

# Deuterium Isotope Effect on Volume Phase Transition of Polymer Gel: Temperature Dependence

Hideaki Shirota,<sup>\*,†</sup> Nozomi Kuwabara,<sup>‡</sup> Kazuya Ohkawa,<sup>§</sup> and Kazuyuki Horie<sup>\*</sup>

Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo,  
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Received: July 14, 1999; In Final Form: October 5, 1999

The deuterium isotope effects on the volume phase transition of a typical temperature-sensitive polymer gel (poly(*N*-isopropylacrylamide) (PNIPAM) gel) and the phase separation of the linear polymer (PNIPAM) have been investigated. For the comparison between the bulk change and the microenvironment change, the deuterium isotope effects of PNIPAM gel and linear PNIPAM solution have also been investigated by using a fluorescence probe. Both the transition temperature of the polymer gel and the phase separation temperature of the linear polymer in heavy water are about 0.7 °C higher than those in water. However, the deuterium isotope effects on the difference of the transition temperatures in the heating process and the cooling process in the microenvironments of PNIPAM gel and linear PNIPAM solution are not observed.

## 1. Introduction

Many experimentalists and theoreticians have studied the volume phase transition in polymer gels.<sup>1–3</sup> The study of the volume phase transition in polymer gels was motivated by the theoretical prediction of Dušek and Patterson in 1968.<sup>4</sup> They suggested that the net repulsion between the segments of a polymer network and a poor solvent can cause a phase transition due to a sudden change in the degree of swelling. Their work was based on the theory analogous to the coil–globule transition of a linear polymer in a solution, which was predicted by Ptitsyn et al.<sup>5</sup> Tanaka discovered the discontinuous volume change of a partially ionized acrylamide gel against the continuous change of the solvent composition in a water/acetone mixture in 1978.<sup>6</sup> Such a volume phase transition was recognized to be generated not only by solvent composition changes but also by temperature, ionic, and pH changes, light irradiation, electronic field, and others.<sup>1,2</sup>

Poly(*N*-isopropylacrylamide) (PNIPAM) gel (and linear PNIPAM) in water, the subject of the present work, is one of the ideal systems for detailed studies of the phase transitions. This polymer gel and linear polymer (with a neutral polymer network and with water molecules) undergo a phase transition as a function of temperature around 34 °C.<sup>7–28</sup> Thus, the PNIPAM gel has been investigated in a large number of studies from different aspects.<sup>29,30</sup>

The volume phase transition (and the phase state) of polymer gels is determined by the following four fundamental interactions: van der Waals, hydrophobic, electrostatic, and hydrogen-bonding interactions. In the present work, we focus on hydrogen-bonding interactions. Although the bond energy is not very large compared to a covalent bond, it plays a key role in many chemical and biological systems. In addition, the physical

properties of water molecules are changed by their environment. Specifically, the properties of the water molecules hydrated with proteins and polymers are rather different from those of the normal water molecules in the bulk media of liquid water.<sup>31–43</sup>

Deuterium isotopic substitution is a very useful method to study the properties of the hydrogen-bonding interactions. Because of the heavier mass of deuterium, the hydrogen bond of deuterium is more stabilized than that of hydrogen. The differences between the features of deuterated and undeuterated hydrogen-bonded systems arise from the stabilized hydrogen-bonding interaction for deuterium in comparison with hydrogen. For example, the viscosity of heavy water is higher than that of water, and the melting and boiling points of heavy water are higher than those in water.<sup>44</sup> The features of water and heavy water were also compared by molecular dynamics and theoretical calculations.<sup>45–47</sup> Although the physical properties of water and heavy water are different, some studies of the volume phase transition of hydrogel (e.g., NMR, neutron scattering) ignore this effect. Since critical phenomena are very sensitive to the environmental conditions, it is very important to compare the critical phenomena measured in undeuterated and deuterated media.

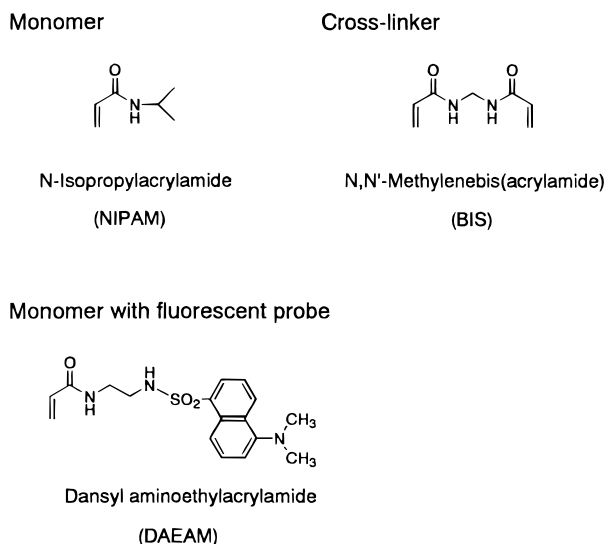
Recently, we investigated the deuterium isotope effect on the volume phase transition of PNIPAM gel in water.<sup>20</sup> The 1 °C increase in the transition temperature of PNIPAM gel in heavy water compared with water was previously observed,<sup>15</sup> although the origin of the deuterium isotope effect in the volume phase transition of PNIPAM gel was not clear. From the simulation based on a simple modified Flory–Rehner model proposed by Shibayama et al.,<sup>19</sup> we showed that the deuterium isotope effect in the swelling–shrinking state of PNIPAM gel arose from the difference in the polymer–solvent interactions (enthalpies) in water and heavy water.

The deuterium isotope effect on the swelling process of PNIPAM gel and polyacrylamide (PAAM) gel was also reported.<sup>48</sup> The deuterium isotope effect in the swelling kinetics of both the PNIPAM gel and PAAM gel is correlated with higher viscosity in heavy water than in water. In the paper, the

<sup>†</sup> Present address: Department of Chemistry, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854.

<sup>‡</sup> Present address: IBM Japan Ltd., 19-21 Nihonbashi Hakozaiki-cho, Chuo-ku, Tokyo 103-8510, Japan.

<sup>§</sup> Present address: Nippon Sheet Glass Corporation Ltd., 5-8-1 Nishi-hashimoto, Sagami-hara, Kanagawa 229-1189, Japan.



**Figure 1.** Structures of the monomer, the cross-linker, and the fluorescent-labeled monomer used in the present study. Abbreviations of the names of the molecules are also given.

deuterium isotope effects on the size of the aqueous gels in the equilibrium state were also reported.<sup>48</sup> Interestingly, the size in the swelling equilibrium state of PNIPAM gel in heavy water was larger than that in water. In contrast to the PNIPAM gel, the size in the equilibrium state of PAAM gel in heavy water was smaller than that in water. The different features of the deuterium isotope effects on the size in the equilibrium state of PNIPAM gel and PAAM gel should be coming from the different polymer-solvent interactions caused by the isotopic substitution of the hydrogen-bonding network. Since PNIPAM in water has a lower critical solution temperature (LCST) and PAAM in water has an upper critical solution temperature (UCST), the deuterium isotope effects in the polymer-solvent interaction of PNIPAM and PAAM should be different.

In this work, we have investigated the deuterium isotope effects on the volume phase transition of PNIPAM gel and the phase separation of linear PNIPAM. To compare with the macroscopic picture of the transitions of the polymer gel and the linear polymer, we have also investigated the microenvironments for transitions of the gel and the linear polymer in water and heavy water by using fluorescent-labeled polymer gel and linear polymer. It is interesting to compare the macroscopic picture and the microscopic picture of the deuterium isotope effects on the transitions of polymer gel and on the phase separation of the linear polymer. To observe the deuterium isotope effects on the volume phase transition of PNIPAM gel and the phase separation of linear PNIPAM solution, we measured several samples at different concentrations.

