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Effects of Saccharides on Fibrinogen Gelation Induced by Low Temperature

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When fibrinogen solution is incubated at a low temperature, it converts to gel state without the action of thrombin. The fibrinogen gel induced by low temperature is called "cryogel" and is associated with some diseases such as thromboembolic disorders, Raynaud's disease and rheumatoid arthritis. In order to clarify the details of fiber structure composing of cryogel network: mass/length ratio (μ), radius (r) and density (μ/r^2) of the fibers, turbidity measurements were carried out over the wavelength range of 400 to 800 nm at 2°C. Furthermore, the additive effects of saccharides, glucose, mannose and dextrans with various molecular weights, on the cryogelation were examined. The addition of glucose and mannose retarded the gelation and produced very thin and loose fibers with small values of μ, r and μ/r^2. Stereo isomeric effects were observed between the two saccharides, and mannose showed the large effects than glucose. On the contrary, the gelation was enhanced by the addition of dextrans especially with high molecular weights more than 42000. These findings obtained for the cryogel were essentially consistent with those obtained for fibrin gel induced by the action of thrombin, suggesting that there may exist a common mechanism between these two different gel formations.

Key words: fibrinogen, cryogel, low temperature, turbidity, saccharides

1. INTRODUCTION

Fibrinogen is a fibrous protein with a molecular weight of 3.4×10^5 and is an essential factor leading to blood coagulation. By the action of thrombin, fibrinogen is converted to fibrin monomer and forms a three-dimensional gel network in a stepwise manner [1,2]. In a previous paper, we reported the additive effects of linear saccharides, mono-, oligo- and poly-saccharides, on the process of fibrin gelation. It was found that the fibrin gelation was retarded by the addition of mono- and oligo-saccharides but accelerated by the addition of poly-saccharides. These saccharides do not interact with thrombin but interact with fibrinogen and/or fibrin [3]. On the other hand, fibrinogen forms a complex with fibronectin and heparin at low temperature [4]. This cold-insoluble complex is called "cryogel" and it is related with some diseases such as thromboembolic disorder [5], Raynaud's disease [6], rheumatoid arthritis [7], and so on. In a few decades, therapeutic plasmapheresis techniques, called "cyrofiltration", have been developed to remove the cryogel from patient's plasma [8]. Although fibrinogen is a main component of cryogel, little is known about its behavior at low temperature.

When a fibrinogen solution is incubated for a few hours at a temperature lower than 4°C, it converts to gel state without the action of thrombin [9]. Figure 1 shows the network structure of fibrinogen gel induced by a low temperature. The image convinces that fibrinogen molecules form fibrous assemblies at a low temperature as well as by the action of thrombin.

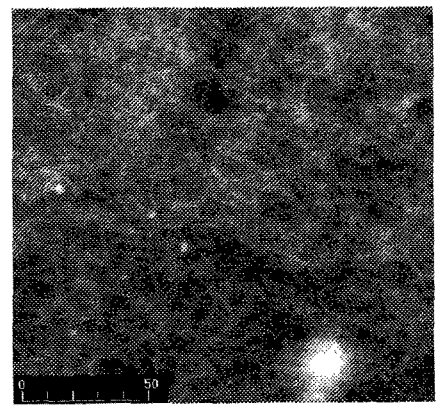

Fig. 1 Confocal laser scanning microscope image of FITC-labeled fibrinogen gel network induced by a low temperature. 6.0 mg/ml fibrinogen solution was incubated at 4°C overnight. The scale length is 50 μm.

In this study, we have measured the wavelength dependence of turbidity of fibrinogen solution at a low temperature in order to clarify the details of fiber structure composing of cryogel network such as radius and density. Furthermore, the additive effects of glucose and dextran on cryogelation were examined, because glucose exists as energy source in blood at a concentration of *ca.* 1.1 mg /ml and dextran has been used as a plasma expander for more than 50 years.

2. EXPERIMENTAL

Bovine fibrinogen was purchased from Sigma-Aldrich Co. and was dissolved into a phosphate buffered saline solution PBS (155 mM NaCl, 3.9 mM K_2HPO_4, 0.7 mM KH_2PO_4, pH 7.4) without further purification. After dialysis against PBS overnight, a final concentration of the solution was adjusted to 6.0 mg/ml, which was determined from an absorbance at 280nm using an extinction coefficient of 1.5 ml/(mg·cm). Saccharides used in this experiment were glucose, mannose (Sigma-Aldrich) and dextrans with nominal molecular weights of 4300 (Sowa Science), 9500, 42000 and 71000 (Sigma-Aldrich). These saccharides dissolved into the PBS were added to the fibrinogen solution at final concentrations of 0.1, 0.5 and 1.0 %.

