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# Temperature Induced Gelation of Fibrinogen

Michiko Kaya, Yoshiharu Toyama, Akiko Yamada, Akio Sakanishi and Kenji Kubota
Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8518, Japan
Fax: 81-277-30-1428, e-mail: toyama@bce.gunma-u.ac.jp

In order to clarify thermoreversible aggregation and gelation of fibrinogen, turbidity of aqueous fibrinogen solution was measured over the temperature range from 4 °C to 35 °C. In addition, the dynamic process of aggregation was investigated by dynamic light scattering at 4.9 °C. Urea was added to the fibrinogen solution at various concentrations to examine the effect of hydrogen bonding on the gelation. Without urea, turbidity increased significantly below 7 °C and was reversible on heating. The temperature dependence of turbidity showed a remarkable hysteresis in the cooling and heating processes. With the addition of urea more than 0.2 M, no significant increase of turbidity was observed. Even with the addition of urea, thermal change of turbidity was reversible. The scattered light intensity for the fibrinogen solution without urea increased rapidly and fluctuated after an elapsed time of 700 min. Temporal evolution of characteristic decay time distribution indicated that fibrinogen formed large aggregating clusters, which should convert to a gel at sufficiently low temperature. These results suggest that fibrinogen gel induced by low temperature was caused by the self aggregation due to hydrogen bonding.

Key words: fibrinogen, gelation, turbidity, dynamic light scattering

## 1. INTRODUCTION

It is well known that fibrinogen is a fibrous protein with a molecular weight of $3.4 \times 10^5$ and is an essential factor leading to blood coagulation and erythrocyte aggregation. Fibrinogen forms an irreversible fibrin gel by the action of thrombin. Growth kinetics of fibrin gel has been studied in details by the light scattering method [1,2]. On the other hand, fibrinogen forms a complex with other plasma proteins at low temperature. This cold-insoluble precipitant is called "cryofibrinogen or cryogel" [3] and it is associated with some diseases such as thromboembolic disorders [4], Raynaud's disease [5], rheumatoid arthritis [6], and so on. Recently, Miyamoto et al. reported that cryogelation was facilitated by both an extra domain A containing fibronectin – heparin interaction and the structural changes of fibrinogen induced by plasma fibronectin [7]. Although fibrinogen is one of the essential components of cryogel, little is known about its behavior at low temperatures.

When the fibrinogen solution was incubated for a few hours at a lower temperature than the cloud point temperature, it converts to a gel state as shown in Fig. 1. The further extension of incubation time brought a syneresis to the fibrinogen gel. That is, the fibrinogen gel thus formed was a very weak one in structure. It is probable that the network structure of fibrin gel formed at a low temperature should differ from that formed by thrombin. It is of interest to clarify such a structural characteristics.

In this report, the turbidity of fibrinogen solution was examined in a wide temperature range to clarify the thermoreversible aggregation and gelation of fibrinogen. Furthermore, we studied the dynamic light scattering for the fibrinogen solution near the cloud point and discussed the dynamic process of self-aggregation of fibrinogen.

## 2. EXPERIMENTAL

Swine fibrinogen was purchased from Sigma-Aldrich Co. and was dissolved in saline solution (155 mM NaCl, 3.9 mM $K_2HPO_4$, 0.7 mM $KH_2PO_4$, pH 7.4) without further purification. Concentration of the solution was adjusted to 0.6 g/dl, which was determined spectrophotometrically using the absorbance at 280 nm. Turbidity of the fibrinogen solutions containing urea with the concentrations of 0.1, 0.15, 0.2 and 0.25 M were measured by using a spectrophotometer at a wavelength of 540 nm as a function of temperature. Temperature was controlled by a programmable water bath with the accuracy of ± 0.01 °C, varying from 16 °C to

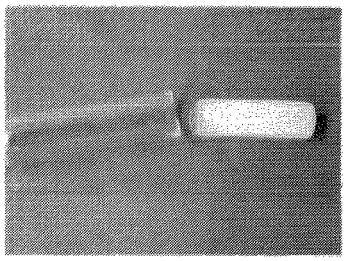


Fig. 1 Fibrinogen gel induced by lowering the temperature. The gel was obtained by incubating 3.3 g/dl fibrinogen solution for 6 hours at 1.5 °C.

