Thermodynamic Study of a Pressure-Temperature Phase Diagram for Poly(N-Isopropylacrylamide) Gels

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ABSTRACT: The volume change, $\Delta V_{h'}$, accompanying the hydrophobic hydration associated with the volume phase transition in Poly(*N*-isopropylacrylamide) gels was measured by a simple method. The hydration accompanies a negative $\Delta V_{h'}$ −2.5 cm^3/mol. The *P-T* phase diagram, the coexistence curve, for the gels was determined from the swelling ratio-pressure curves up to 350 MPa for various constant temperatures. The contour of the coexistence curve is shaped like an ellipsoid on the *P-T* plain, which is a feature peculiar to the reversible pressure-temperature denaturation of a protein. The thermodynamic analysis of the Clausius–Clapeyron relation for the measured ΔV_h elucidates that the obtained coexistence curve represents the phase boundary between thermodynamic different phases like the two phases, native and denatured, of a protein and gives the transition enthalpy, ΔH, 5.2kJ/mol by estimate, which well coincides with the transition heat measured by a calorimetric method. Considering the volume-dependent free energy, $\Delta v_{mi} \cdot P$, for the mixing free energy of the gel, we can fit the calculated curve to the measured swelling ratio-pressure curve of PNIPA gels. The value of Δv_{mi} changes the sign from negative to positive above around 100MPa. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 405–412, 2005

Key words: poly(N-isopropylacrylamide) gel; P-T phase diagram; high pressure; transition volume and heat; Clausius–Clapeyron relation

INTRODUCTION

A poly(*N*-isopropylacrylamide) (PNIPA) gel is a well known thermosensitive hydrogel that undergoes a volume phase transition in response to an infinitesimal change in temperature.[1] Thermodynamically, the volume phase transition has been explained by considering the osmotic pressure of a gel derived from its free energy.[2] It has been known that the mixing free energy due to association between polymer chains and water, which is given by the corresponding enthalpy and entropy changes, plays an important role in the phase transition.[3] The volume phase transition has also been understood as a phenomenon of hydration and dehydration of the polymer chains.[4] The swelling and shrinking of the gel, that is, hydration and dehydration of the chains, will be accompanied by some volume change as well as the entropy change. Up to now, there have been many investigations concerning the temperature-induced volume phase transition from static and dynamic points of view.[5–10] However, investigation that reveals the volumetric character in the phase transition scarcely has been done. Study on the volumetric character will give new information about the pressure-induced phase transition of the gel.

Recently, the author reported that hydrogels, such as PNIPA gels in water[11] and poly(acrylamide) gels in acetone-water mixtures,[12] underwent volume phase transitions by a change in hydrostatic pressure, the pressure-induced volume phase transition. The pressure-induced volume phase transition can be explained by taking account of the free energy difference derived from the volume change accompanying the hydration of the network polymer chains. However, as far as I know, the value of this volume change has not been measured in the volume phase transition.

It has been reported that the underlying mechanism of the volume phase transition of a gel resembles that of a coil-globule transition of a polymer.[13, 14] And the coil-globule transition of PNIPA polymers has been studied to understand the mechanism of the denaturation of proteins.[15] Kunugi and colleagues reported a temperature-pressure diagram for the cloud point of a PNIPA solution and discussed it in terms of the thermodynamic theory of denaturation of proteins by Hawley.[16] Some proteins undergo a reversible transition from a native state to a denatured state by changes in stimuli, such as solvent composition, temperature, and pressure.[17–19] The denaturation has been understood as disruption of a higher structure of proteins into a random-coil state. The disruption results in hydration of polar and nonpolar groups of proteins

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and is accompanied by the decrease of the partial molar volume of protein. This implies that there are some similarities between the volume phase transition of gels and the denaturation of proteins.

For our study, the experiment of the volume changeΔV_h accompanying the hydration in the volume phase transition of PNIPA gels was carried out by using a simple apparatus. The swelling ratio of PNIPA gels in water was measured as a function of hydrostatic pressure up to 350 MPa at various constant temperatures to obtain the coexistence curve in a pressure-temperature plain for the volume phase transition of the gels. The obtained coexistence curve is examined in terms of the Clausius–Clapeyron relation and discussed in terms of the thermodynamic theory of the reversible pressure-temperature denaturation of proteins by Hawley.[16] By using the extended Flory-type free energy considering$\Delta V_h \cdot P$, the obtained pressure-induced volume phase transition of PNIPA gels is discussed thermodynamically.

