Preparation and thermoresponsive behaviors of UV-crosslinked gelatin nanogels

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Abstract  UV-crosslinked gelatin nanogel was prepared without using chemical crosslinking agents and its thermoresponsive behaviors were investigated. The particle size of the nanogels decreased on heating, which is attributed to the helix-to-coil transition of gelatin. On the other hand, the increase in the particle size on cooling was not observed in the quiescent solutions, but was noticeably accelerated by the application of shear stress.

Keywords  gelatin, nanoparticle, thermoresponsive behavior, drug delivery

Introduction

In recent years, substantial efforts have been devoted to the development of colloidal drug-delivery systems [1]. Nanoparticles typically below the 1000-nm size range fit into colloidal drug-delivery carriers, which offer the following advantages: the ability to travel through small capillaries, the ability to avoid clearance by phagocytes, the drug targeting by modified body distribution, and the enhancement of the cellular uptake [2–4]. The carrier should be nontoxic and able to be degraded in vivo. Nanoparticles of gelatin gel (gelatin nanogels), therefore, can be attractive candidates as colloidal drug carriers due to their biodegradability, biocompatibility, and low immunogenicity/antigenicity [2–4].

Gelatin is a protein obtained by the heat denaturation and partial hydrolysis of collagen. Gelatin is soluble in water at temperatures higher than the helix melting temperature and the gelatin chains assume a random coil state, and on cooling, the gelatin chains partly revert to a collagen-like triple-helical state [5, 6]. This coil-helix transition is thermoreversible and depends on various factors such as concentration, temperature, solvent, stress, and the presence of chemical-crosslinking [5–12]. The coil-helix transition of gelatin has been investigated for bulk solution and gel [5–12], but recently Gandhi et al. observed the melting of the helices in the gelatin nanogels with low chemical-crosslink density [13]. Moreover, they, and another group, reported that deswelling of the nanogels coincided with the melting of the helices [13, 14]. The thermoresponsive behaviors may contribute to a better regulation of drug release from the nanogels for a colloidal drug carrier.

There are two common methods for the preparation of gelatin nanogels, namely, emulsification and desolvation [3, 4]. In the emulsification process, an aqueous phase containing gelatin is added to an oil phase comprising organic solvent and surfactant under stirring or homogenization, resulting in the formation of very small droplets of gelatin. After the crosslinking of the droplets, the organic solvent is removed [3, 4]. The major disadvantage of this method is the need to add organic solvent and then to remove it. In the desolvation process, a salting-out agent such as sodium sulfate or a nonsolvent such as an alcohol is added dropwise to a gelatin solution to trigger the coacervation, followed by the crosslinking of the gelatin coacervates [1, 3, 4].

In the crosslinking of gelatin nanogels, chemical reagents such as glutaraldehyde (GA) have been commonly used, although the residual crosslinking agents may cause toxicity problems. Recently, UV-induced crosslinking of gelatin has been utilized for increasing thermal stability of gelatin microspheres for a cell culture scaffold [15]. The crosslinking reaction is thought to be initiated by the radical formation on aromatic amino acid residues such as tyrosine and phenylalanine due to the UV irradiation, and the subsequent bonding of these radicals will result in the crosslink formation [16–18]. The UV-crosslinking is a simple and nontoxic method in contrast to the methods using the crosslinking agents and hence is more suitable for materials of pharmacological and
medical use. In the present study, we prepared novel gelatin nanogels by the desolvation process and the UV-induced crosslinking, and investigated the thermoresponsive behaviors of the obtained nanogels.

Experimental

Gelatin (Type A from porcine skin, 250 Bloom) was provided by Nitta Gelatin Inc., Japan. Sodium acetate, sodium sulfate, isopropanol, and GA were obtained from Wako Pure Chemical Industries, Ltd., Japan. Sodium metabisulfite and Tween 20 (polyoxyethylene sorbitan monolaureate) were obtained from Kanto Chemical Co., Inc., Japan and Tokyo Chemical Industry Co. Ltd., Japan, respectively.