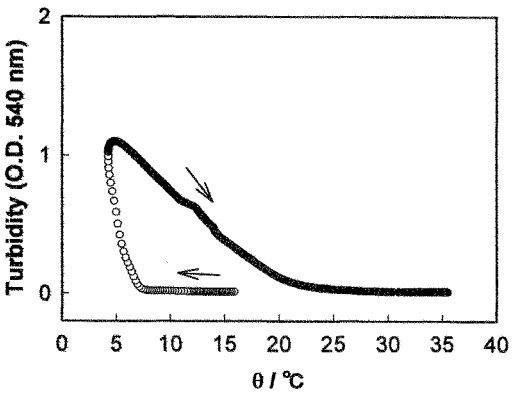
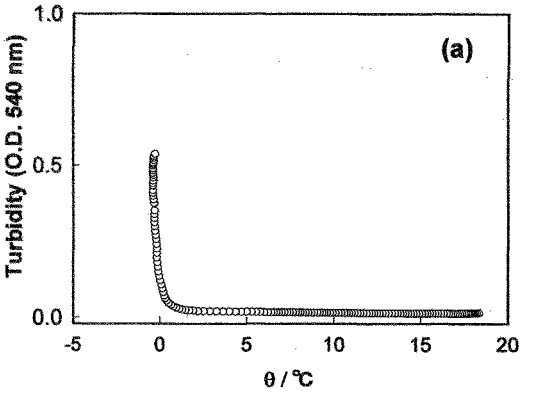



Fig. 2 Temperature dependence of the turbidity of a 0.6 g/dl fibrinogen solution. Cooling (○) and heating (●) rate was *ca.* 0.1°C/min.



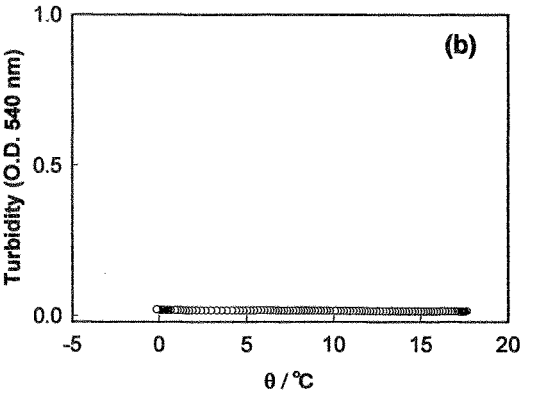




Fig. 3 Effect of the addition of urea on the turbidity. Urea concentrations were 0.15 M (a) and 0.20 M (b), respectively.

4°C during the cooling and from 4 °C to 35 °C during the heating processes at a scanning rate of *ca.* 0.1 °C / min.

Dynamic light scattering was carried out using a homemade spectrometer and an ALV-5000 multiple-tau digital correlator to obtain the correlation function of scattering light intensity $g^{(2)}(t)$, where $t$ is delay time, and the averaged scattering light intensity <I> simultaneously [8]. The decay time $\tau$ distribution function $G(\tau)$ was obtained from $g^{(2)}(t)$ using a CONTIN program. The light source was a He-Ne laser and the details of the apparatus was described elsewhere [8]. Fibrinogen solution was passed through a membrane filter of 0.8μm pore size and was poured into a cylindrical cell of 6 mm inner diameter. The preparation was carried out in a clean box in order to prevent contamination of impurities. Immediately after quenching to 4.9 °C, the measurements of correlation function were starteded by using the homodyne mode at a scattering angle of $\theta = 30°$.

## 3. RESULTS AND DISCUSSION

Figure 2 shows the turbidity of fibrinogen solution on cooling and heating at a scanning rate of *ca.* 0.1 °C / min. Turbidity increased significantly below 7 ℃ and returned to the initial state reversibly by heating. The temperature dependence of turbidity showed a definite hysteresis in the cooling and heating processes.