## 2. Experimental Section

**2.1. Sample Preparation.** *2.1.1. Linear Poly(N-isopropylacrylamide).* Linear PNIPAM was synthesized from *N*-isopropylacrylamide (NIPAM, Tokyo Kasei, Figure 1). The monomer was used after recrystallization (hexane/benzene solution (2/1, v/v)). The concentration of NIPAM was kept at 0.35 M. The aqueous solution containing NIPAM and 6.57 mM ammonium persulfate (initiator) was stirred for about 15 min in an ice bath under nitrogen atmosphere. *N,N,N',N'*-Tetramethylethylenediamine (accelerator) was then added to give a concentration of 13.3 mM. The solution was stirred for about 20 h at ambient temperature. The polymer was purified by reprecipitation (first

time, methanol (good solvent)/diethyl ether (poor solvent); second time, THF (good solvent)/hexane (poor solvent)). The polymer was dried at 145 °C under vacuum for more than 6 h. The molecular weight of the polymer,  $M$ , was estimated from the intrinsic viscosity,  $[\eta]$ , of the polymer in water, using the viscosity-molecular weight relationship:  $[\eta] = 2.26 \times 10^{-4} M^{0.97} \text{ cm}^3 \text{ g}^{-1}$ .<sup>49</sup> The value of  $M$  of the linear PNIPAM was  $1.7 \times 10^6$ .

*2.1.2. Poly(N-isopropylacrylamide) Gel.* PNIPAM gels were synthesized from NIPAM and the cross-linker *N,N'*-methylenebisacrylamide (BIS, Tokyo Kasei, Figure 1). The concentrations of NIPAM were kept at 0.3, 0.7, and 1 M, respectively. The concentration of BIS was kept at a molar ratio of [NIPAM]/[BIS] = 79.8. The pregel solution, containing NIPAM, BIS, and 1.75 mM ammonium persulfate (initiator) in water, was stirred for about 2 min in an ice bath under nitrogen atmosphere. *N,N,N',N'*-Tetramethylethylenediamine (accelerator) was then added to give a concentration of 8 mM. After being stirred for about 1 min, the solutions were kept in micropipets (1.20 mm in diameter,  $l_0$ ) at 25 °C for more than 30 h. The undeuterated sample gels were rinsed with water, and the deuterated sample gels were rinsed with heavy water. After the rinse procedures, the undeuterated sample gels were immersed in water and the deuterated sample gels were immersed in heavy water. The isotopic purity of the amide proton of PNIPAM gel in heavy water was estimated to be around 95% by <sup>1</sup>H NMR measurement.

*2.1.3. Fluorescent-Labeled Monomer.* Dansylaminoethylacrylamide (DAEAM, Figure 1) was prepared by the procedure reported by Shea et al.<sup>50</sup> A solution of dansyl chloride (0.94 g, 3.5 mmol) in THF (75 mL) was dropwise added to the solution of ethylenediamine (2.4 mL, 35 mmol) in THF (150 mL) at 0 °C. The solution was stirred at 0 °C for 3 h, and 10 mL of 1 M KOH solution was added. The THF was evaporated, and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 × 100 mL). The organic layer was dried using MgSO<sub>4</sub> and evaporated. The residue was recrystallized from benzene/hexane (5/1, v/v) solution. An amount of 0.38 g (1.3 mmol) of dansylethylenediamine was obtained (37% yield). To a solution of dansylethylenediamine (0.2 g, 0.68 mmol) in THF (25 mL) at room temperature was added acryloyl chloride (0.07 mL, 0.85 mmol), triethylamine (0.1 mL, 0.71 mmol), and hydroquinone (0.1 g, 1 mmol). The solution was stirred at room temperature for around 12 h. The salts were then filtered and washed with THF. After the solvent was evaporated, water and CH<sub>2</sub>Cl<sub>2</sub> were added. The organic layer was dried using MgSO<sub>4</sub> and evaporated. The residue was chromatographed on silica with CH<sub>2</sub>Cl<sub>2</sub>/ether (4/1, v/v) as an eluent to provide DAEAM (0.11 g, 0.32 mmol, 47% yield). The <sup>1</sup>H NMR and IR spectra of the products (dansylethylenediamine and DAEAM) were the same as the result reported by Shea et al.<sup>50</sup>

*2.1.4. Fluorescent-Labeled Linear Poly(N-isopropylacrylamide).* The concentration of purified NIPAM was kept at 350 mM. A solution of DAEAM in DMF (6.7 mM) is added to the monomer solution. A monomer solution (water/DMF: 47/3, v/v) containing NIPAM, DAEAM (0.21 mM, [NIPAM]/[DAEAM] = 99.94/0.06), and 6.57 mM ammonium persulfate (initiator) was stirred for about 15 min in an ice bath under a nitrogen atmosphere. *N,N,N',N'*-Tetramethylethylenediamine (accelerator) was then added to give a concentration of 13.3 mM. The solution was stirred for about 20 h at ambient temperature. The polymer was purified by reprecipitation (first time, methanol (good solvent)/diethyl ether (poor solvent); second time, THF (good solvent)/hexane (poor solvent)). The linear polymer purified was

dried at 145 °C in a vacuum for more than 6 h. The molecular weight of the linear polymer,  $M$ , was estimated from the intrinsic viscosity,  $[\eta]$ , of the polymer in water, using the viscosity–molecular weight relationship:  $[\eta] = 2.26 \times 10^{-4} M^{0.97} \text{ cm}^3 \text{ g}^{-1}$ .<sup>49</sup> The value of  $M$  of the dansyl-group-labeled linear PNIPAM was  $1.8 \times 10^6$ .

**2.1.5. Fluorescent-Labeled Poly(*N*-isopropylacrylamide) Gel.** The fluorescent-labeled PNIPAM gels were synthesized from NIPAM, DAEAM, and BIS. The concentration of the monomer was kept at 0.7 M. The concentrations of the cross-linker were kept at a molar ratio of  $[\text{NIPAM}]/[\text{BIS}] = 79.8$ . A solution of DAEAM in DMF (6.7 mM) is added to the monomer solution. The monomer solution (water/DMF: 47/3, v/v) containing NIPAM, BIS, DAEAM (0.21 mM,  $[\text{NIPAM}]/[\text{DAEAM}] = 99.94/0.06$ ), and 1.75 mM ammonium persulfate (initiator) was stirred for about 2 min in an ice bath under a nitrogen atmosphere. *N,N,N',N'*-Tetramethylethylenediamine (accelerator) was then added to a concentration of 8 mM. After being stirred for about 1 min, the solutions in micropipets could be kept at 25 °C for more than 30 h. The gels crushed were rinsed with water or heavy water.

