Swelling Behavior of γ-Irradiation Cross-Linked Elastomeric Polypentapeptide-Based Hydrogels

Jonghwi Lee,*,†,‡ Christopher W. Macosko,† and Dan W. Urry†,‡,§,⊥

Chemical Engineering and Materials Science, The University of Minnesota, Minneapolis, Minnesota 55455; Biological Process Technology Institute, The University of Minnesota, St. Paul, Minnesota 55108; and Bioelastic Res. Ltd., Suite 386, 2800 Milan Ct., Birmingham, Alabama 35211

Received September 8, 2000; Revised Manuscript Received March 20, 2001

ABSTRACT: The dynamic and equilibrium swelling behavior are reported for four γ-irradiation cross-linked elastic protein-based polymers comprised of repeating pentapeptide sequences. The elastomeric polypentapeptides, previously prepared using recombinant DNA technology, are $(GVGVP)_{251}$, $(GVGIP)_{260}$, $(GVGVP\ GVGVP\ GEGVP\ GVGVP\ GVGVP\ GVGVP)_{35}$](GVGVP), and (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP)$_{35}$](GVGVP), where G = glycine, V = valine, P = proline, I = isoleucine, F = phenyalanine, and E = glutamic acid. These polymers had been γ-irradiation cross-linked into elastic matrices using doses ranging from 6 to 34 Mrad. Fick's law was able to explain their initial dynamic swelling behavior. Thermal history was found to be a variable affecting dynamic swelling. The equilibrium swelling ratio was dependent on both temperature and pH (for the E-containing polymers) and analyzed using the Flory−Huggins interaction parameter. The interaction parameter could be related with the T_t mechanism developed for polypeptides. Different continuities found in the phase transitions of these polymers and poly(*N*-isopropylacrylamide) were discussed in terms of cooperative units.

Introduction

Protein-based polymers are defined as macromolecules having repeating peptide sequences, where the size of repeating units can vary over a wide range from two to hundreds of amino acid residues.[1] When the hydrophobicity[2] and structure of chains are carefully controlled, an interesting phase transition can be found, which is the hydrophobic folding and assembling transition. For example, if poly(GVGVP) is dissolved into water and the solution is heated above 30 °C, phase separation can be observed. This LCST (lower critical solution temperature) type phase transition has been found to mediate the energy transduction of protein-based polymers.[1] Many different types of energy could be interconverted, including mechanical, thermal, electrical, chemical, pressure−volume, and electromagnetic radiation.[1,3]

The phase transition behavior has been studied using various techniques such as DSC (differential scanning calorimetry), light and X-ray scattering, dielectric relaxation, NMR, circular dichroism, etc. [ref 1 and references therein]. From these studies, a simple and satisfactory understanding has been established. In water, a protein-based polymer such as poly(GVGVP) has a significant number of hydrophobic hydration sites. Water molecules surrounding hydrophobic moieties (waters of hydrophobic hydration) are thought to be in a more ordered structure than bulk water.[2,4−7] This relatively ordered structure of water has been variously called a "clathrate", "caged", or "cathedral" structure. When the solution is heated, the entropically unfavorable structure becomes less ordered bulk water. As a result, polymers lose hydrophobic hydration and increase contacts within and between chains. Thus, inter- and intramolecular folding and assembly follow. Input energies other than temperature changes shift the temperature at which the phase transition occurs, depending on how they affect the free energy of hydrophobic hydration. This can result in energy conversion.

When poly(GVGVP) chains are hydrophobically folded and assembled at a temperature above their phase transition temperature, it is found that they form a more organized structure. The structure has been described as containing twisted filaments of β-spirals.[8−11] Each pentamer of the β-spiral contains type II β-turns, 10-atom hydrogen-bonded rings involving the C−O of the valine preceding the proline and the NH of the subsequent valine between the two glycine residues. There are described approximately three pentamers per turn of β-spiral with the β-turns functioning as hydrophobic spacers between the turns of the helical β-spiral. Electron micrographs of negatively stained initial aggregates suggest that three β-spirals combine to form the twisted filament. Thus, the hydrophobic folding and assembly transition on raising the temperature, or the hydrophobic unfolding and disassembly on lowering the temperature, has characteristics of the hydrophobic folding of natural proteins,[12] which unfold and disassemble on lowering the temperature, a process known as cold denaturation. In fact, the amino acid sequence of GVGVP was found by Sandberg and colleagues[13,14] in tropoelastin, which is the precursor protein of mammalian elastin.[15−18]

The LCST type phase transition has been reported in aqueous solutions of a number of petroleum-based polymers. Among them, the aqueous solutions of poly(*N*-isopropylacrylamide) (PNIPAM)[19,20] and similar poly-

* To whom correspondence should be addressed. Current address: Merck Research Laboratories, Sumneytown Pike, WP78A-31, West Point, PA 19486. Fax 215-993-3730, e-mail jonghwi-lee@merck.com.
† Chemical Engineering and Materials Science, University of Minnesota.
‡ Biological Process Technology Institute, University of Minnesota.
§ Bioelastic Res. Ltd.
⊥ E-mail urryx001@tc.umn.edu. Fax 205-943-6591.

10.1021/ma0015673 CCC: $20.00
Published on Web 05/03/2001

mers, e.g., poly(*N*-vinylcaprolactam)[21−23] are worth comparison with the protein-based polymers. The phase transition temperature of PNIPAM depends on the types of cross-linkers and reactions, but it is usually 30−34 °C. This is similar to the transition temperature of poly(GVGVP), which is ca. 28−30 °C. The chemical constituents of a PNIPAM repeating unit are one amide bond, two CH, one CH_2, and two CH_3. These constituents are similar to those of a poly(GVGVP) repeating unit, i.e., five amide bonds, five CH, five CH_2, and four CH_3 in a pentamer. PNIPAM has more alkyl units per amide bond than poly(GVGVP), but because proline is an imino acid rather than an amino acid, one of the five amide bonds in poly(GVGVP) has a disubstituted amide nitrogen. Thus, their overall hydrophobicities are quite similar.

An important difference between them exists in structural characteristics. Protein-based polymers such as poly(GVGVP) have regular amino acid sequences and stereoregularities (L-amino acid residues). Above their phase transition temperature, there is the stabilization of secondary structure, a 10-atom hydrogen-bonded ring called a β-turn, as occurs on the folding of globular proteins. The phase transition of these protein-based polymers can be described in terms of an increase in order. On raising the temperature cyclic analogues reversibly crystallize, and the linear high polymers form parallel aligned filaments with a twisted filament substructure.[24] Both occur due to hydrophobic folding and assembly. Because of this increase in order on increasing the temperature of the polypeptide component of the water−polymer system, the phase transition of these protein-based polymers has been called an inverse temperature transition, and the onset temperature on raising the temperature has been designated as T_t. On the other hand, most petroleum-based polymers including PNIPAM do not show such increases in order during the phase transition. Coil-to-globule transition is a proper description for the changes in their macromolecular chains.[25,26] Thus, the LCST behavior of these polymers has been explained using various random chain models.[20,27]

Cross-linking can add elasticity to the properties of artificial protein-based polymers with the result that stimuli responsive elastic hydrogels can be prepared.[1] Recently, these hydrogels have attracted a considerable interest, not only because of their interesting phase behavior but also because of their promising applications.[28] Protein-based polymers can be precisely designed to obtain desirable energy conversion properties or even to mimic natural protein functions. They can be environmentally friendly from production to disposal, and their production cost using the recombinant DNA technology is continually decreasing due to rapid progress in biotechnology. Because of this progress, bulk characterizations of microbially prepared polymers such as the present swelling study now become possible.

