Swelling Behavior and Elasticity of Protein-Based Hydrogels

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1. Introduction

Protein-based polymers were found to have interesting physical properties,[1] which were not commonly found in conventional petroleum-based polymers. Among them, energy conversion ability (responsive behavior)[1] seems to be the most useful for the future development of the polymers. It is possible because of the existence of Tt transition[1] and its sensitivity to various inputs such as thermal energy. The Tt transition found in elastomeric polypentapeptides is somewhat similar to natural protein folding and lower critical solution temperature type phase transitions. Various different energies, i.e. thermal, mechanical, pressure, chemical, and electrical energies, have been found to be inter-convertible. These polymers are usually composed of mainly hydrophobic amino acids, such as glycine (G), valine (V), proline (P), and isoleucine (I). Thus, hydrophobic hydration has been considered the major interaction to understand their phase transition in water.[1] Since this class of polypeptides has the repeating sequences of amino acids analogous to the repeats commonly found in elastins, understanding their energy conversion mechanisms has also been an interesting subject.

If thermal energy is provided to the solution of a protein-based polymer, the polymer tends to be hydrophobically folded and assembled at a certain temperature, resulting in the possible output of mechanical energy. The temperature can be named the lower critical solution temperature (LCST) of the polymer. However, to remind that the phase transition has many characteristic aspects[1] of globular proteins, the phase transition temperature will be designated T_t in this study, which has been proposed and used by Urry *et al.*[1]. In this study, we cross-linked two polypentapeptides, poly(GVGVP) and poly(GVGIP) [designations after cross-linking: γ-V and γ-I, respectively], and studied their swelling and elastic properties in different stress states.

2. Experimental

The two kinds of cross-linked polypeptides were provided from Bioelastics Research, Ltd. First, both the high molecular weight polymers were expressed by *Escherichia coli* using the biosynthesis technique.[2] After purification using T_t transition,[2] they

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were cross-linked in water (concentration = 1g/mL) by γ-irradiation at 23 ℃. The irradiation dose was changed from 6 to 34 Mrad (0.3 Mrad/hr). After cross-linking, uncross-linked molecules were removed by cleaning in water for more than two weeks. Details on the basic characterizations of the polymer synthesis can be found elsewhere.[1,2]

Hydrogels were stored in double-distilled water bath. They were cleaned by simply changing water more than 3 times. It was expected that no impurities other than uncross-linked polymers exist. Equilibrium swelling ratio was obtained by measuring the weight of fully swollen and completely dried gels. Specimens of $\approx$50 mm^2 (above T_t) were cut and equilibrated in double-distilled water for more than 2 days. Then, they were heated at 2 ℃ step and 12 hours were given for them to equilibrate at each temperature. Swelling measurements were also performed during cooling following the same procedure. In weight measurements of swelling, specimens had to be taken out of a water bath, and experience a different ambient temperature. Thus, specimen weight could possibly change during the measurements. However, it was found that the weight measurement of a sample was normally done in less than 15 seconds, and during this time, the amount of water diffused out of the specimen was found to be insignificant.

Tensile tests of rectangular strips (gauge section = 5x10x0.7 mm) were performed using a MTS MicroBionix (crosshead speed = 3 mm/min) at room temperature in water. For dynamic shear (1 rad/sec) and compression tests (displacement rate = 3 mm/min), disk specimens (diameter = 0.8 mm) were tested in water at room temperature using a Rheometrics ARES rheometer. Shear strain was 3% and circular parallel plates 7.9 mm in diameter were used.

3. Results and discussion

Equilibrium swelling ratio is one of important physical properties to understand the phase behavior of hydrogels. The equilibrium swelling ratio results of cross-linked $(GVGVP)_{251}$ and $(GVGIP)_{260}$ showed relatively reproducible data with small error ranges. The equilibrium swelling ratio of both polymer systems is found to decrease, as the dose of γ-irradiation increases. However, this difference in equilibrium swelling ratio becomes negligible as temperature increases. Above the T_t of two polymers (ca. 30 ℃ for $(GVGVP)_{251}$ and ca. 12 ℃ for $(GVGIP)_{260}$), the effect of γ-irradiation on equilibrium swelling ratio disappears.

The equilibrium swelling of hydrophobic polypeptide gels is determined by the two competing factors, free energy of mixing (ΔG_{mixing}) and elastic energy of rubber ($\Delta G_{elastic}$).

$$\Delta G = \Delta G_{mixing} + \Delta G_{elastic} \quad (1)$$

As temperature increases over ca. 30 ℃, all the different curves of swelling ratio start to overlap with the spinodal line of the uncross-linked linear polymer. Therefore, it seems to be the case that the phase transition observed in cross-linked hydrogels is basically the same phenomena as uncross-linked polymers have. In fact, the transition temperature seemed to be independent on the dose of γ-irradiation. According to the phase behavior of uncross-linked polymers, the contribution of the first term in equation 1 will decrease with an increase in temperature. Consequently, as equilibrium swelling ratio decreases, the effect of γ-irradiation dose will decrease too.

