

Fractal Analysis of the Elasticity of BSA and β -Lactoglobulin Gels

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The fractal structure of aggregates in heat-induced BSA and β -lactoglobulin gels prepared with and without CaCl_2 was examined. From the concentration dependence of the gel elasticity determined from a uniaxial compression test of the gel, the fractal dimensions D_f of the aggregates in the gels were evaluated, using the theory of Shih *et al.* It was confirmed that the gels with and without CaCl_2 showed weak- and strong-link behavior, as defined in the theory of Shih *et al.*, respectively. The obtained values of D_f were about 2 for strong-link gels and about 2.7 for weak-link gels. In addition, for the β -lactoglobulin gels containing CaCl_2 (weak-link type), from the analysis of the gel image obtained with confocal scanning laser microscopy of the gels, the fractal dimensions were also evaluated, the value being close to that found from the gel elasticity measurements. These results indicate that the elastic behavior of the aggregate gels is a reflection of the fractal structure of the aggregates in the gels.

Keywords: Fractal; aggregate; elasticity; confocal scanning microscopy; food protein

INTRODUCTION

Heating a globular protein solution causes aggregates of protein molecules by mechanisms such as hydrophobic interaction, disulfide bond formation, or electrostatic interaction, and a gel is often formed at high concentration (Clark and Lee-Tuffnell, 1986). Macroscopic physical properties such as elasticity for protein gels are important properties to be controlled. The aggregate structure in the gel would influence the macroscopic physical properties for gel. To systematically understand the behavior of the macroscopic physical properties of protein gels, the relationship between the structure of the aggregates and the macroscopic physical properties should be investigated. However, the structure of the aggregates in protein gels was difficult to characterize due to their disordered shape.

Recently, fractal analysis has attracted attention as a quantitative analytical method that can characterize many kinds of disordered shapes (Mandelbrot, 1982). A fractal is a self-similar structure which can be characterized by a noninteger dimension; the fractal dimension D_f (Mandelbrot, 1982; Viscek, 1989). It has been shown that colloid aggregate formed in a dilute system such as gold and silica can be characterized as a fractal (Weitz and Oliveria, 1984; Lin *et al.*, 1990). On the other hand, the studies on the fractal structure of gels formed by aggregation have not been well done, especially on proteins. Bremer *et al.* (1989, 1990, 1993) examined the fractal structure of a caseinate gel prepared by addition of glucono- δ -lactone from the concentration dependence of the elasticity of the gel. Shih *et al.* (1990) evaluated the fractal dimension of boehmite alumina colloidal gels from the measurement of the elasticity of the gels, using a theory different from that of Bremer *et al.*

We have shown that the value of the fractal dimension of the aggregates formed by heating dilute bovine serum albumin (BSA) solutions was 1.8 or 2.1 with light scattering methods, being close to those predicted by the cluster-cluster aggregation model (Hagiwara *et al.*,

1996). We have also performed fractal analysis of the aggregates in BSA gels by two different methods; one involves measurement of concentration dependence of gel elasticity using the theory of Shih *et al.* (1990) and the other involves analysis of the images obtained with confocal scanning laser microscopy (Hagiwara *et al.*, 1997). Consequently, it was concluded that the values of the fractal dimensions obtained from the rheological measurement agreed with those from the image analysis, indicating that the elastic behavior of the BSA gels is a reflection of the fractal structure of the aggregates in the gels. In addition, the BSA gels showed the behavior of the weak-link gel in the theory of Shih *et al.*, although the weak-link-type gels had not been found then.

Many studies have been reported in which the macroscopic properties of protein aggregate gels vary with the aggregation conditions; e.g., pH and ionic strength (Egelandsdal, 1980; Richardson *et al.*, 1981; Hatta *et al.*, 1986); however, only the correlation between the conditions and the macroscopic physical properties has been repeatedly discussed so far.

In this study, the fractal structure of aggregates in heat-induced BSA and β -lactoglobulin (β -LG) gels prepared under different conditions was examined, and the results were compared with those in the preceding work (Hagiwara *et al.*, 1997).

THEORY

Theory of Shih *et al.* (1990). The structure of a colloidal gel (aggregate gel) is approximated as closely packed fractal flocs, and the elastic property of the gel is dominated by that of the flocs (Shih *et al.*, 1990). Depending on the strength of the links between the neighboring flocs in comparison with that in the flocs, the links are classified into two types; strong and weak. In the strong-link regime, the links between the neighboring flocs have a higher elasticity than those in the flocs. For the gel with a strong link (hereafter referred to as a strong-link gel), the dependence of the elasticity E and the limit of linearity γ_0 (γ_0 is the upper limit value of strain (γ) where the stress σ is proportional to γ) of the gels on the particle (in this study, protein) concen-

