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Videomicroscope Study on Temperature Dependence of Brownian Motion of Microparticle in Gelatin Aqueous Solution

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Type-A porcine gelatin was studied by videomicroscope and image analysis to understand how 3D network is formed or disassembled in sol-gel transition. This report demonstrates measurements of local fluctuation in 2 wt% gelatin aqueous solution in thermal cycles at rate of approximate 0.15-0.3 ℃/min. The fluctuation was monitored by Brownian motion of a micron-sized particle with spatial accuracy of 10-20 nm at interval of 1/30 s. For quantitative analysis, 2D mean-square-displacement of the particle was calculated to evaluate a diffusion coefficient *D*. By determining *D* at various temperatures, hysteresis could be observed. In addition, pH-dependence of the hysteresis was measured and two characteristic temperatures, T_{gel} for gelation of the solution and T_{melt} for melting of the gel, were evaluated. We observed that T_{gel} which was 17 ℃ in pH=2 increased to 20 ℃ in pH=10 (ΔT=+3 ℃). On the other hand, T_{melt} was increased from 25 ℃ to 34 ℃ (ΔT=+9 ℃) for the same pH-defference. However, as pH became larger than 10, both temperatures were reduced. Above pH=12, we did not see a significant change in *D* in the cycle any longer. Relationship between the local fluctuation and the gelation will be discussed.
Key words: gelatin, sol-gel transition, videomicroscope, image analysis

1. INTRODUCTION

In sol-gel transition in gelatin aqueous solution, parts of randomcoils assemble into triplehelices [1, 2] which make intermolecular and intramolecular cross-links. Currently, it has been supposed that the gel network is formed by percolation of such cross-links through the whole solution. However, details about its process has not been well understood, yet.

Herning et al. have reported in [3, 4] that small clusters (size ~ 75 nm) exist in the gelatin solution. Therefore, cross-linking in such clusters is likely to be preferred especially in the early stage of gelation. This microscopic heterogeneity may play an important role on the network formation.

In this study, using microscope and image analysis we have tried to investigate the local fluctuation in the gelatin solution in the sol-gel transition. However, the gelatin molecule is too fine (≪ wave length) to observe with an optical microscope. Therefore, the fluctuation was indirectly monitored by Brownian motion of a micron-sized particle dispersed in the sample. Because such diffusion is supposed to reflect on the local structural dynamics, we can expect to acquire information about growth of the network.

In experiment, we have used a thermoregulated microscope, which equipped with a CCD camera and other electronic devices, so called a videomicroscope. By using this apparatus, the 2D motion of the microbead (diameter ~ 1 μm), could have measured with accuracy of 10-20 nm at interval of 1/30 s. The fluctuation of the bead has been analyzed by 2D mean-square-displacement (MSD) whose initial slope is proportional to diffusion coefficient *D*. In thermo cycling experiment, temperature dependence of *D* has been investigated. In addition, we have also studied an effect of pH variation on local fluctuation.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Type-A gelatin which extracted from acid-cured porcine skin (bloom number=300, Sigma Co.) was moistened with small amount of distilled water. As optical probe, polystyrene microbead (diameter = 1.07 μm, standard deviation = 0.019 μm, Polyscience Co.) was added. The sample was diluted with warm water (<50 ℃) so that final concentration of the gelatin is equal to 2.0 wt%. The pH, which was initially 4.8, was varied from pH=2 to 12 with H_2SO_4 and NaOH dilute solutions.

Sample chamber was composed of NO.0 slide glass and cover slip as spacer of both sides tape (thickness ~ 0.2 mm). Edge of the cover slip was perfectly sealed by white vaseline to prevent from water evaporation. In order to improve thermal coupling between the chamber and a thermo stage, crevice was filled up with immersion oil of the microscope.

2.2 Microscope and Thermoregulating System

Figure 1 shows the schematic diagram of the videmicroscope apparatus used in this study.

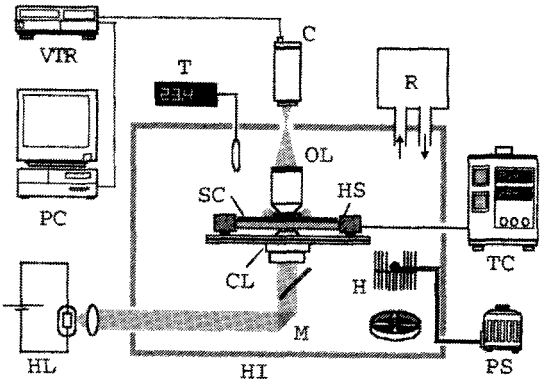

Fig.1: Schematic diagram of thermoregulating videomicroscope system. VTR: videotape recorder, C: half-inch CCD camera, T: thermometer, R: refrigerator, OL: objective lens (x40), SC: sample chamber, HS: heat stage, PC: personal computer, CL: condenser lens, TC: thermo controller, H: heater, M: mirror, HI: heat-insulating box, PS: AC power sourse, HL: 150W halogen lamp.

