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Mechanical Properties of Cross-Linked Synthetic Elastomeric Polypentapeptides

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ABSTRACT: Mechanical properties in equilibrium swelling states were examined for two elastomeric polypentapeptides, namely, $(GVGVP)_{251}$ and $(GVGIP)_{260}$ where G = glycine, V = valine, P = proline, and I = isoleucine. They had been prepared using recombinant DNA technology and cross-linked by γ-irradiation over the range of doses from 6 to 30 Mrad. Moduli were measured in uniaxial tensile tests. The specific work of fracture was determined as a measure of toughness. Increased doses of γ-radiation increased the moduli (cross-link density) of hydrogels. The hydrophobic folding and assembly phase transition that occurred on raising the temperature was demonstrated to have significant effects on moduli and fracture properties. Thermoelasticity experiments suggest that the phase transition involves changes in elasticity mechanism. Above the transition temperature, hydrogels were shown to become more viscoelastic.

Introduction

Tropoelastin is the precursor protein of the mammalian elastic fiber,[1,2] which is the major elastic protein of ligaments, arteries, skin, and lung. One of the most prominent amino acid sequences of tropoelastin was found by Sandberg and co-workers to be $(GVGVP)_n$, where n is 11 in porcine,[3−5] and bovine tropoelastins[6] (G = glycine, V = valine, P = proline, I = isoleucine). It has been demonstrated that, upon raising the temperature, solutions of synthetic high molecular weight poly(GVGVP) self-assemble into filamentous and fibrous structures.[7−11] Subsequently, cross-linked poly(GVGVP) was shown to be a dominantly entropic elastomer.[12] With an understanding of the nature of the self-assembly process on raising the temperature, it then became possible to design polymers capable of diverse energy conversions.[10,13] Beginning with the polymer, poly(GVGVP), hundreds of analogues such as poly(GVGIP) have also been synthesized and studied. When the hydrophobicity and structure of the polypentapeptides are carefully considered, polymers capable of diverse free energy transductions and varied elastic moduli are the results.

In a series of studies, various types of energy have been shown to be interconvertible using the polypeptides.[10] When thermal energy input is added into cross-linked poly(GVGVP), mechanical energy output can be obtained by shrinking of the materials. If mechanical energy is put into the materials modified with a small number of glutamic acid residues (e.g., 3.4 mol %), a pK_a shift can be observed, which is an example of mechanical to chemical energy transduction. Other forms of energy including electromagnetic, electrical, and pressure have been proved interconvertible, too. These experiments show the potential of these materials as future smart materials for medical and also nonmedical applications.

The most important underlying phenomenon for the energy transduction is a hydrophobic folding and assembling transition. The polypeptides have a significant amount of hydration of nonpolar groups,[14] the water structure of which (variously referred to as "clathrate", "cage", or "cathedral") is believed not to be entropically favored.[15−17] When thermal or other energy input raises the free energy of the hydration, the hydrophobic folding and assembling transition can result. For example, if poly(GVGVP) solution is heated above the transition temperature (T_t), the hydration of nonpolar groups becomes unstable, and the polymer will reduce the amount of the hydration of nonpolar groups with an accompanying increase of inter- or intramolecular contacts and thus an increase in chain density. At temperatures above the hydrophobic folding and assembling transition, poly(GVGVP) is believed to form twisted filament structures of β-spirals, which are a helical arrangement of type II β-turns.[8,18−20] Following previous work,[10] the temperature for the onset of the phase transition on heating will be designated as T_t and the phase transition will be called the T_t transition.

These polypeptides can be prepared by two quite distinct methods: chemical synthesis and biosynthesis using recombinant DNA technology to produce the genes and transform the cells of animals and plants.[21] Biosynthesis has the potential to produce well-defined polymers, with precise molecular weight, stereochemistry, and amino acid sequence. By protein engineering it becomes possible to design polypeptides for specific applications.

The microbially prepared polypeptides, $(GVGVP)_{251}$ and $(GVGIP)_{260}$, have been cross-linked using γ-irradiation and used as hydrogels in the previous studies on the T_t transition.[10] Although systematic studies have been carried out on the T_t transition and free energy transduction, the mechanical properties of the bulk

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Table 1. Designations and Effective Cross-Link Densities of Polymers

γ-V (MW = 102 kg/mol)								
dose of γ-irradiation (Mrad)	6	10	14	18	20	22	26	30
effective cross-link density (mol/m^3)	2.8	8.5	14.8	24.6	29.2	31.2	32.9	35.1
γ-I (MW = 109 kg/mol)								
dose of γ-irradiation (Mrad)	10	14	18	22	26	30		
effective cross-link density (mol/m^3)	2.2	7.6	13.6	10.5	21.6	30.3		

hydrogel materials have yet to be adequately studied. To the best of our knowledge, due to previously limited quantities, there has been no systematic study on the bulk mechanical properties of microbially prepared polypeptides. When a material is to be selected for a given application, the mechanical properties of a specific hydrogel become critical. Fortunately, the recent developments of recombinant DNA technology enable preparation of protein-based polymers in large quantities for bulk mechanical studies. It will also hasten the time when these materials become commonplace in our daily life.

