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Study on the Fractal Structure of Clustered Protein by Light Scattering

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Using static and dynamic light scattering, the fractal dimension and the kinetics of the aggregation for a solution of protein particles were investigated under various conditions of pH, the weight percentage of precipitation ($(NH_4)_2SO_4$), the concentration of protein, and temperature. Our results show that the radius of cluster with time at pH=3 grows with power law $R \sim t^b$ with b being 1.56 ± 0.03, which agrees with diffusion-limited cluster-cluster aggregation (DLCA) model. It is shown that the fractal dimension decreases with the increase of the protein concentration, but it increases with the weight percentage of precipitation. In addition, it is also shown that the cluster of aggregate does not grow with time at low temperature ($\leq 60°C$). However, the radius of cluster grows with $R \sim e^{\alpha t}$ at 65°C, which agrees with reaction-limited cluster-cluster aggregation (RLCA) model.

I. INTRODUCTION

In the past decade, many studies of the kinetics of the irreversible aggregation process have been made [1–13] in considerable detail for gold, silica, polystyrene, and protein colloids from both experimental and theoretical point of view, in order to understand their nonequilibrium phenomena in terms of the properties of the scale invariance of the resulting clusters. The clusters are usually characterized by their fractal dimension d that relates the total mass M of the aggregate to its typical size R, i.e., $M \sim R^d$.

It is well known from the theoretical point of view that, for irreversible aggregation in which clusters can not be separated into constituent monomers after sticking together, two regions of aggregation, characterized as diffusion-limited cluster-cluster aggregation (DLCA) and reaction-limited cluster-cluster aggregation (RLCA), appear in terms of the apparent rate-limiting step in the process. In the former case, individual particles or clusters stick with a high probability upon contact and yield clusters with the fractal dimension $d = 1.75 \pm 0.05$. This rapid DLCA process is found to exhibit power law kinetics with $R \sim t^{1/d}$, where R is the hydrodynamic radius of the cluster and t is the time [14–16]. On the other hand, in the slow RLCA process, the particles or the clusters have a low sticking probability and produce clusters with the fractal dimension $d = 2.05 \pm 0.05$. The RLCA process exhibits exponential kinetics with $R \sim e^{\alpha t}$, where α depends on the experimental conditions[17, 18].

Aggregation processes for various colloids have been investigated by means of many experimental techniques including computer simulation, X-ray scattering, neutron scattering, and light scattering [1–10]. Among these, light scattering has been provided as a powerful tool for characterizing the kinetics of aggregation because the measurements of the scattered intensity and of the linewidth in a quasi-elastic experiment give us information on the fractal dimensions and the hydrodynamic radius of the clusters[19–24]. A series of very interesting results using the dynamical light-scattering technique have been recently reported on gold, silica, and polystrene colloids under various experimental conditions. However, less work has been done on the fractal dimension and the kinetics of protein. Therefore, we will investigate the kinetics and the fractal dimension of the aggregation process of protein under various experimental conditions by using dynamic and static light scattering. Our results will be compared with other experimental and theoretical results.

The rest of the paper is organized as follows: In Sec. II, we review the theoretical background related to the light scattering experiment and present the sample preparation and our experimental results, where the pH dependence, the protein concentration dependence of the fractal dimension with and without the weight percentage of precipitation, and the temperature dependence of the property and structure of protein are discussed. Conclusion will be given in the last section.