The videomicroscope was constructed from an overhead-type microscope (BH-50, Olympus Co.), a CCD camera, a videotape recorder, and a personal computer (PC).

Sample temperature was regulated by a heat stage (MATS-52SF, Tokai Hit Co.) from 17 to 40 ℃ within ± 0.1 ℃. For thermal stabilization, the microscope was enclosed in a heat-insulating box which is connected to a refrigerator. In addition, a heater with a fan was placed in this box to reduce the temperature gradient between the sample and the surroundings.

For remote-focusing, a geared stepping motor (20,000 pulses/round) was equipped on a knob of the microscope. The height of the stage was manually controlled in submicron range without open the box. The image of the bead could be focused during recording.

2.3 Thermo Cycling Experiment

Prior to the experiment, in order to stabilize the temperature in sol state, the chamber was kept at 40 ℃ for 20 min.

In the first half of the cycle, the temperature was decreased to 17-18 ℃ step by step at intervals of 1-2 ℃. After 5 min at rest, the videoimage of the microbead was recorded for 1 min. Average rate of the temperature change was 0.15-0.3 ℃/min.

In the latter half of the cycle, the sample was heated back to 40 ℃ as mentioned above, and the motion of the bead was measured in each step.

After the cycle, we checked a reproducibility in gelation behavior by falling down the temperature to 20 ℃ step by step at intervals of 10 ℃.

2.4 Analysis

In Fig.2 we show a flow chart of analysis in our study.

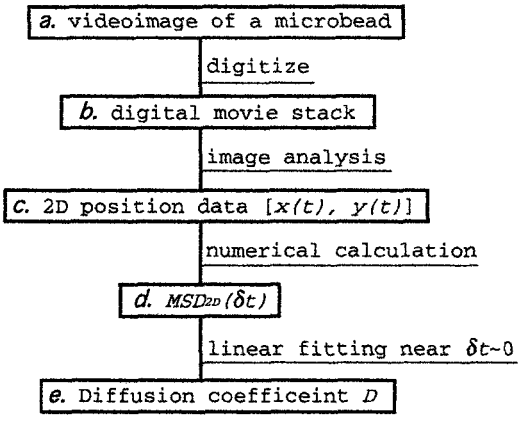

Fig.2: Block diagram of data analysis in the present study.

In order to transfer the videoimage to PC, a picture of the bead for about 20 s (600 frames) was cut and digitized (Fig.2a-b) by a built-in videocard. By means of image analyzing software (National Institute of Health Image version 1.62) and its macro programs (not described in detail), 2D position $[x(t), y(t)]$ of the bead was measured for each frame within 10-20 nm of spacial accuracy (Fig.2c).

For statistical analysis, 2D mean-square-displacement (MSD_{2D}) defined as

$$MSD_{2D}(\delta t) \equiv \langle (x(t+\delta t)-x(t))^2+(y(t+\delta t)-y(t))^2 \rangle_t$$

where $\langle\rangle_t$ indicates an average about accessible t, was calculated from $[x(t), y(t)]$ (Fig.2d). Here, we introduced following assumptions for simplicity. First, the local fluctuation was isotropic. Therefore, the information in depth $z(t)$ was not necessary for understanding the dynamics. Secondary, the boundary condition near the wall did not effect on the motion of the bead. Last, the single diffusion mode was dominant in the interesting timescale (1/30~0.5 s) and the other could be negligible. Thus, MSD_{2D} can be simply approximated as follows.

$$MSD_{2D} \fallingdotseq 4D \times \delta t$$

We determined D at various temperatures (Fig.2e) by least square fitting algorithm. This linear function was fitted to MSD_{2D} plots in δt=0-0.5 s, where the rate of increase was found to be nearly uniform. Fitting error was smaller than several percent.

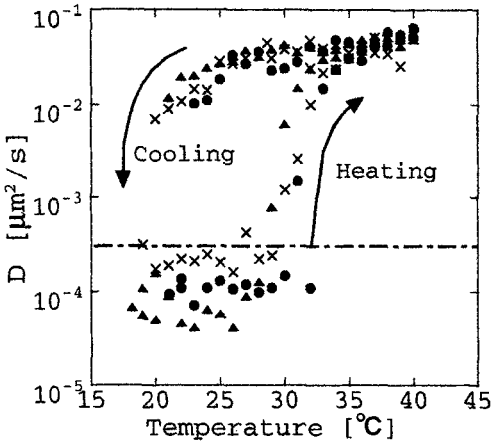

Fig3: Temperature dependence of diffuision coefficient D of a microbead in 2 wt% gelatin solution. Each plot indicated by differenet symbols (● , × , ▲) shows the measurement value for equivalently prepared samples. Broken line (D=3.0 × 10^{-4} μm²/s) was drawn for criterion of noise level (See text).