EXPERIMENTAL

Sample preparation

Sample NIPA gels were prepared by a free radical copolymerization in water. They were slightly ionized. 3.88 g of NIPA monomer, 0.067 g of *N,N′*-methylenebisacrylamide as a crosslinker, and 0.064 g of sodium acrylate (SA) to ionize the network were dissolved in distilled water by bubbling with N_2 gas (a total volume of 50 mL). The mole ratio of NIPA to SA is 50 : 1. After that, 20 mg of ammonium persulfate and 120 μL of *N,N,N′,N′*,-tetramethylethylenediamine were added to the solution as an initiator and an accelerator for gelation (pregel solution). The solution was transferred to a glass beaker that contained glass tubes of 1.63 mm inner diameter. Gelation was completed for more than several hours at 20°C; after which, the gel was taken out of the tubes and immersed in distilled water to remove residual substances. The remaining bulky gel was crushed in fragments of small sizes. These fragments were immersed in a large amount of distilled water. The former cylindrical gel was cut into approximately 5 mm length for the use of the high-pressure experiment. The latter gel fragments were dried and evacuated by a vacuum pump. These dried hard fragments were ground into grain sizes with a mortar for the use of the measurement of the volume change of water, ΔV_h.

Measurement of volume change

The volume change, ΔV_h, of water accompanying the volume phase transition in a PNIPA gel, hydration, or dehydration of the gel chains, is measured by using a pycnometer-type glass bottle (100 mL) having a capillary tube with a scale. The volume change is determined by comparing the volume change caused by thermal expansion of the mixture of PNIPA gels and water with that of distilled water of the same volume contained in the same bottle. The difference between these volume changes can be regarded as the volume change that accompanies the volume phase transition, that is, hydration. The pycnometer is put in a thermostat water bath controlled within $\pm$ 0.1°C. By using this apparatus, one can measure the volume change in accuracy of $\pm$ 0.002 cm^3. First of all, the glass bottle is filled with only distilled water, and the mouth of the bottle is closed with the capillary tube. The volume change of the water is measured as a function of temperature by reading the level of water in the capillary. After the measurement, a small amount (a few mg) of dried PNIPA gel grains was put into the bottle, and it was filled with distilled water. The mixture was degassed to remove the air. The volume change of the mixture was measured as a function of temperature. From these measurements, one obtains two thermal expansion curves. One is of water and the other is of the mixture of PNIPA gels and water. The volume change accompanying the volume phase transition can be estimated from the excess volume change derived from the deviation of the latter curve from the former curve occurring around the transition temperature. The measurements were also carried out for the neutral gels.

Measurement of swelling ratio

An optical high-pressure cell used in the present measurements was the same as reported in a previous paper.[12] The gel was placed on the bottom of the cylinder, 15 mm in diameter, which was filled with distilled water. The water was compressed through the piston, and the pressure was measured with a Heise Bourdon gauge. The temperature of the pressure cell was controlled in accuracy within $\pm$ 0.2°C by circulating temperature-regulated water around the cell. A diameter d of the gel was measured with a microscope through a sapphire window as a function of pressure. The measurement was carried out up to pressures of 350 MPa for various temperatures. The equilibrium swelling ratio V/V_0 is calculated from the relation $V/V_0 = (d/d_0)^3$, where V is the volume of the gel, and V_0 and d_0 are the volume and the diameter when the gel was prepared, respectively.

RESULTS AND DISCUSSION

Volume change

The amount of 2.0 g of the dried ionized PNIPA gel grains was put in the pycnometer-type glass bottle and filled with distilled water. The mixture was de-