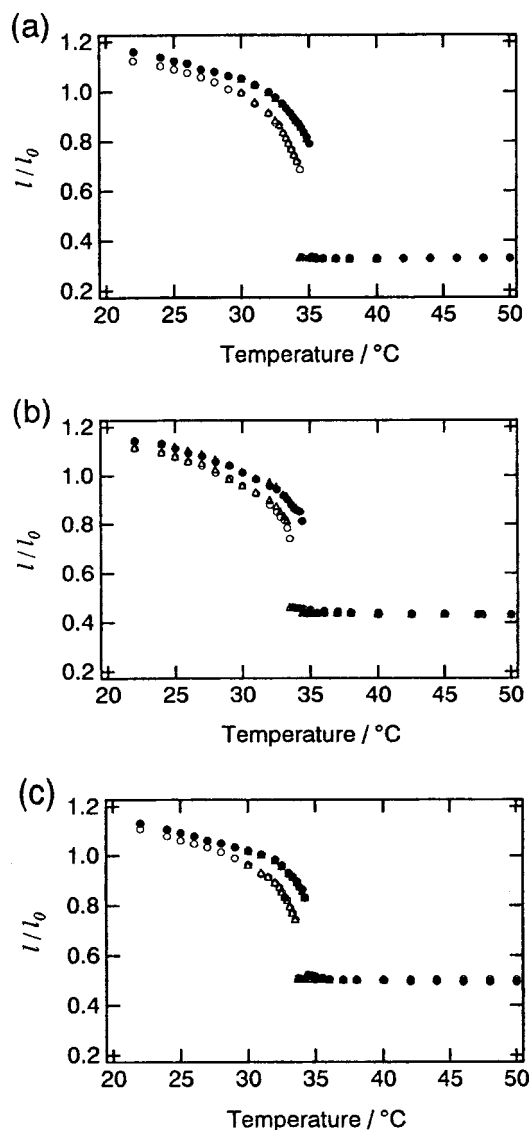
**2.2. Measurements of Cloud Point of Linear Polymer Solution and Degree of Swelling of Polymer Gel.** The cloud points of the linear polymer solutions were determined by transmittance measurements (Jasco, V-570 UV/vis/NIR spectrophotometer). The temperature of the sample solution was adjusted by a thermocontroller (Jasco, ETC-505T). The optical path length of the sample was 10 mm. The wavelength of the light for transmittance measurements was fixed at 500 nm. The diameter of the sample gels,  $l$ , was measured with a microscope (Olympus, BX50). The temperature of the sample gels in water or heavy water was regulated with a thermocontroller (Linkam, LK600PM). Both measurements were made after reaching thermal equilibrium.

**2.3. Fluorescence Measurements of Fluorescent-Labeled Polymer Gel and Linear Polymer.** The fluorescence spectra of the dansyl-group-labeled PNIPAM gels and the dansyl-group-labeled linear PNIPAM solutions were measured by a fluorescence spectrophotometer (Hitachi, 850). The temperature of the samples was adjusted by a thermocontroller (Jasco, ETC-272T). The optical path length of the sample was 10 mm. The excitation wavelength was fixed at 330 nm, where the dansyl group absorbs. The gel crushed was used for the fluorescence measurements. The fluorescence spectra of the samples were measured after reaching thermal equilibrium. The concentration of the linear PNIPAM solution was kept at 2 g/L.

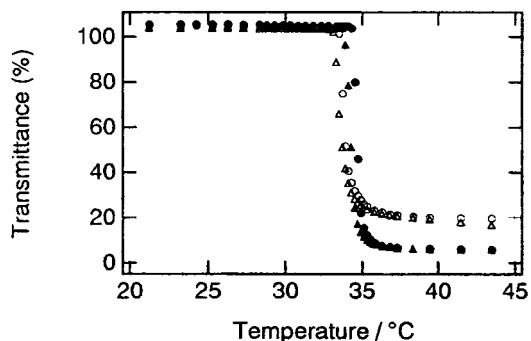
### 3. Results

**3.1. Bulk Change: Size of Gel and Transmittance of Polymer Solution.** Figure 2 shows the temperature dependence of the swelling ratio,  $l/l_0$ , for PNIPAM gel in water (open marks) and in heavy water (filled marks) with different initial monomer concentrations,  $C_0$  ((a)  $C_0 = 0.3 \text{ M}$ , (b)  $C_0 = 0.7 \text{ M}$ , and (c)  $C_0 = 1 \text{ M}$ ). The  $l$  is the diameter of the gel at each temperature, and  $l_0$  is the initial diameter of the gel (the inner diameter of a micropipet,  $l_0 = 1.2 \text{ mm}$ ). Circles show the heating process, and triangles show the cooling process. The transition temperatures of PNIPAM gel in water are around 34 °C as were reported by several groups.<sup>7–20</sup>

A comparison of the polymer gel with the linear polymer is relevant for a good understanding of the transition phenomena of PNIPAM because the volume phase transition of polymer gels and the coil–globule transition (and probably intermolecular aggregation) of linear polymers behave similarly.<sup>4</sup> Figure

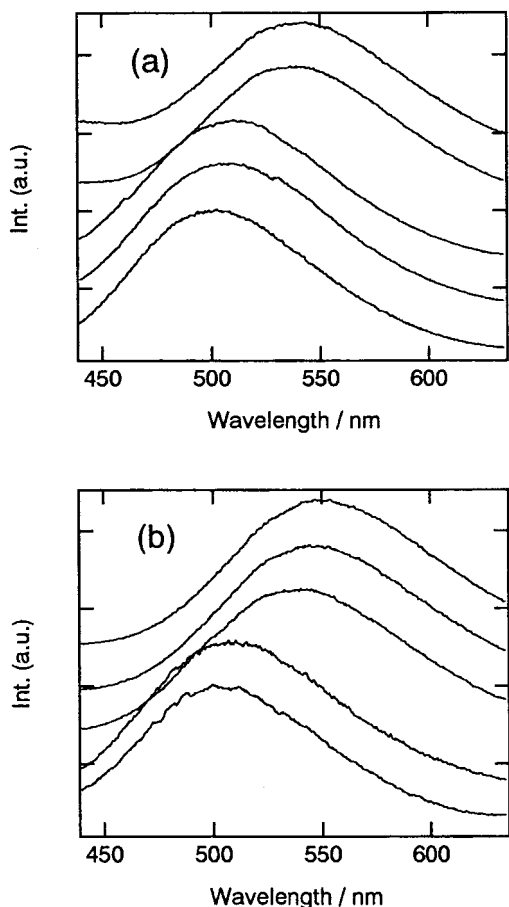


**Figure 2.** Temperature dependence of the swelling ratio,  $l/l_0$ , for PNIPAM gel in water (open symbols) and PNIPAM gel in heavy water (filled symbols) with different initial monomer concentrations ((a) 0.3 M, (b) 0.7 M, and (c) 1 M). Circles show the heating process, and triangles show the cooling process.



**Figure 3.** Temperature dependence of the transmittance for linear PNIPAM in water (open symbols) and linear PNIPAM in heavy water (filled symbols). Circles show the heating process, and triangles show the cooling process.

3 shows the temperature dependence of the transmittance for linear PNIPAM in water (open symbols) and heavy water (closed symbols) with 2 g/L of the polymer concentrations. Circles show the heating process, and triangles show the cooling process. The transition temperatures of linear PNIPAM in water



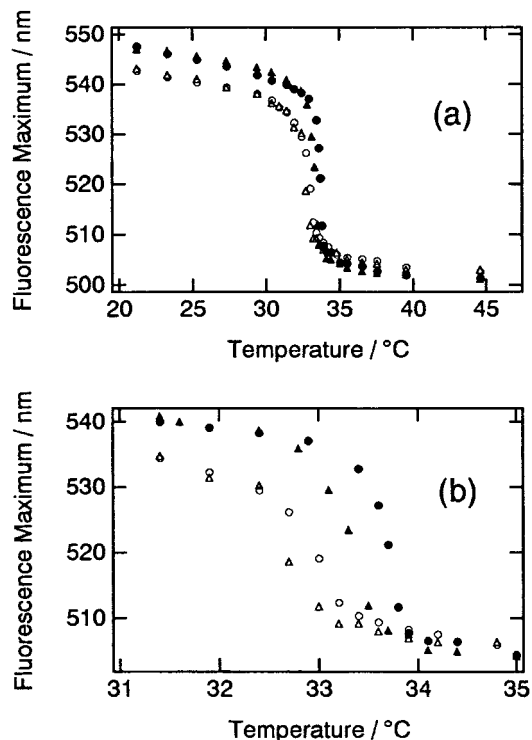
**Figure 4.** Fluorescence spectra of the dansyl-group-labeled PNIPAM gel in water (a) and heavy water (b) at several temperatures. The fluorescence spectra from the top are measured at 23.3, 29.4, 33.4, 34.4, and 44.6 °C. The wavelength of the excitation light is 330 nm.

are around 33–34 °C as were reported by several groups.<sup>21–28</sup> Different concentrations of linear PNIPAM solutions (0.5 g/L and 5 g/L) also showed similar behavior.