3. RESULTS AND DISCUSSION

3.1 Temperature Hysteresis of Diffusion Coefficient

Results of the thermo cycling measurement in gelatin solution were summarized in Fig.3. As shown in the figure, we found that the experiment was carried out with good reproducibility.

While the temperature was changed from 40 ℃ to 25 ℃, we can see that D decreased gradually. However, at temperature below 25 ℃ the Brownian motion of the beads became slow significantly and the falling rate of D rose up rapidly. At 20 ℃, the bead turned out to be almost at reset, and D reached 1.0-3.0 × 10^{-4} μm²/s.

In subsequent heating, Fig.3 shows that D did not depend on temperature lower than 30 ℃. Its value was less than 3.0 × 10^{-4} μm²/s. We think that since the position was determined within 10-20 nm, a precision of D is estimated as 1.0-4.0 × 10^{-4} μm²/s. Therefore, it is suitable to consider the broken line in Fig.3 as a criterion of noise level. When the temperature exceeded 30 ℃, the Brownian motion become appeared and D began to ascend. At temperature above 35 ℃, increasing rate of D slowed down. At 40 ℃, D coinsided with the initial value.

Finally, the sample was cooled down once more (See 2.3). We examined that D traced the result in the first cycle (data not shown in the figure).

3.2 pH-Dependence on Diffusion Coefficient

The examples for pH-dependence in the temperature hysteresis of D are shown in Fig.4 and Fig.5.

In pH between 2 and 9 (See Fig.4a-c), we can see that the transition curves guided by solid line moved to the right as pH increased: the transition region

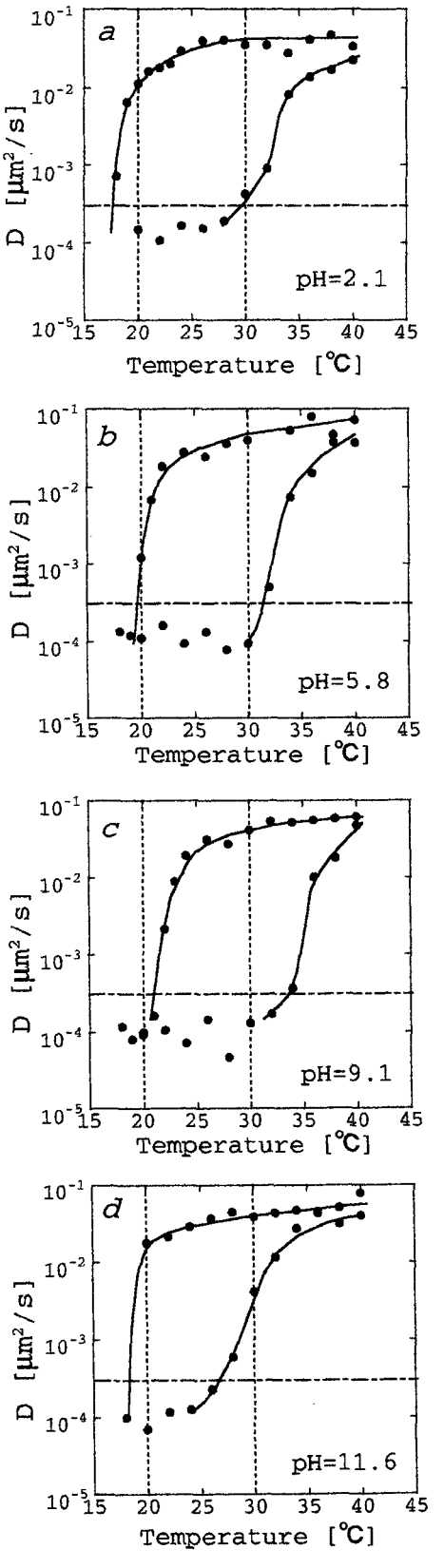

Fig.4: Examples for temperature dependence of D measured in various pH: a) pH=2.1, b) pH=5.8, c) pH=9.1, and d) pH=11.6. Solid lines were drawn for the guides to the eye. Horizontal broken line shows a level of D=3.0 × 10^{-4} μm²/s. Virtical dotted lines indicate the standard temperatures 20 ℃ and 30 ℃.