In this study, the mechanical properties of γ-irradiation cross-linked $(GVGVP)_{251}$ and $(GVGIP)_{260}$ are examined. Elastic and viscoelastic properties and their dependence on environmental conditions are our primary interests. Additionally, fracture behavior is examined using the work of fracture approach.[22,23] This study will provide useful insight into this new class of materials in addition to the previous studies on micron-scale samples of polypeptides.[24,25]

Experimental Section

Materials. The elastic protein-based polymers, $(GVGVP)_{251}$ ($T_t \approx 28$ °C) and $(GVGIP)_{260}$ ($T_t \approx 12$ °C), as well as their γ-irradiation cross-linked matrices, were obtained from Bioelastics Research, Ltd., as part of a coordinated research effort funded by the Office of Naval Research. Briefly, by means of recombinant DNA technology, the polypentapeptides were synthesized in *Escherichia coli* and purified using their T_t transition.[26] After purification, they were freeze-dried and stored dry. Their synthesis was confirmed using MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry and one-dimensional and two-dimensional ^{1}H NMR following the same methods as described in ref 27. Details of the biosynthesis and purification technique can be found elsewhere.[26,28] Deionized ultrafiltered water from Fisher Scientific and mineral oil from the Aldrich Co. were used in this experiment.

The biosynthesis technique is able to produce materials with a monodisperse molecular weight. However, after synthesis, microbes and their enzymes can degrade the polymers, resulting in polydispersity and decreased molecular weight. During our experiments, it was observed that the polymer, $(GVGVP)_{251}$, seems to be quite resistant to biodegradation whereas $(GVGIP)_{260}$ is quite susceptible. Degradation was difficult to completely prevent or control. Thus, instead of controlling it with antimicrobial agents and enzyme poisons that could alter the mechanical properties to be characterized, the tensile moduli (or equilibrium swelling ratios) of hydrogels below T_t were checked after the material's characterizations and compared to their initial values. When a significant difference was found, samples were considered no longer intact. All linear polymers were stored in a dried state at room temperature and hydrogels at 5 °C.

Preparation of Hydrogels. In general, for preparation of hydrogels, polymer solutions at temperatures below T_t are placed in molds in a centrifuge and heated above T_t during centrifugation to obtain a uniformly dense polymer phase (≈500 mg/mL). Then, the phase-separated polymer still in its mold is cross-linked at 23 °C using γ-irradiation of 6−36 Mrad (0.3 Mrad/h). The cross-linked hydrogels were purified in water for more than 2 weeks with changes of water every 24 h. Additional details on cross-linking can be found elsewhere.[9] After γ-irradiation cross-linking, $(GVGVP)_{251}$ and $(GVGIP)_{260}$ are designated γ-V and γ-I, respectively (Table 1), followed by the radiation dose in Mrad, e.g., γ-V-20 Mrad. Before performing the mechanical tests described below, samples were equilibrated for more than 24 h under the testing conditions. All the characterizations were performed on specimens immersed in water.

Uniaxial Tensile Tests. A MTS MicroBionix instrument with a 4 N load cell was used for uniaxial tensile tests at a crosshead speed of 3 mm/min. Because of limited materials, small rectangular strips (gauge section = 0.7 × 5 × 10 mm) of hydrogels were used [ASTM-D412-98a]. Their dimensions were measured using a micrometer, NSK digitrix II (±1 μm). During the tests, entire samples and gripping fixtures were kept in a temperature-controlled water bath. Young's modulus was obtained from the initial linear part of the stress−strain curve (usually <0.2 strain). Consecutive loading and unloading tests were done at the same crosshead speed. Maximum displacement was 5 mm. For the thermoelasticity experiments, samples of the same size were first stretched to a certain strain (within their linear elastic regions) in a medium (water or mineral oil), and keeping the displacement constant, changes in force were measured as a function of temperature.

Work of Fracture. The work of fracture concept[22,23] was used to evaluate the toughness of hydrogels using a limited amount of samples. When a double-edge-notched (DEN) specimen undergoes fracture, the total work of fracture, W_t (J), is the sum of an essential work of fracture, W_e, dissipated in the process zone of fracture and a nonessential plastic work, W_p, dissipated outside of process zone.

$$W_t = W_e + W_p \tag{1}$$

$$W_t = w_e lt + bl^2 t w_p \tag{2}$$

where l and t are the initial ligament (load-carrying part between two initial notches) length and specimen thickness, respectively, and b is a shape factor. Dividing the terms in eq 2 by lt (ligament area) gives

$$w_t = w_e + blw_p \tag{3}$$

The specific work of fracture, w_t (J/m^2), was measured using DEN tensile specimens (10 × 30 × 0.7 mm) in water at a crosshead speed of 3 mm/min using the same MTS MicroBionix instrument. Sharp notches were introduced by cutting a specimen using a fresh razor blade. Critical crack tip opening displacement was experimentally found to be much larger than the initial crack tip radius produced by razor blades.[29]

Results and Discussion

To discuss the mechanical properties of protein-based hydrogels, knowledge of some of their physical properties is useful.[10] As their chemical structures suggest, γ-I ($T_t \approx 10$ °C) is more hydrophobic than γ-V ($T_t \approx 28$ °C), because the GVGIP pentamer contains one additional alkyl unit in the side chain of the isoleucine residue.[10] Thus, when a mechanical test is performed at room temperature, γ-V is below its T_t and γ-I is above T_t. Above T_t, both the hydrogels have equilibrium weight swelling ratios (Q_{we}) of around 2.[30] As temperature decreases below T_t, Q_{we} increases up to 35. The T_t transition in the plots of Q_{we} vs temperature is continuous and reversible like a second-order phase