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tration ϕ can be described as follows:

$$E \propto \phi^{(3+x)/(3-D_f)} \quad (1)$$

$$\gamma_0 \propto \phi^{-(1+x)(3-D_f)} \quad (2)$$

where D_f is the fractal dimension of the flocs ($D_f \leq 3$), and x is the backbone fractal dimension of the flocs, which varies between 1.0 and 1.3 (Shih *et al.*, 1990). On the other hand, in the weak-link regime, the links in the flocs have a higher elasticity than those between the neighboring flocs: for the gel with a weak link (hereafter referred to as a weak-link gel), the dependence of the E and γ_0 on particle concentration ϕ can be expressed as follows:

$$E \propto \phi^{1/(3-D_f)} \quad (3)$$

$$\gamma_0 \propto \phi^{1/(3-D_f)} \quad (4)$$

EXPERIMENTAL PROCEDURES

Protein Samples. β -LG was obtained from Sigma Chemical Co. (St. Louis, MO, ref L-6879) and was a mixture of genetic variants A and B. BSA was obtained from Boehringer Mannheim GmbH (Mannheim, Germany; ref 238040).

Preparation of Gels for Elasticity Measurement. β -LG was dissolved in two different types of buffers 50 mM HEPES buffer (pH 7.0, no salt added) and 50 mM HEPES buffer (pH 7.0, CaCl_2 added to make the concentration of the calcium ion 30 mM). The solutions were degassed under vacuum for 3 min. The pH of the solutions was then adjusted to the pH of the buffer, using NaOH solutions. The solutions prepared above were poured into a glass tube (28 mm in outer diameter, 20 mm in inner diameter, 40 mm in height) precoat with Sigmacoat (Sigma Chemical Co.) and each end was closed with a Teflon cap. To prevent the production of bubbles, the samples were preheated at 40 °C for 60 min. Then, preheated samples were heated in a water bath at 95 °C for 10 min. The samples were immediately cooled to 25 °C in a water bath. The β -LG gels were removed from the tube, cut into cylindrical shapes (15 mm in height), and stored in silicon oil at 25 °C for 24 h before measurement.

BSA was dissolved in 50 mM HEPES buffer (pH 7.0, no salt added), and the BSA gels were prepared by a method similar to that of β -LG gels, preheating temperature being 50 °C.

Elasticity Measurements. The elasticity of the gels was determined from a uniaxial compression test with Rheoner RE-3305 (Yamaden Co., Tokyo, Japan) by the same procedure in the preceding study (Hagiwara *et al.*, 1997). A cylindrical gel was vertically compressed with a flat plunger (30 mm diameter) at a compression rate of 1.0 mm/s. The strain γ of the gel was determined as the ratio of the deformation to the initial height of the gel. The elasticity E was calculated from the linear part of the stress-strain curve at $\gamma < 0.01$. The elasticity measurements were performed in triplicate.

Estimation of the Limit of Linearity γ_0 . It is difficult to determine γ_0 accurately; however, only the sign of the slope of the $\log \gamma_0$ vs $\log \phi$ plot is necessary to determine the value of D_f , as explained later. Therefore, the limit of linearity γ_0 was estimated by the same method used in the preceding study (Hagiwara *et al.*, 1997); as the limit of linearity γ_0 , the strain value where the deviation was 5% between the ordinate value of the stress-strain curve σ and γE was taken.

Evaluation of the Fractal Dimension from Elasticity Measurements. The same procedure as in the preceding study (Hagiwara *et al.*, 1997) was used. First, γ_0 was plotted against ϕ . Because γ_0 decreases with increasing ϕ for a strong-link gel and increases for a weak-link gel as shown in eqs 2 and 4, the link type for the gel can be identified from the sign of the slope of the plot for $\log \gamma_0$ vs $\log \phi$. For the weak-link gels, the value of the fractal dimension D_f is evaluated from the slope of the $\log E$ vs $\log \phi$ plot, using eq 3 for a weak-link gel. For the strong-link gel, to determine the value of D_f from

eq 1, the value of the fractal dimension for the effective backbone of the aggregate x is necessary. However, because x took a value from 1.0 to 1.3 (Shih *et al.*, 1990), the obtained value of D_f varies little in the range of the value of $x = 1.0$ –1.3, as shown later. Therefore, in this study, the minimum and maximum values of D_f were estimated from the slope of the $\log E$ vs $\log \phi$ plot, using the value of x as 1.0–1.3, for strong-link gels.

Preparation of the Gels for Confocal Scanning Microscopy. The preheated protein solutions, as explained before, were poured into a gap of 0.18 mm between two glass plates equipped with a spacer made from Niftron tape (Nitto Denko Co., Osaka, Japan). The samples were heated at 95 °C for 10 min. Thereafter, the sample was cooled to 25 °C and stored for 24 h. Then, the gel strips were removed and cut into 5 mm squares. The obtained gels were immersed in the buffer containing 0.001 wt % fluorescein isothiocyanate (FITC, a fluorescent labeling agent for proteins (Brelje *et al.*, 1993)) for 1 h with gentle shaking and subsequently washed in fresh buffer for 1 h. The stained gels were then mounted on a slide glass with a spacer around the gels. Then, a cover glass was placed on top of the spacers and fixed with clear nail polish.

