a 4% (v/v) glutaraldehyde solution at 4°C for 16 h.15) The gel pieces were then dehydrated by immersing in a series of ethanol solutions of 30, 50, 70, 85, 95 and 100% (v/v), and finally in isoamyl acetate (97%, v/v).33) After this dehydration, critical-point drying was performed in liquid CO\(_2\) in an HCP-2 pressurized chamber (Hitachi Koki Co., Tokyo, Japan). The dried sample was coated with carbon in an E1020 spotter (Hitachi, Tokyo, Japan). Each sample was then examined and photographed with an S-2250N scanning electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 15 kV and a magnification of 2000.

3. Results and Discussion

As mentioned in earlier studies (He et al., 2006a; He et al., 2006b; Kanno et al., 2002), it appears that pressure-induced gelation of a WPI solution is accompanied by phase separation. In the first set of experiments, the dynamic process of pressure-induced gelation was examined by conducting static light scattering experiments.

The early stage of phase separation is often analyzed on the basis of the Cahn-Hilliard linear theory (Cahn & Hilliard, 1958; Cahn & Hilliard, 1959), which suggests that scattered light intensities show exponential increase with time in the early stage of spinodal decomposition and can be described by

\[
I(q,t) = I(q,0)\exp[2R(q)t]
\]

(1).
Figure 1. Time evolution of the light scattering intensity profile for a 28% WPI solution pressurized at 250 MPa and 25°C.

Here, \( q \) is the scattering vector, and \( R(q) \) is the rate of growth of concentration fluctuation given by

\[
q = \frac{4\pi}{\lambda} \sin(\theta/2)
\]

(2) and

\[
R(q) = D_{\text{app}} q^2 \left[ 1 - \frac{q^2}{2q^2_{m0}} \right]
\]

(3),

where \( \lambda \) is incident wavelength and \( \theta \) is the scattering angle. \( D_{\text{app}} \) is the apparent diffusivity, and \( q^2_{m0} \) is the scattering vector corresponding to the maximum growth rate of fluctuations. The apparent diffusivity can be calculated from plots of \( R(q)/q^2 \) as the limiting value of \( q \) approaches zero. In other words,

\[
D_{\text{app}} = \lim_{q \to 0} \{ R(q)/q^2 \}
\]

(4).

Firstly, the solution of WPI with a concentration from 20% to 28% (w/v) was pressurized at 250 MPa and 25°C, respectively. Coincidentally, the scattered light intensity at various scattering vectors was real time measured. From these results, it can be seen that the \( \ln(I) \) versus \( t \) relationship is indeed linear after the induction time (data not shown). Furthermore, the value of \( R(q)/q^2 \) was calculated and had a fairly linear relationship with \( q^2 \) (data not shown). Thus, based on these results, it appears that the early stage of scattering behavior of WPI solution during gel formation under high pressure can be described by the Cahn-Hilliard linear theory.

As phase separation proceeds beyond the early stage of spinodal decomposition, the range of concentration fluctuations becomes significant (Cahn, 1965; Cahn et al., 1958; Cahn et al., 1959). The intermediate and late stages of spinodal decomposition are often described in terms of power-law relationships of the following type (Binder & Stauffer, 1974; Langer, Bar-on, & Miller, 1975):

\[
I_m \sim t^\beta
\]

(5) and

\[
q_m \sim t^{-\alpha}
\]

(6).

These relationships provide a scaling description, in terms of the exponents \( \beta \) and \( \alpha \) of time evolution, of the scattered light intensity maximum \( I_m \) and the corresponding wavenumber \( q_m \).

Figure 1 shows the scattering pattern as a function of time during the gelation of a 26% WPI solution pressurized at 250 MPa and 25°C. First, the position of the scattering maximum does not change and actually decreases with time. Finally, both \( I_m \) and \( q_m \) become steady. A similar scattering pattern was also obtained for other WPI solutions with the different concentrations (data not shown). Under this condition, uniform power-law behavior is not observed, and there is a time period during which \( \ln(q_m) \) decreases linearly with \( \ln(t) \). These results indicate that the scattering behavior of pressure-induced gelation from a WPI solution might be partially described by the scaling law.
Figure 2. Time evolution of the microstructure of 20% WPI solution in the presence of 20% lactose pressurized at 8000 MPa and 25°C for different holding time from 30 min to 12 hours.

At a microscale level, the pressure-induced gel of a WPI has a three-dimensional network structure (He et al., 2006a; He et al., 2006b; Kanno et al., 1998). Based on the earlier results, we had suggested that the microstructure of a WPI gel is formed by phase separation (He et al., 2006a; He et al., 2006b).

The data showed that the exponential increase in the scattered intensity with time could be described by the Cahn-Hilliard linear theory. With increasing time, the maximum scattered position \( q \) starts to decrease, and the scattered intensity starts to deviate from the exponential increase with time. The concentration fluctuation reaches a value equal to that in the coexistence curve, and the phase separation domains continue to grow in size in order to reduce the interfacial energy between the coexisting phases (Hashimoto, Itakura, & Shimidzu, 1986; Tanaka, 2000). Therefore, nonlinear behavior of time evolution of the structure function becomes obvious and leads to a crossover from exponential growth to power-law behavior for the enlargement of amplitudes.

The maximum value of the wavenumber \( q_m \) is related to the domain size scale \( (L) \), which is expressed as \( L = 2\pi q_m \), so that, it can be inferred that the domain size estimating from time \( L \sim t^\alpha \)

Indeed, these inferences in the domain size of microstructure of gel samples have been confirmed by electron microscopic analysis. Figure 2 shows the SEM photographs of gel samples from a 20% WPI (w/v) solution in the presence of 20% lactose pressurized at 800 MPa and 30°C for different holding time from 30 min to 12 hours. Qualitatively, it is clearly seen that the domain size increased following the morphology coarsens. The scaling characteristics of the structure exhibit self-similar growing behavior.

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4. Conclusion
The crosslinking of proteins is accompanied by phase separation during pressure-induced gelation of WPI, and the formation of microstructures may be due to the time evolution of phase separation.

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