In this study, previously prepared and γ-irradiation cross-linked elastomeric polypentapeptides—$(GVGVP)_{251}$, $(GVGIP)_{260}$, (GVGVP GVGVP GEGVP GVGVP GVGVP GVGVP)$_{35}$](GVGVP), and (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP)$_{35}$](GVGVP), where G = glycine, V = valine, P = proline, I = isoleucine, F = phenyalanine, and E = glutamic acid—are characterized as to the swelling properties associated with their hydrophobic folding and assembly transition. The weight swelling ratio of resulting hydrogels was measured as a function of time (dynamic swelling), temperature, and pH (equilibrium swelling), which is the ratio of the weight of fully swollen hydrogel (W_s) to that of dried gel (W_d). Because the processes involved in LCST or heat renaturation (cold denaturation),[2] or heat-driven crystallization, or heat-driven fiber assembly all involve hydrophobic association, a general terminology will be used in this study. The temperature for the onset of the hydrophobic folding and assembly transition will be designated T_t, and the inverse temperature transition itself will be called the "T_t transition", consistent with the previous work on these protein-based polymers.[1]

Experimental Section

Materials. The elastic protein-based polymers and their cross-linked matrices were obtained from Bioelastics Research, Ltd., as part of a coordinated research effort funded by the Office of Naval Research. Briefly, experimental details on the biosynthesis and purification of protein-based polymers can be found elsewhere in details.[29,30] Following the same technique, protein-based polymers were expressed from recombinant DNA in *Escherichia coli*. In the subsequent purification after biosynthesis, the phase separation property of the T_t transition was used.[30] After purification, polymers were lyophilized. MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry and H NMR were used to confirm the synthesis following the same experimental methods in ref 31. The molecular weights before cross-linking of $(GVGVP)_{251}$, $(GVGIP)_{260}$, (GVGVP GVGVP GEGVP GVGVP GVGVP GVGVP)$_{35}$] (GVGVP), and (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP)$_{35}$](GVGVP) are 102, 109, 88, and 88 kg/mol, respectively.

The initial product of gene expression can be a singular molecular weight. However, degradation by microbes and enzymes occurs. Thus, the decrease of molecular weight with broadening of polydispersity could become significant with time. In this experiment, all linear polymers were stored in dried states and hydrogels at 5 °C. Before each measurement, the tensile modulus or equilibrium weight swelling ratio (Q_{we}) of hydrogels below T_t was checked and compared with their initial values measured after preparation, to make sure that the degradation effect was insignificant.

Water used in this experiment was the deionized ultrafiltered water from the Fisher Scientific (specific conductance at 25 °C = 2.0 μmhos/cm). For the preparation of phosphate buffer solution (PBS), monobasic monohydrate sodium phosphate (enzyme grade) was purchased from the Fisher Scientific and dibasic heptahydrate sodium phosphate from ICN Biomedicals Inc. To prepare the buffers of pH below 4 and above 8, a small amount of NaOH (5 mM) or HCl (5 mM) aqueous solution was added.

Preparation of Hydrogels. γ-Irradiation cross-linking used the same method as described elsewhere in detail.[32] Polymer solution was first prepared and heated above T_t. The resulting dense polymer phase was separated by centrifugation and cross-linked in a mold at 23 °C by γ-irradiation (intensity = ca. 0.3 Mrad/h). Before characterization, hydrogels were cleaned in a shaking water bath for more than 2 weeks with changing water at every 24 h.

Protein-based hydrogels prepared are listed in Table 1 with their designations and effective cross-link densities as determined in the present report. Reaction conditions, which specify the type of samples, will follow the designations, e.g., γ-V-20 Mrad.

Characterizations. For dynamic weight swelling ratio measurement, hydrogels of ca. 100 mm^2 × 0.7 mm (above T_t) were first equilibrated in water at a temperature for 3 days and dried under vacuum at the same temperature for more than a week. Then, they were suspended by a copper wire (diameter = 0.8 mm) vertically attached to a Sartorius B120S balance (standard deviation ≤ ±0.1 mg, mounted on a shock-absorbing table (LKB Ultrastable 8870)). After the immersion

Table 1. Designations and Cross-Link Densities of Polymers

Designations; Amino Acid Sequence								
γ-V; $(GVGVP)_{251}$								
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-V(E); $(GVGVP\ GVGVP\ GEGVP\ GVGVP\ GVGVP\ GVGVP)_n\ GVGVP$								
Dose of γ-Irradiation (Mrad)	20							
Effective Cross-link Density (mol/m^3)	10.5							
γ-V(E5F); $(GVGVP\ GVGFP\ GEGFP\ GVGVP\ GVGFP\ GFGFP)_n\ GVGVP$								
Dose of γ-Irradiation (Mrad)			14		26		30	34
Effective Cross-link Density (mol/m^3)			4.2		19.2		32.3	55.8
γ-I; $(GVGIP)_{260}$								
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		

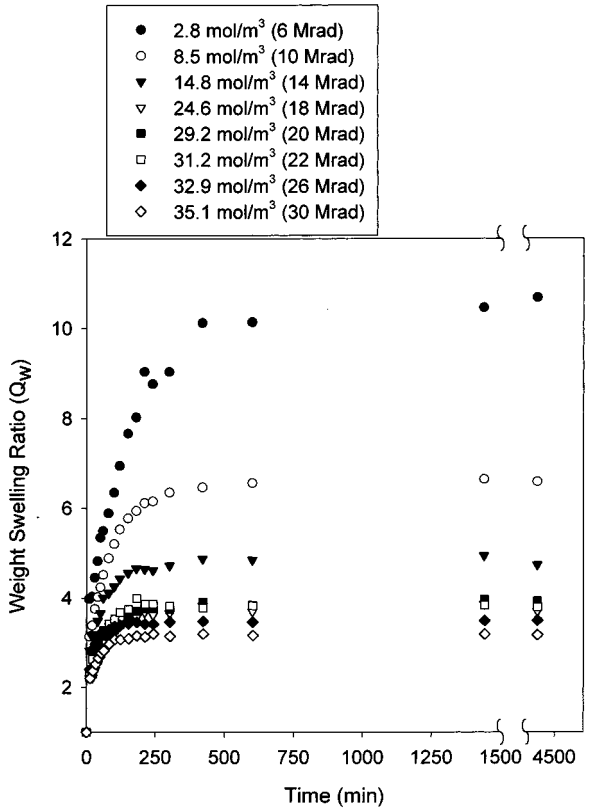

Figure 1. Dynamic swelling of γ-V in water at 21 °C. The polymers dried at 21 °C were immersed in water, and their weight swelling ratio was measured as a function of time.

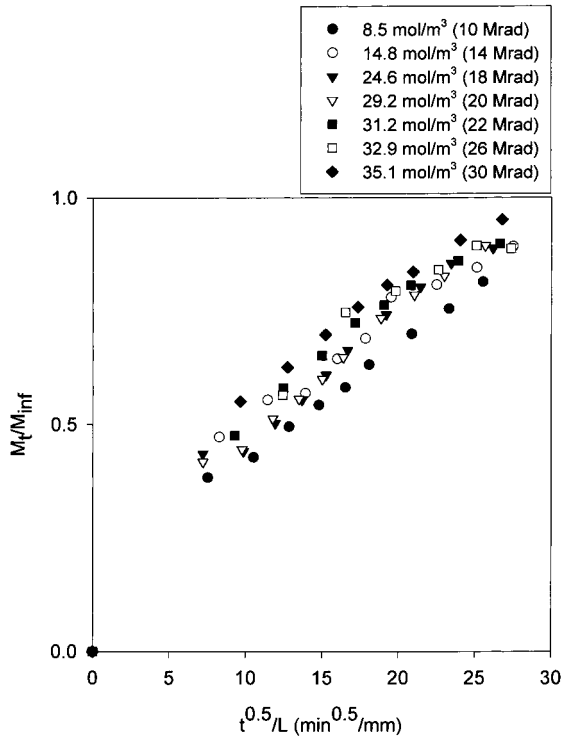

Figure 2. Applicability of Fick's law to the dynamic swelling of γ-V in water at 21 °C.

of the specimens into a water bath, their weight swelling ratio (Q_w) was measured by temporarily removing the water bath.

Thin disk specimens of the same size as used in the above were used for the measurements of equilibrium weight swelling ratio ($Q_{we} = W_s/W_d$) as a function of pH. They were first immersed in PBS at 22 °C for more than 24 h, and their W_s was measured. W_d was measured after drying of more than 1 week under vacuum at room temperature. Compared to the amount of absorbed water in swollen states, the amount of residual water after this drying was found to be insignificant. This was confirmed by a comparison between the dried and lyophilized specimens.