The notable points from Figures 2 and 3 are as follows. (i) Deuterium isotope effects on both the volume phase transition of PNIPAM gel and the phase separation of linear PNIPAM are clearly observed. (ii) The transition temperature of PNIPAM gel and the phase separation temperature of linear PNIPAM in heavy water are about 0.6–0.8 °C higher than those of PNIPAM gel and linear PNIPAM in water, as previously reported.<sup>15,20</sup> (iii) In the swollen state,  $I/I_0$  of PNIPAM gel in heavy water is larger than that of PNIPAM gel in water at each temperature. (iv) The magnitudes of the hysteresis of the transition temperatures (the difference between the transition temperatures in the heating process and the cooling process) are not affected or are only weakly affected by isotopic substitutions.

### 3.2. Microenvironment Change: Fluorescence Study.

Figure 4 shows the fluorescence spectra of the dansyl-group-labeled PNIPAM gel ( $C_0 = 0.7$  M) in water (a) and in heavy water (b) at several temperatures. The measurement temperatures of the fluorescence spectra in the figure from top to bottom are 23.3, 29.4, 33.4, 34.4, and 44.6 °C. These fluorescence spectra in the figure were measured in the heating process. It is clear from the figure that the transition temperature of PNIPAM gel in heavy water is higher than in water. Figure 5 compares the temperature dependence of the fluorescence wavelength maximum of the dansyl-group-labeled PNIPAM gel in water (open symbols) and heavy water (filled symbols). Circles express the heating process, and triangles express the cooling process.



**Figure 5.** Temperature dependence of the fluorescence maximum of the dansyl-group-labeled PNIPAM gel in water (open symbols) and heavy water (filled symbols). Circles show the heating process, and triangles show the cooling process. (a) is for the large range, and (b) is for the small range.

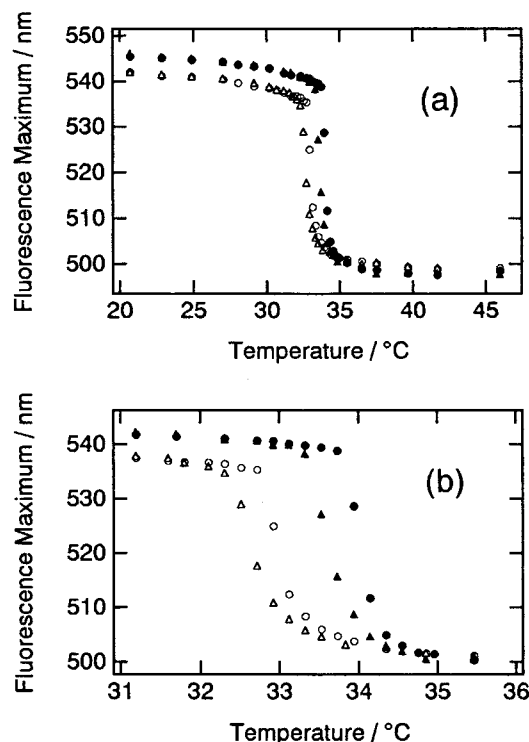
The important points to be noted from Figures 4 and 5 are as follows. (i) The transition temperature of PNIPAM gel in heavy water is about 0.8 °C higher than that in water. (ii) In the swollen state (<32 °C), the wavelength of the fluorescence maximum of the dansyl-group-labeled PNIPAM gel in heavy water is longer than that in water. (iii) In the shrunken state (>35 °C), the wavelengths of the fluorescence maximum of dansyl-group-labeled PNIPAM gel in water and heavy water are identical within experimental error. (iv) The transition temperature of the gel in the heating process is about 0.3 °C higher than that in the cooling process. (v) The magnitudes of the hysteresis of the transition temperatures (the difference between the transition temperatures in the heating process and in the cooling process) in water and heavy water are almost same.

To see the generality of the deuterium isotope effect on the transition of PNIPAM, Figure 6 shows the temperature dependence of the dansyl-group-labeled linear PNIPAM in water (open symbols) and in heavy water (filled symbols). Circles show the heating process, and triangles show the cooling process. The qualitative features of the deuterium isotope effects of the microenvironments in the volume phase transition of PNIPAM gel (Figure 5) and in the phase separation of linear PNIPAM solution (Figure 6) are quite similar.

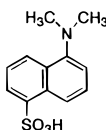
### 4. Property of Fluorescence Probe in Water and Heavy Water

The dansyl group has been widely used as a fluorescence probe to study the microenvironments of the conformational transitions of proteins,<sup>51,52</sup> polypeptides,<sup>53–55</sup> chemical polymers,<sup>56,57</sup> and polymer gels<sup>58–63</sup> because the dansyl group is very solvatochromic. When the polarity of the solvent increases, the fluorescence maximum of the dansyl group shifts to longer





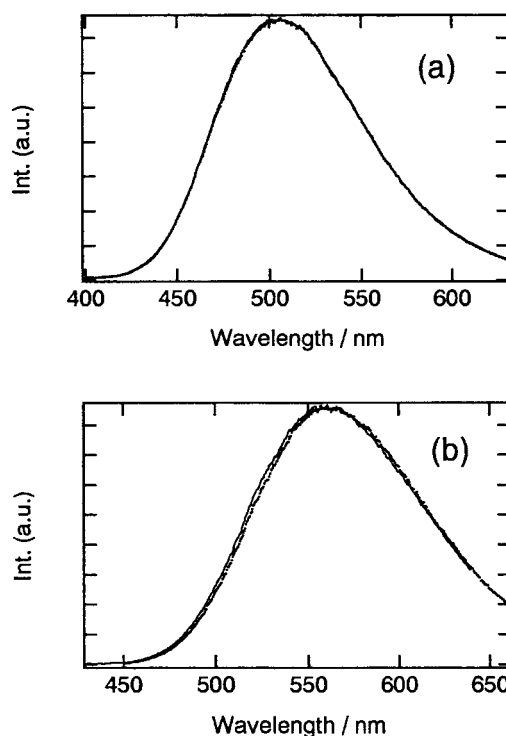
**Figure 6.** Temperature dependence of the fluorescence maximum of the dansyl-group-labeled linear PNIPAM in water (open symbols) and heavy water (filled symbols). Circles show the heating process, and triangles show the cooling process. (a) is for the large range, and (b) is for the small range.



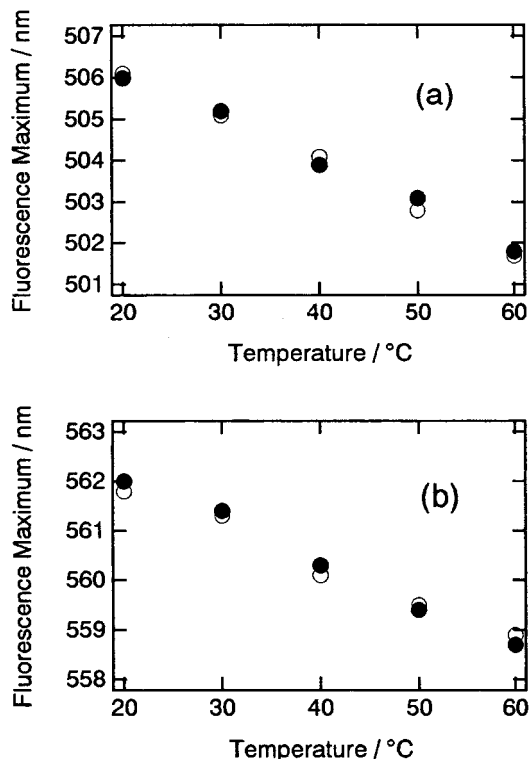
**Figure 7.** Chemical structure of dansyl acid.

wavelengths.<sup>64</sup> In this work, the dansyl group is used to study the deuterium isotope effects on the microenvironments of the volume phase transition of PNIPAM gel and the phase separation of linear PNIPAM solution. It is very important to compare the characteristics of the fluorescence of dansyl group in water and heavy water in order to discuss the deuterium isotope effect on the transitions of the polymer.