In this study, the UV-crosslinked gelatin nanogels and the GA-crosslinked gelatin nanogels for comparison were prepared. The GA-crosslinked gelatin nanogels were prepared by a desolvation method partially modified from the method shown in [13]. First, gelatin was dissolved in Milli-Q water and prepared a stock solution of gelatin of 5%. The gelatin stock solution (2 mL), 16.5% sodium acetate solution (1 mL), and Tween 20 (100 μL) were mixed with Milli-Q water (7 mL) by stirring at 600 rpm for 10 min in the water bath thermostated at 40°C. The mixture was taken out from the water bath and the desolvation process was induced by the dropwise addition of 20% sodium sulfate solution (5.4 mL) under stirring at 600 rpm, resulting in a faintly turbid coacervate solution. The coacervate was redissolved by the addition of isopropanol (1 mL) until a clear solution was formed, by which the desolvation process was controlled to be near the coacervate point so that nanoparticles are obtained rather than larger aggregates [1]. The solution temperature was then lowered in a 10°C water bath. After stirring at 1600 rpm for 5 min, 6.25% GA solution (400 μL) was added and stirred at 1600 rpm for 5 min, while keeping the temperature at 10°C. Finally, 12% sodium metabisulfite solution (5 mL) was added for quenching the cross-linking reaction and stirred at 1600 rpm for 1.5 hours at 10°C. The method for the preparation of the UV-crosslinked nanogels was identical to that of the GA-crosslinked nanogels except for the addition of Milli-Q water (400 μL) followed by the UV irradiation at 254 nm for 3 hours at 10°C under stirring at 1600 rpm, instead of the addition of the GA solution. In the UV irradiation, a UV lamp of 4 W (UVG-11, Funakoshi Co. Ltd.) was used. The distance from the lamp to the top and the bottom of the sample was 3 cm and 7 cm, respectively. The solutions of gelatin nanogels were diluted at desired ratios and stored at 3°C.

The dynamic light scattering (DLS) experiment was carried out to characterize the gelatin nanogels and investigate their thermoresponsive behaviors. The DLS measurements were performed with Zetasizer Nano ZS (Malvern), and the hydrodynamic diameter ($d_h$) distribution and the average hydrodynamic diameter $\langle d_h \rangle$ were obtained by the CONTIN analysis.

Results and Discussion

For both UV-crosslinked and GA-crosslinked gelatin nanogels, the nanogel solutions diluted at 1:10 and 1:100 remained transparent during storage at 3°C for more than two weeks after the preparation, but in the undiluted solutions the formation of precipitation was observed in a few days, which is due to the aggregation of nanogels in the undiluted solutions.

The size distribution of nanogels in the diluted and undiluted solutions measured immediately after the preparation is shown in Fig. 1. The size distribution in the solutions diluted at 1:10 coincided with those in the solutions at the dilution of 1:100, which was not shown in Fig. 1. The average...
hydrodynamic diameters \( <d_h> \) of the UV-crosslinked and GA-crosslinked nanogels in the diluted and undiluted solutions are summarized in Table 1. The data in Fig. 1 and Table 1 show that the sizes of the UV-crosslinked nanogels were comparable to those of the GA-crosslinked nanogels for the same dilution ratios, so that the impact of the crosslinking method to the particle size was not very significant. The particle size of the gelatin nanogels in the undiluted solutions was larger than that in the diluted solutions for both UV-crosslinked and GA-crosslinked samples, indicating that the gelatin nanogels were highly dispersed in the diluted solutions but formed the aggregates in the undiluted solutions. The aggregation of nanogels in the undiluted solutions would be caused by the higher concentration of sodium sulfate as a salting-out agent. In the present study, therefore, the nanogel solutions diluted at 1:10 was used for the measurement of the thermoresponsive behaviors of gelatin nanogels, although the low-molecular weight species such as sodium sulfate could be removed efficiently by gel filtration [1]. The particle size of the UV-crosslinked and GA-crosslinked nanogels in the solution diluted at 1:10 was unchanged during storage at 3°C for two weeks (Fig. 2), which confirms the high dispersity and stability of the gelatin nanogels in the diluted solutions.

The temperature dependence of \( <d_h> \) of the UV-crosslinked and GA-crosslinked gelatin nanogels measured on heating was shown in Fig. 3. For the both samples, \( <d_h> \) was constant at temperatures below 25°C, and \( <d_h> \) decreased with temperature between 25 and 35°C, and \( <d_h> \) became almost constant at temperatures above 35°C. This thermoresponsive behavior is qualitatively the same as that of the GA-crosslinked gelatin nanogels reported in [13]. The degree of deswelling by heating for the UV-crosslinked nanogels was larger than that for the GA-crosslinked nanogels; a threefold decrease in the particle size for the UV-crosslinked nanogels and a twofold decrease for the GA-crosslinked nanogels.

The observed decrease in the particle size with increasing temperature can be explained in terms of helix-to-coil transition of gelatin chains, as is reported for the GA-crosslinked nanogels [13, 14] and the UV-crosslinked gelatin microspheres [19]. In the present study, the nanogels were crosslinked at 10°C, which is below the coil-helix transition temperature, and the gelatin chains in the particles assumed partial helix structure when the crosslinking reaction started. If substantial amount of the helix structure was remained unchanged during the crosslinking process, the gelatin chains in the as-prepared nanogels exhibited transition from helix structure to random coil on heating. The helix-to-coil transition induces the decrease in the persistence length \( q \) of the chains, resulting in the decrease in the mesh size \( \xi \) of the gel network as \( \xi \sim (q\lambda)^{1/2} \), where \( \lambda \) is the contour length of the chain between the crosslinking. Assuming that the particle size of the nanogels is proportional to the mesh size and the persistence length of the chains in the nanogels at high temperatures is represented by that of gelatin in the random coil state, \( q = 2 \) nm [20], the persistent length of the chains in the as-prepared nanogels is estimated to be a few tens of nanometers from the