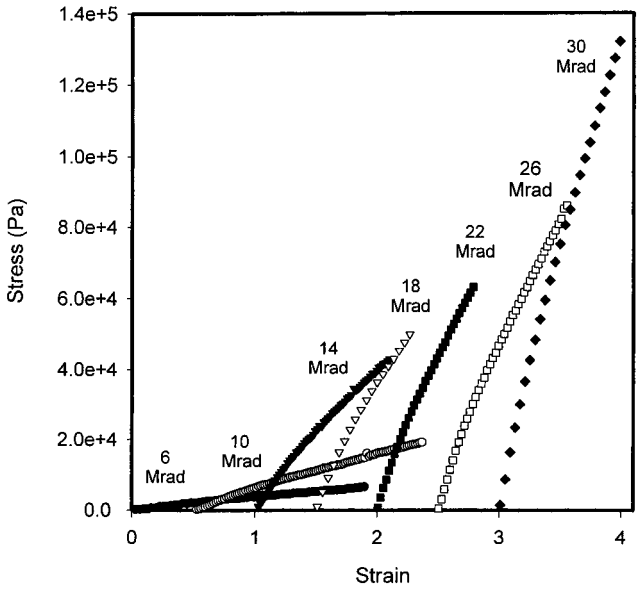

Figure 1. Typical stress−strain curves of uniaxial tensile tests of γ-V hydrogels at 21 °C. The strain of curves was shifted to separate each curve.

transition.[30] The glass transition temperatures (T_g) of dried γ-V and γ-I, measured using a Perkin-Elmer DSC 7 at a heating rate of 10 °C/min, were 199 and 175 °C, respectively. The additional alkyl unit, i.e., CH_2 moiety, is capable of decreasing T_g.

Uniaxial Tensile Modulus. An essential test to understand the mechanical performance of materials is the uniaxial tensile test. Figure 1 shows the typical engineering stress−strain curves of γ-V. (Other hydrogels showed the similar trends as found in this figure.) The first obvious finding is that they are linear elastic in a range of strain, i.e., ca. 0−0.2. This corresponds to the lower limit that Yannes proposed as characteristic of rubbery networks.[31] As γ-irradiation dose increases, Young's modulus increases, too. Tangential modulus decreases as strain increases.

The uniaxial tensile tests used rectangular strips without direct strain measurement. Thus, the strength of materials may not be a reliable value in Figure 1. This limits further analyses of strength data, i.e., strain, stress, and energy to break. However, general observations found in this experiment are worth mentioning. As γ-irradiation dose increases, strain at break decreases and stress at break increases (Figure 1). The energy to break, which is a function of both parameters, seems to have a maximum at a certain γ-irradiation dose. As cross-link density increases with radiation dose, higher load can be carried by the resulting hydrogels, but extensibility decreases because of the shorter chain length between cross-links. When a hydrogel reaches its maximum extensibility, fracture will follow mainly through chain scission.

The dependence of stress−strain curves on temperature is shown in Figure 2A−C. The energy to break appears to increase with temperature by the accompanying increase of strain and stress at break. The same trend was also found in γ-I series. This can be expected from the equilibrium swelling ratio of hydrogels.[30] Since the swelling ratio is higher at lower temperature, chains at low temperature are already significantly extended from their reference state due to swelling, resulting in smaller extensibility in tensile tests.

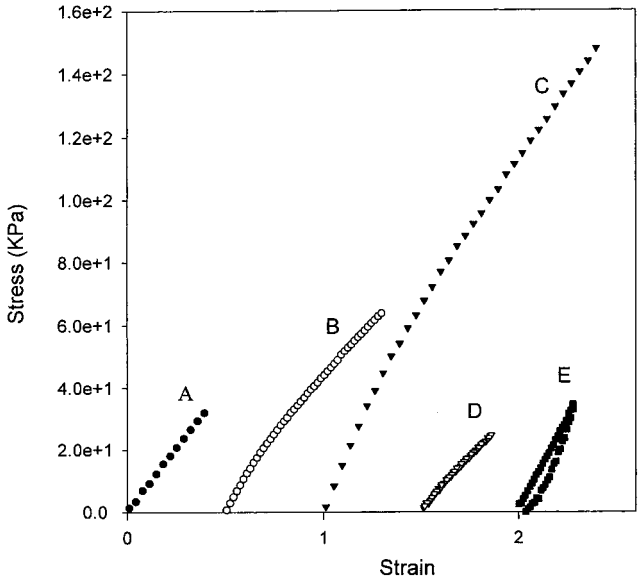

Figure 2. Typical stress−strain curves of uniaxial tensile tests of γ-V and γ-I hydrogels. The strain of curves was shifted to separate each curve. Curves D and E were taken upon consecutive loading−unloading: (A) γ-V-20 Mrad at 7 °C; (B) γ-V-20 Mrad at 21 °C; (C) γ-V-20 Mrad at 35 °C; (D) γ-I-26 Mrad at 5 °C; (E) γ-I-22 Mrad at 21 °C.