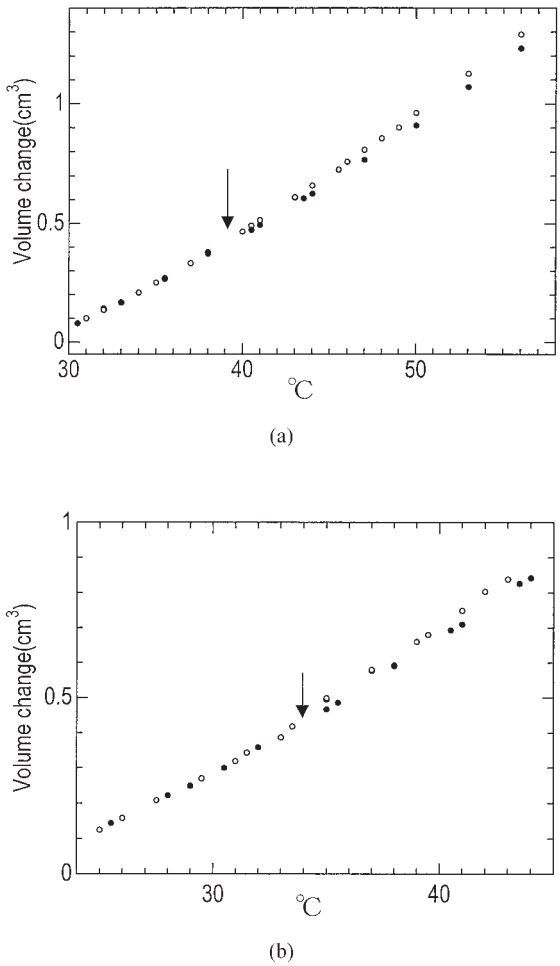

Figure 1 Volume change as a function of temperature. The vertical axis shows the scale (cm^3) on the capillary at the surface of the mixture or the water. The scale of the mixture coincides with that of the water at 31°C for the ionized gel (a) and at 25°C for the neutral gel (b). ○ : the mixture, and ●: the water.

gassed to remove the air, and the mouth of the bottle was closed with the capillary tube. The volume change of the mixture due to thermal expansion was measured by reading a scale (cm^3) on the capillary at the surface of the mixture. The same measurement was also done for the water. For the mixture, more than one hour was taken to reach an equilibrium value due to relaxation time in the volume change of the gels. The measured scales for increasing temperature are plotted in Figure 1a. The measurements were set so that the scale of the surface of the mixture coincides with that of the water at 31°C. In the Figure, the open circles represent the data of the mixture, and the solid circles those of the water. From the latter curve, one can estimate a thermal expansion coefficient of water, 4.1×10^{-4}/°C around 40°C, which nearly equals the value listed in the literature. It is seen that the data of the open circles deviate from the solid ones around the transition temperature, 38°C. Below that temperature, the swollen gel grains occupied a great part of the glass bottle, and above that temperature, all the gel grains collapsed and water excluded from the gel grains occupied a great part of the bottle. Though the deviation is very small, no more than 10% of the volume change due to thermal expansion of water, the obtained data are reproducible. The deviation gives the observed excess volume change of 0.048 cm^3 for 2.0g PNIPA gels, namely, 2.7 cm^3/mol for the shrinking of the gel, dehydration. The hydration, swelling, accompanies the decrease of the volume, -2.7 cm^3/mol. The same measurements were carried out for the neutral gels of 1.8 g. The obtained data are plotted in Figure 1b. It is seen that a similar deviation between the curves appears around the transition temperature, 35°C, of the neutral PNIPA gels. This deviation gives approximately a similar excess volume change, 2.4 cm^3/mol, associated with the volume phase transition (shrinking of the PNIPA gel), namely, -2.4 cm^3/mol for hydration of the polymer network. Since the difference between the values of ionized and neutral PNIPA gels is not so large compared with experimental error, we do not consider the difference here. So, we obtained the mean value, -2.5 cm^3 /mol, as the tentative value of ΔV_h for the hydration of the PNIPA gel network.

As mentioned in the Introduction, proteins undergo denaturation induced by changes in concentration of solution, temperature, and pressure. The thermodynamic mechanism of the denaturation has been explained by considering the volume change accompanying the hydration of polar and nonpolar groups of proteins. This volume change is derived from the structure change that free water molecules are ordered around the groups. It has been reported that the denaturation of proteins is usually accompanied by the volume change $-(1 \sim 7) \times 10$ cm^3/mol.[20] Sawamura and coworkers reported that hydrophobic hydration was accompanied by a volume change of $-(4 \sim 8.5)$ cm^3/mol at atmospheric pressure, and it changed the sign from negative to positive at high pressures above 100 MPa.[21] The value obtained by the present simple measurements is nearly equal to these values. This implies that the volume phase transition of the gels and the denaturation of proteins relate to a similar thermodynamic mechanism.