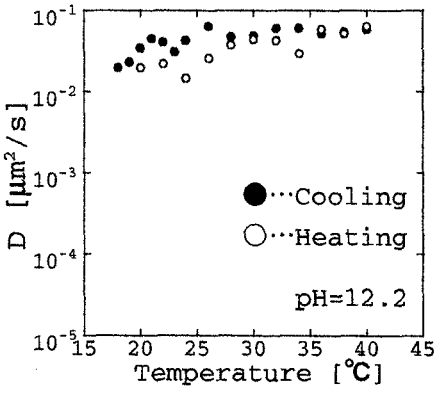

Fig.5: Temperature dependence of D in pH=12.2. Closed and opened circles show the data in cooling and heating process, respectively.

shifted to higher temperature. In addition, the heating process seems to be more sensitive to pH than cooling that.

In pH above 9 which is close to an isoelectric point of type-A gelatin (pH=8.9), the transition behavior become different significantly from that in pH less than 9 (See Fig.4c and d). When the temperature was lowered from 19 ℃ to 18 ℃, D dropped suddenly from 10^{-2} μm²/s to 10^{-4} μm²/s. By contrast, in heating process, D increased at slower rate than that in pH less than 9.

In pH=12.2, D was independent on temperature between 18 ℃ and 40 ℃ as shown in Fig.5. Probably, this sample did not change into the gel any longer. In this solution, the forming of triplehelix might occur very slowly as compared with temperature scanning velocity. Otherwise, the diffusion coefficient in the semidilute solution should change significantly as the coil-helix transition advances. In fact, if the same solution was kept in refrigerator a night, it showed a sol-gel transition.

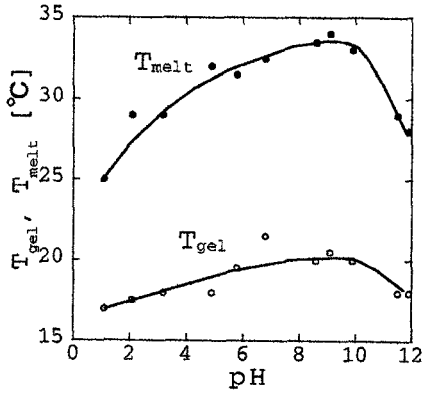

Fig.5: pH-dependence of T_{gel} and T_{melt}. T_{gel} is characteristic temperature where D become lower than 3.0×10^{-4} μm²/s and T_{melt} is that where D exceeded 3.0×10^{-4} μm²/s. Opened circles (○) show gelation temperatures and closed those (●) show melting temperatures. Solid lines were drawn for criterion.

For convenience, we shall introduce two temperatures: T_{gel} for gelation of the solution, and T_{melt} for melting of the gel. The former is defined as the temperature where D has fallen below 3.0×10^{-4} μm²/s. The latter is defined as that where D has exceeded 3.0×10^{-4} μm²/s. In Fig.5, we summarized T_{gel} and T_{melt} which was evaluated for various pH.

As shown in Fig.5, we can see that when pH varied from 1 to 9 both T_{gel} and T_{melt} increased gradually with difference of ΔT_{gel}= +3 ℃ and ΔT_{melt}= +9 ℃, respectively. That is, pH-sensitivity of T_{melt} was found to be three times higher than that of T_{gel}. Simultaneously, we can also see that width of the hysteresis gap increased from 8 ℃ (pH=2.1) to 19 ℃ (pH=9.1).

On the contrary, when pH increased from 9 to 12, both T_{gel} and T_{melt} reduced rapidly with difference of ΔT_{gel}= − 1.5 ℃ and ΔT_{melt}= − 7 ℃, respectively. We suppose this reason as that an electrostatic repulsion was strengthened by rise in electric charge as pH goes away from the isoelectric point.

4. CONCLUSION

In this study, by videomicroscope and image analysis, sol-gel transition in gelatin solution was investigated. We also studied on pH-effect on the transition. Transition behavior in gelatin solution is very complicated. However, the present videomicroscope enables us to investigate it in intermediate scale (submicron ~ microns). We believe that this approach will bring us helpful information about the network formation in gelatin solution.

References

[1] M. Djabourov, J.P. Lechaire, and F. Gaill, *Biorheology*, **30**, 191-205 (1993).
[2] K. Takahashi, *Hikaku Kagaku*, **40**, 121-132 (1994).
[3] T. Herning, M. Djabourov, J. Leblond, and G. Takerkart, *Polymer*, **32**, 3211-3217 (1991).
[4] I. Pezron, M. Djabourov, and J. Leblond, *Polymer*, **32**, 3201-3209 (1991).

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