Table 2. Residual Strains, E_{res}, in Consecutive Loading−Unloading Tensile Tests at Different Temperatures

	hysteresis (e_{res})[a]			hysteresis (e_{res})[a]	
temp (°C)	γ-V	γ-I	temp (°C)	γ-V	γ-I
40	0.01		17	0	0.02
38	0.01		14	0	0
33	0.02	0.04	11	0	0
28	0.02	0.03	8	0	0
24	0	0.02	6	0	0

[a] e_{res} is defined as the residual strain at zero load after a consecutive loading−unloading test (crosshead speed = 3 mm/min, maximum strain = 0.5).

The curves, D and E, were taken during consecutive loading and unloading. Significant hysteresis was found in E, but not in D. In many repeated tests, significant hysteresis was found only above T_t. In a detailed study, the residual strain after unloading, e_{res}, was measured at a displacement rate and given in Table 2. The e_{res} value is the average of at least three different tests. As can be seen, hysteresis is noticeable only above T_t. γ-I have more significant hysteresis than γ-V, if any. This hysteresis actually increases error in our modulus measurements, because strain rate is difficult to be kept exactly the same from test to test. Since it is well-known that viscoelastic hysteresis depends on strain rate, the e_{res} values in Table 2 will vary if a different displacement rate is applied.

Figure 3 shows the Young's moduli of γ-V and γ-I hydrogels below T_t. γ-Irradiation can successfully stiffen the hydrogels. The modulus almost increases linearly with the dose of γ-irradiation. If protein-based hydrogels can be assumed to be random Gaussian chain networks, the tensile test data can be related to cross-link density. Under the rubber elasticity assumptions, effective cross-link density (ν) can be calculated from the equation[32−34]

$$\tau = \varphi^{1/3} R T \nu \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (4)$$

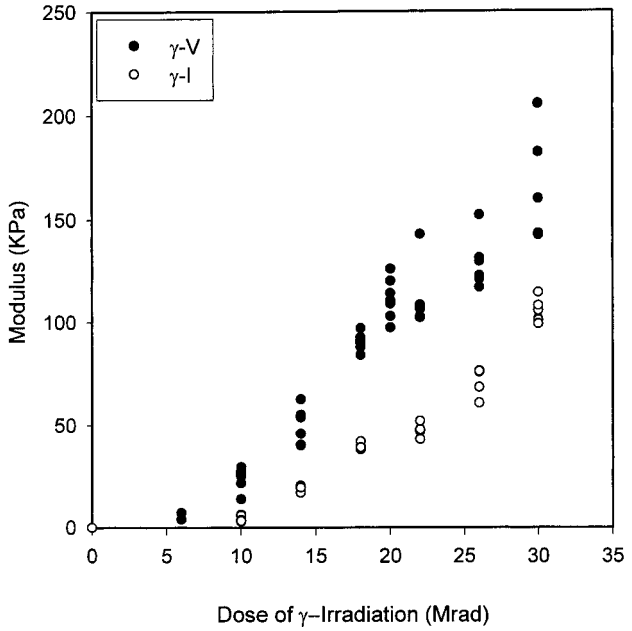

Figure 3. Uniaxial tensile modulus of γ-V and γ-I as a function of γ-irradiation dose. The γ-V and γ-I data were taken at 21 and 7 °C, respectively.

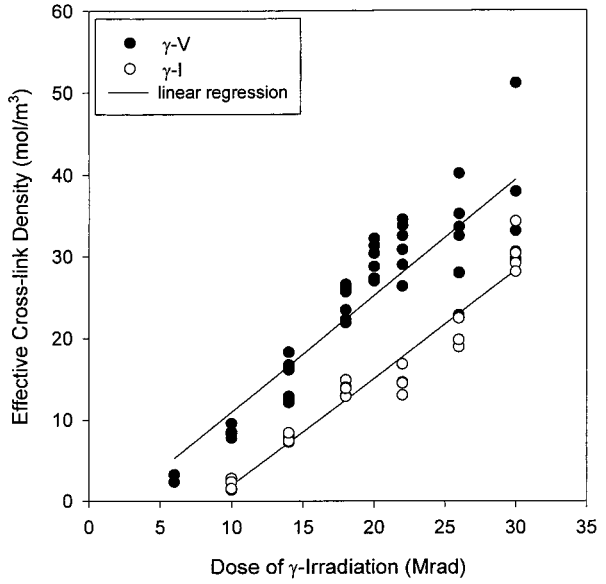

Figure 4. Effective cross-link density of γ-V and γ-I as a function of γ-irradiation dose. Linear regression: $y = -11.21 + 1.32x$ for γ-V and $y = -3.27 + 1.42x$ for γ-I.

where τ is the tensile stress, φ the volume fraction of polymers, R the gas constant, T temperature, and α the extension ratio, i.e., current length divided by the initial length. Since the polypeptide chains were found to have random conformations below T_t, it is proper to use eq 4 and the tensile data below T_t for the calculation of ν. The φ values were measured separately, and details are available elsewhere.[30]

The calculated ν is presented in Figure 4. It increases with γ-irradiation dose. The slopes of both curves seem to be similar. In our experiment, the dose was controlled by time, and so the similar slopes indicate the similar generation rates of cross-links. The rate depends on not only cross-linking but also chain scission kinetics.[35,36] An interesting finding is that the critical dose of γ-irradiation to reach gel points appears to be smaller in γ-V (≈2 and 8 Mrad for γ-V and γ-I, respectively).