Swelling ratio as a function of pressure

The swelling ratio of the cylindrical PNIPA gel is plotted as a function of pressure up to about 350 MPa, for increasing pressure at various constant temperatures, in Figure 2. The ratio was calculated from an equilibrium diameter of the gel. For an increment of pressure, the gel needed more than a few hours to reach the equilibrium diameter. At atmospheric pressure, the measured gels underwent the discontinuous

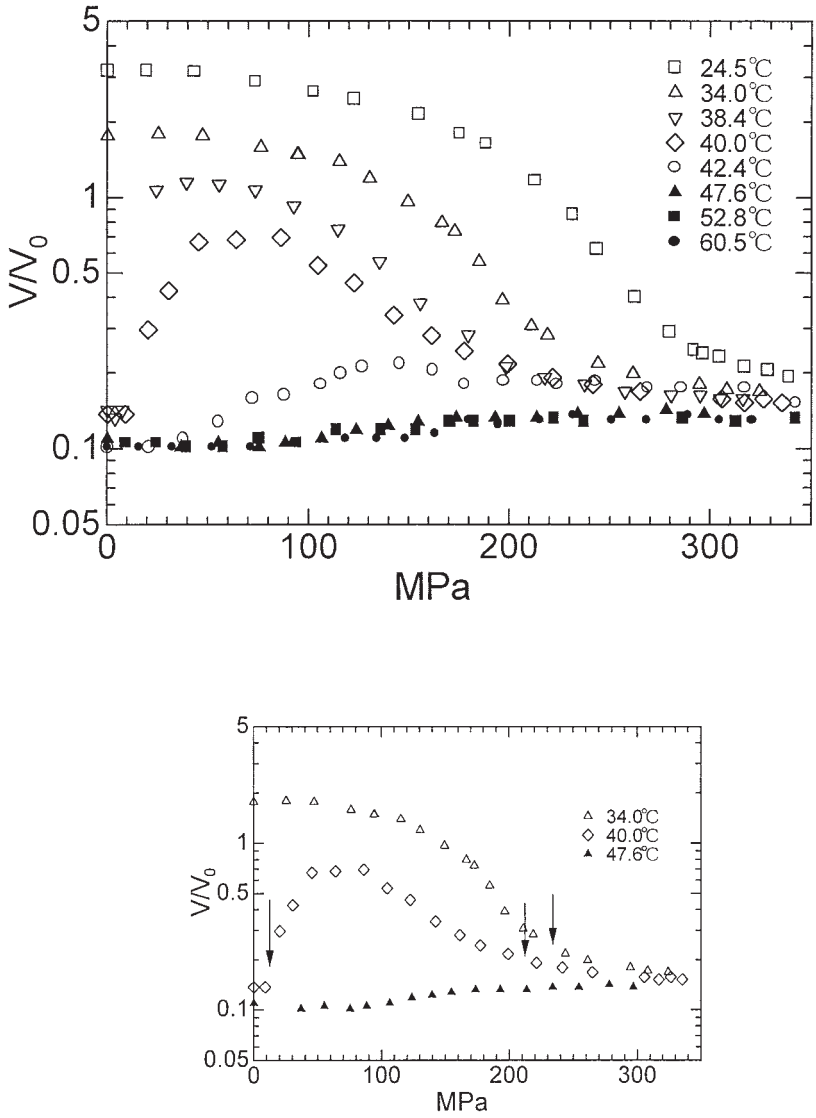

Figure 2 Swelling ratios of PNIPA gels as a function of hydrostatic pressure for various constant temperatures. The arrows inserted in the lower figure represent the transition pressures for the corresponding temperatures.