Q_{we} was also measured with changing temperature. Samples of the same size were first immersed in 40 mL water bottles, and the bottles were preconditioned in a temperature-controlled water bath (Neslab endocal refrigerated circulating bath, RTE-5DD) at 1 or 2 °C for more than 2 days. Then, the samples were heated at 2 °C step per 12 h, and their weight was measured after every 12 h of equilibration time. This weight measurement was usually done in 15 s, and water loss during this time lapse was found to be insignificant. This is a

reasonable observation because the diffusion coefficient (D) of our hydrogels was found to be on the order of 10^{-8} cm^2/s (Figure 2). Time for a certain length change, L, is roughly L^2/D.[33,34] Thus, the length change during 15 s is only about 0.4 μm.

Optical measurement of specimen dimension in a water bath can give the same information as Q_{we} data, since $L_s/L_d \approx (W_s/W_d)^{1/3}$, where L_s and L_d are the dimensions (e.g., length) of swollen and dried hydrogels, respectively. The optical measurement was tried using a 35 mm camera, but its results were not better than those of the direct weight measurement. This is mainly because of the similar refractive index of our hydrogels to that of water. Additionally, the small inherent curvature of "slab" specimen was found to change with temperature, producing a significant error in the measurement of 2-D images.

Results

The effective cross-link density, ν, in Table 1 was obtained under the assumptions of the rubber elasticity theory of random Gaussian chain network. Uniaxial tensile test data[35] below T_t were used for the following equation.[36−38]

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

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.

Dynamic Swelling Measurement. Dried protein-based hydrogels are allowed to absorb water at 21 °C, and the change of their Q_w is shown in Figure 1. In the initial regime, they rapidly absorb water, and then their absorbing rates level off. After ca. 400 min, the swelling ratios reach their plateau values, i.e., Q_{we}. Similar swelling behavior to Figure 1 has been seen in other hydrogels,[39−41] too. This dynamic swelling result is the basis for the equilibration time of 12 h in the Q_{we} experiment in the next section. In the Q_{we} experiment, hydrogels did not start swelling from their dried states, and so equilibrium swelling will be reached earlier than in the experiment of Figure 1. Thus, the 12 h seems to be ample time.

As the dose of γ-irradiation increases, a higher swelling ratio results from the initial fast swelling stage. The initial swelling region needs further analyses. In particular, it is useful to know whether the diffusion is Fickean or not. Fick's law predicts that flux, j_x, linearly depends on concentration gradient, $\Delta c/\Delta x$; $j_x = -D\Delta c/\Delta x$, where D is the diffusion coefficient.[41] The weight of water, M_t, absorbed by a membrane at a time t is[41]

$$\frac{M_t}{M_{inf}} = 8\left(\frac{D}{\pi}\right)^{1/2}\left(\frac{t^{1/2}}{L}\right) \qquad (2)$$

where M_{inf} is the weight of equilibrium water uptakes and L the thickness of samples.

Figure 2 has the plot of M_t/M_{inf} vs $t^{1/2}/L$ for initial swelling stages. First of all, a fairly linear relationship can be found between them. Thus, Q_w in the initial swelling stages can be predicted using Fick's law. In the plot, the slopes of curves indicate the magnitude of D, which is calculated to be around 10^{-8} cm^2/s. The accuracy of this experiment may not be adequate to study the effect of cross-link density on D. However, this plot shows that the effect may not be significant. It appears reasonable because the cross-link density of our hydrogels is rather small, as can be seen in Table 1. The most significant error in this experiment comes from water on the surface of hydrogels, which is difficult to control. It causes that the M_t/M_{inf} data in Figure 2 does not approach zero swelling ratio at $t = 0$. It can shift M_t/M_{inf} data along the y-axis.

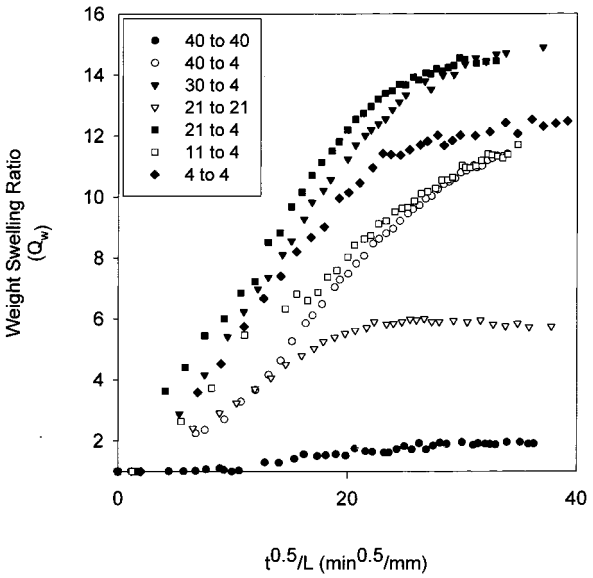

Figure 3. Dynamic swelling of γ-V-14 Mrad in water at different temperatures: e.g., the "30 to 4" indicates that the hydrogel was dried at 30 °C, and then its dynamic swelling ratio was measured at 4 °C.

Complicated swelling behavior caused by thermal history was found in this experiment. If a hydrogel was dried at a temperature, which is different from that of the measurement, an interesting overshoot behavior can be found in dynamic swelling curves. Figure 3 shows typical curves. The samples for three curves, "40 to 40", "21 to 21", and "4 to 4", did not have any significant temperature change in their thermal history. They all have the typical swelling behavior, which was seen in Figures 1 and 2. When hydrogels were dried at 40 and 11 °C ("40 to 4" and "11 to 4"), which are far from their T_t (ca. 28−30 °C), a smooth transition can be found in dynamic swelling curves. The curves are between the "4 to 4" curve and the "21 to 21" or "40 to 40" curves.

When hydrogels were dried at 30 and 21 °C ("30 to 4" and "21 to 4"), an overshoot can be found in the swelling curves. The two swelling curves gradually overlap with the "4 to 4" curve at $t^{0.5}/L > 40$. After swelling for more than 2 days, the swelling ratios of all hydrogels became the same, regardless of thermal history. Thus, it is the case in this experiment that thermal shock should be avoided during sample preparation for the dynamic swelling study. The swelling overshoot phenomenon might occur due to the density fluctuation caused by the T_t transition.[42,43] The nonequilibrium structure generated by the density fluctuation might be partially stored by drying and later induce excess swelling in the initial stage of dynamic swelling. The coarse parts of the nonequilibrium structure will be the main source for the excess swelling, because of their low friction with water.[39] Another possible clue to understand the phenomenon might be found in the previous NMR study on poly(GVGVP).[44] The study shows that the carbonyl mobility of amide bonds reaches a maximum at 20−30 °C.[44] The overshooting phenomenon is similar to what Peppas et al. observed,[45] although it is not clear whether they had the same cause or not.

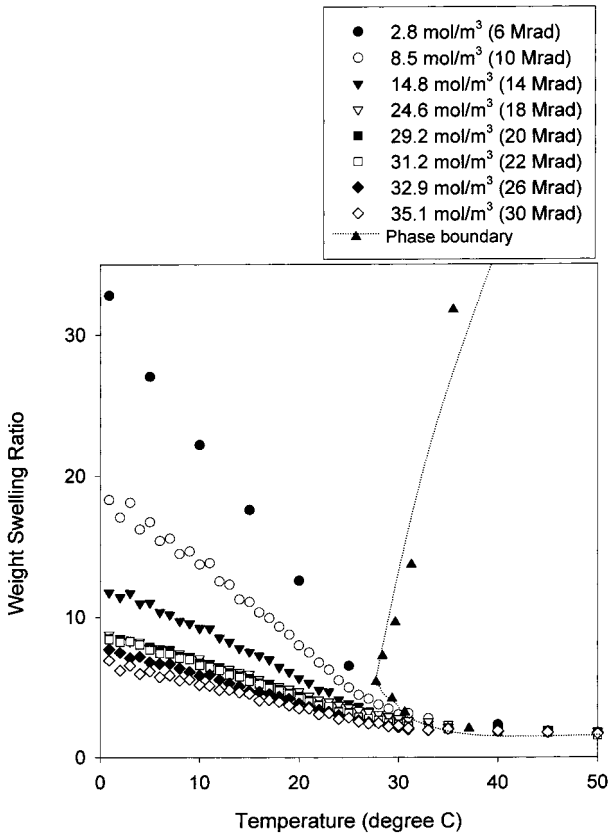

Figure 4. Equilibrium weight swelling ratio of γ-V as a function of temperature for a series of γ-irradiation cross-linking doses ranging from 6 to 30 Mrad. The phase boundary was taken from ref 47.