II. EXPERIMENT

A Dynamic Light Scattering Measurement

In a homodyne dynamic light scattering experiment, the normalized autocorrelation function $C(\tau)$ is related to the normalized scattered-field autocorrelation function $g(\tau)$ as [25, 26]

$$C(\tau) = 1 + A\,|g(\tau)|\,, \tag{1}$$

where A is constant depending on the measurement system and τ denotes the delay time. For monodispersed colloid particles, $g(\tau)$ is given by

$$g(\tau) = \exp(-Dq^2\tau). \quad (2)$$

Here, $q = (4\pi n/\lambda)\sin(\theta/2)$ denotes the scattering vector with λ being the wavelength of the light, n the refractive index of the solution, θ the scattering angle, and D the translational diffusion coefficient. For a polydispersed system, the normalized autocorrelation function can be analyzed by the cumulants[26–28]. In general, the cumulant expression is expressed as

$$C(\tau) = 1 + A\exp(-2K_1\tau + \frac{2K_2}{2!}\tau^2 - \frac{2K_3}{3!}\tau^3 + ...), \quad (3)$$

where K_n denotes the n-th cumulant of $C(\tau)$. The first three cumulants are obtained from a third order polynomial least-squares fit of the logarithm of the normalized autocorrelation function given by

$$\ln(C(\tau) - 1) = \ln A - 2(K_1\tau - \frac{K_2}{2!}\tau^2 + \frac{K_3}{3!}\tau^3 + ...) \quad (4)$$

and the first cumulant $K_1(= Dq^2)$ is used to obtain the translational diffusion coefficient D, which can be related to the hydrodynmic radius R in terms of the Stokes-Einstein relationship; $D = kT/6\pi\eta R$ with k being the Boltzman constant, T the absolute temperature, and η the viscosity of the solution. Thus, the hydrodynamics radius R can be calculated if K_1 is given by the dynamic light-scattering experiment.

In our experiment, we used a photon correlation spectroscopy apparatus that consists of a BIC (Brookharven Instrument Co.). In the goniometer, the incident radiation is supplied by a spectra-physics 10 mW He-Ne laser. The sample holder is centered in a thermostated index-matching vat which is mounted on the pivot point of the optical rail holding the detector and which is filled with the Declain as an index-matching liquid. The scattered light is collected by an iris diaphragm and then focused by a 100 mm forcal length lens onto a selected pinhole (100, 200, 400 μm). The light that passes through the pinhole is focused by the detector optics onto a PMT. The output of the PMT is sent to an amplifier-discriminator, and the resultant signal is sent to the Brookhaven BI9000AT 232 channel correlator. Figure 1 shows the schematic experimental set-up.

B Sample preparation

The protein used in this experiment is Albumin having spherical shape, which was purchased from the Sigma Chemical Co. The samples with the concentrations of protein being 2, 4, 6, 8, and 10 g/dl were used. NaOH and HCl were used to control the pH value of samples and the pH values of each sample were obtained as 3, 5, 7, 9, and 11, respectively. In order to form colloid, the amonium sulfate $(NH_4)_2SO_4$ was used for precipitation. The weight percentages of precipitation were given by 7.5, 15, 25, and 30 wt%, respectively. In addition, the temperatures of the sample were controlled at 25, 30, 40, 50, 60, and 65°C, respectively, to understand the temperature dependence of protein colloid. The particles were filtered with a filter of pore size 0.45 μm in order to remove any dust.

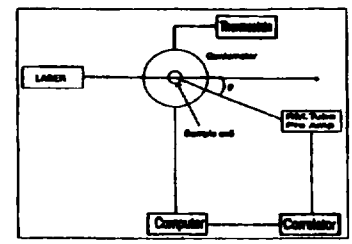

FIG. 1: Block diagram of experimental system.

C Experimental results and discussion

The time averaged autocorrelation function of the scattered intensity for the concentration of protein 2 g/dl is shown in Fig. 2, as a function of time. The diffusion coefficient D obtained from COTIN program is $1.08 \times 10^{-7}\text{cm}^2/\text{s}$ and the radius of protein calculated from the Einstein-Stokes relation ($D = kT/6\pi\eta R$) is given by 7.31 nm. It is to be noted that the hydrodynamic radius of protein for other concentrations of protein except for 2 g/dl can be obtained by same procedure.