volume phase transition around 38°C. As seen in the Figure, the behaviors of the obtained swelling ratio-pressure curves strongly depend on the temperature, namely, whether the gels swell or shrink at atmospheric pressure. For the curves of 24.5°C and 34.0°C, the gels swollen at atmospheric pressure gradually shrink above 100MPa with an increase of pressure and approach the shrunken state above ~ 250 MPa (high-pressure shrunken state). For 38.4°C, 40.0°C, and 42.4°C, just above the transition temperature at atmospheric pressure, the gels shrink at atmospheric pressure, and as the pressure is increased they undergo the volume change twice. The first change is the abrupt volume transition from the shrunken state to the swollen state, occurring at a low pressure of several tens MPa. This abrupt volume transition disappears as the temperature is increased. The second change is the gradual shrinking occurring at a higher pressure. Such a convex type volume transition is very similar to a reentrant phase transition of PNIPA gels in methanol-water mixtures, which shows two discontinuous swelling transitions induced by a change in the solvent composition.[3] For the gels at 47.6°C, 52.8°C, and 60.5°C, it seems that the swelling ratios (V/V_0) scarcely change with pressure and the gels take the shrunken state in the measured pressure region. However, it is worthwhile to mention that V/V_0 at 47.6°C, 52.8°C, and 60.5°C exhibits a slight increase at around 140 MPa. At around that pressure, a phenomenon that the gel becomes opaque was observed. The result may suggest that the PNIPA gel has two shrunken states below and above the pressure, namely, a low pressure shrunken state and a high pressure shrunken state. The pressure does not induce the volume transition in

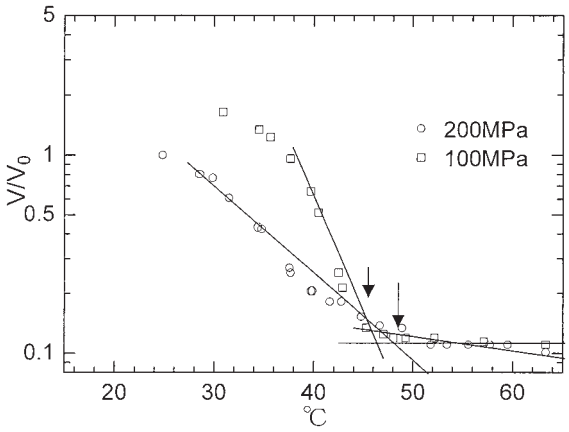

Figure 3 The swelling ratios of ionized PNIPA gels as a function of temperature at constant pressures. The arrows inserted in the figure represent the transition temperatures.

the gels above 47.6°C. At high pressures above 250 MPa, all the gels are in the shrunken state in contrast to the case at low pressures.

From these curves, one can obtain the coexistence curve of the PNIPA gel in the pressure-temperature plain. The volume changes occurring above 100 MPa are continuous in contrast to the transitions at low pressures. So, one cannot regard that the volume change observed at the high pressure is the first-order phase transition in a thermodynamic point of view. However, taking into account the similarity between the pressure-induced volume transition and the reentrant transition and the fact that the gel became opaque around the transition point, we regard that these continuous changes also reflect the volume transitions at the high pressure region.

Figure 2 also shows three swelling curves obtained for the temperatures 34.0°C, 40.0°C, and 47.6°C as representatives. One can regard the pressures at which the gradients of the curves abruptly change as the transition pressures for the continuous transition. The arrows denoted on these curves represent the transition pressures for the corresponding temperature. The curve of 47.6°C shows that the gel is in the shrunken state at the whole pressure region. The swelling ratios at constant pressures were also measured as a function of temperature. The data measured at 100 MPa and 200 MPa are plotted in Figure 3. The gradients of these swelling curves also change at particular temperatures. They represent the transition temperatures for the corresponding pressures. There was no significant difference between the transition points obtained from the temperature and pressure scannings.

Figure 4 shows more detailed investigation of the volume phase transition at low pressures for the three representative temperatures. The data are those obtained for increasing pressure. It is found that the gel at 38.5°C undergoes the discontinuous volume phase transition at ~ 8.0 MPa, just above the transition temperature of the gel at atmospheric pressure. This pressure-induced volume phase transition shows hysteresis like the volume phase transition induced by temperature. The transition pressure when pressure is decreased is about 5.0 MPa lower than that for increasing pressure. One finds that the pressure-induced volume phase transition at low pressures is the first-order phase transition. As the temperature is increased, the transition pressure increases, and the transition becomes continuous. One can expect the existence of a critical point on the pressure-volume plain for the pressure-induced volume phase transition. The obtained transition pressures are plotted as a function of temperature in Figure 5 It is seen that the transition pressure is approximately linear to temperature as approximated by a straight line. The region above the line is the swollen state, and the region below the line the shrunken state. The line seems to end at around 24 MPa and 47°C.