Equilibrium Weight Swelling Ratio (Q_{we}). Figure 4 shows the Q_{we} of γ-V as a function of temperature. The data at a temperature of even number were taken during heating and the others during cooling. The two sets of data appear to be generally consistent with each other, although there are small differences between them. (The error range of the data can be estimated.) Thus, the T_t transition in this case is reversible. It is also continuous, such as a second-order phase transition. These results are similar to those of an initial study[46] on 20 Mrad cross-linked poly(GVGVP) and bovin ligamentum nuchae elastin. The continuous phase transition is in contrast to the discontinuous or at least sharper phase transitions exhibited by PNIPAM.[20]

Below T_t (ca. 30 °C), as cross-link density increases from 2.8 to 35.1 mol/m^3, Q_{we} decreases (Figure 4). However, the effect of cross-link density becomes smaller with an increase in temperature. Above T_t, there is no significant difference among the swelling ratios of hydrogels. At about 30 °C, all hydrogels reach the swollen state of $Q_{we} \approx 2$. This temperature agrees well with the T_t of un-cross-linked linear polymers. For comparison, the phase boundary for aqueous solutions of linear poly(GVGVP),[47−49] obtained from dynamic light scattering, is plotted in Figure 4. This is a spinodal line determined by the extrapolation procedure of the scattering method based on the Ornstein−Zernicke−Debye theory.[47] All the swelling curves of hydrogels start to overlap with the phase boundary curve at ca. 30 °C. Thus, we conclude that the phase transition of the hydrogels cross-linked by different doses of γ-irradiation is basically the same phenomenon as that of linear polypeptides. Changes in γ-irradiation dose do not appear to affect significantly the transition temperature of ca. 30 °C.

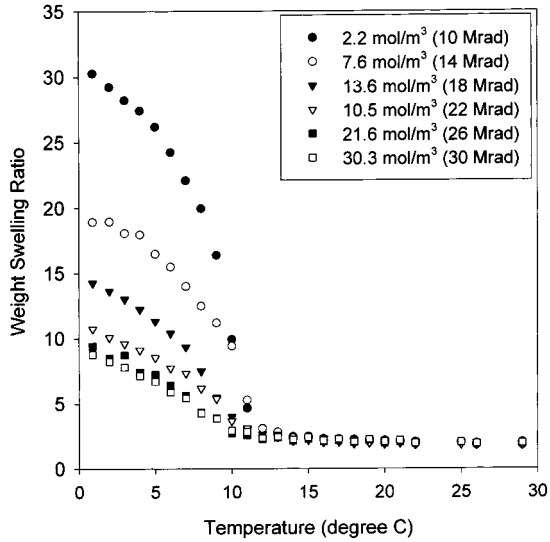

Figure 5. Equilibrium weight swelling ratio of γ-I as a function of temperature for a series of γ-irradiation cross-linking doses ranging from 10 to 30 Mrad.

Similar observations can be made in the swelling behavior of γ-I (Figure 5), where a continuous and reversible phase transition can also be found. The T_t transition temperature is about 10 °C. There is about a 2 °C difference between the T_t of the 2.2 and 7.6 mol/m^3 samples with that of the others. However, it is unclear whether this difference is significant. Cross-link density affects Q_{we} below T_t, but not above T_t. The difference between Figures 4 and 5 is the slope of swelling curves. While Q_{we} decreases almost linearly with temperature in the case of γ-V, it decreases more rapidly in the other case. Because of the existence of isoleucine, γ-I is more hydrophobic than γ-V, as can be indicated by its lower T_t. Thus, the increased hydrophobicity may increase the cooperativity of the transition,[1] resulting in more rapid changes in Q_{we} curves.

The temperature dependence of Q_{we} is not the only stimuli responsive behavior of our protein-based hydrogels. If a glutamic acid residue (E) replaces a valine residue every 30th residue of poly(GVGVP) backbone (γ-V(E) in Table 1), a strong pH dependence of Q_{we} can be found (Figure 6). A continuous but sharp volume phase transition can be found in γ-V(E)-20 Mrad at around pH = 5.5. This corresponds to the pK_a of carboxylic acids (γ-CO_2H) of glutamic acids. In addition to a glutamic acid residue, γ-V(E5F) has five phenylalanine residues (F) in each 30-residue repeating unit. The pK_a of γ-CO_2H is increased by the hydrophobic F residues, to approximately 6.5 in Figure 6, but also appears to increase at the higher cross-linking doses of 30 and 34 Mrad. This influence of hydrophobicity on pK_a has been described as a competition between hydrophobic and charged residues and labeled an apolar−polar repulsive free energy of hydration.[1]

On the other hand, it is obvious in Figure 6 that the swelling ratios of γ-V and γ-I do not depend on pH in a range of pH = 2.5−8.5. It is reasonable because both polypeptides do not have any ionizable groups on their side chains. γ-Irradiation does not seem to produce any significant number of functional groups either. A glutamic acid residue content of only 3.4 mol % such as in γ-V(E) and γ-V(E5F) causes the strong pH dependence exhibited by these polypeptides.

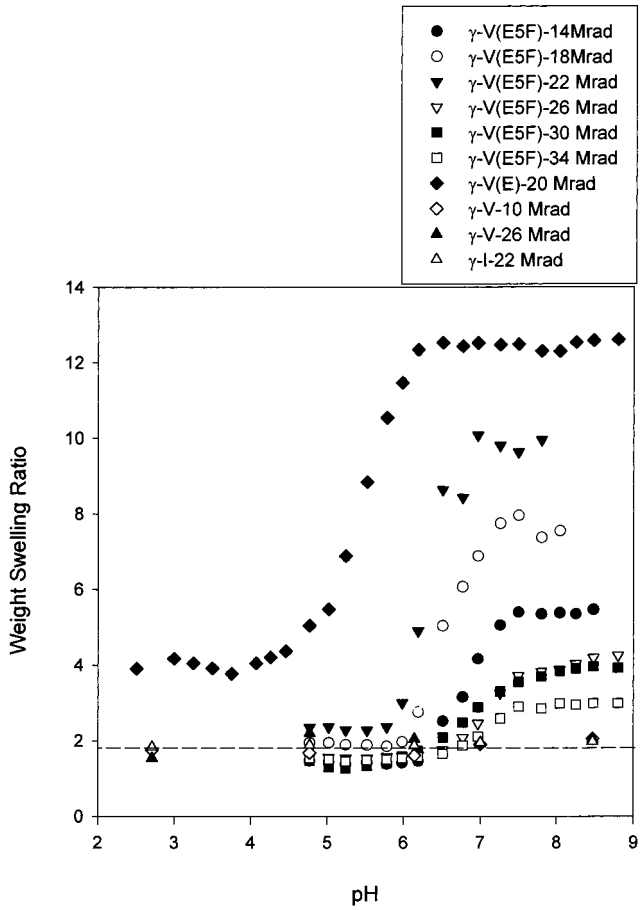

Figure 6. Equilibrium weight swelling ratio of γ-V(E5F), γ-V(E), γ-V, and γ-I as a function of pH at room temperature. The data of γ-V and γ-I at five different pH values lie along the dashed line. The data on the E-containing polymers are limited due to their tendency to become contaminated and biodegrade.

An interesting difference between the phase transitions driven by pH and temperature changes is that while γ-V and γ-I hydrogels exhibit the same swelling ratio in their collapsed states above T_t, γ-V(E5F) hydrogels have different swelling ratios. Figure 6 shows that the Q_{we} of collapsed states depends on the dose of γ-irradiation.