Figure 3 shows the effect of addition of urea on the turbidity of fibrinogen solution. For the solutions with the urea concentrations below 0.15M, turbidity was significantly increased at low temperatures, whereas above 0.2M, almost no change in turbidity was observed indicating the strong inhibition of aggregation by urea. The secondary structure of fibrinogen molecules was

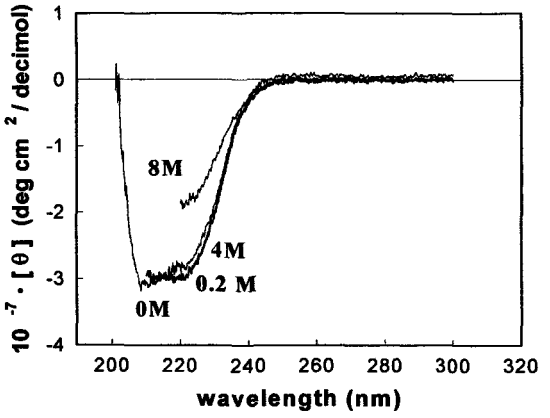



Fig. 4 CD spectra of the fibrinogen solution containing urea at various concentrations. The measurements were carried out using a model J-600 spectrometer (Jasco, Japan) at a room temperature.

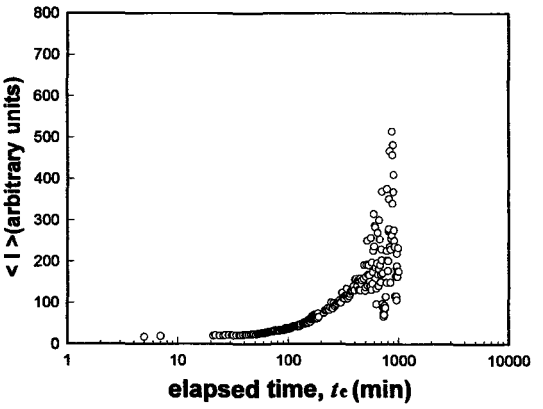



Fig. 5 Time evolution of the scattering light intensity of fibrinogen solution at the scattering angle of 30°. The measurement was started from immediately after the fibrinogen solution was quenched.

examined by circular dichroism, and the results indicated that there occurred no structural change up to a urea concentration of 0.2 M as shown in Fig 4. These results suggest that fibrinogen gel induced by a low temperature was formed dominantly by hydrogen bonding. It should be noted that the aggregating process is very slow, and the present turbidity measurements were not carried out at an equilibrium state but at the transient state.

Figure 5 shows the scattered light intensity <I>

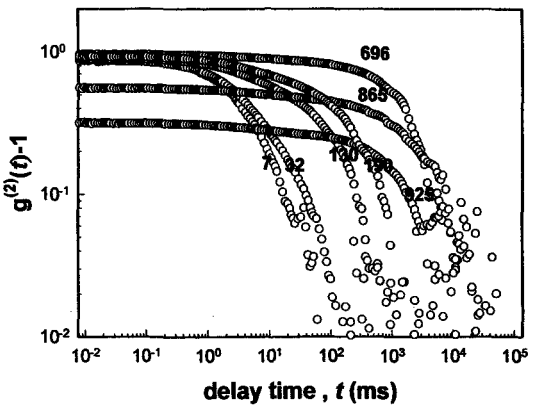



Fig. 6 Correlation function $g^{(2)}(t)$ -1 at various elapsed time. Numbers in the figure denote the elapsed time in min.