Confocal Scanning Microscopy. A confocal laser scanning microscope Model MRC600 (Bio-Rad Laboratories, Inc., Hercules, CA) was used.

Evaluation of Fractal Dimension D_f from the Images of Protein Aggregates in a Gel. The same procedures as those in a preceding study (Hagiwara *et al.*, 1997) were used. Clear images of the aggregates in the β -LG and the BSA gels prepared with 50 mM HEPES buffer (no salt added) as a solvent were not obtained using confocal scanning microscopy; therefore, the image analysis was done only for the β -LG gel prepared with 50 mM HEPES buffer containing 30 mM CaCl_2 . The obtained confocal microscopy images were digitized with the public domain NIH Image program ver.1.59 (Rasband, 1996) on the Macintosh platform. From the digitized image, the fractal dimension D_f was calculated by the box counting method (Kaye, 1989; Bourke, 1993) as follows.

A square mesh of size L is laid over the object on the digitized image. The fractal dimension of the protein aggregates on the image, D_f , is determined using eq 5 from the slope of the double-logarithmic plot for $N(L)$ vs L ; computer software for fractal analysis (Bourke, 1993) was used.

$$N(L) \propto L^{-D_f} \quad (5)$$

where $N(L)$ is the number of mesh boxes that contain part of the image. The fractal dimension of protein aggregates of three dimensions D_f can be calculated from the following equation (Vicsek, 1989):

$$D_f = D + 1 \quad (6)$$

RESULTS

Figure 1 shows the stress-strain curves for β -LG gels (no salt added) (A), BSA gels (no salt added) (B), and β -LG gels (CaCl_2 concentration 30 mM) (C). The stress-strain curves for β -LG and BSA gels without salt addition curved upward ((A) and (B)), while those for β -LG gels with CaCl_2 curved downward. In appearance, the β -LG without salt addition (A) and the BSA gels without gel addition (B) were transparent, and the β -LG gel with CaCl_2 addition was turbid (C).

Figure 2 shows the examples of the estimation of γ_0 for the β -LG gels prepared with 50 mM HEPES buffer (no salt added) (A) and 50 mM HEPES buffer containing 30 mM CaCl_2 (B) from each stress-strain curve, as explained before. The values of γ_0 were estimated to be 0.062 (A) and 0.051 (B), respectively.

Figure 3A shows the double-logarithmic plot of the limit of linearity γ_0 vs protein concentration ϕ for the β -LG gels prepared with 50 mM HEPES buffer (no salt

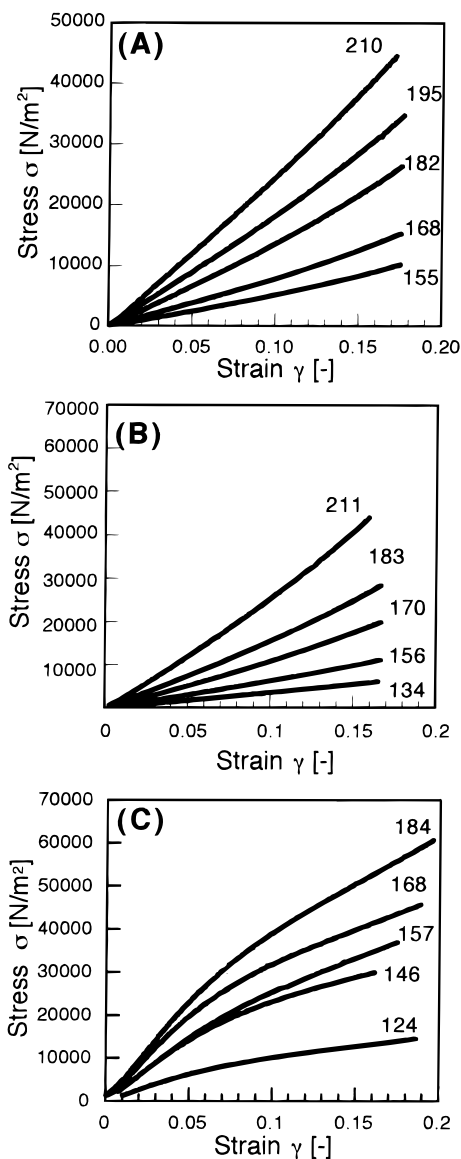


Figure 1. Stress–strain curves for the gels prepared with 50 mM HEPES buffer (pH 7.0): (A) β -LG (no salt added); (B) BSA (no salt added.) (C) β -LG (CaCl_2 , 30 mM). The numbers in the figures represent the concentration of the gels (kg/m^3).