In the Q_{we} data of γ-V(E5F), the effect of γ-irradiation dose is not straightforward. As the dose increases, Q_{we} initially increases and then decreases. Cross-link density is expected to follow the trends of Q_{we}. This is distinctly different from the trends in γ-V and γ-I (Figures 4 and 5). It might be caused by the different competition between cross-linking and chain scission kinetics.[50,51] Because of the phenylalanine and glutamic acid residues, this could be a possible reason. However, it was found that the degradation rates of γ-V(E5F) and γ-V(E) hydrogels were significantly faster than those of the others. Thus, the data in Figure 6 might be affected by the effect of biodegradation, even though they were measured within about 2 months after their preparation. Accordingly, while of interest and notable, the relationship between γ-irradiation dose and Q_{we} for γ-V(E5F) and γ-V(E) hydrogels must be considered to be qualitative at best.

The hydrogels exhibiting a pH dependence also showed temperature responsive behavior as can be seen in Figure 7. Q_{we} decreases with temperature and reaches

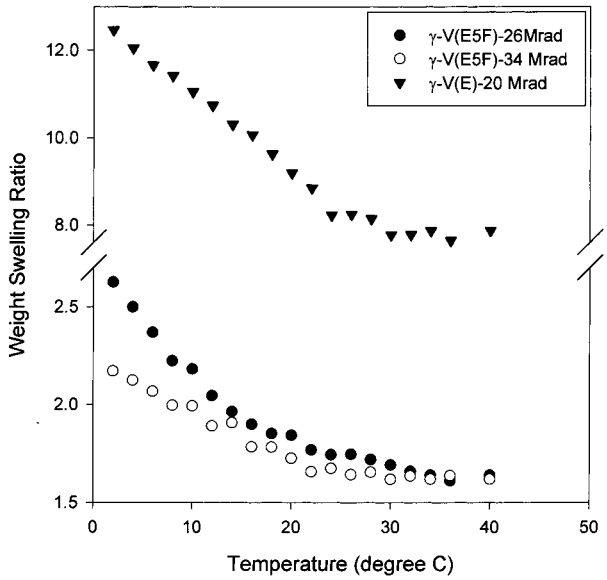

Figure 7. Equilibrium weight swelling ratio of γ-V(E5F) and γ-V(E) as a function of temperature. The γ-V(E) is in a PBS solution of pH = 5.5 and the others in pH = 6.5.

plateau values above ca. 25 °C (T_t). Since the Q_{we} of the hydrogels depends on both temperature and pH, Q_{we} above T_t is not always ca. 2 like the cases in Figures 4 and 5 but varies depending on the pH of medium. The Q_{we} data of 26 and 34 Mrad hydrogels cannot be compared in here, because of the same degradation problem. The data of Figures 6 and 7 will be excluded in the following Discussion section. However, despite the problem, Figures 6 and 7 have been included as they demonstrate the general phase transition behavior found in protein-based hydrogels having ionizable functional groups.

Discussion

The equilibrium swelling of polymer networks is determined by the competition of three free energy terms, free energy of mixing (ΔG_m), rubber elasticity (ΔG_r), and ionizable group's osmotic pressure terms (ΔG_i).

$$\Delta G = \Delta G_m + \Delta G_r + \Delta G_i \tag{3}$$

Thus, water diffusion into a polymer network driven by the free energy of mixing term will be counterbalanced by the other two terms. The simplest approach for equilibrium swelling is based on the Flory−Rehner equation.[36] Using the mean-field approximation, the Gibbs free energy of hydrogels of random Gaussian chain networks is

$$\Delta G = kT[n \ln(1-\varphi) + \chi n\varphi] + \frac{3\nu kT}{2}(\alpha^2 - 1 - \ln\alpha) - \nu fkT \ln\left(\frac{V_0\alpha^3}{n\nu_1}\right) \tag{4}$$

where n is the number of solvent molecules in the gel, χ the polymer−solvent interaction parameter, V_0 the volume of the gel when its network has a random walk configuration, v_1 the molar volume of the solvent, k the Boltzmann constant, N the Avogadro number, and f the number of counterions per chain. For nonionizable hydrogels such as γ-V and γ-I, the last term can be

neglected ($f = 0$). Under conditions of equilibrium, the total osmotic pressure should be zero as follows,

$$-\frac{N}{v_1}\left(\frac{\partial \Delta G}{\partial n_1}\right)_{T,p} = 0 = -\frac{NkT}{v_1}[\varphi + \ln(1-\varphi) + \chi\varphi^2] + \nu kT\left[\frac{\varphi}{2\varphi_0} - \left(\frac{\varphi}{\varphi_0}\right)^{1/3}\right] \quad (5)$$

where φ_0 is the reference state of zero rubber elastic force. Thus, once we know the interaction parameter, χ, and effective cross-link density, ν, an equilibrium swelling ratio can be determined.

For applying the equations above to our protein-based hydrogels, one caution should be taken into account. Unlike the LCST type phase separation of conventional petroleum-based polymers, the phase transition exhibited by these elastic protein-based polymers is a hydrophobic folding and assembling process having structural transition to increased order. As temperature increases above T_t, polypeptide chains become more regularly structured. For polypeptide chains of regular structures, the assumption of random Gaussian chain networks seems to be an oversimplification. Analysis on swelling behavior and mechanical performance *above* T_t needs to consider the structural characteristics of the polypeptides. The proposed peptide librational motion[52−54] as the major source of entropic elasticity is an example. In an extreme case, there is the folding phenomenon of natural globular proteins having more complicated amino acid sequences, which would be far outside of the scope of the approach using eqs 4 and 5. The physical insights obtained from such an analysis *above* T_t would be very limited.

Fortunately, below the T_t of our hydrogels, the macromolecular chains are thought to have more random conformations, and so the approach based on random chain network may be applicable. Thus, the simple description of swelling will be applied to the swelling data of protein-based hydrogels below T_t.

To compare the hydrophobicities of protein-based hydrogels and the amounts of hydrophobic hydration, a T_t-based hydrophobicity scale has been developed.[1] By simply comparing T_t transition temperatures of polypeptides, their hydrophobicity and related phenomena can be qualitatively predicted. For the substitution of 20 amino acids and their modified forms, the T_t temperature of poly(GXGVP) is experimentally determined by extrapolation to $f_x = 1$ of the data for poly(f_v(GVGVP)-f_x(GXGVP)), where X is the substituted or guest amino acid residue; f_v and f_x are the mole fractions for the specific composition for which T_t is determined.[1,55]

The value of T_t appears to be related to the interaction parameter in eqs 4 and 5. In the free energy of mixing term, the interaction parameter is a key factor remaining after the combinatorial terms are separated. Thus, the correlation between T_t temperature and the interaction parameter (χ_{sp}) predicted from the group contribution approach of solubility parameter components[56,57] was tried. An almost linear relationship between T_t and the interaction parameter, χ_{sp}, was found when X is a neutral (nonionized) amino acid. The result suggests that the interaction parameter may be useful in understanding the hydrophobic folding and assembling transition. However, when the value of T_t and χ_{sp} for all 31 different substituents reported in ref 55 were plotted, no systematic relationship was found. It is known that