Figure 5 also shows the transition pressures versus temperature curve (coexistence curve) obtained for the measured pressure and temperature region. It is seen that the contour of the obtained coexistence curve is shaped like an ellipsoid on the pressure-temperature plain. The inside of the counter represents the swollen state, and the outside the shrunken state. The contour appears from the atmospheric axis at around 38°C and has a positive gradient, $dP/dT > 0$. At around 100 MPa and 45°C, dP/dT becomes zero. Above that pressure, the contour changes its gradient, and dP/dT becomes negative. Below 38°C, the gels undergo the volume change once, from the swollen state to the high-pressure shrunken state at higher pressures. Between 38°C and 45°C, the gels undergo the volume transition twice, that is, from the low-pressure shrunken state to the swollen state at lower pressures, and from the swollen state to the high-pressure shrunken state at higher pressures. Above 45°C, the

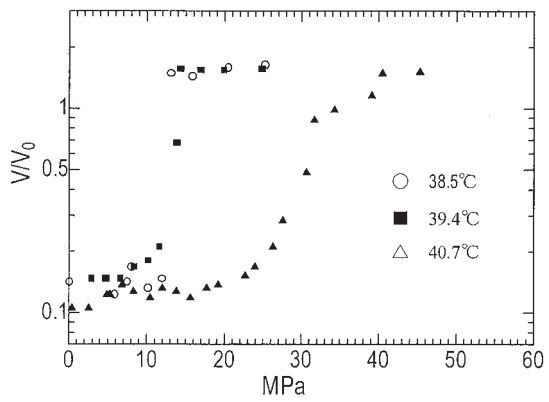

Figure 4 Detailed investigation of the swelling ratios at lower pressures.

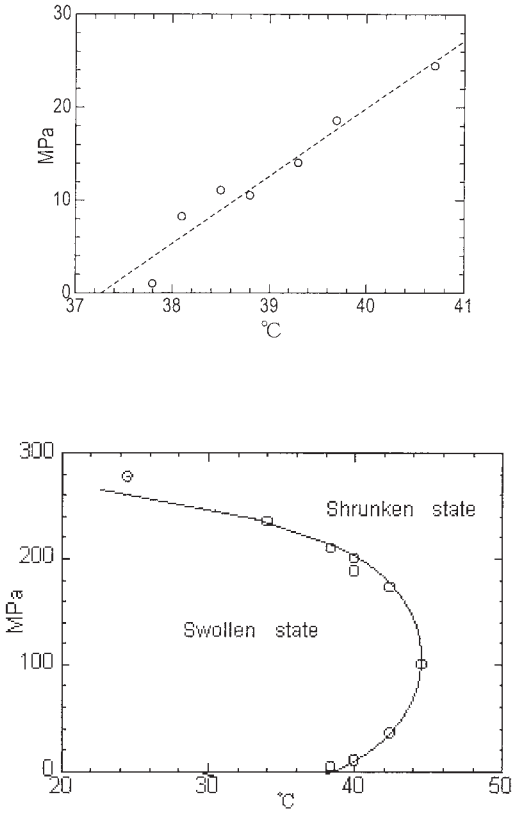

Figure 5 Transition pressure versus temperature at low pressures for ionized PNIPA gel, and the coexistence curve on the pressure-temperature plain for the pressure-induced volume phase transition. For the upper figure, the dependence is approximated to the straight line.

gels do not undergo the volume transition, and they are in the shrunken state for the whole measured pressures. Here, we emphasize that the curve of the positive gradient, $dP/dT > 0$, represents the first-order transition, and the curve of $dP/dT < 0$ the continuous volume transition.

Kunnugi and colleagues obtained an elliptical temperature-pressure diagram for the cloud point of PNIPA solution and discussed the behavior in terms of the thermodynamic interpretation of the denaturation of proteins. It is found that the obtained coexistence curve of PNIPA gel is shaped like the same ellipsoid. The pressure at $dP/dT = 0$ is nearly equal to that for the cloud point curve of the solution, but the temperature is about 5°C higher. The region of $dP/dT > 0$ in the cloud point curve corresponds to the discontinuous volume transition of the gel, and the region of $dP/dT < 0$ corresponds to the continuous transition.