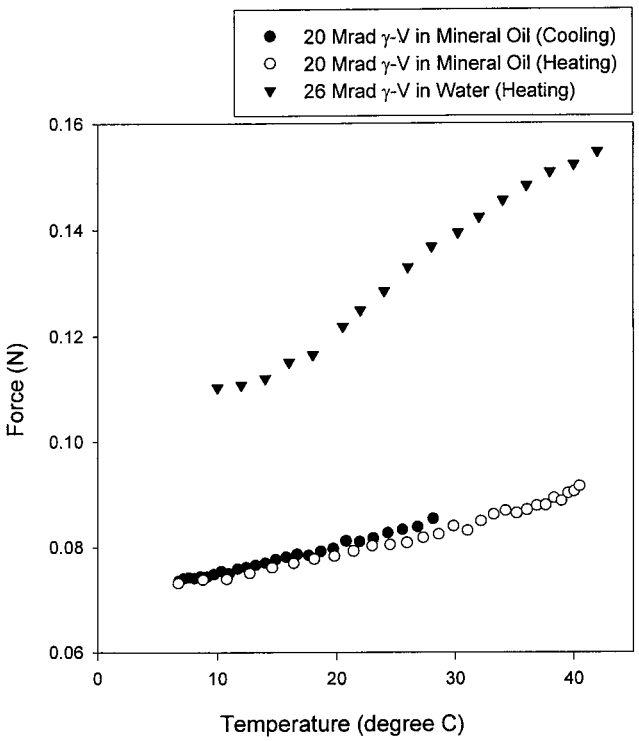

Figure 5. Thermoelasticity data (force vs temperature) of stretched γ-V in water and mineral oil. The heating curve of γ-V-20 Mrad was obtained after its cooling experiment with no time lag (initial strain = 0.5, cooling and heating rate = 0.4 °C/min).

This may result in part from the partial degradation of $(GVGIP)_{260}$ during purification with the appearance of a smear of lower molecular weight polymers in SDS−PAGE gels[26] before cross-linking, whereas $(GVGVP)_{251}$ appears to remain monodisperse. The cross-link density range in Figure 4 corresponds to the molecular weight between cross-links, M_c, of 15−50 kg/mol, which is inversely proportional to the dose of γ-irradiation. Thus, the hydrogels prepared are lightly cross-linked, compared to most petroleum-based hydrogels. γ-Irradiation doses higher than 30 Mrad may lead to stiffer hydrogels with higher cross-link densities. However, higher cross-link density was found to blur the volume phase transition behavior of hydrogels,[30] because it decreases equilibrium swelling ratio below transition temperature.

Elasticity of Hydrogels. Protein-based hydrogels were found to be entropic elastic.[9,42] Thus, their modulus is expected to increase with temperature. Furthermore, polymer volume fraction increases with temperature, because of the T_t transition. This will further accelerate the increase of modulus with temperature. Typical thermoelasticity experiments easily reveal this trend, as can be seen in Figure 5. The γ-V-26 Mrad hydrogel shows nonlinear force development in the temperature range 10−40 °C. Entropic elasticity usually produces a linear increase with temperature, and so the more rapid increase in Figure 5 might be caused by the T_t transition.

An interesting comparison can be made using the thermoelasticity results obtained in mineral oil (Figure 5, two curves of γ-V-20 Mrad). Mineral oil is expected to prevent water diffusion into hydrogels throughout the experiment. The hydrogel was equilibrated at 30 °C before the test, and its cooling curve was taken first. Thus, it seems to be able to keep its water content

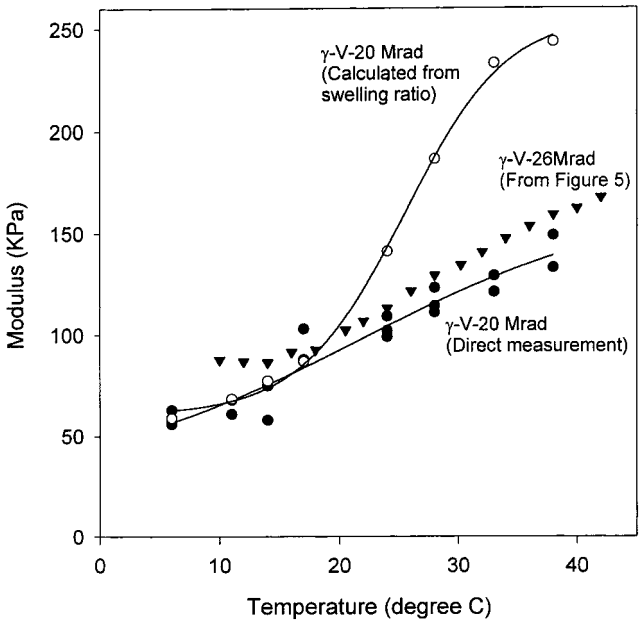

Figure 6. Tensile modulus of γ-V in water as a function of temperature. The γ-V-26 Mrad curve was obtained from the thermoelasticity data of Figure 5.