added). Because γ_0 tended to decrease with increasing ϕ , this gel is confirmed to be a strong-link gel, as explained before. Figure 3B is the double-logarithmic plot of the gel elasticity E vs ϕ for the same gels as those in (A). From the slope of the plot, using eq 1 for strong-link gels, the fractal dimension D_f was estimated to be 2.14–2.20, considering that the fractal dimension of the effective backbone, x , takes a value of 1–1.3 (Shih *et al.*, 1990). Panels A and B of Figure 4 show the double-logarithmic plots of γ_0 vs ϕ and E vs ϕ , respectively, for the BSA gels prepared with 50 mM HEPES buffer (no salt was added). From the slope of the $\log \gamma_0$ vs $\log \phi$ plot, this gel is also confirmed to show strong-link behavior, and the value of the fractal dimension D_f was estimated to be 2.00–2.07, using eq 1. Panels A and B of Figure 5 are the double-logarithmic plots of γ_0 vs ϕ and E vs ϕ , respectively, for the β -LG gels prepared with 50 mM HEPES buffer containing 30 mM CaCl_2 . From the slope of the $\log \gamma_0$ vs $\log \phi$ plot, this gel was confirmed to be a weak-link gel, and the fractal dimension D_f was evaluated to be 2.69, using eq 3.

Table 1 summarizes the results of the fractal analysis of the β -LG and the BSA gels from the elasticity

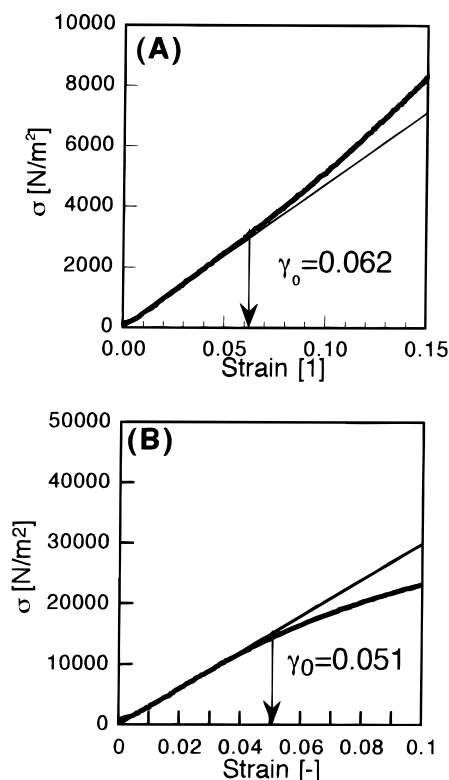


Figure 2. Examples of estimation of the limit of linearity γ_0 from stress–strain curves: (A) 155 kg/m^3 β -LG gels prepared with 50 mM HEPES buffer (no salt added); (B) 146 kg/m^3 β -LG gels prepared with 50 mM HEPES buffer containing 30 mM CaCl_2 .

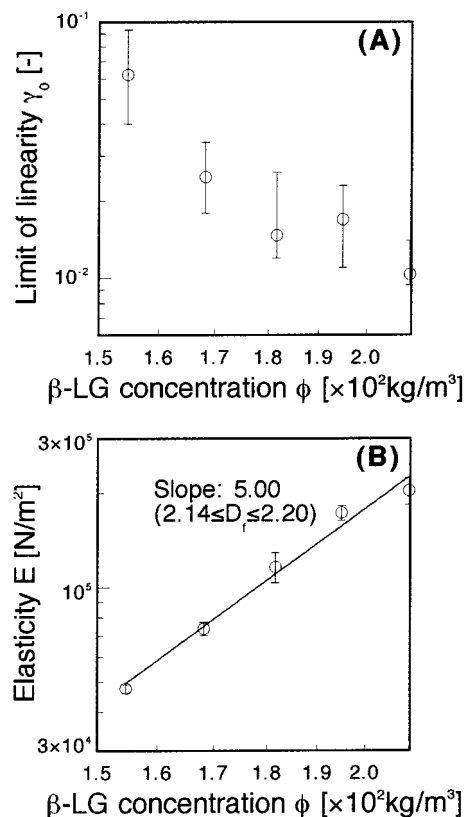


Figure 3. Double-logarithmic plots of the limit of linearity γ_0 vs β -LG concentration ϕ (A) and elasticity E vs ϕ (B). Buffer condition: No salt was added.

measurements, including the results of that for the preceding study (Hagiwara *et al.*, 1997).