Since both the polymers were cross-linked above T_t, it may be difficult for the equilibrium swelling ratio of ca. 2 above T_t to generate significant elastic energy, resulting in no effect of γ-irradiation dose

on swelling. In other word, above T_t, only ΔG_{mixing} may determine the swelling ratio of hydrogels, and the contribution of $\Delta G_{elastic}$ may be negligible. However, this series of events may not be important to understand the swelling behavior of our hydrogels. The simple rubber elasticity theory may not be applicable to the hydrogels above T_t. This is because both the polypeptides are likely to have helical structures above T_t. As mentioned above, it has been reported[1] that these polymers have a tendency to be hydrophobically folded and assembled above T_t. Therefore, the effect of cross-links on swelling above T_t could be overwhelmed by the inter- and intra-molecular interactions.

According to the mean field theory of equilibrium swelling, a simple equation for the molecular weight between cross-links (M_c) can be derived from the equation 1, as follows.

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\frac{\overline{v}}{V_1}\left[\ln(1-v_2)+v_2+\chi v_2^2\right]}{\left[v_2^{1/3}-\frac{v_2}{2}\right]} \quad (2)$$

where $\overline{M}_n$ is the number-average molecular weight of uncross-linked polymer, $\overline{v}$ is the specific volume of bulk polymer, V_1 is the molar volume of solvent (18 cm^3/mol), v_2 is the equilibrium volume fraction of polymer, and χ is the polymer-solvent interaction parameter. This equation makes it obvious that the M_c of hydrogels decreases with the increase of γ-irradiation dose. On the other hand, the cross-link density of hydrogels will increase, because it is inversely proportional to M_c. However, it should be noted that the existence of elastically ineffective chains except chain ends and non-Gaussian properties of chains are not considered in equation 2.[8]

The rubber elasticity theory was employed for the characterization of the $\Delta G_{elastic}$ term only below T_t. Using this theory, M_c values can be obtained from the uniaxial tensile test data.

$$\tau = v_2^{1/3}\frac{\rho RT}{\overline{M}_C}\left(1-\frac{2\overline{M}_C}{\overline{M}_n}\right)\left(\lambda-\frac{1}{\lambda^2}\right) \quad (3)$$

where τ is the tensile stress, ρ is the density of hydrogels, R is the gas constant, T is temperature, λ is the extension ratio. M_c was inversely proportional to the dose, i.e. $M_c \propto 1/(dose)^n$. The n values were found to be about 0.6. Volume shrinkage related with phase transitions was found to become smaller as cross-link density increases. Therefore, increasing the dose of γ-irradiation may result in less noticeable phase transition, although it can effectively stiffen hydrogels.

In the three mechanical tests, i.e., tensile, compression, and dynamic shear tests, it was found that the modulus of hydrogels increased as the dose of γ-irradiation increased.[3] This relation was obvious only below the T_t temperature of polymers. Below T_t, the cross-linked polymers can be assumed as random chain networks. According to the rubber elasticity theory, the increase of modulus indicates the increase of cross-link density. It is obvious in the comparison of the three different tests that the modulus of hydrogels depends on the stress conditions of measurements. Modulus decreases in the order of tensile, shear, and compressive modulus, while a linear relationship can be found between any two sets of modulus data. However, the relationship between tensile (E) and shear storage moduli (G') gave an unusually small Poisson's ratio.

The normal compressive strain dependence of G' might explain the unusual Poisson's ratio value. Before significant normal stress, N, develops (normal strain < ca. 0.05), G' rapidly increases with normal strain, because of greater contact area between specimen and parallel plates. Thus, the data above the normal strain of ca. 0.05 are our interest.

As a model of petroleum-based hydrogels, a poly (methacrylic acid) (PMAA) hydrogel was prepared and its behavior was compared with that of protein-based systems. On the other hand, no significant normal strain dependence of G' is found within the normal strain range in petroleum-based hydrogels (PMAA gels).

While there is no significant normal strain dependence in the PMAA case, the G' of all protein-based hydrogels prepared in this experiment shows significant dependence. About 15% decrease in G' from its maximum value can be found in the normal strain range of 0.05-0.3. This interesting dependence is currently difficult to explain. Compression perpendicular to shear direction may induce an anisotropic structural change of polypeptide chains, with the result that the shear modulus changes. Compression can decrease the volume of materials, and water inside hydrogels may be squeezed out. However, this effect should increase G' instead of decrease it. The decrease in volume of materials may have a different mechanism. According to the studies on the pressure dependence of the phase transition of poly(GVGVP),[1] the transition temperature was found to increase with an initial increase in pressure. Thus, compression may induce the same effect by decreasing the volume of hydrogels, which might be related with the normal compressive strain dependence.

4. Conclusions

The phase transition of hydrogels based on hydrophobic amino acids, which were cross-linked by γ-irradiation, was studied by measuring equilibrium swelling ratio and tensile modulus. Regardless of cross-link density (dose of γ-irradiation), a phase transition temperature was observed for a polymer in equilibrium swelling data. Below the temperature, equilibrium swelling ratio was shown to depend on the dose of γ-irradiation. As the dose of γ-irradiation increased, equilibrium swelling ratio was found to decrease and tensile modulus increase. Both the results showed the decrease of molecular weight between cross-links. However, above the phase transition temperature, the d ependence on the dose of γ-irradiation was not noticeable. Linear relationship was found between any two kinds of moduli among the tensile, compressive, and dynamic shear storage moduli of polypentapeptide hydrogels. Tensile modulus was found be the largest, followed by the dynamic shear storage and the compressive moduli. The significant normal compressive strain dependence of dynamic shear storage modulus was found, which is one of responsive behavior.

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