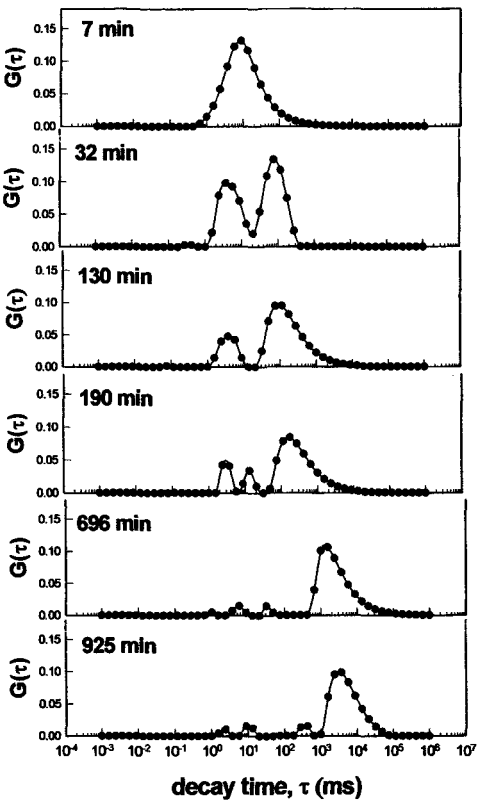



Fig. 7 Time evolution of the decay time distribution function G(τ).

as a function of the elapsed time $t_e$ after the quench. The <I> increased gradually with increasing $t_e$, and then increased steeply and drastically at around $t_e$ = 700 min. This result indicates that very large aggregating clusters were formed subsequently to the self-aggregation of fibrinogen below the cloud point temperature. Kita et al. have investigated the dynamic process of fibrin gel induced by the addition of thrombin [2]. The elapsed time corresponding to a drastic change in <I> was about 50 min, and was much shorter than that obtained by the present measurement. It was revealed that the aggregating process of fibrinogen induced by low temperature was considerably slow.

Figure 6 shows double-logarithmic plots of the correlation function $g^{(2)}(t)$ - 1 as a function of the delay time $t$. The $g^{(2)}(t)$ - 1 exhibited characteristic behaviors showing the slowing down of concentration fluctuation with the increase of elapsed time. There was not observed a clear power law type decay behavior in $g^{(2)}(t)$ - 1 with a drastic shape change as observed for fibrinogen – thrombin system by Kita et al. On the other hand, the initial value of $g^{(2)}(t)$ - 1 corresponding to the coherence factor of detection decreased remarkably for $t_e \geq$ 865 min, which means the existence of considerable inhomogeneity (nonergodicity). That is, the aggregation of fibrinogen grew up to a macroscopic level in this elapsed time region, although self-similar space-time structure was not developed well. Syneresis may occur simultaneously with the cluster growth. Figure 7 shows the time evolution of the decay time distribution function $G(\tau)$. The $G(\tau)$ showed a unimodal distribution in the short decay time region at the elapsed time $t_e$ = 7 min. The $\tau$ value corresponding to the peak of distribution was 10 ms and was obviously larger than that for fibrinogen monomers. Fibrinogen oligomers might have already been formed even at this stage. At $t_e$ = 32 min, a slower decay mode appeared at around $\tau = 10^2$ ms and it shifted to a longer decay time region with an increase of the elapse time. For $t_e \geq$ 696 min, the fast mode, which appeared at $t_e$ = 7 min, disappeared, and finally almost only a single decay mode was observed at about $\tau = 4 \times 10^3$ ms. This elapsed time corresponds well to that of the steep increase of the scattered light intensity as shown in Fig. 5. These facts indicate that macroscopically grown fibrinogen aggregates are connected (or correlated) very weakly and loosely with each other, and the Brownian motions of respective clusters are possible almost independently. Such a heterogeneous structure results in syneresis. In fact, the sample solution after the measurement (about 15 hrs after the quench) flowed quite slowly by tilting the sample cell upside down, and an aggregate which is visible even by eye is formed by weak shear stress.

In conclusion, we investigated the thermoreversible fibrinogen gelation by means of turbidity and dynamic light scattering. The solution of fibrinogen converted to a very weak gel at a low temperature. It was revealed by dynamic light scattering that large aggregating clusters were formed subsequently to self-aggregation of fibrinogen and finally resulted in a gel network. It is suggested that gel formation of fibrinogen at a low temperature should be dominated by hydrogen binding.

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