Table 1. Summary of the Fractal Analysis of BSA and β -Lactoglobulin Gels Prepared with Various Buffers as Solvent

protein	buffer conditions	link type	fractal dimens	gel appearance
β -LG	pH 7.0, no salt was added	strong	2.14–2.20	transparent
	pH 7.0, 30 mM CaCl ₂	weak	2.69	turbid
BSA	pH 7.0, no salt was added	strong	2.00–2.07	transparent
	pH 7.0, 30 mM CaCl ₂ ^a	weak	2.82	turbid
	pH 7.0, 5 mM CaCl ₂ ^a	weak	2.82	turbid
	pH 5.1 ^a	weak	2.61	turbid

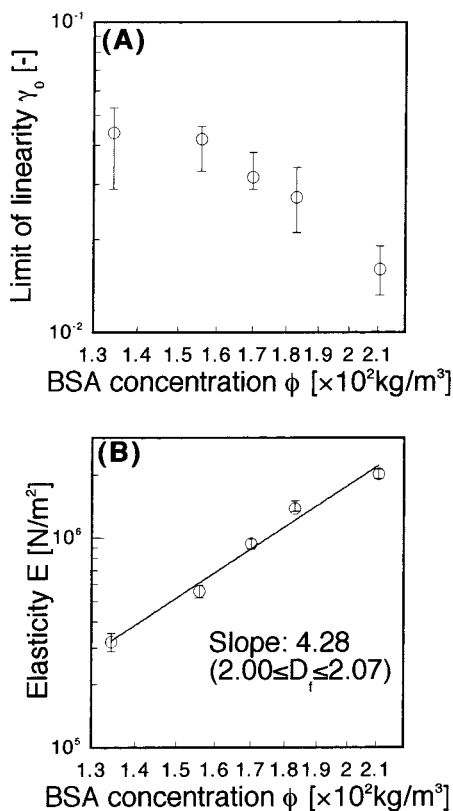
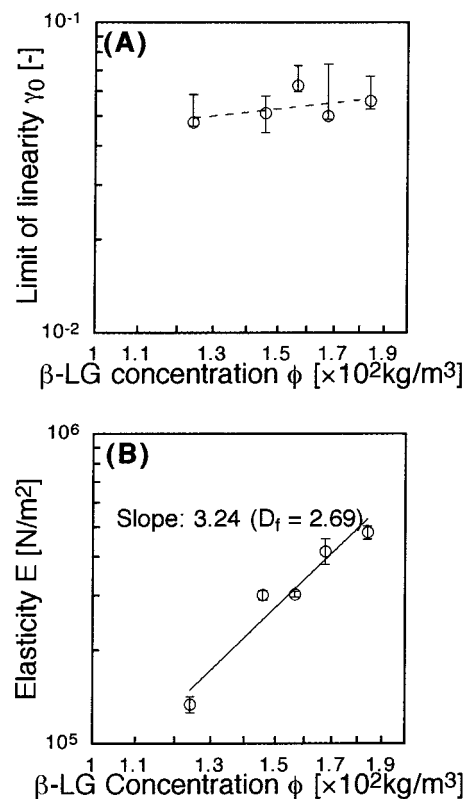
^a Hagiwara *et al.*, 1997.**Figure 4.** Double-logarithmic plots of the limit of linearity γ_0 vs BSA concentration ϕ (A) and elasticity E vs ϕ (B). Buffer condition: No salt was added.

Figure 6 shows typical images taken by confocal scanning microscopy for the β -LG gels prepared using 50 mM HEPES buffer without salt addition (A) and the BSA gels prepared by using 50 mM HEPES buffer without salt addition (B). Aggregates of micrometer order were not observed in these gels, suggesting that the order of the aggregate size was smaller than a micrometer. Figure 7 shows a typical original image (A) and the digitized image (B) obtained with confocal scanning microscopy for a β -LG gel prepared with 50 mM HEPES buffer containing 30 mM CaCl₂. Aggregates of micrometer order were observed in the β -LG gel containing 30 mM CaCl₂.

Figure 8 shows the double-logarithmic plot of the box number $N(L)$ vs box size L for the same gels as Figure 7. The plot shows the power law dependence of the box number $N(L)$ on box size L , as predicted by eq 5. From the slope of the plot, the value of the fractal dimension on the image, D , in eq 5 for the gel was 1.70. From eq 6, the value of D_f was calculated to be 2.70. The obtained value of D_f was close to that obtained from the concentration dependence of the gel elasticity ($D_f = 2.69$), indicating that the elastic behavior of this gel is a reflection of the fractal structure of the aggregates in the gels, as confirmed for the BSA gels investigated in the preceding study (Hagiwara *et al.*, 1997). In addition,

**Figure 5.** Double-logarithmic plots of the limit of linearity γ_0 vs β -LG concentration ϕ (A) and elasticity E vs ϕ (B). Buffer condition: CaCl₂ concentration was 30 mM.

irrespective of the β -lactoglobulin concentration in the concentration range examined, each value of D_f obtained from the image of the gels was almost constant ($D_f = 2.70 \pm 0.02$).