Dansyl acid (Figure 7) and DAEAM (Figure 1) were used to characterize the fluorescence spectra in undeuterated hydrogen-bonding liquid and deuterated hydrogen-bonding liquid. Since DAEAM is insoluble in water and heavy water, methanol/water and methanol-OD/heavy water mixtures (2/98, v/v) were used as solvents. Water and heavy water are used as solvents for the fluorescence measurements of dansyl acid because dansyl acid is soluble in water and heavy water. The wavelength of the excitation light was fixed at 330 nm where dansyl acid and DAEAM both absorb. Figure 8a shows the fluorescence spectra of dansyl acid in water (solid line) and heavy water (dotted line) at 20 °C, and Figure 8b shows the fluorescence spectra of DAEAM in the methanol/water mixture (solid line) and the methanol-OD/heavy water mixture (dotted line) at 20 °C. It is clear from Figure 8 that the fluorescence spectra of dansyl acid and DAEAM in normal hydrogen-bonding solvents and in deuterated hydrogen-bonding solvents are almost identical. The fluorescence probe used in the present study is insensitive to isotopic substitutions of hydrogen-bonding liquids. The fluorescence spectrum of another solvatochromic molecule (cou-



**Figure 8.** (a) Fluorescence spectra of dansyl acid in water (solid line) and heavy water (dotted line) and (b) DAEAM in methanol/water (solid line) and methanol-OD/heavy water (dotted line) mixtures (2/98, v/v) at 20 °C. The wavelength of the excitation light is 330 nm.



**Figure 9.** (a) Temperature dependence of the fluorescence maximum of dansyl acid in water (open circles) and heavy water (filled circles) and (b) DAEAM in methanol/water (open circles) and methanol-OD/heavy water (filled circles).

marin 153) in hydrogen-bonding liquid also shows the absence of a deuterium isotope effect.<sup>65</sup>

Figure 9 shows the temperature dependence of the fluorescence maxima of dansyl acid in water (open circles) and heavy water (filled circles) (a) and DAEAM in a methanol/water

mixture (open circles) and a methanol-OD/heavy water mixture (filled circles) (b). The small blue shifts of the fluorescence maximum of dansyl acid and DAEAM with increasing temperature are monotonic (no discontinuous change), and the temperature dependence of the fluorescence maxima of dansyl acid and DAEAM in normal and deuterated hydrogen-bonding solvents is quite similar. The experimental results of the fluorescence measurements of the dansyl-group-labeled PNIPAM gel (Figure 5) and linear dansyl-group-labeled PNIPAM solution (Figure 6) reflect the deuterium isotope effect of the micro-environments in the conformational change of the polymer network in the volume phase transition of the polymer gel and the phase separation of the linear polymer solution.

### 5. Simulation of Temperature Dependence of Swelling Ratio of PNIPAM Gel: Modified Flory–Rehner Model

Recently, Shibayama et al. reproduced the experimental results of the temperature dependence of the volume of PNIPAM gel in water using a simple modified Flory–Rehner model.<sup>19</sup> In their modified model, the initial monomer concentration dependence of the effective entanglements of a polymer network is added to the original Flory–Rehner model. This model reproduced the swelling–shrinking curve of PNIPAM gel in the swollen state. Although the model is very simple, we used this model to simply estimate the origin of the deuterium isotope effect on the volume phase transition of PNIPAM gel.<sup>20</sup> In this section, we briefly introduce the theoretical background of the model.

When a neutral polymer gel is in swelling equilibrium, the net osmotic pressure,  $\Pi$ , becomes zero. According to the original Flory–Rehner theory,<sup>66–68</sup>  $\Pi$  consists of the mixing free energy and the elastic free energy.

$$\Pi = -\frac{k_B T}{V_s} [\phi + \ln(1 - \phi) + \chi \phi^2] + \nu k_B T \left[ \frac{1}{2} \left( \frac{\phi}{\phi_0} \right) - \left( \frac{\phi}{\phi_0} \right)^{1/3} \right] \quad (1)$$

where  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature,  $V_s$  is the molar volume of the solvent,  $\nu$  is the number of cross-links per unit volume,  $\chi$  is Flory's interaction parameter, and  $\phi$  and  $\phi_0$  are the network volume fractions at swelling equilibrium and the reference states, respectively. Although the concentration-dependent  $\chi$  parameter is very complex,<sup>13,69–71</sup> for simplicity it is assumed to be linearly dependent on concentration and can be expressed by<sup>19,72</sup>

$$\chi = \chi_1 + \phi \chi_2 \quad (2)$$

where

$$\chi_1 = \frac{\Delta H - T \Delta S}{k_B T} \quad (3)$$

and  $\chi_2$  is a constant.  $\Delta H$  and  $\Delta S$  are the enthalpy and entropy per monomeric unit of the network related to the volume phase transition.

In the modified Flory–Rehner model proposed by Shibayama et al.,  $\nu$  is replaced by<sup>19</sup>

$$\nu \frac{C_0}{C_{0,\text{ref}}} \quad (4)$$

where  $C_{0,\text{ref}}$  is the lowest initial monomer concentration at which a uniform gel can be formed. According to their experimental result, the  $C_{0,\text{ref}}$  of NIPAM is 0.3 M.<sup>19</sup> In the case of the lower

initial monomer concentration of the monomer, the polymer gel expands in the swollen state because of the dilution of the monomer concentration. We assume that the number of effective cross-links is proportional to the initial monomer concentration. Thus, eq 1 can be rewritten as

$$-\frac{1}{V_s} [\phi + \ln(1 - \phi) + \chi \phi^2] + \nu \frac{C_0}{C_{0,\text{ref}}} \left[ \frac{1}{2} \left( \frac{\phi}{\phi_0} \right) - \left( \frac{\phi}{\phi_0} \right)^{1/3} \right] = 0 \quad (5)$$

To reproduce the swelling–shrinking curves of PNIPAM gels, the parameters in eq 5 are determined in the following way.<sup>19</sup>  $\phi_0$  is defined as

$$\phi_0 = V_{\text{NIPAM}} C_0 \quad (6)$$

where  $V_{\text{NIPAM}}$  is the molar volume of the NIPAM monomeric unit (0.103 L/mol).<sup>73</sup> The  $\nu$  is fixed to be the stoichiometric value.

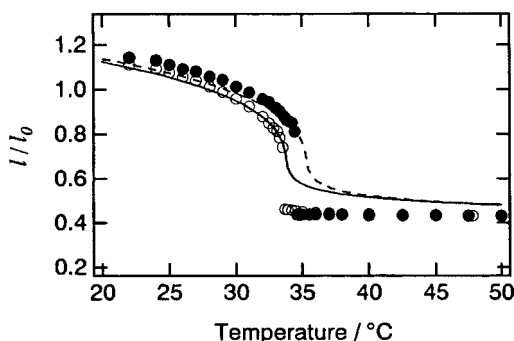
$$\nu = \frac{2C_{\text{BIS}}}{C_0} \frac{\phi_0}{V_{\text{NIPAM}}} = 2C_{\text{BIS}} \quad (7)$$

where  $C_{\text{BIS}}$  is the concentration of the cross-linker (BIS). Here, it is assumed that the molar volume of BIS is the same as that of NIPAM. The  $V_s$  values of water and heavy water at 20 °C are 18.05 and 18.12 mL/mol, respectively.<sup>74</sup> Though the densities of water and heavy water change with a change of temperature, the swelling–shrinking curve is not largely affected by the different values of the densities in the experimental range (22–50 °C). We neglect the effect of the temperature dependence of the water and heavy water densities.