Clausius–Clapeyron relation

Such an elliptical contour of the coexistence curve is a feature peculiar to the reversible pressure-temperature denaturation of a protein, as mentioned. The contour represents the thermodynamic transition surface, phase boundary, between a native state and a denatured state on the pressure-temperature plain. The above results show that the obtained coexistence of the PNIPA gel is very similar to the transition surface of a protein.

For the reversible denaturation of proteins, the coexistence (transition) curve represents a phase boundary between thermodynamically different phases (native and denatured).

According to the Clausius–Clapeyron relation, dP/dT is written as

$$dP/dT = \Delta H/(T\Delta v), \tag{1}$$

where ΔH and Δv are changes of enthalpy and volume in the phase transition. For the volume phase transition in PNIPA gels, Δv corresponds to the volume change $\Delta \boldsymbol{V}_{\mathbf{h}}$, 2.5 cm^3/mol.

By calculating the gradient of the straight line in Figure 5, one obtains 6.7 MPa/K as the value of dP/dT. By adopting these values into eq. (1), one can estimate the value of ΔH for the volume phase transition in PNIPA gel at 310 K. Thus, one obtains 5.2 kJ/mol as the value of ΔH. Otake and coworkers measured the transition heat of the volume phase transition of PNIPA gels.[22] They reported that the transition heat of collapse of the gel is endothermic and the value is 3.3–4.5 kJ/mol. The obtained ΔH well coincides with those values. This implies that the transition curve for the volume phase transition in PNIPA gels represents the phase boundary between thermodynamic different phases, which is similar to the boundary between the native and denatured states of a protein.[17]

The obtained values of ΔV_{h} and ΔH can be compared with the value for free PNIPA polymer reported in reference 16. The reference gives the ratio, $\Delta S_0/\Delta V_0 = 1.24[\mathrm{cal}/(\mathrm{Kcm}^3)]$, for the free polymer. The obtained values for the PNIPA gel give the ratio, $\Delta S/\Delta V = 0.63[\mathrm{cal}/(\mathrm{Kcm}^3)]$. It is found that the ratio for the gel is two times smaller than that for the free polymer.

Volume dependent free energy for the swelling curve

The volume phase transition in a polymer gel has been explained by the osmotic pressure exerted on a gel network that is expressed by the free energy of a gel consisting of the terms of mixing, rubber elasticity, and ions. The free energy for the association between the polymer segment and the solvent ΔF is important in the volume phase transition. In the usual treatment (lattice theory), ΔF does not have a volume-dependent term. To explain the pressure-induced volume phase transition, however, one needs to consider a volume dependent term. When the association between the

polymer segment and the solvent is accompanied by a volume change Δv_{mi}, one needs to consider the energy term $\Delta v_{mi} \cdot P$, which is not taken into account in the lattice theory, for ΔF, that is,

$$\Delta F = \Delta F_0 + \Delta v_{mi} \cdot P \quad (2)$$

where P is pressure and ΔF_0 is the volume independent term that depends on temperature.

The equation of a state of a gel is given by the condition of zero osmotic pressure,[23]

$$-\frac{N_A kT}{v}\left[\phi + \ln(1-\phi) + \frac{\Delta F}{2kT}\phi^2\right] + \nu kT\left[\frac{1}{2}\left(\frac{\phi}{\phi_0}\right) - \left(\frac{\phi}{\phi_0}\right)^{\frac{1}{3}}\right] + f\nu kT\left(\frac{\phi}{\phi_0}\right) = 0 \quad (3)$$

where N_A is Avogadro's number, k the Boltzmann's constant, T temperature, v_{mi} the molar volume of the solvent, ν the number of constituent chains per unit volume, and f is the number of dissociated counter ions per effective chain. φ is the volume fraction of the network, φ_0 is that when the gel was prepared, and they are related to the swelling ratio

$$\frac{\phi}{\phi_0} = \frac{V_0}{V}.$$

By using eqs. (2) and (3), one can calculate V/V_0 as a function of P. In the calculation, one needs to know the values of the parameters ν, φ_0, f, ΔF_0, and Δv_{mi} in advance. In these parameters, the values of two parameters, f and ΔF_0, are unknown. But a maximum value of ν can be estimated from the amount of crosslinking agent added when the gel was prepared, and the value of φ_0 from the ratio of the volume of a dried gel to that of a prepared gel. And the value of ΔV_{mi} is derived from the volume difference between the molar volumes of free water and ordered water caused by the association. Since the volumes of these waters depend on pressure, Δv_{mi} depends on pressure. Assuming that Δv_{mi} is given by a linear function of pressure P, one obtains the relation,

$$\Delta v_{mi} = \Delta v_{mi,0} + \Delta v_{mi,p} \cdot P. \quad (4)$$

Since the free water is more compressible than the ordered water, the value of $\Delta v_{mi,p}$ is assumed to be a small positive. So it is expected that the value of Δv_{mi} is negative at low pressures but its sign changes to a positive at high pressures.