DISCUSSION

Bremer *et al.* (1989, 1990, 1993) proposed a theory, which related gel elasticity to the fractal structure of aggregates in a colloidal gel. In their theory, they classified gels into type 1 and type 2. For a type 1 gel, the strands composing the network in the gel are stretched or shrunk under applied stress, while the strands of the type 2 gels are bent under applied stress. Equations for evaluating the fractal dimensions are different between the two types of gels. It is, however, difficult to identify experimentally such strand properties. Therefore, from the theory of Bremer *et al.*, one can only guess the types of a gel (type 1 or type 2) by comparing the value of the fractal dimension determined with that found by another method such as image analysis. On the other hand, the cross-link type in the theory of Shih *et al.* (1990) used in the present study is identified from the dependence of γ_0 on ϕ . Thus, the fractal dimension D_f can be determined from rheological measurement only, the theory of Shih *et al.* used in this study having an advantage over that of Bremer *et al.*

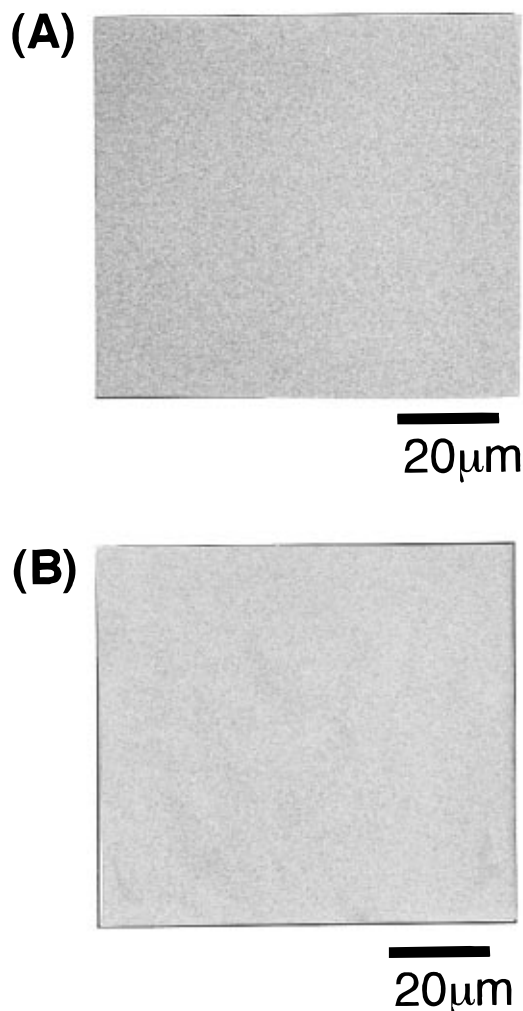


Figure 6. Photographs obtained using confocal scanning laser microscopy for the aggregates in β -lactoglobulin gels (no salt added) (A) and BSA gels (no salt added) (B). Gel concentration: (A) 182 kg/m^3 ; (B) 183 kg/m^3 .

The stress-strain curves for β -LG and BSA gels without salt addition curved upwards (Figure 1A,B), while those for β -LG gels with CaCl_2 curved downward. We have shown that those for the BSA gels with CaCl_2 curved upward in the preceding study (Hagiwara *et al.*, 1997). These differences suggest that the mechanism causing nonlinearity of the elasticity (for example, breaking bonds) was different between these gels. Tracing the deviation of the theory of Shih *et al.* (1990), however, it is found that the eqs 1-4 in this study are derivable without assuming a specific mechanism causing nonlinearity of the elasticity. Therefore, the equations are applicable, independent of the mechanism causing nonlinearity. In addition, not only for the β -LG gels in Figure 1C of this study (the stress-strain curves bent downward) but also for the BSA gels in the preceding study (the stress-strain curves bent upward), the values of D_f obtained from the elasticity measurement were close to those evaluated from the image analysis, demonstrating the applicability of the theory of Shih *et al.*, irrespective of the shape of the stress-strain curves.

We have shown that the elastic behavior of the BSA gels prepared in the preceding study was a reflection of the fractal structure of the protein aggregates on the scale length of micrometers (Hagiwara *et al.*, 1997). Therefore, to understand the elastic behavior among the gels at various metal ion concentrations from the point