1 pH dependence

To study the change of cluster size according to the pH dependence of sample, the samples with different pH values for each concentration of protein were prepared. Table 1 shows the hydrodynamic radius of protein resulting from the dynamic light scattering. It is clearly seen from the table that the hydrodynamic radius of protein increases with the decrease of the concentration of sample, especially for the concentration of protein, 2 g/dl. In the case of pH = 3, we can see that the clusters of protein stick together and they form the fractal structure. Figure 3 shows the hydrodynamic radius of protein for pH = 3 as a function of time. As shown in the figure, the clusters of protein grow with time. There exists a little growth of

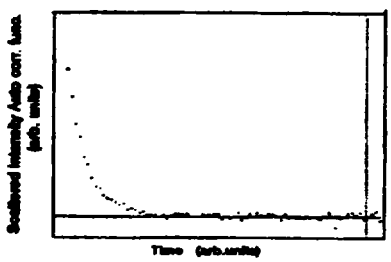

FIG. 2: Time averaged autocorrelation function of the scattered intensity as a function of time.

TABLE I: The effective diameter of bovin serum albumin concentration.

Concentration (g/dl)	2	4	6	8	10	Average Diameter
Effective Diameter (nm)	7.31	7.01	7.41	6.81	7.01	7.11 ± 0.03

cluster for 80 minutes from aggregation. After then, the growth of cluster with time shows the form $R \sim t^b$. This growth form is similar to that of DLCA, but the experimental value of b given by 1.56 ± 0.03 is different from the value of $b = 0.57$ presented by the DLCA theoretical model.

2 *Protein concentration dependence without precipitation*

Also, to understand the dependence of the fractal dimension on the protein concentration for a fixed pH value of 3, the fractal dimension of samples with different concentrations was measured by static light scattering. Figure 4 shows the relationship between $\ln(I)$ and $\ln(q)$ with time for the concentration of protein, 6 g/dl, where I and q denote the light intensity of scattering and the scattering vector, respectively. The fractal dimension, D_f, can be obtained from the linear slope of $\ln(I) - \ln(q)$ plot since the light intensity of scattering depends on the scattering angle and the fractal dimension, that is, $I(q) \propto q^{-D_f}$. The fractal dimensions of cluster at 80, 180, and 345 minutes after aggregation are, respectively, given by 1.30 ± 0.05, 1.71 ± 0.07, and 1.79 ± 0.04 from the slopes of the figure. The average fractal dimension between 150 minutes and 345 minutes is found to be 1.78 ± 0.08. In addition, the average fractal dimension of other samples with the concentrations of protein, 8 and 10 g/dl, is given by 1.77 ± 0.08. It is shown that our results are in good agreement with the theoretical value

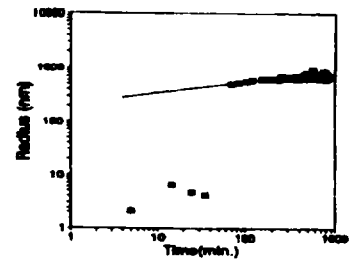

FIG. 3: Radius of cluster as a function of time (pH=3). Here, the solid line represents the fit of $R \sim t^b$ with 1.56 ± 0.03.

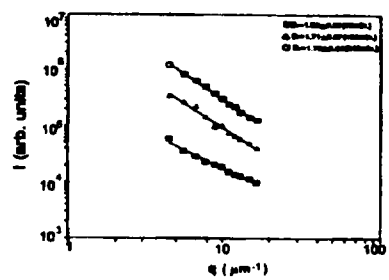

FIG. 4: ln(I) vs ln(q) for a specific protein concentration of 6 g/dl in the case of pH=3.

of 1.75 presented by the DLCA model.