According to Hirotsu, the values of the fit parameters consisting of  $\chi$  in PNIPAM in water are  $\chi_2 = 0.518$ ,  $\Delta H = -1.246 \times 10^{-20}$  J, and  $\Delta S = -4.717 \times 10^{-23}$  J/K.<sup>14</sup> The values of the parameters for PNIPAM gel in heavy water are estimated as  $\chi_2 = 0.518$ ,  $\Delta H = -1.252 \times 10^{-20}$  J, and  $\Delta S = -4.717 \times 10^{-23}$  J/K.<sup>20</sup> These values of the parameters consisting of  $\chi$  are used in the present study. The theoretical result based on this model will be shown in section 6.1.

## 6. Discussion

**6.1. Deuterium Isotope Effect on Phase State of PNIPAM Gel.** As shown in Figures 2–5, the transition temperatures of the volume phase transition and phase separation of PNIPAM in heavy water are 0.6–0.8 °C higher than those in water. In some transitions of aqueous macromolecules (protein, polypeptide, and cellulose), the deuterium isotope effects on the transition temperature were reported.<sup>75–79</sup> The higher transition temperatures in the unfolding process of some proteins in heavy water compared with that in water were observed.<sup>75,76</sup> In polypeptide<sup>77</sup> and cellulose<sup>78,79</sup> having a LCST feature, lower transition temperatures in heavy water were observed in comparison with water. These results were well explained by the analogy of the different magnitudes of the hydrophobic interaction in water and heavy water. Namely, if the polymer solution has a UCST feature, the transition temperature in heavy water is higher than that in water, and if the polymer in solution has a LCST feature, the transition temperature in heavy water is lower than that in water. The results reported previously<sup>72–76</sup> are the opposite of the deuterium isotope effect observed in PNIPAM systems. Although the contribution of a hydrophobic interaction may exist, this effect should not be a dominant factor for the deuterium isotope effect on the transition temperatures



**Figure 10.** Comparison of the swelling–shrinking curves of PNIPAM gel with  $C_0 = 0.7$  M obtained by the experiment (symbols) and predicted by the modified Flory–Rehner model (lines). Open circles and solid line show PNIPAM gel in water, and filled circles and broken line show PNIPAM gel in heavy water.

of the volume phase transition of PNIPAM gel and the phase separation of linear PNIPAM solution.

Van Hook and co-workers discussed the deuterium isotope effect of the polymer solutions using the Bigeleisen equation that connects the transfer free energy isotope effects with molecular properties.<sup>80–85</sup>

$$\frac{\delta\Delta\mu_0}{RT} = \frac{A}{T^2} + \frac{B}{T} \quad (8)$$

$$A = \frac{1}{24} \left( \frac{hc}{k_B} \right)^2 \sum_i [(\nu_{H,i}^2 - \nu_{D,i}^2)_\infty - (\nu_{H,i}^2 - \nu_{D,i}^2)_0] \quad (9)$$

$$B = \frac{1}{2} \left( \frac{hc}{k_B} \right) \sum_j [(\nu_{H,j} - \nu_{D,j})_\infty - (\nu_{H,j} - \nu_{D,j})_0] \quad (10)$$

where  $\delta\Delta\mu_0 = (\mu_\infty - \mu_0)_H - (\mu_\infty - \mu_0)_D$ . The subscript  $\infty$  refers to infinite dilution and the subscript 0 to the pure reference state.  $h$  is the Planck constant,  $c$  is the velocity of the light, and  $k_B$  is the Boltzmann constant. The  $A$  term represents contributions from low-frequency modes (intermolecular modes), and the  $B$  term represents contribution from high-frequency modes (intramolecular modes). In the present system, it is necessary to consider the hydroxyl group (solvent) and amide group (polymer):<sup>85</sup>

$$\frac{\delta\Delta\mu_0}{RT} = 2 \left( \frac{B_{OH}}{T} \right) - \frac{B_{NH}}{T} + 4 \left( \frac{A_{OH}}{T^2} \right) - 2 \left( \frac{A_{NH}}{T^2} \right) \quad (11)$$

For simplicity, we assume uniform intermolecular and intramolecular modes in the aqueous PNIPAM system. By use of the values of the stretching frequencies of hydroxyl groups ( $\nu_H^0 \approx 3350$   $\text{cm}^{-1}$ ,  $\nu_D^0 \approx 2600$   $\text{cm}^{-1}$ ,  $\nu_H^\infty \approx 3450$   $\text{cm}^{-1}$ , and  $\nu_D^\infty \approx 2690$   $\text{cm}^{-1}$ ), the librational frequencies of hydrogen bonds ( $\nu_{Hlib}^0 \approx 650$   $\text{cm}^{-1}$ ,  $\nu_{Dlib}^0 \approx 460$   $\text{cm}^{-1}$ ,  $\nu_{Hlib}^\infty \approx 250$   $\text{cm}^{-1}$ , and  $\nu_{Dlib}^\infty \approx 180$   $\text{cm}^{-1}$ ), and the effective masses, we find that  $A = -3.13 \times 10^4$   $\text{K}^2$  and  $B = 7.2$   $\text{K}$ .<sup>44,85</sup> From this crude estimate, the deuterium isotope effect in PNIPAM systems should arise from the intermolecular contribution. The different features in the deuterium isotope effects on the transitions of the biopolymers and PNIPAM may arise from the different magnitudes of the solvent–polymer interaction parameters.

Figure 10 shows a comparison of the swelling–shrinking curves of PNIPAM gel prepared by the  $C_0 = 0.7$  M condition in water and heavy water predicted by the modified Flory–Rehner theory (water, solid line; heavy water, broken line) and

obtained by experiment (water, open circles; heavy water, filled circles). Only the experimental results from the heating process are shown in the figure. The simulation result was obtained using eq 5. As reported previously,<sup>20</sup> the deuterium isotope effect in the swelling–shrinking curve of PNIPAM gel obtained by the experiment is well reproduced by the simple modified Flory–Rehner model. Namely, the transition temperature of PNIPAM gel in heavy water is higher than in water, and the size at the swollen state of PNIPAM gel in heavy water is larger than that in water.

From a comparison between the experimental result and the theoretical prediction, the deuterium isotope effect in the swelling–shrinking curve in PNIPAM gel should arise from  $\Delta H$  including  $\chi$ . The value of  $\Delta H$  of PNIPAM gel in water is  $-1.246 \times 10^{-20}$  J and that of PNIPAM gel in heavy water is  $-1.252 \times 10^{-20}$  J. In PNIPAM systems, the solvent–polymer interaction becomes stronger because of the stabilized hydrogen-bonding network by isotopic substitution.

## 6.2. Comparison between Bulk and Microenvironment.

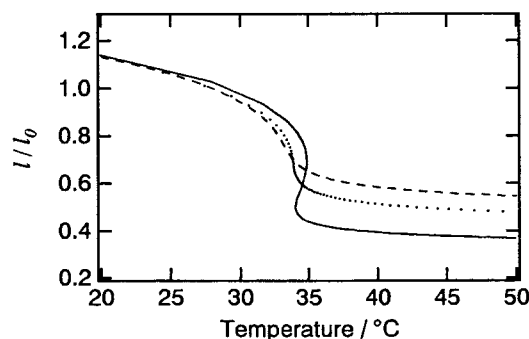
It is interesting to compare the deuterium isotope effects of the macroscopic features (Figures 2 and 3) and the microenvironment features (Figures 5 and 6) in the transitions of PNIPAM. For lack of information about the transmittance of the linear PNIPAM in the low-temperature region ( $<30$   $^\circ\text{C}$ ), it is difficult to directly compare the microscopic and the macroscopic observations of the phase separation of linear PNIPAM solution. However, it is clear from the comparisons of the bulk changes and the microenvironment changes that (i) the overall features of the deuterium isotope effects in both macroscopic and microscopic observations are qualitatively similar (see the difference in the transition temperatures in water and heavy water and the features in both the low-temperature region ( $<30$   $^\circ\text{C}$ ) and the high-temperature region ( $>35$   $^\circ\text{C}$ ) in water and heavy water) and (ii) the transition temperatures for the macroscopic observable are about  $0.5$   $^\circ\text{C}$  higher than those for the microscopic observable.