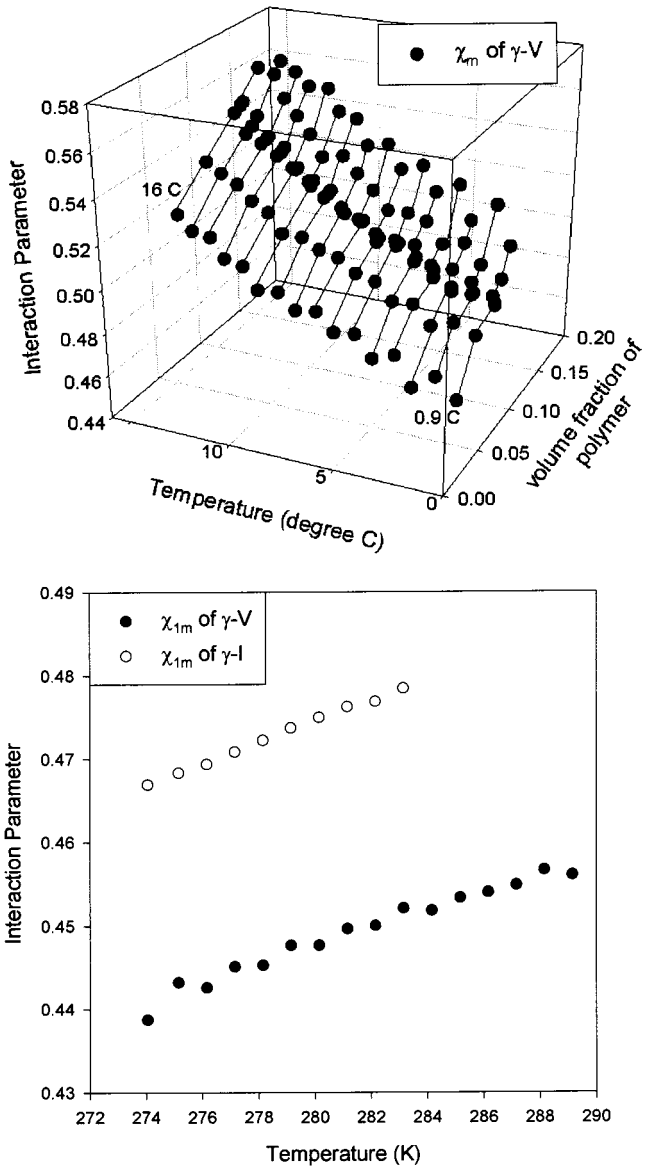

Figure 8. Interaction parameters of γ-V and γ-I as a function of temperature and volume fraction of polymer. The χ_m and χ_{1m} are obtained from uniaxial tensile test data.

the polar and hydrogen-bonding interactions are too complicated to obey simply additive rules such as the group contribution.

Departing from the original Flory treatment,[36] the interaction parameter, χ, is more likely to be an empirical parameter left after the separation of the combinatorial parts. It depends on temperature and also on composition of the hydrogels. The same dependences could be found in experimentally determined values in Figure 8. They were obtained by inserting the cross-link density data of Table 1 and the Q_{we} data into eq 5 (χ_m). Another set of χ data was obtained from the curve fitting of Q_{we} data. Its interaction parameter will be designated χ_{sw}. Except that χ_m is found to be ca. 0.02 larger, the two sets of data basically agree with each other. In both calculations, φ_0 was assumed to be 0.5 (collapsed states in Figures 4 and 5).[58]

Figure 8 shows that significant temperature and composition dependences of χ_m: As temperature or volume fraction of the polymer increases, the interaction parameter also increases. The data of the first row (a series of small polymer volume fractions (ca. 0.05−0.08))

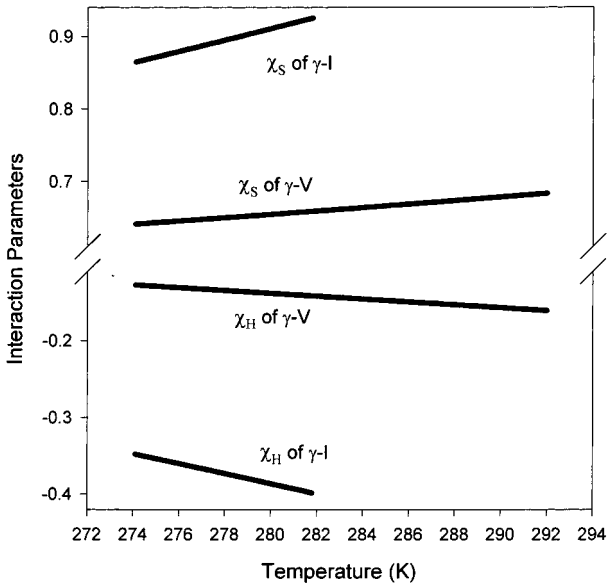

Figure 9. Enthalpic (χ_H) and entropic (χ_S) contributions of χ_m of γ-V and γ-I are plotted as a function of temperature.

is for γ-V-10 Mrad. From this row, as polymer volume fraction increases, the dose of γ-irradiation increases from 10 to 30 Mrad. Thus, the volume fraction dependence in this figure may be just radiation dose dependent. However, no significant radiation dose dependence of T_t found in the previous section suggests that the effect of volume fraction will dominate that of radiation dose on χ_m.

The composition dependence of χ_m in Figure 8 has quite complicated aspects to analyze. It also includes the composition dependence of rubber elasticity, since the rubber elasticity term in eq 5 does not consider possible transition from phantom to affine deformations with a change in polymer volume fraction.[59] Thus, the increase of interaction parameter with an increase in polymer volume fraction might be reduced if the contribution of the rubber elasticity term is subtracted.

Fortunately, instead of composition dependence, the temperature dependence of χ_m is our major interest. The composition dependence can be considered as follows[60]

$$\chi = \chi_1 + \varphi\chi_2 + \varphi^2\chi_3 + \varphi^3\chi_4 \ldots \quad (6)$$

where χ_1, χ_2, ... are functions of temperature. In this study, only the first two terms are considered, and χ_2 is assumed to be independent of temperature. Although this empirical approach appears too simplistic, its result turns out to be of interest. The composition dependence can be separated using the χ_2 term on leaving the volume fraction dependence of χ_1 insignificant in both χ_m and χ_{sw} data. Figure 8 shows the χ_1 term of the χ_m data (χ_{1m}). (The $\chi_{1,sw}$ plot showed the same results as χ_{1m}.)

An interesting analysis can be tried when the interaction parameter, χ_m, in Figure 8 is divided into enthalpic (χ_H) and entropic (χ_S) contributions as follows:[36,62]

$$\chi = \chi_H + \chi_S, \quad \chi_H = -T(\mathrm{d}\chi/\mathrm{d}T), \quad \chi_S = \chi + T(\mathrm{d}\chi/\mathrm{d}T) = \mathrm{d}(\chi T)/\mathrm{d}T \quad (7)$$

The results are given in Figure 9, which were obtained using the polynomial series fit of χ_m. While χ_S is found to increase, χ_H decreases with temperature. The actual partial molar enthalpy of dilution ($\bar{\Delta}H_1$, J mol^{-1}) and partial molar entropy of dilution ($\bar{\Delta}S_1$, J K^{-1} mol^{-1}) are related with χ_H and χ_S:[36]

$$\chi_H = \frac{\bar{\Delta}H_1}{RT\varphi^2}, \quad \chi_S = 0.50 - \frac{\bar{\Delta}S_1}{R\varphi} \quad (8)$$

In our systems, both $\bar{\Delta}H_1$ and $\bar{\Delta}S_1$ are negative, since χ_S is >0.5 and χ_H is negative. Although the enthalpy term on dilution in water decreases with the increase of temperature, the swelling ratio of our hydrogels is found to become smaller. This is because of the negative entropy effect. The negative entropy effect in this analysis is consistent with the hydrophobic hydration water explanation.[1] As temperature increases, the number of waters of hydrophobic hydration (bound water) around the hydrophobic moiety of polypeptides should be reduced, because it is entropically less favorable. Similar observations have been reported for petroleum-based polymer systems.[62,63]

Up to now, the analysis of interaction parameter obtained from eq 5 has been the focus. It gives successful linkage to a fundamental mechanism for protein function, i.e., the T_t mechanism.[1] Yet, it still lacks the capacity to predict interaction parameters as a function of temperature. If it were possible, even above T_t, our understanding could move significantly forward. The equilibrium swelling behavior of PNIPAM was successfully predicted using the modified double-lattice model and proposed universal constants.[64] The model was based on the double-lattice model of Hu et al.,[65,66] which was developed using Freed et al.'s exact solution of the Flory−Huggins lattice model. To predict the equilibrium swelling behavior of our protein-based hydrogels, we try to use the same model with the universal constants. However, the prediction we obtained was rather poor. Without support from the independent measurement of parameters, using the modified double-lattice model with the universal constants may be an oversimplification for our materials.

Cooperativity of T_t Transition. As mentioned above, the solution behavior of PNIPAM is similar to that of protein-based hydrogels used in this experiment. However, there is a distinct difference between them. It is the continuity of phase transition. The equilibrium swelling curve of PNIPAM usually shows a nonlinear and sharp phase transition occurring over a 3−5 °C range.[19,20] Sometimes, discontinuous transitions can be found, too. On the other hand, all the swelling curves of our protein-based hydrogels show almost linear and broad phase transitions (Figures 4, 5, and 7).