Turbidity (τ) measurements were carried out by using a spectrophotometer (Shimadzu, UV-1600) at 2°C. Wavelength (λ) dependence of turbidity was obtained scanning over 400 to 800 nm at every 10 minutes, and was analyzed according to the procedures employed by Carr et al [10]. For a very long thin fiber with a radius smaller than the incident wavelength, the following relation is obtained:

$$\tau = (c/A)\,\mu\lambda^{-3} \qquad (1)$$

where c is a fibrinogen concentration, μ is a mass/length ratio of fiber and A is a constant. When the turbidity increases and the radius of the fibers is no longer small relative to the incident wavelength, Eq. (1) is replaced by the following equation:

$$(c/\tau\lambda^3) = A\mu^{-1} + B(r^2/\mu)\lambda^{-2} \qquad (2)$$

where r is the radius of fiber and B is a constant.

3. RESULT AND DISCUSSION

Figure 2 shows a typical example of wavelength dependence of turbidity of the fibrinogen solution without any saccharides. The data were plotted as τ vs. λ^{-3} (a) and $c/\tau\lambda^3$ vs. λ^{-2} (b). After quenching to 2°C, the turbidity increased with increasing elapse time (t). In the initial stage of gelation, $t \leq 50$ min, the wavelength dependence of turbidity were well expressed by Eq. (1) as shown in Fig. 2 (a). These plots were linear and approached an intercept of zero. The plots did not extrapolate to zero at $t \geq 60$ min, and then these data were potted as $c/\tau\lambda^3$ vs. λ^{-2} in accordance with Eq. (2) as shown in Fig. 2 (b). The linearity is very good, suggesting the validity of the above equations. In other wards, fibrinogen molecules assemble into a rod shape at low temperature. From the intercepts and the slopes of these plots, mass/length ratio (μ), radius (r), and density (μ/r^2) of the fiber were obtained. The values of μ, r and μ/r^2 were 1.1×10^{13} Da/cm, 1.5×10^{-5} cm and 5.0×10^{22} Da/cm^3, respectively. The fibrinogen fibers of cryogel were thick and loose compared with fibrin fibers ($\mu=1.7\times10^{13}$ Da/cm, $r=8.4\times10^{-6}$ cm and $\mu/r^2=2.4\times10^{23}$ Da/cm^3) induced by thrombin [3].

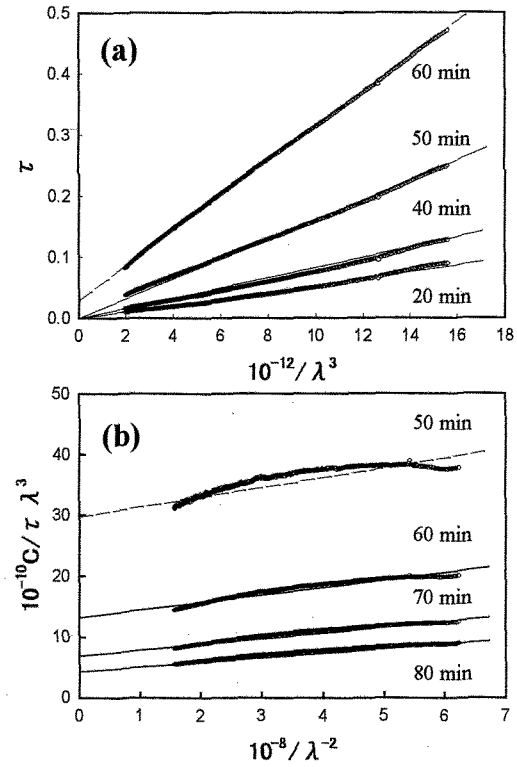

Fig. 2 Wavelength dependence of the turbidity of fibrinogen solution without any saccharides. The data were plotted as τ vs. λ^{-3} (a) in accordance with Eq. (1) and $c/\tau\lambda^3$ vs. λ^{-2} (b) in accordance with Eq. (2). The numbers in the figure mean the elapse time after quenching to 2°C.

Figures 3 and 4 show the additive effects of glucose and mannose on the values of τ, μ, r and μ/r^2, respectively. τ value increased with increasing the elapse time t and did not reached a plateau level within the measuring time of 6 hours. The cryogelation was retarded by the addition of glucose and mannose, and the retardation effects were increased with increasing a concentration of these saccharides. All values of τ, μ, r and μ/r^2 were smaller than those obtained for the control sample without saccharides at a same t. The fibrinogen