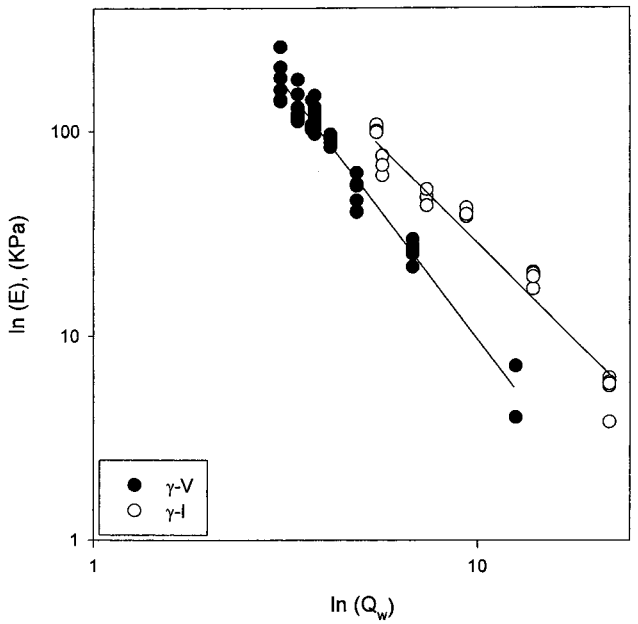

Figure 7. Relationship between uniaxial tensile modulus (E) and equilibrium weight swelling ratio (Q_{we}) at 21 °C (γ-V) and 7 °C (γ-I). Linear regression: $y = 3.44 - 2.46x$ for γ-V and $y = 3.35 - 1.88x$ for γ-I.

during the tests, although there is a small hysteresis between the cooling and heating curves. Comparison between the data taken in water and in mineral oil shows that preventing the changes of water content removes the effect of the T_t transition in the thermoelasticity experiments, resulting in linear force changes. This experiment reflects the phase separation characteristic of T_t transition.

The force data of γ-V-26 Mrad in Figure 5 can be converted to secant modulus data, since the dimensional change of the hydrogel is known from equilibrium swelling ratio measurements.[30] The results obtained are given in Figure 6 (γ-V-26 Mrad). A continuous increase in modulus is obvious. The secant modulus was found to decrease with strain in Figures 1 and 2. Thus, the difference between Young's and secant moduli will increase with temperature. However, the difference seems to be small. In Figure 6, the temperature dependence of secant modulus data agrees well with that of Young's modulus data of γ-V-20 Mrad measured as a function of temperature (closed circles).

In Figure 6, there is a significant difference between the actual modulus change and the change expected from equilibrium swelling ratio. The γ-V-20 Mrad curve calculated from swelling ratio was obtained as follows. First, the average modulus value of γ-V-20 Mrad at 5 °C was taken as a reference, and then using its dimensional change data,[30] the increase of modulus with an increase in temperature was calculated, assuming there is no change in the elasticity of polymer phase. The hydrogel was considered as a simple mixture of two phases, water and polymer. Then, the modulus of hydrogel, E_h, is φE_2, where E_2 is the modulus of polymer phase obtained from E_h at 5 °C. As can be seen in Figure 6, modulus change is much smaller in the actual data than in the calculated data.

Obviously, the assumption above needs to be corrected in order to predict the actual behavior of these hydrogels. E_2 should depend on temperature and composition. According to the scaling law based on the rubber elasticity theory, modulus, E, should scale to φ as follows:[43]

$$E \cong T\varphi^{2.25}(v^{3/4}a^{3/2}) \qquad (5)$$

where v is the excluded-volume parameter ($= a^3(1 - 2\chi)$) and a is effective length per monomer. Since the interaction parameter, χ, was found to increase with temperature,[30] the resulting decrease in v can reduce the degree of modulus increase with temperature. However, in our polypeptide hydrogels, the change of φ is far more dominant. From 1 to 14 °C, while φ was found to increase ca. 400%, χ was found to increase ca. 2%.[30] Thus, eq 5 predicts more rapid increase of modulus with temperature than the curve of the simple rule of mixtures in Figure 6. Thus, the experimental data cannot be explained by both the equations.

As hydrogels undergo the T_t transition, they become more organized into the twisted filament forms of β-spirals.[10,20,44,45] Thus, the structural transformation can make the idea of the equations no longer useful for our hydrogels. To predict the elasticity of hydrogels above T_t, an advanced theory based on the structure of polypeptide chains will be needed. This may need to consider the librational model proposed for the entropic elasticity of poly(GVGVP) molecules.[46]

In eq 5, if a temperature and a solvent are chosen, a simple relationship is expected to result between E and φ. Using the Q_{we} data[30] and the uniaxial tensile modulus data, the correlation was examined. Figure 7 shows the relationship. The strong Q_{we} dependence of modulus can readily be seen. In this plot, the slopes of γ-V and γ-I curves are -2.46 and -1.88, respectively. According to eq 5, the modulus of hydrogels should scale as $Q_w^{-2.25}$. Considering the precision of our current tensile tests, the discrepancy here is not surprising.

Toughness Assessments. Up to now, our study has focused on the modulus of hydrogels. For the basic understanding of the mechanical performance of these materials, fracture (rupture) properties[48,49] should be examined in addition to modulus. Toughness assessment using notched specimens is usually better than