As mentioned in section 4, the fluorescence spectra of the dansyl group in water and heavy water are identical. The difference between the fluorescence spectra of the dansyl-group-labeled PNIPAM in water and heavy water at the low-temperature region ( $<30$   $^\circ\text{C}$ ) (Figures 5 and 6) occurs because of the difference of the microenvironments of the polymer network in water and heavy water. Because the longer wavelength of the fluorescence maximum of the dansyl group occurs for higher polarity media, the present results indicate that the medium of PNIPAM in heavy water at the low-temperature region ( $<30$   $^\circ\text{C}$ ) is more polar than that in water. The polarity of the PNIPAM chain should be lower than that of water. Although the information observed in the microenvironment and the bulk is different, the results of the microenvironment in PNIPAM are somewhat correlated with the bulk results.<sup>86</sup>

The small disagreement of the transition temperatures in the bulk change and the microenvironment change is not unreasonable. This is because the microenvironment change is the conformational changes of the functional group and the main chain of polymer network, and the bulk change is the overall change of the polymer gel. However, the deuterium isotope effects on the volume phase transition of the gel and the phase separation of the linear polymer solution in both the bulk change and the microenvironment change are quite similar. Namely, the deuterium isotope effect on the bulk change of the whole system might be reflected by the deuterium isotope effect on the microenvironment change of the polymer network.

Another interesting point in comparing the macroscopic and





**Figure 11.** Simulated swelling–shrinking curves of PNIPAM gel with different initial monomer concentrations,  $C_0$ , in water. Broken line shows  $C_0 = 1$  M, dotted line shows  $C_0 = 0.7$  M, and solid line shows  $C_0 = 0.3$  M.

microscopic pictures is the discontinuous feature in the volume phase transition. Although the bulk change of the PNIPAM gel shows a discontinuous change (Figure 2), the microscopic environment of the PNIPAM gel shows a continuous change and does not show any critical behavior (Figure 5). Although the discontinuous feature in the microenvironment may be hidden by the inhomogeneous broadening of the fluorescence spectrum, this possibility should be less. This is because the remarkable change of the width of the fluorescence spectrum was not observed. Since the probe used in the present work is sensitive to the polarity of the medium, the polarity in the microenvironment of the polymer network should change gradually.

**6.3. Difference of Transition Temperatures in Heating Process and Cooling Process: Hysteresis of Transitions.** The hysteresis of the volume phase transition (the difference of the transition temperatures in the heating process and the cooling process) of the PNIPAM gel was observed by several groups.<sup>7,12</sup> Hirokawa and Tanaka observed the hysteresis of 0.2 °C in the volume phase transition of PNIPAM gel in water.<sup>7</sup> The magnitude of the hysteresis reported by Hirokawa and Tanaka is in good agreement with the results in the present study. The discontinuous feature of the volume phase transition of the polymer gel mainly arises from the polymer–solvent interaction parameter. Otake et al. proposed the importance of the hydrophobic interaction for the discontinuous feature of the volume phase transition of hydrogels.<sup>87</sup> Sasaki and Maeda presented a simple theory for the volume phase transition of hydrated gels.<sup>88</sup> The uniqueness of their theory is the inclusion of the coupling effect of the volume change of hydrogels and the dehydration (and hydration) of the polymer network. They also suggested that the degree of the swelling of the PNIPAM gel is a function of the degree of hydration, which is regulated by the chemical potential of water molecules.<sup>89</sup> In the present experimental results, however, the magnitudes of the hysteresis of the transition temperatures of PNIPAM in water and heavy water are almost same.

In the simple Flory–Rehner model, the discontinuous feature is also sensitive to the initial monomer concentration  $C_0$ . Figure 11 shows the simulated  $C_0$  dependence of the swelling–shrinking curve of PNIPAM gel (broken line shows  $C_0 = 1$  M, dotted line shows  $C_0 = 0.7$  M, and solid line shows  $C_0 = 0.3$  M). The smaller  $C_0$  gel shows the larger discontinuous feature and the larger magnitude of the hysteresis in the volume phase transition. The experimental result (Figure 2) and the theoretical result are qualitatively similar. No hysteresis of  $C_0 = 1$  M PNIPAM gel in water and heavy water was observed (Figure 2c), and the hysteresis of 0.2 °C was observed in  $C_0 = 0.3$  M and  $C_0 = 0.7$  M PNIPAM gels in water and heavy water.

However, the magnitude of the hysteresis of  $C_0 = 0.3$  M PNIPAM gel is not as large as the result of the theoretical prediction. The quantitative disagreement between the experimental result and the theoretical prediction may arise from the imperfect description of the shrunken state of the theoretical model. Previously, we showed the deuterium isotope effect on the swelling–shrinking curve of PNIPAM gels with  $C_0 = 1$  M and  $C_0 = 0.3$  M predicted by the simple Flory–Rehner model.<sup>20</sup> Interestingly, the magnitude of the hysteresis in the transition was not affected or was weakly affected by the change of  $\Delta H$  consisting  $\chi$ . Both the experimental result and the theoretical prediction show that though the transition temperature of PNIPAM gel is very sensitive to isotopic substitution, the magnitude of the hysteresis is insensitive to isotopic substitution.

## 7. Conclusions

We have investigated the deuterium isotope effects on the volume phase transition of poly(*N*-isopropylacrylamide) (PNIPAM) gel and on the phase separation of linear PNIPAM in solution. The transition temperatures of PNIPAM gel and linear PNIPAM in heavy water are about 0.7 °C higher than those in water. To compare the macroscopic picture of the deuterium isotope effect on the transitions of PNIPAM, we have also investigated the microenvironment picture of the deuterium isotope effect on the transitions of PNIPAM using a fluorescence probe, DAEAM. The features of the deuterium isotope effects in the macroscopic picture and the microenvironment picture of the transitions of PNIPAM are quite similar. From a comparison with the simulation based on a simple Flory–Rehner model, the origin of the deuterium isotope effect on the transitions of PNIPAM should arise from the enthalpy of the polymer–solvent interaction. The polymer–solvent interaction of PNIPAM in the heavy water system may be stronger than that of PNIPAM in the water system because of the more stable hydrogen-bonding interactions in heavy water compared to those in water. The deuterium isotope effect of the polymer–solvent interaction also seems to affect the size of the PNIPAM gel at the equilibrium swollen state. However, the difference in the transition temperatures in the heating process and the cooling process is not affected by isotopic substitutions of the hydrogen-bonding network.

**Acknowledgment.** We thank Ms. Noriko Endo of University of Tokyo for helpful discussion and valuable advice. We are grateful to Mr. Tokiji Kawamura of University of Tokyo for his kind help in <sup>1</sup>H NMR measurements. We are also grateful to Mr. Jionghao He of University of Tokyo for his kind help in the characterization of the linear polymers. Further, we thank Prof. Edward W. Castner, Jr. of Rutgers University for a critical reading this manuscript. This work is partly supported by a Grant-in-Aid for Scientific Research (No. 08405060) from Ministry of Education, Science, Sports and Culture of Japan.