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**Table 1** Average hydrodynamic diameters \( <d_h> \) of the UV-crosslinked and GA-crosslinked nanogels in the diluted and undiluted solutions

| Crosslinking | Dilution ratio | \( <d_h> \) [nm] |
|--------------|----------------|-----------------|
| UV           | undiluted      | 271             |
| UV           | 1:10           | 116             |
| UV           | 1:100          | 130             |
| GA           | undiluted      | 300             |
| GA           | 1:10           | 117             |
| GA           | 1:100          | 152             |

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**Fig. 2** Storage-time dependence of \( <d_h> \) for the UV-crosslinked nanogels (open circles) and GA-crosslinked nanogels (filled circles), respectively, in the solutions diluted at 1:10. The samples were stored at 3°C until the measurements, and the DLS measurements were carried out at 25°C.

**Fig. 3** Temperature dependence of \( <d_h> \) of the UV-crosslinked nanogels (open circles) and GA-crosslinked nanogels (filled circles), respectively, measured on heating.
ratio of the size at low temperatures to that at high temperatures. The larger deswelling degree of the UV-crosslinked nanogels than that of the GA-crosslinked nanogels indicates more helix structure remained in the UV-crosslinked nanogels during the crosslinking process.

Because coil-helix transition of gelatin is reversible, the particle size of gelatin nanogels would increase on cooling owing to the renaturation of the triple helical structure. In the preceding study [13], however, an asymmetric thermoresponsive behavior was reported for the GA-crosslinked gelatin nanogels; the deswelling of the nanogels occurred immediately on heating, but the nanogel size was almost unchanged by cooling. Moreover, the size of the nanogels increased slowly while the solutions was vigorously stirred at low temperatures. These behaviors indicate that the swelling process of the nanogels is much slower than the deswelling process and accelerated by the application of shear stress, namely, the vigorous stirring [13]. In this study, we verified if the UV-crosslinked gelatin nanogels showed this asymmetric thermoresponsive behavior which is dependent on the shear application. Figs. 4 (a) and (b) show the change in $<d_h>$ for the GA-crosslinked and UV-crosslinked gelatin nanogels, respectively, when the solution temperature was changed under the defined stirring condition as shown in the insets of Figs. 4(a) and (b). The both samples showed qualitatively the same behaviors. The as-prepared gelatin nanogels deswelled within an hour after the temperature rise to 45°C. When the solution was cooled to 10°C and kept at the temperature under stirring at 1400 rpm, the particle size was almost unchanged within an hour after the temperature change, but it noticeably increased and became much larger than that of the as-prepared nanogels within a day. The size distributions of the nanogels in the solution which was kept at 45°C for an hour and at 10°C under stirring for ~20 h were shown in Fig. 5. The fast deswelling on heating and the slow swelling on cooling under the stirring was reproducibly observed. For the heating of the solution at 45°C for 1 hour and subsequent cooling at 10°C without stirring, the deswelling of the nanogels occurred at 45°C but the swelling at 10°C was not observed at least within 2 days after the temperature change. These results confirmed the asymmetric and shear-dependent thermoresponsive behaviors of the gelatin nanogels. The particle size of the UV-crosslinked nanogels kept at 10°C for a day under stirring was approximately 10 times larger than that at 45°C. The corresponding persistence
length of the chains is estimated to be ~200 nm, which is comparable to that of triple helical collagen, 180 nm [21]. The helix formation would occur markedly in the gelatin nanogels when the solution was cooled under vigorous stirring.

Since swelling and deswelling of the gelatin nanogels are induced by renaturation and melting of the triple helix structure [13], the observed asymmetric and shear-dependent thermoresponsive behaviors indicates that the renaturation of the helix from the random coil on cooling is much slower than the helix melting on heating in the nanogels and is accelerated by the shear stress due to the stirring. In the bulk gelatin solutions and gels, the helix formation occurs very slowly when compared to the helix melting [5–10]. This slow helix formation, called “aging”, is due to the increasing geometric frustration, that is, the growing internal tensions on the network caused by the growth of helices which is interconnected with each other [10]. Recently, Ronsin et al. reported that the aging of the gelatin gel was accelerated by the shear-induced texturing effect [10]. The stirring-assisted slow swelling of the gelatin nanogels would be also attributed to the helix formation accelerated by the shear application.

Conclusions

Novel gelatin nanogels were prepared by the UV-induced crosslinking of gelatin. The thermoresponsive behaviors of the obtained UV-crosslinked gelatin nanogels were qualitatively the same as those of the GA-crosslinked nanogels. The thermoresponsive crosslinker-free gelatin nanogels have potential applications for novel colloidal drug delivery systems.

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