3 *Protein concentration dependence with precipitation*

First, we consider the case where the protein is aggregated with different protein concentrations at a fixed weight percentage of precipitation of 7.5 wt%. In this case, the relationship between $\ln(I)$ and $\ln(q)$ obtained from the static light scattering is shown in Fig. 5, where the fractal dimensions of the different protein concentrations, 2, 4, 6, and 10 g/dl, are given by 2.08 ± 0.09, 1.89 ± 0.09, 1.76 ± 0.09, and 1.75 ± 0.08, respectively. It is clearly seen from the figure that the fractal dimension decreases as the protein concentration increases. This phenomena can be understood by the sticking probability. The sticking probability of colloid decreases as the protein concentration decreases and the resulting cluster has more dense structure due to the reconstruction of colloids. In this case, the clusters are formed, as in

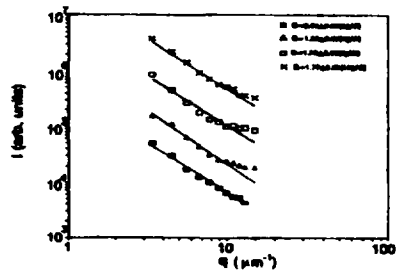

FIG. 5: ln(I) vs ln(q) for different protein concentrations in the case of the ammonium sulfate of 7.7 wt%.

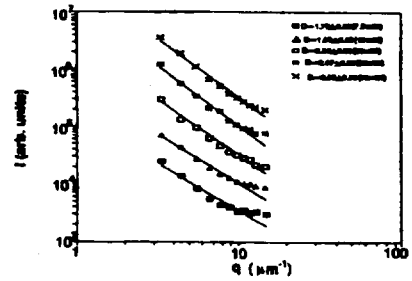

FIG. 6: ln(I) vs ln(q) for different ammonium sulfate concentrations in the case of the protein concentration of 6 g/dl.

the RLCA model. For high protein concentration, however, clusters stick with a high probability upon contact, so that the resulting cluster has less dense structure. In this case, the clusters are formed, as in the DLCA model.

Next, we investigate the fractal dimension for a fixed protein concentration by changing the weight percentage of precipitation. The relationship between $\ln(I)$ and $\ln(q)$ obtained from the static light scattering is shown in Fig. 6, where the fractal dimensions for different weight percentages of precipitation of 7.5, 15, 25, and 30 wt% at a fixed protein concentration of 6 g/dl are given by 1.76 ± 0.14, 1.88 ± 0.17, 2.10 ± 0.10 and 2.05 ± 0.04, respectively. It is shown from the figure that the fractal dimension increases as the weight percentage of precipitation increases. This can be understood from the reaction time of aggregation and diffusion time giving an influence on the fractal dimension, but more studies are needed to see the detailed mechanism associated with their relationship.

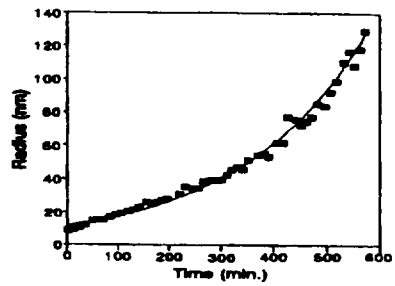

FIG. 7: Radius of cluster as a function of time at T=65°C. Here, the solid line represents the fit of $R_H \sim exp[\alpha t]$ and the value of α is 0.0042 ± 0.0001.

4 *Temperature dependence*

The property and structure of protein are affected by many factors, so that the activity of protein can be changed or decreased. One of main factors is temperature. The fractal dimension and hydodynamic radius with time are measured under various temperatures to understand the property of protein according to the temperature, where the temperature is controlled from 30°C to 65°C with the step increment of 5°C. For temperatures below 60°C, no aggregation is observed, but the property of protein is changed at 65°C, the clusters are aggregated, and the fractal structure is formed. The effective hydrodynamics radius of protein with time obtained from dynamic light scattering is shown in Fig. 7. The growth of cluster has the form $R_H \sim e^{\alpha t}$, and the value of α from our experiment is given by 0.0042 ± 0.0001. The relationship between $\ln(I)$ and $\ln(q)$ obtained from the static light scattering is shown in Fig. 8, where the average fractal dimension is given by 2.05 ± 0.06, which is in good agreement with the theoretical value of RLCA model. It is seen from this result that the aggregation progress of protein by temperature is very slow and the structure is dense.