For simple analysis, one assumes that the value of Δv_{mi} is a negative constant at low pressures, that is, -2.5 cm^3/mol, and a certain positive constant at high pressures.

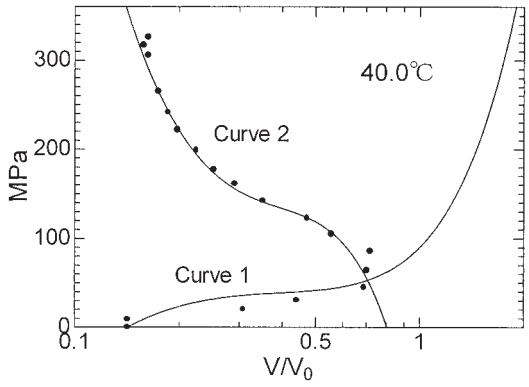

Figure 6 Fitting of the calculated swelling curves to the measured curve at 40°C.

Thus, one obtains 1.0×10^{19}/cm^3, 0.072, and -0.00048 MPa^{-1} as the values of ν, φ_0, and $\Delta v_{mi}/2kT$ (at 40°C) at low pressures. The former two values are somewhat ambiguous because the network structure of a real gel deviates from the ideal one, that is, there is inhomogeneity in real gels.[24,25] Figure 6 shows the comparison between the calculated curves and the measured swelling ratio-pressure curve for 40°C. In the Figure, Curve 1 is the calculated one best fitting the data in the lower pressure region below 100 MPa, and Curve 2 is the one in the higher pressure region above the pressure. For Curve 1, the values of the parameters, $\nu = 1.9 \times 10^{18}$/cm^3, $\varphi_0 = 0.020$, $f = 0.64$, $\Delta F_0/2kT = 0.57$, and $\Delta v_{mi}/2kT = -0.00048$ MPa^{-1}, were used. For Curve 2, $\nu = 1.9 \times 10^{18}$/ cm^3 , $\varphi_0 = 0.020$, $f = 0.64$, $\Delta F_0/2kT = 0.54$, and $\Delta v_{mi}/2kT = 8.5 \times 10^{-5}$ MPa^{-1} were used. It is seen that at low pressures below 100 MPa, Curve 1 calculated by using the present negative ΔV_h, which was obtained by the simple experiment, well fits the measured swelling curve. Curve 2 well fits the data for the positive Δv_{mi} also. These results confirm that ΔV_h changes the sign from the negative value to a positive one at higher pressures above ~ 100 MPa and that the gradient, dP/dT, of the coexistence curve in Figure 5 changes the sign above the pressure.

CONCLUSIONS

The volume change, ΔV_h, accompanying the hydration of PNIPA chains in the volume phase transition of the gels was directly measured by a simple experimental method. The coexistence curve of the volume phase transition on the pressure-temperature plain was obtained from the measurements of the swelling-ratios at high pressures up to 350 MPa. The curve is shaped like an ellipsoid, which is a feature peculiar to the reversible pressure-temperature denaturation of a

protein. Thermodynamic analysis with the Clausius–Clapeyron relation using the measured ΔV_h elucidates that the obtained coexistence curve represents the phase boundary between thermodynamic different phases like two states (native and denatured) of a protein. The estimated enthalpy change, ΔH, well coincides with the transition heat measured by a calorimetric method. Considering the free energy, $\Delta v_{mi} \cdot P$, which changes the sign above 100MPa, we can fit the calculated curve to the measured swelling ratio-pressure curve. These suggest that there is a resemblance between the underlying mechanisms of the pressure-induced volume phase transition of PNIPA gels and the reversible pressure-temperature denaturation of a protein in the thermodynamic point of view.

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