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Liquid Crystalline Gelation of Aqueous Solutions of Structure Proteins

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We have newly prepared liquid crystalline gels (LCGs) from concentrated aqueous solutions of structure proteins, such as gelatin, silk fibroin, and collagen with dialysis into concentrated aqueous solution of chemical cross-linkers. The condition for forming LCGs was examined. From the experimental results, the mechanism for forming LCGs is discussed.

Key words: Liquid Crystalline Gels (LCGs), dialysis, structure protein

1. INTRODUCTION

Recently, elastomers and gels having anisotropic network, which are called liquid crystalline elastomers (LCEs) and liquid crystalline gels (LCGs), respectively, have attracted great interest in fields of materials science, medical science, and pharmaceutical because of their anisotropic optical property and mechanical property [1-10]. Since the anisotropic network induces anisotropic actuating (or swelling) behavior of such LCEs and LCGs, they could be utilized as artificial muscles and artificial skins. Thomsen III et al. have shown that nematic liquid crystalline elastomers can exhibit muscle-like mechanical properties [4]. On the other hands, Kaneko et al. have reported the potential to control the direction of cell extension by cell cultivating on the uniaxially oriented hydroxyapatite and polyvinyl alcohol (Hap-PVA) hybrid gel [5]. In the previous study, we have prepared unique LCGs which was made from semi-flexible or rigid biopolymers such as curdlan and DNA by using simple dialysis into multivalent metal cations solutions [6-10]. Anisotropic gel networks are formed by coupling the intermolecular cross-linking that is induced by an electrostatic interaction between the biopolymers and metal cations and the orientation of the biopolymers that is induced by a cylindrically symmetric flow of the metal cations. Because of the mechanism of liquid crystalline gelation of biopolymers is simple, the method for forming the LCGs can be applied to the systems consisting of other intradialytic rigid or semi-flexible polymer solutions and extradialytic cross-linking agent solutions separated with a dialysis membrane.

In this study, we have newly prepared liquid crystalline gels (LCGs) from concentrated aqueous solutions of structure proteins, such as gelatin, silk fibroin, and collagen with dialysis into concentrated aqueous solutions of glutaraldehyde (GA), ethanol, and buffer. We have also discussed the LCG formation mechanism for each structure protein. The experimental results suggest universality of mechanism of forming liquid crystalline gelation of biopolymer solutions induced by dialysis into cross-linking agent solutions.

2. EXPERIMENTS

Materials

Type A gelatin (M=300,000, pI~9) derived from porcine skin was kindly provided from Nitta gelatin. The gelatin was dissolved into milliQ water at 20 wt%. The *Bombyx mori* silkworms were kindly provided by Gunma Sericultural Technology Center. The liquid silk solution was extracted by gently dispersing 1 g of the full grown larvae of the *Bombyx mori* silkworms into 10 g of the milliQ water at room temperature for 1 day. The bovine dermis pepsin-solubilized type I atelocollagen solution containing 0.5 mg/mL of atelocollagen (pH 3.0) was purchased from KOKEN Co., Ltd..

Liquid Crystalline Gelation of structure protein solutions

(A) The gelatin solution was sandwiched between two circular glass plates with the radius R=12mm, and then put into a refrigerator at 4°C for 10 min. After cooling, the gelatin gel films were immersed into 25 % GA solution at different temperatures in the range of T=10 - 40 °C.

(B) In order to prepare the liquid silk LCG beads, the liquid silk solution was dropped into ethanol at room temperature (25°C).

(C) The atelocollagen solution was sandwiched between two circular glass plates with the radius R=12mm, and then immersed into phosphate buffer at the different pH in the range of pH 5.20 - pH 9.15.

3. RESULTS AND DISCUSSION

Gelatin LCG films observed under crossed nicols were shown in Figure 1. The gelatin LCG film was an alternately layered composite consisting of outer LCG layer, amorphous gel (AG) layer, and inner LCG layer. The birefringence of the gelatin LCG films decreased with increasing the immersing

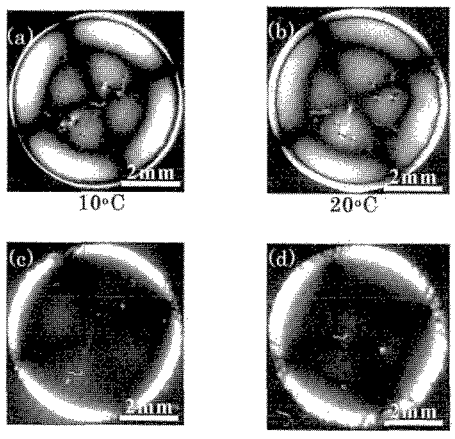

Figure 1 Gelatin liquid crystalline gel films prepared by immersing gelatin solution into glutaraldehyde solution at temperatures T = 10 °C (a), 20°C (b), 30°C (c), and 40 °C (d), observed under crossed nicols.