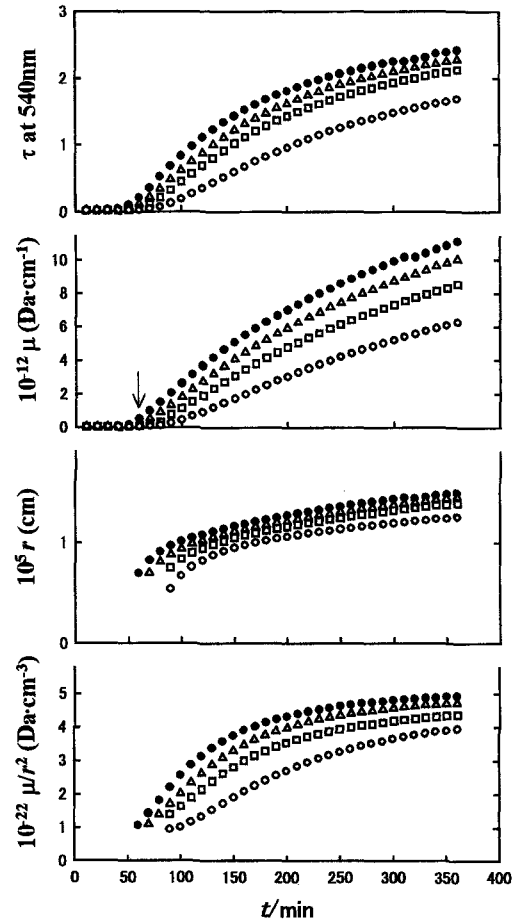

Fig. 3 Temporal evolution of turbidity (λ), mass/length ratio (μ), radius (r) and density (μ/r^2) of fibrinogen fibers in the presence of glucose at various concentrations. The final concentrations of added glucose were 0 (●), 0.1 (△), 0.5 (□) and 1.0 % (○). The equation used for analysis was shifted from Eq. (1) to Eq. (2) at the arrow.

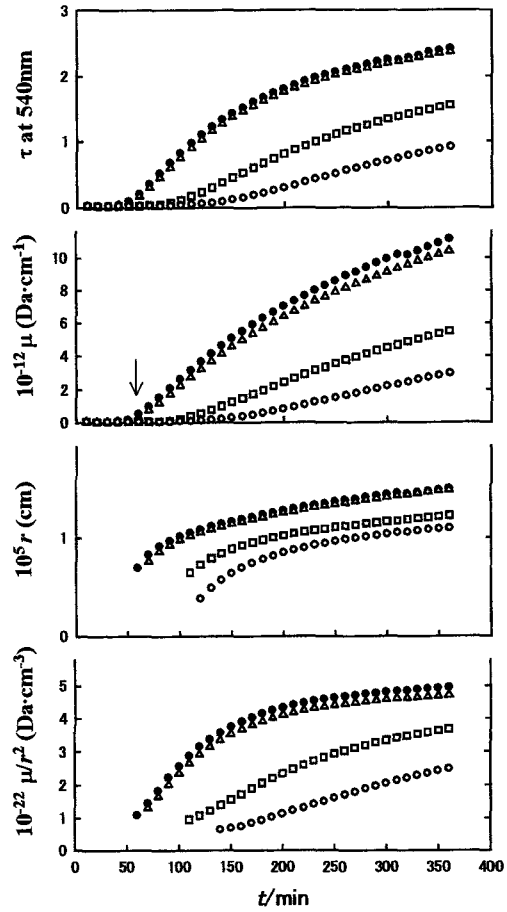

Fig. 4 Temporal evolution of turbidity (λ), mass/length ratio (μ), radius (r) and density (μ/r^2) of fibrinogen fibers in the presence of mannose at various concentrations. The final concentrations of added mannose were 0 (●), 0.1 (△), 0.5 (□) and 1.0 % (○). The equation used for analysis was shifted from Eq. (1) to Eq. (2) at the arrow.

fibers formed in the presence of these saccharides were very thin and loose. The lateral aggregation of fibrils did not sufficiently proceed, as evidenced by the decrease in μ. This finding means that these saccharides interact with a part of fibrinogen molecule, which is closely related to the lateral aggregation. The cryogel obtained after the measurements was very weak and further incubation at 2°C brought syneresis. Furthermore, stereo isomeric effects were observed. As compared with glucose, mannose, which is the C-2 epimer of glucose, showed large retardation effect on the cryogelation, and the values of μ, r and μ/r^2 were fairly less. These results suggest that there may be a lectin-like domain on a fibrinogen molecule.

Figure 5 shows the additive effects of dextrans with various molecular weights on the values of τ, μ, r and μ/r^2. The cryogelation was enhanced with increase in the molecular weight of added dextran, and τ was almost saturated within 6 hours. μ and r showed significantly high values for high molecular weight dextrans of 42000 and 71000. This result indicates that there exists a critical chain length of dextran to enhance the lateral aggregation. The critical value is *ca.* 5 nm which is a hydrodynamic radius corresponding to a dextran with molecular weight of 40000. The saturate values of μ/r^2 were almost unaffected by the addition of dextrans. These results are essentially consistent with those obtained for fibrin gelation induced by the action of thrombin through the release of fibrinopeptides A and B [3,11]. It is suggested that there may exist a common mechanism of gelation between the fibrinogen gel induced by low temperature and the fibrin gel induced by thrombin.