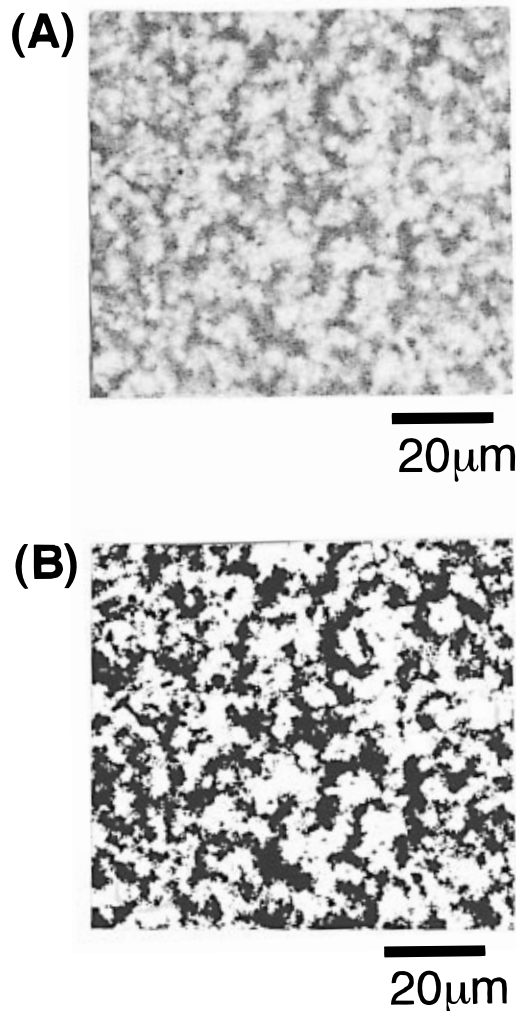


Figure 7. Photographs obtained using confocal scanning laser microscopy for the aggregates in β -lactoglobulin gel prepared with 50 mM HEPES buffer containing 30 mM CaCl_2 : (A) original image; (B) digitized image. Gel concentration, 157 kg/m^3 .

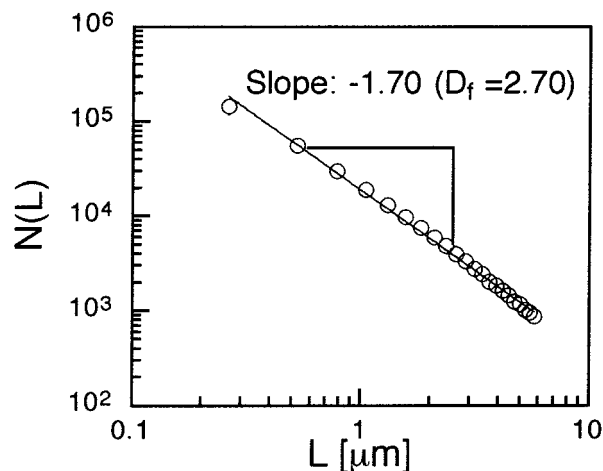


Figure 8. Estimation of fractal dimension D_f for aggregates in β -LG gels prepared with 50 mM HEPES buffer (pH 7.0, 30 mM CaCl_2) as a solvent. β -LG concentration, 157 kg/m^3 .

of view of the correlation between the macroscopic physical properties of the gels and the microscopic structure of the gels, it is necessary to observe the micrometer-scale structure of the aggregates in the gel. Many research studies investigated the microstructure of protein aggregates using electron microscopy (Clark *et al.*, 1981; Koseki *et al.*, 1989; Murata *et al.*, 1993);

however, in these studies only the microstructure on a scale range of a few nanometers to hundreds of nanometers was observed, not a scale range of micrometers. There were few studies that observed the microstructure for the protein aggregate gels containing metal salts in a solvent with confocal scanning microscopy. In this study, using confocal scanning microscopy, the difference in the aggregate structure among the gels prepared at different metal ion concentrations was observed. Confocal scanning microscopy is a promising tool to understand the behavior of physical properties of other food gels relating to the microstructure on a micrometer at scale.

Shih *et al.* (1990) reported that boehmite alumina colloid gels showed strong-link behavior. However, a gel with weak-link behavior had not been reported until we found that BSA gels prepared with 50 mM HEPES buffer (pH 7.0) containing 5 mM CaCl₂ or that containing 30 mM CaCl₂ showed weak-link behavior (Hagiwara *et al.*, 1997). In the present study, it was shown that the BSA gel without salt addition (Table 1) showed strong-link behavior. In addition, as shown in Table 1, the β -LG gels also showed both strong- and weak-link behavior, by controlling the concentration of CaCl₂. That is, it was confirmed that the protein aggregate gels showed both strong- and weak-link behavior by varying the aggregation conditions in this study.

Shih *et al.* (1990) stated that aggregate gels could be both the strong- and weak-link types, according to the rigidity of the aggregates in the gel; in the case where the aggregate deforms flexibly against applied force, the gel shows strong-link behavior, while in the case where the aggregate has a rigid structure, the gel shows weak-link behavior. As for the β -LG gels and the BSA gels, a clear explanation of the existence of both strong- and weak-link behavior does not exist; however, the following reason can be considered: From electron microscopic observation of heat-induced protein aggregates such as BSA, the aggregates prepared at low ionic strength and neutral pH were linear, similar to polymer chains in form (Murata *et al.*, 1993). The aggregates in the gels revealed to be strong-link gels in this study (prepared with 50 mM HEPES buffer, pH 7.0, no salt was added) also probably developed a similar structure. On the other hand, regarding the gels showing weak-link behavior in the preceding and this study, the aggregate structures were revealed from confocal scanning microscopy to be massive rather than linear (Hagiwara *et al.*, 1997). Therefore, it is suggested that these differences in shape among the aggregates caused the difference in rigidity among the aggregates, showing either strong- or weak-link behavior, in accordance with the degree of the rigidity of the aggregates. Furthermore, the table shows that the gels which were strong-link gels had a transparent appearance, while those which were weak-link gels had a turbid appearance. Transparency and turbidity of the protein aggregate gels might be universal characteristics for strong- and weak-link gels, respectively.