For the analysis of this difference, a "cooperative unit" idea can be useful.[67] A cooperative unit is thought to have its phase transition at the same time as an "all-or-not" process.[67] A macromolecular chain can be considered to consist of these cooperative units. Then, it can be assumed that a phase transition involves equilibrium of two states of cooperative units, where the equilibrium constant $K = \theta/(1 - \theta)$ and θ is the number of moles of cooperative units in one of the two states.[67,68] The heat absorbed by the conversion of 1 mol of cooperative units, ΔH_{unit}, can be related to the specific heat capacity of polymers, $C_p(T)$, and the heat of transition for 1 g of polymer, Q_{total}, as follows:[67]

$$\frac{\mathrm{d}\ln K}{\mathrm{d}T} \equiv \frac{\mathrm{d}H_{\mathrm{unit}}}{RT^2} = \frac{1}{\theta(1-\theta)}\frac{\mathrm{d}\theta}{\mathrm{d}T},$$

$$\Delta H_{\mathrm{unit}} = \frac{RT^2}{\theta(1-\theta)}\frac{C_p(T_{\mathrm{m}})}{Q_{\mathrm{total}}} \quad (9)$$

ΔH_{unit} can be obtained as follows. Since θ is $^1/_2$ in the middle of the transition,

$$\Delta H_{\mathrm{unit}} = 4RT^2\frac{C_p(T_{\mathrm{m}})}{Q_{\mathrm{total}}} \approx 4RT^2\frac{1}{\Delta T} \quad (10)$$

An effective number of cooperative units per polymer molecule can be obtained by the ratio of $\Delta H_{\mathrm{whole}}/\Delta H_{\mathrm{unit}}$, where $\Delta H_{\mathrm{whole}}$ is the enthalpy for the conversion of 1 mol of polymer chains. $\Delta H_{\mathrm{whole}}$ can be measured from the area under the absorption peak, i.e., $\Delta H_{\mathrm{whole}} = MQ_{\mathrm{total}}$, where M is molecular weight.

In the microcalorimeteric studies on poly(GVGVP), the temperature interval (ΔT) is ca. 18 °C.[69] ΔH_{unit} is calculated to be 40 kcal/mol, and $\Delta H_{\mathrm{whole}}$ is 270 kcal/mol.[69] Thus, the cooperative unit of poly(GVGVP) molecules is ca. 36 pentamers (15 kg/mol). The cooperative unit of PNIPAM has been reported to be ca. 60 kg/mol. This is 4 times larger than that of poly(GVGVP). This is an expected result since PNIPAM exhibits a narrower temperature range for its phase transition.[67,68,70] Furthermore, compared to that of PNIPAM, $\Delta H_{\mathrm{whole}}$ of poly(GVGVP) is smaller as well. From these results, the difference in continuity becomes apparent when viewed in terms of the cooperative unit.

The size of a cooperative unit can be affected by chain rigidity,[58] broadness of molecular weight between cross-links,[71] specific interactions such as hydrogen bonding,[72] etc. Poly(GVGVP) chains would appear to be more rigid than the PNIPAM chain, because of the polypeptide backbone. If this were the case, poly(GVGVP) would have more discontinuous transitions (longer cooperative unit). Obviously, it is not true in reality. However, the precise comparison of their rigidities (persistence lengths) is not currently available.

Since different cross-linking methods do not produce any significant changes in continuity in our studies,[35] the molecular weight distribution argument might be improper. Furthermore, there is no reason why our condensation and radical cross-linking methods should produce much broader polydispersity than the radical cross-linking of PNIPAM.

In the two polymers, the positions of amide bonds are different. Thus, the different amounts of hydrogen bonding between polymers and water molecules may result. The regular structure of poly(GVGVP) can make this difference more significant. The existence of proline will also reduce the number of possible hydrogen bonds as would the existence of the 10-atom hydrogen-bonded ring of the β-turn, which while stabilized above T_{t} is still present below T_{t}.[73] Thus, the phase transition of PNIPAM could have more contributions from hydrogen bonding, resulting in a sharper phase transition.

In fact, the amount of water of hydrophobic hydration for PNIPAM has been reported to be ca. 1.4 and 0.2 g/g (water molecules/dried polymers) at below and above the T_{t} transition, respectively. These values were obtained from both an experiment and a theoretical calculation using the extended lattice-fluid hydrogen-bond theory.[74] On the other hand, those of poly(GVGVP) were reported to be ca. 4.3 and 0.9 g/g, respectively.[1] In the case of poly(GVGIP), the contents were 10.7 and 0.4 g/g.[1] Thus, more hydrophobic hydration and less hydrogen bonding appears to be the case for our polypeptides. However, the answer is still unclear, since more hydrophobic hydration was found to increase the discontinuity in the study on poly(*N*-cyclopropylacrylamide), poly(*N*-isopropylacrylamide), and poly(*N*-*n*-propylacrylamide).[75] More hydrophobic hydration was also found to increase the cooperativity of T_{t} transition of protein-based polymers.[1]

Conclusions

The dynamic and equilibrium swelling ratio study revealed essential features of protein-based hydrogels. The initial dynamic swelling of protein-based hydrogels was found to follow Fick's law. Depending on their thermal history, hydrogels exhibited an overshoot phenomena in their dynamic swelling curves. Continuous and reversible phase transitions observed in equilibrium swelling ratio data as a function of temperature were explained in terms of the interaction parameter. It was found to follow the increase of temperature. The entropic component, χ_{S}, increased and the enthalpic component, χ_{H}, decreased with an increase in temperature. This was explained in connection with the effect of hydrophobic hydration. As expected, γ-I was shown to have higher interaction parameters than γ-V, indicating its more hydrophobic nature.

The phase transition of protein-based polymers was compared with that of PNIPAM; the former was more continuous than the latter. The cooperative unit was found to be smaller in protein-based polymers. In addition to the temperature dependence of equilibrium swelling ratio, pH dependence was also observed for carboxylate containing polymers, which imparted different characteristics to the phase transition.

Acknowledgment. The authors acknowledge the support of the Office of Naval Research (ONR) through N0001498-1-0656 and also Bioelastic Research Laboratory, BPTI, and Prof. F. Prochazka.

References and Notes

(1) Urry, D. W. *J. Phys. Chem. B* **1997**, *101*, 11007.
(2) Dill, K. A. *Biochemistry* **1990**, *29*, 7133.
(3) Urry, D. W.; Peng, S. Q. *J. Am. Chem. Soc.* **1995**, *117*, 8478.
(4) Stackelberg, M. V.; Müller, H. R. *Naturwissenschaften* **1951**, *38*, 456.
(5) Snadberg, L. B., Gray, W. R., Franzblau, C., Eds.; *Elastin and Elastic Tissue*; Plenum: New York, 1976; p 645.
(6) Urry, D. W.; Peng, S.; Xu, J.; McPherson, D. T. *J. Am. Chem. Soc.* **1997**, *119*, 1161.
(7) Muller-Plathe, F. *Macromolecules* **1998**, *31*, 6721.
(8) Volpin, D.; Urry, D. W.; Pasquali-ronchetti., I.; Gotte, L. *Micron* **1976**, *7*, 193.
(9) Cook, W. J.; Einspahr, H.; Trapane, T. L.; Urry, D. W.; Bugg, C. E. *J. Am. Chem. Soc.* **1980**, *102*, 5502.
(10) Urry, D. W.; Trapane, T. L.; Sugano, H.; Prasad, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 2080.
(11) Urry, D. W.; Chang, D. K.; Krishna, N. R.; Huang, D. H.; Trapane, T. L.; Prasad, K. U. *Biopolymers* **1989**, *28*, 819.
(12) Pain, R. H. *Mechanisms of Protein Folding*; Oxford University Press: New York, 1991.
(13) Foster, J. A.; Bruenger, E.; Gray, W. R.; Sandberg, L. B. *J. Biol. Chem.* **1973**, *248*, 2876.
(14) Gray, W. R.; Sandberg, L. B.; Foster, J. A. *Nature* **1973**, *246*, 461.
(15) Sandberg, L. B.; Soskel, N. T.; Leslie, J. G. *N. Engl. J. Med.* **1981**, *304*, 566.
(16) Sandberg, L. B.; Leslie, J. G.; Leach, C. T.; Alvarez, V. L.; Torres, A. R.; Smith, D. W. *Pathol. Biol.* **1985**, *33*, 266.
(17) 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.