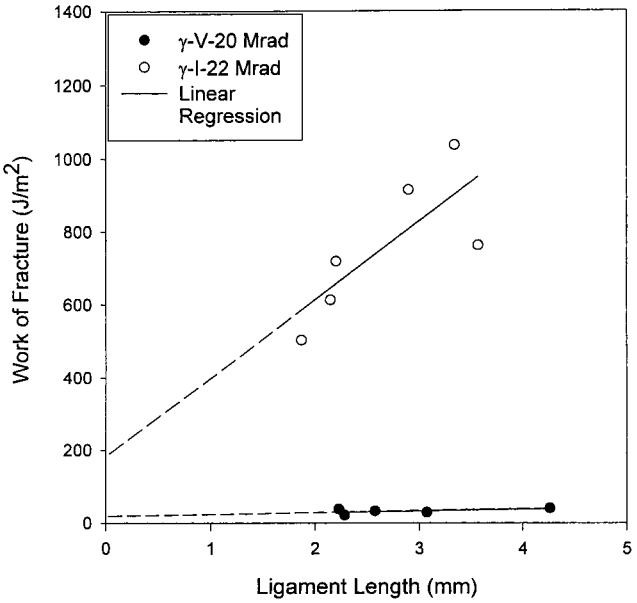

Figure 8. Specific work of fracture of γ-V and γ-I as a function of ligament (load-carrying part between two initial notches) length of DEN specimens at 21 °C. Linear regression: $y = 18.7 + 4.7x$ for γ-V-20 Mrad and $y = 183.7 + 215.4x$ for γ-I-22 Mrad.

toughness estimation using tensile specimens, because of the controllability of crack initiation.[50] Since the hydrogels prepared in this experiment show a wide range of elasticity from linear elastic to viscoelastic, the standard J_{IC} (critical J integral, i.e., a line integral related to energy in the vicinity of a crack) approach is appropriate, instead of the critical stress intensity measurement.[50] In this study, the essential work of fracture approach[22,23] was employed as a method to cover the wide range of elasticity, which is similar to J_{IC}.

The most serious obstacle in this mechanical performance study is the limited amount of microbial prepared polymers. The biosyntheses supporting this experiment produced sufficient quantities of polymers that this mechanical performance study on bulk specimens became possible. However, the number of specimens that can be prepared is still very limited. Thus, only six specimens were prepared and tested.

While γ-I is not distinctly different from γ-V in the modulus studies above, it was noticed from handling the protein-based hydrogels during experiment that γ-I is much "stronger" at room temperature. This difference can be easily confirmed in Figure 8. The specific work of fracture of γ-I is more than 30 times larger than that of γ-V. This difference in specific work of fracture reflects the phenomenological observation. It may be caused predominantly by the different water contents (different hydrophobicity of two polymers). Since γ-I is above its T_t, its water content is relatively small, and it might be close to its primary relaxation (glass transition) region, resulting in significant viscoelastic energy dissipation. This dissipation will mainly contribute to the nonessential part of work.

As explained before, the total work of fracture involves the essential work of fracture and the nonessential plastic work that significantly depends on specimen geometry. By subtracting the nonessential contribution from the total work of fracture, the essential work of fracture, considered a fundamental material property, can be obtained. In Figure 8, it can be estimated by extrapolating the linear regression curves. The essential work of fracture of γ-I may be larger than that of γ-V. Unfortunately, the number of samples is insufficient to confirm this conjecture. This will be a matter for the future studies.

Conclusions

Uniaxial tensile tests were performed on protein-based hydrogels that achieved elasticity by cross-linking. As the dose of γ-irradiation increases, the modulus of hydrogels was demonstrated to increase, indicating an increase in cross-link density. Thermoelasticity data suggested that different elasticity mechanisms might be working in two regions, one below and the other above T_t. When water diffusion into hydrogels was prevented, the T_t transition was not distinct in thermoelasticity experiments, which was otherwise obvious. When temperature increased above T_t, significant hysteresis in tensile tests is observed. It was conjectured that the reduction of water content by increasing temperature above T_t could induce the primary relaxation in hydrogels, which could explain the viscoelastic behavior. The specific work of fracture at 21 °C was shown to be larger in our γ-I specimens than in γ-V, possibly because of different hydrophobicity.

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References and Notes

(1) Sandberg, L. B.; Soskel, N. T.; Leslie, J. G. *N. Engl. J. Med.* **1981**, *304*, 566.
(2) Debelle, L.; Alix, A. J. P. *Biochimie* **1999**, *81*, 981.
(3) Foster, J. A.; Bruenger, E.; Gray, W. R.; Sandberg, L. B. *J. Biol. Chem.* **1973**, *248*, 2876.
(4) Gray, W. R.; Sandberg, L. B.; Foster, J. A. *Nature* **1973**, *246*, 461.
(5) Sandberg, L. B.; Leslie, J. G.; Leach, C. T.; Alvarez, V. L.; Torres, A. R.; Smith, D. W. *Pathol. Biol.* **1985**, *33*, 266.
(6) Yeh, H.; Ornstein-Goldstein, N.; Indik, Z.; Sheppard, O.; Anderson, N.; Rosenbloom, J. C.; Cicila, G.; Yoon, K. G.; Rosenbloom, J. *Collagen Relat. Res.* **1987**, *7*, 235.
(7) Urry, D. W.; Long, M. M.; Cox, B. A.; Ohnishi, T.; Mitchell, L. W.; Jacobs, M. *Biochim. Biophys. Acta* **1974**, *371*, 597.
(8) Volpin, D.; Urry, D. W.; Pasquali-Ronchetti, I.; Gotte, L. *Micron* **1976**, *7*, 193.
(9) Urry, D. W.; Henze, R.; Harris, R. D.; Prasad, K. U. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 1082.
(10) Urry, D. W. *J. Phys. Chem. B* **1997**, *101*, 11007.
(11) McMillan, R. A.; Caran, K. L.; Apkaraian, R. P.; Conticello, V. P. *Macromolecules* **1999**, *32*, 9067.
(12) Urry, D. W.; Wood, S. A.; Harris, R. D.; Prasad, K. A. In *Polymers as Biomaterials*; Shalaby, S. W., Horbett, T., Hoffman, A. S., Ratner, B., Eds.; Plenum Publishing: New York, 1984; pp 17−32.
(13) Urry, D. W.; Peng, S. Q. *J. Am. Chem. Soc.* **1995**, *117*, 8478.
(14) Dill, K. A. *Biochemistry* **1990**, *29*, 7133.
(15) Urry, D. W.; Long, M. M. In *Elastin and Elastic Tissue;* Adv. Exp. Med. Biol. Vol 79; Snadberg, L. B., Gray, W. R., Franzblau, C., Eds.; Plenum: New York, 1977; pp 685−714.
(16) Urry, D. W.; Peng, S. Q.; Xu, J.; McPherson, D. T. *J. Am. Chem. Soc.* **1997**, *119*, 1161.
(17) Muller-Plathe, F. *Macromolecules* **1998**, *31*, 6721.
(18) Cook, W. J.; Einspahr, H.; Trapane, T. L.; Urry, D. W.; Bugg, C. E. *J. Am. Chem. Soc.* **1980**, *102*, 5502.
(19) Urry, D. W.; Trapane, T. L.; Sugano, H.; Prasad, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 2080.
(20) Urry, D. W.; Chang, D. K.; Krishna, N. R.; Huang, D. H.; Trapane, T. L.; Prasad, K. U. *Biopolymers* **1989**, *28*, 819.