temperature. The temperature dependence of birefringence of the gelatin LCG films could be attributed to the coil - helix transition of the gelatin molecules. When the concentrated gelatin solution is cooled below the gelation temperature ($T_{gel} \approx 30°C$), the gelatin solution gelled by forming the triple helix that plays the role of cross-linking points. In the physical gelation process of gelatin solution, many dangling chains are formed in the gelatin gel network. The dangling chains form triple helix below the coil - helix transition temperature, which is approximately equal to gelation temperature. The dialysis of gelatin physical gel films into GA solution induces a cylindrical symmetric inflow of GA molecules in the physical gel films. The gelatin molecules in effective gel network and dangling chains are cross-linked by the cross-linking reaction between amino groups in gelatin molecules and GA molecules. The dangling chains are also radially orientated by the cylindrical symmetric inflow of GA molecules. On the other hands, the conformation of gelatin molecule is random coil above T_{gel}. Thus, the gelatin gel network formed by the dialysis of the gelatin solution into the GA solution above T_{gel} is mainly composed of random coil chains. Since the rigidity of the triple helix is much higher than that of the random coil, the triple helix structure composed of gelatin molecules could play role of the mesogens. Because of the amount of mesogens in the gelatin physical gel are larger than those in the gelatin sol, a more anisotropic (ordered) structure is formed by the dialysis of gelatin physical gel into the GA solution than that of gelatin sol.

Figure 2 shows that the silk fibroin gel beads were observed under the crossed nicols. We have found that the silk fibroin gel beads have a birefringence. The result suggests that the liquid crystalline gelation of silk fibroin solution is induced by the dialysis into ethanol. The silk fibroin is one of fibrous proteins which are synthesized and reserved into the silk gland in the silk worm. The silk fibroin aqueous solution is easily insolubilized by applying shear stress. The insolubilization of silk fibroin solution is due to random coil to β-sheet conformational transition. The conformational change of silk fibroin can be also induced by adding ethanol into the silk fibroin solution [11]. Thus, the liquid crystalline gelation of liquid silk is attributed to the random coil to β-sheet transition of silk fibroin.

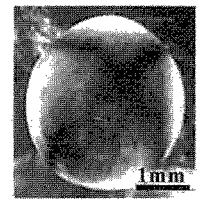

Figure 2 Silk fibroin liquid crystalline gel bead observed under crossed nicols.

Figure 3 shows atellocollagen LCG film observed under the crossed nicols. The condition for forming atellocollagen LCG is examined by means of insolubilization reaction with buffered solution at various pH in the range of 5.20<pH<9.15. In the pH range from 5.29 to 6.05, precipitations of atellocollagen are formed by dropping atellocollagen solution into buffer solutions. At pH 6.54, amorphous gel beads are formed by dropping atellocollagen solution into buffer solution. In the pH range from 7.00 to 9.15, atellocollagen LCG beads are successfully produced by dropping the atellocollagen solution into the buffer solutions. Atellocollagen is one of typical rod like biopolymers, and can be dissolved in acidic buffer solution (3<pH<5). On the other hand, atellocollagen cannot be dissolved in neutral buffer solution and basic buffer solution (7<pH<9), which is close to the isoelectric point of collagen, because of the reduction of electrostatic repulsion between atellocollagen molecules induces intermolecular aggregation. Thus, the concentrated acidic atellocollagen solution can be gelled by neutralization reaction. From the experimental results, it is found that the critical pH value for forming atellocollagen liquid crystalline gel is pH=7.0. Since the critical pH value of atellocollagen liquid crystalline gelation is close to the isoelectric point of collagen (7.0<pI<9.2), the liquid crystalline gelation of atellocollagen solution could be attributed to the intermolecular aggregation of atellocollagens.

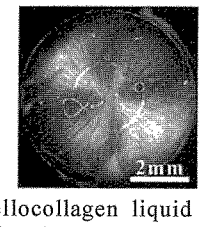

Figure 3 Atellocollagen liquid crystalline gel film observed under crossed nicols.

Many biological tissues, such as skins, muscles, bones, and veins which mainly consist of structural proteins, have anisotropic network structures. The anisotropic biomechanical properties such as anisotropic extension and construction of muscles and anisotropic mechanical property of vein are attributed to the

anisotropic network structures. In this study, we have introduced a simple method for producing biomimetic anisotropic materials by a dialysis of concentrated structural protein solutions into concentrated cross-linking agent solutions. It is hoped that the liquid crystalline gelation of the biopolymer solutions induced by this method is utilized as one of methodologies for producing easily and economically biomimetic gels.

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