(18) Debelle, L.; Alix, A. J. P. *Biochimie* **1999**, *81*, 981.
(19) Shibayama, M.; Norisuye, T.; Nomura, S. *Macromolecules* **1996**, *29*, 8746.
(20) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(21) Mikheeva, L. M.; Grinberg, N. V.; Mashkevich, A. Y.; Grinberg, V. Y.; Thanh, L. T. M.; Makhaeva, E. E.; Khokhlov, A. R. *Macromolecules* **1997**, *30*, 2693.
(22) Makhaeva, E. E.; Tenhu, H.; Khokhlov, A. R. *Macromolecules* **1998**, *31*, 6112.
(23) Liu, Y.; Velada, L.; Huglin, M. B. *Polymer* **1999**, *40*, 4299.
(24) Cunningham, L. W., Frederiksen, D. W., Eds.; *Methods in Enzymology*; Academic Press: New York, 1982; p 673.
(25) Wang, X.; Qiu, X.; Wu, C. *Macromolecules* **1998**, *31*, 2972.
(26) Wang, X.; Wu, C. *Macromolecules* **1999**, *32*, 4299.
(27) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.
(28) Urry, D. W.; Nicol, A.; McPherson, D. T.; Xu, J.; Shewry, P. R.; Harris, C. M.; Parker, T. M.; Gowda, D. C. In *Encyclopedic Handbook of Biomaterials and Bioengineering, Part A Materials, Vol. 2*; Marcel Dekker: New York, 1995; p 1619.
(29) Guda, C.; Zhang, X.; McPherson, D. T.; Xu, J.; Cherry, J. H.; Urry, D. W.; Daniell, H. *Biotechnol. Lett.* **1995**, *17*, 745.
(30) McPherson, D. T.; Xu, J.; Urry, D. W. *Protein Expression Purif.* **1996**, *7*, 51.
(31) Urry, D. W.; Pattanaik, A.; Xu, J.; Woods, T. C.; McPherson, D. T.; Parker, T. M. *J. Biomater. Sci., Polym. Ed.* **1998**, *9*, 1015.
(32) Urry, D. W.; Henze, R.; Harris, R. D.; Prasad, K. U. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 1082.
(33) Tanaka, T. *Macromolecules* **1987**, *20*, 1342.
(34) Hirose, Y.; Amiya, T.; Hirokawa, Y.; Tanaka, T. *Macromolecules* **1987**, *20*, 1342.
(35) Lee, J.; Macosko, C. W.; Urry, D. W., submitted to *Macromolecules*.
(36) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: London, 1971.
(37) Peppas, N. A.; Merrill, E. W. *J. Polym. Sci.* **1976**, *14*, 459.
(38) Peppas, N. A.; Merrill, E. W. *J. Appl. Polym. Sci.* **1977**, *21*, 1763.
(39) Tanaka, T.; Fillmore, D. J. *J. Chem. Phys.* **1979**, *70*, 1214.
(40) Li, Y.; Tanaka, T. *J. Chem. Phys.* **1990**, *92*, 1365.
(41) Neogi, P. *Diffusion in Polymers*; Marcel Dekker: New York, 1996.
(42) Sciortino, F.; Palma, M. U.; Urry, D. W.; Prasad, K. U. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 1061.
(43) Tanaka, S.; Norisuye, T.; Shibayama, M. *Macromolecules* **1999**, *32*, 3989.
(44) Urry, D. W.; Trapane, T. L.; Iqbal, M.; Venkatachalam, C. M.; Prasad, K. U. *Biochemistry* **1985**, *24*, 5182.
(45) Keys, K. B.; Andreopoulos, F. M.; Peppas, N. A. *Macromolecules* **1998**, *31*, 8149.
(46) Urry, D. W.; Haynes, B.; Harris, R. D. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 749.
(47) Sciortino, F.; Prasad, K. U.; Urry, D. W.; Palma, M. U. *Chem. Phys. Lett.* **1988**, *153*, 557.
(48) Sciortino, F.; Urry, D. W.; Palma, M. U.; Prasad, K. U. *Biopolymers* **1990**, *29*, 1401.
(49) Scortino, F.; Prasad, K. U.; Urry, D. W.; Palma, M. U. *Biopolymers* **1993**, *33*, 743.
(50) Dole, M. *The Radiation Chemistry of Macromolecules*; Academic Press: New York, 1972.
(51) Moad, C. L.; Winzor, D. J. *Prog. Polym. Sci.* **1998**, *23*, 759.
(52) Henze, R.; Urry, D. W. *J. Am. Chem. Soc.* **1985**, *107*, 2991.
(53) Buchet, R.; Luan, C.; Prasad, K. U.; Harris, R. D.; Urry, D. W. *J. Phys. Chem.* **1988**, *92*, 51.
(54) Chang, D. K.; Urry, D. W. *J. Comput. Chem.* **1989**, *10*, 850.
(55) Mark, J. E., Ed.; *Polymer Data Handbook*; Oxford University Press: New York, 1999; p 78.
(56) Vankrevelen, D. W.; Hoftyzer, P. J. *Properties of Polymers*; Elservier Scientific: New York, 1976; p 152.
(57) Brandrup, J.; Immergut, E. H. *Polymer Handbook*; John Wiley & Sons: New York, 1989; p VII519.
(58) Khokhlov, A. R. *Polymer* **1980**, *21*, 376.
(59) Gottlieb, M.; Macosko, C. W.; Benjamin, G. S.; Meyers, K. O.; Merrill, E. W. *Macromolecules* **1981**, *14*, 1039.
(60) Erman, B.; Flory, P. J. *Macromolecules* **1986**, *19*, 2342.
(61) Wang, F.; Saeki, S.; Yamaguchi, T. *Polymer* **1999**, *40*, 2779.
(62) Huglin, M. B.; Rehab, M. M. A.-M.; Zakaria, M. B. *Macromolecules* **1986**, *19*, 2986.
(63) Warren, T. C.; Prins, W. *Macromolecules* **1972**, *5*, 506.
(64) Oh, K. S.; Oh, J. S.; Choi, H. S.; Bae, Y. C. *Macromolecules* **1998**, *31*, 7328.
(65) Hu, Y.; Liu, H.; Soane, D.; Prausnitz, J. M. *Fluid Phase Equilib.* **1991**, *67*, 65.
(66) Hu, Y.; Lambert, S. M.; Soane, D. S.; Prausnitz, J. M. *Macromolecules* **1991**, *24*, 4356.
(67) Tiktopulo, E. I.; Bychkova, V. E.; Rièka, J.; Pitsyn, O. B. *Macromolecules* **1994**, *27*, 2879.
(68) Tiktopulo, E. I.; Uversky, V. N.; Lushchik, V. B.; Klenin, S. I.; Bychkova, V. E.; Pitsyn, O. B. *Macromolecules* **1995**, *28*, 7519.
(69) Luan, C.; Parker, T. M.; Prasad, K. U.; Urry, D. W. *Biopolymers* **1991**, *31*, 465.
(70) Grinberg, N. V.; Dubovik, A. S.; Grinberg, V. Y.; Kuznetsov, D. V.; Makhaeva, E. E.; Grosberg, A. Y.; Tanaka, T. *Macromolecules* **1999**, *32*, 1471.
(71) Wu, C.; Zhou, S. *Macromolecules* **1997**, *30*, 574.
(72) Kokufuta, E.; Wang, B.; Yoshida, R.; Khokhlov, A. R.; Hirata, M. *Macromolecules* **1998**, *31*, 6878.
(73) Thomas, G. J., Jr.; Prescott, B.; Urry, D. W. *Biopolymers* **1987**, *26*, 921.
(74) Lele, A. K.; Hirve, M. M.; Badiger, M. V.; Mashelkar, R. A. *Macromolecules* **1997**, *30*, 157.
(75) Inomata, H.; Goto, S.; Saito, S. *Macromolecules* **1990**, *23*, 4887.

MA0015673