In conclusion, we examined the details of fiber

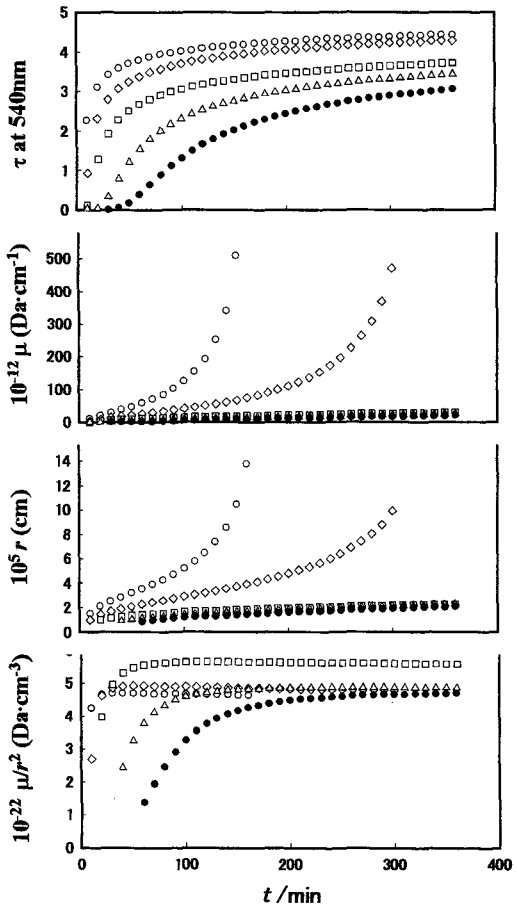

Fig. 5 Temporal evolution of turbidity (λ), mass/length ratio (μ), radius (r) and density (μ/r^2) of fibrinogen fibers in the presence of dextrans with various molecular weights. The molecular weights of added dextrans were 4300 (△), 9500 (□), 42000 (◇) and 71000 (○). Closed circles (●) indicate the values for control sample in the absence of dextran. All data were analyzed by using Eq. (2).

structure composing of cryogel network such as radius and density by turbidity measurements. Fibrinogen molecules assembled into a rod shape and formed the fibrinogen fiber, which was thick and loose compared with fibrin fiber induced by thrombin. Furthermore, the additive effects of glucose, mannose and dextran on cryogelation were investigated. The addition of glucose and mannose retarded the cryogelation, and the stereo isomeric effects were observed. In contrast, the cryogelation is enhanced by dextrans, and its effects increased with increase in the molecular weight of added dextrans. These findings obtained for the cryogel were essentially consistent with those obtained for fibrin gel induced by the action of thrombin. These results will give useful clues to the elucidation of cryogelation mechanism.

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REFFERENCES

[1] R. Hantgan, W. Fowler, H. Erickson and J. Hermans, Thromb. Haemost., 44, 119-24 (1980).

[2] R. Kita, A. Takahashi, M. Kaibara and K. Kubota, BioMacromolecules, 3, 1013-20 (2002).

[3] Y. Masuda, Y. Toyama, H. Kogure, K. Kubota and M. Ochiai, Trans. MRS-J., 29, 3331-35 (2004).

[4] K. Miyamoto, M. Tokita, T. Komai, K. Miyashita and E. Sakashita, Int. J. Biol. Macromol., 28, 183-89 (2001).

[5] S. Sankarasubbaiyan, G. Scott and J. L. Holley, Am. J. Kidney Dis., 32, 494-98 (1998).

[6] K. B. Goyle and J. A. Dormandy, Lancet, 19, 1317-18 (1976).

[7] R. Umemoto, S. Asakura, Y. Kariya, M. Yonekawa, A. Kawamura, T. Komai, K. Sekiguchi, H. Hirano, K. Hino, E. Sakashita and M. Matsuda, Blood Coagul. Fibrinol., 4, 127-31 (1993).

[8] G. A. Siami, F. S. Siami, Therapeutic Apheresis, 5, 283-86 (2001).

[9] M. Kaya, Y. Toyama, A. Yamada, A. Sakanishi and K. Kubota, Trans. MRS-J., 27, 597-600 (2002).

[10]M. E. Carr, Jr. and J. Hermans, Macromolecules, 11, 46-50 (1978).

[11] M. E. Carr and D. A. Gabriel, Macromolecules, 13, 1473-77 (1980).

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