III. CONCLUSION

So far, we have investigated the fractal dimension and the kinetics of the aggregation for a solution of protein particles under various conditions of pH, the weight percentage of precipitation ($(NH_4)_2SO_4$), the concentration of protein, and temperature, by using static and dynamic light scattering. The samples with the concentrations of protein being 2, 4, 6, 8, and 10 g/dl were used. NaOH and HCl were used to control the pH of samples and the pH values of each sample were obtained as 3, 5, 7, 9,

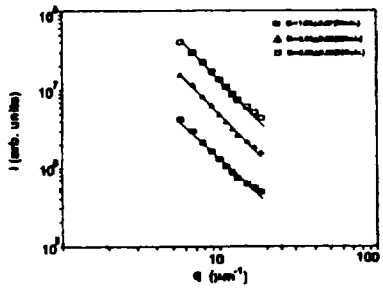

FIG. 8: ln(I) vs ln(q) for aggregation time at T=65°C.

and 11, respectively. For pH = 3 the clusters of protein stick together and they form the fractal structure. The growth of cluster with time shows the form $R \sim t^b$, which is similar to that of DLCA, but the experimental value of b given by 1.56 ± 0.03 is different from the value of $b = 0.57$ presented by the DLCA theoretical model. In the case of pH = 3, the average fractal dimension for different protein concentrations, 6, 8, and 10 g/dl, are given by 1.78 ± 0.08, 1.77 ± 0.08, and 1.77 ± 0.08, respectively. The results are in good agreement with the theoretical value of 1.75 presented by the DLCA model.

In the case where the weight percentage of precipitation was given by 7.5 wt%, the fractal dimensions for different protein concentrations, 2, 4, 6, and 10 g/dl, are given by 2.08 ± 0.09, 1.89 ± 0.09, 1.76 ± 0.09 and 1.75 ± 0.08, respectively. It is clearly seen that the fractal dimension decreases as the protein concentration increases. For low protein concentration, the clusters are formed, as in the RLCA model, while for high protein concentration, the clusters are formed, as in the DLCA model. Moreover, the fractal dimensions according to different weight percentages of precipitation, 7.5, 15, 25, and 30 wt% at a fixed protein concentration of 6 g/dl are given by 1.76 ± 0.14, 1.88 ± 0.17, 2.10 ± 0.10 and 2.05 ± 0.04, respectively. It is shown that the fractal dimension increases as the precipitation concentration increases. In addition, the fractal dimension was also investigated for the change of sample temperature. The cluster of aggregate does not grow with time at low temperature($\leq$ 60°C). However, the radius of cluster grow with $R \sim e^{\alpha t}$ at 65°C, and the average fractal dimension is given by 2.05 ± 0.06, which agrees with RLCA model.

In conclusion, the fractal dimension and the kinetics of the aggregation for a solution of properties are very sensitive to the pH value, the weight percentage of precipitation, the concentration of protein, and temperature.

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광산란 실험을 이용한 단백질분자 응고체의 프랙탈 구조에 관한 연구

김영희, 강명화, 유영훈, 류재연, 최치규, 홍성락, 김두철
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요 약

광산란 실험을 이용하여, 용액의 pH, 침전제$(NH_4)_2SO_4$의 wt%, 단백질의 농도 및 온도 변화에 따른 단백질 분자 응고체의 응집현상과 프랙탈 차원을 연구하였다. pH=3인 경우 시간(t)에 따른 응집체의 반경(R)은 멱함수 $Rt\sim_b$인 형태로 성장하였으며, 이는 DLCA모델과 일치하는 결과이다. 프랙탈 차원은 단백질 농도의 증가함에 따라 감소하였으나, 침전제 wt%증가에 따라서는 프랙탈 차원도 증가하였다. 또한 저온(≤60℃)에서는 응집체의 크기가 시간에 따라 변화하지 않았으나, 65℃에서는 응집체의 반경은 RLCA모델인 $Rt\sim_e^{at}$에 따라 성장함을 보였다.