As for the β -LG gel and the BSA gel prepared with 50 mM HEPES buffer with no salt added, from confocal scanning laser microscopy, a clear image of the aggregates was not obtained, suggesting that the size of the aggregates was smaller than the smallest size the confocal scanning microscope used could observe. To analyze the structure of the aggregates for these gels, another experimental method rather than confocal scanning microscopy should be developed.

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LITERATURE CITED

- Bourke, P. *Fractal Dimension Calculator User Manual Version 1.5*; Auckland University, New Zealand, 1993.
- Brelje, T. C.; Wessendorf, W.; Sorenson, R. L. Multicolor Laser Scanning Confocal Immunofluorescence Microscopy: Practical Application and Limitations. *Methods Cell Biol.* **1993**, *38*, 97–181.
- Bremer, L. G. B.; van Vliet, T.; Walstra, P. Theoretical and Experimental Study of the Fractal Nature of the Structure of Casein Gels. *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 3359–3372.
- Bremer, L. G. B.; Bijsterbosch, B. H.; Schrijvers, R.; van Vliet, T.; Walstra, P. On the Fractal Nature of the Structure of Acid Casein Gels. *Colloids Surf.* **1990**, *51*, 159–170.
- Bremer, L. G. B.; Bijsterbosch, B. H.; Walstra, P.; van Vliet, T. Formation, Properties and Fractal Structure of Particle Gels. *Adv. Colloid Interface Sci.* **1993**, *46*, 117–128.
- Clark, A. H.; Lee-Tuffnell, C. D. Gelatin of Globular Proteins. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledward, D. A., Eds.; Elsevier Applied Science: London, 1986.
- Egelandsdal, B. Heat-Induced Gelling in Solutions of Ovalbumin. *J. Food Sci.* **1980**, *45*, 570–573, 581.
- Hagiwara, T.; Kumagai, H.; Nakamura, K. Fractal Analysis of Aggregates Formed by Heating Dilute BSA Solutions Using Light Scattering Methods. *Biosci., Biotechnol. Biochem.* **1996**, *60*, 1757–1763.
- Hagiwara, T.; Kumagai, H.; Nakamura, K. Fractal Analysis of Aggregates in Heat-Induced BSA Gels. *Food Hydrocolloids* **1997**, in press.
- Hatta, H.; Kitabatake, N.; Doi, E. Turbidity and Hardness of a Heat-induced Gel of Hen Egg Ovalbumin. *Agric. Biol. Chem.* **1986**, *50*, 2083–2089.
- Kaye, B. H. Image Analysis Techniques for Characterizing Fractal Structure. In *Fractal Approach to Heterogeneous Chemistry*; Avnir, D., Ed.; Wiley: New York, 1989.
- Koseki, T.; Kitabatake, N.; Doi, E. Irreversible Thermal Denaturation and Formation of Linear Aggregates of Ovalbumin. *Food Hydrocolloids* **1989**, *3*, 123–134.
- Lin, M. Y.; Lindsay, H. M.; Weitz, D. A.; Ball, R. C.; Klein, R.; Meakin, P. Universal Reaction-Limited Colloid Aggregation. *Phys. Rev. A* **1990**, *41*, 2005–2020.
- Mandelbrot, B. B. *The Fractal Geometry of Nature*; Freeman: San Francisco, 1982.
- Murata, M.; Tani, F.; Higasa, T.; Kitabatake, N.; Doi, E. Heat-Induced Transparent Gel Formation of Bovine Serum Albumin. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 43–46.
- Rasband, W. *NIH Image Manual*; National Institutes of Health: Washington, DC, 1996.
- Richardson, R. K.; Ross-Murphy, S. B. Mechanical Properties of Globular Protein Gels II. *Br. Polym. J.* **1981**, *13*, 11–16.
- Shih, W.-H.; Shih, W. Y.; Kim, S.-I.; Liu, J.; Aksay, I. A. Scaling Behavior of the Elastic Properties of Colloidal Gels. *Phys. Rev. A* **1990**, *42*, 4772–4779.
- Viscek, T. *Fractal Growth Phenomena*; World Scientific: Singapore, 1989.
- Weitz, D. A.; Oliveria, M. Fractal Structures Formed by Kinetic Aggregation of Aqueous Gold Colloids. *Phys. Rev. Lett.* **1984**, *52*, 1433–1436.

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