(21) Salamone, J. C., Ed.; *Polymeric Materials Encyclopedia;* CRC Press: Boca Raton, FL, 1996; Vol. 9, p 7263.
(22) Cotterell, B.; Reddel, J. K. *Int. J. Fract.* **1977**, *13*, 267.
(23) Broberg, K. B. *J. Mech. Phys. Solids* **1971**, *19*, 407.
(24) Liivak, O.; Blye, A.; Shah, N.; Jelinski, L. W. *Macromolecules* **1998**, *31*, 2947.
(25) Seidel, A.; Liivak, O.; Jelinski, L. W. *Macromolecules* **1998**, *31*, 6733.
(26) McPherson, D. T.; Xu, J.; Urry, D. W. *Protein Expression Purif.* **1996**, *7*, 51.
(27) Urry, D. W.; Pattanaik, A.; Xu, J.; Woods, T. C.; McPherson, D. T.; Parker, T. M. *J. Biomater. Sci., Polym. Ed.* **1998**, *9*, 1015.
(28) Guda, C.; Zhang, X.; McPherson, D. T.; Xu, J.; Cherry, J. H.; Urry, D. W.; Daniell, H. *Biotechnol. Lett.* **1995**, *17*, 745.
(29) Stading, M.; Langton, M.; Hermansson, A.-M. *J. Rheol.* **1995**, *39*, 1445.
(30) Lee, J.; Macosko, C. W.; Urry, D. W. *Macromolecules* **2001**, *34*, 4114.
(31) Yannes, I. V. *J. Macromol. Sci., Phys.* **1972**, *B6*, 91.
(32) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: London, 1971.
(33) Peppas, N. A.; Merrill, E. W. *J. Polym. Sci.* **1976**, *14*, 459.
(34) Peppas, N. A.; Merrill, E. W. *J. Appl. Polym. Sci.* **1977**, *21*, 1763.
(35) Dole, M. *The Radiation Chemistry of Macromolecules*; Academic Press: New York, 1972.
(36) Moad, C. L.; Winzor, D. J. *Prog. Polym. Sci.* **1998**, *23*, 759.
(37) Turner, J. S.; Cheng, Y. *J. Membr. Sci.* **1998**, *148*, 207.
(38) Urry, D. W.; Hayes, L. C.; Gowda, D. C.; Parker, T. M. *Chem. Phys. Lett.* **1991**, *182*, 101.
(39) Urry, D. W.; Hayes, L. C.; Parker, T. M.; Harris, R. D. *Chem. Phys. Lett.* **1993**, *201*, 336.
(40) Kunugi, S.; Takano, K.; Tanaka, N.; Suwa, K.; Akashi, M. *Macromolecules* **1997**, *30*, 4499.
(41) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(42) Urry, D. W.; Trapane, T. L.; Prasad, K. U. *Biopolymers* **1985**, *24*, 2345.
(43) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
(44) Venkatachalam, C. M.; Khaled, M. A.; Sugano, H.; Urry, D. W. *J. Am. Chem. Soc.* **1981**, *103*, 2372.
(45) Venkatachalam, C. M.; Urry, D. W. *Macromolecules* **1981**, *14*, 1225.
(46) Chang, D. K.; Urry, D. W. *J. Comput. Chem.* **1989**, *10*, 850.
(47) Park, Y.; Ko, J.; Ahn, T.; Choe, S. *J. Appl. Polym. Sci.* **1997**, *66*, 807.
(48) Rivlin, R. S.; Thomas, A. G. *J. Polym. Sci.* **1972**, *10*, 291.
(49) Foegeding, E. A.; Bowland, E. L.; Hardin, C. C. *Food Hydrocolloids* **1995**, *9*, 237.
(50) Williams, J. G. *Fracture Mechanics of Polymers*; Ellis Harwood Limited: New York, 1984.

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