## References and Notes

- (1) Dušek, K., Ed. *Responsive Gels: Volume Transitions I*; Advances in Polymer Science 109; Springer-Verlag: Berlin Heidelberg, 1993.
- (2) Dušek, K., Ed. *Responsive Gels: Volume Transitions II*; Advances in Polymer Science 110; Springer-Verlag: Berlin Heidelberg, 1993.
- (3) Lifshitz, I. M.; Grosberg, A. Y.; Khokhlov, A. R. *Rev. Mod. Phys.* **1978**, *50*, 683.
- (4) Dušek, K.; Patterson, D. J. *Polym. Sci., A-2* **1968**, *6*, 1209.
- (5) Ptitsyn, O. B.; Kron, A. K.; Eizner, Y. Y. *J. Polym. Sci., Part C: Polym. Symp.* **1968**, *16*, 3509.
- (6) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820.
- (7) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.
- (8) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392.



- (9) Hirotsu, S. *J. Phys. Soc. Jpn.* **1987**, *56*, 233.
- (10) Hirotsu, S. *J. Chem. Phys.* **1988**, *88*, 427.
- (11) Li, Y.; Tanaka, T. *J. Chem. Phys.* **1989**, *90*, 5161.
- (12) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283.
- (13) Inomata, H.; Goto, S.; Saito, S. *Macromolecules* **1990**, *23*, 4887.
- (14) Hirotsu, S. *J. Chem. Phys.* **1991**, *94*, 3949.
- (15) Shibayama, M.; Tanaka, T.; Han, C. C. *J. Chem. Phys.* **1992**, *97*, 6829.
- (16) Shibayama, M.; Morimoto, M.; S. Nomura, S. *Macromolecules* **1994**, *27*, 5060.
- (17) Shibayama, M.; Mizutani, S.; Nomura, S. *Macromolecules* **1996**, *29*, 2019.
- (18) Shibayama, M.; Norisuye, T.; Nomura, S. *Macromolecules* **1996**, *29*, 8746.
- (19) Shibayama, M.; Shirota, H.; Hirose, H.; Nomura, S. *Macromolecules* **1997**, *30*, 7307.
- (20) Shirota, H.; Endo, N.; Horie, K. *Chem. Phys.* **1998**, *238*, 487.
- (21) Eliasaff, J.; Silberberg, A. *J. Polym. Sci.* **1959**, *41*, 33.
- (22) Heskins, M.; Guillet, J. E. *J. Macromol. Sci., Chem.* **1968**, *A2*, 1441.
- (23) Yamato, I.; Iwasaki, K.; Hirotsu, S. *J. Phys. Soc. Jpn.* **1989**, *58*, 210.
- (24) Winnik, F. C.; Ringsdorf, H.; Venzmer, J. *Macromolecules* **1990**, *23*, 2415.
- (25) Binkert, T.; Oberreich, J.; Meewes, N.; Nyffenegger, R.; Ricka, J. *Macromolecules* **1991**, *24*, 5806.
- (26) Meewes, M.; J. Ricka, J.; de Silva, M.; Nyffenegger, R. *Macromolecules* **1991**, *24*, 5811.
- (27) Kubota, K.; Fujishige, S.; Ando, I. *J. Phys. Chem.* **1991**, *94*, 5154.
- (28) Wang, X.; Qie, X.; Wu, C. *Macromolecules* **1998**, *31*, 2972.
- (29) Shibayama, M.; Tanaka, T. *Adv. Polym. Sci.* **1993**, *109*, 1.
- (30) Hirotsu, S. *Adv. Polym. Sci.* **1993**, *110*, 1.
- (31) Tamai, Y.; Tanaka, H.; Nakanishi, K. *Mol. Simul.* **1996**, *16*, 359.
- (32) Tamai, Y.; Tanaka, H.; Nakanishi, K. *Macromolecules* **1996**, *29*, 6750.
- (33) Tamai, Y.; Tanaka, H.; Nakanishi, K. *Macromolecules* **1996**, *29*, 6761.
- (34) Müller-Plathe, F.; van Gunsteren, W. F. *Polymer* **1997**, *38*, 2259.
- (35) Müller-Plathe, F. *J. Chem. Phys.* **1998**, *108*, 8252.
- (36) Netz, P. A.; Dorfüller, T. *J. Phys. Chem. B* **1998**, *102*, 4875.
- (37) Terada, T.; Maeda, Y.; Kitano, H. *J. Phys. Chem.* **1993**, *97*, 3619.
- (38) Maeda, Y.; Tsukida, N.; Kitano, H.; Terada, T.; Yamanaka, J. *J. Phys. Chem.* **1993**, *97*, 13903.
- (39) Tsukida, N.; Muranaka, H.; Ide, M.; Maeda, Y.; Kitano, H. *J. Phys. Chem. B* **1997**, *101*, 6676.
- (40) Kuntz, I. D., Jr.; Kauzmann, W. *Adv. Protein Chem.* **1974**, *28*, 239.
- (41) Saenger, W. *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 93.
- (42) Teeter, M. M. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 577.
- (43) Pethig, R. *Annu. Rev. Phys. Chem.* **1992**, *43*, 177.
- (44) Némethy, G.; Scheraga, H. A. *J. Chem. Phys.* **1964**, *41*, 680.
- (45) Guillot, B.; Guissani, Y. *J. Chem. Phys.* **1998**, *108*, 10162.
- (46) Svishchev, I. M.; Kusalik, P. G. *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 1405.
- (47) Sabo, D.; Bacic, Z.; Graf, S.; Leutwyler, S. *J. Chem. Phys.* **1999**, *110*, 5745.
- (48) Shirota, H.; Horie, K. *Chem. Phys.* **1999**, *242*, 115.
- (49) Chiantore, O.; Guaita, M.; Trossarelli, L. *Makromol. Chem.* **1979**, *180*, 169.
- (50) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *23*, 4497.
- (51) Weber, G. *Biochem. J.* **1952**, *51*, 155.
- (52) Klotz, I. M.; Fiess, H. F. *Biochim. Biophys. Acta* **1960**, *38*, 57.
- (53) Iio, T.; Iwashita, Y.; Watanabe, H. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2206.
- (54) Iio, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 335.
- (55) Torii, T.; Yamashita, T.; Horie, K. *Biopolymers* **1994**, *34*, 101.
- (56) Strauss, U. P.; Vesnaver, G. *J. Phys. Chem.* **1975**, *79*, 1558.
- (57) Strauss, U. P.; Vesnaver, G. *J. Phys. Chem.* **1975**, *79*, 2426.
- (58) Hu, Y.; Horie, K.; Ushiki, H. *Macromolecules* **1992**, *25*, 6040.
- (59) Hu, Y.; Horie, K.; Ushiki, H.; Tsunomori, F.; Yamashita, T. *Macromolecules* **1992**, *25*, 7324.
- (60) Hu, Y.; Horie, K.; Ushiki, H. *Polym. J.* **1993**, *25*, 651.
- (61) Asano, M.; Yamashita, T.; Horie, K. *Polym. Gels. Networks* **1995**, *3*, 281.
- (62) Asano, M.; Winnik, F. W.; Yamashita, T.; Horie, K. *Macromolecules* **1995**, *28*, 5861.
- (63) Horie, K.; Kuriyama, M. *Rep. Prog. Polym. Jpn.* **1997**, *40*, 159.
- (64) Li, Y.-H.; Chan, L.-M.; Tyler, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118.
- (65) Pal, H.; Nagasawa, Y.; Tominaga, K.; Yoshihara, K. *J. Phys. Chem.* **1996**, *100*, 11964.
- (66) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
- (67) Flory, P. J.; Rehner, J., Jr. *J. Chem. Phys.* **1943**, *11*, 521.
- (68) Flory, P. J. *J. Chem. Phys.* **1950**, *18*, 108.
- (69) Moerkerke, R.; Koningsveld, R.; Berghmans, H.; Dušek, K.; Šolc, K. *Macromolecules* **1995**, *28*, 1103.
- (70) Schäfer-Soenen, H.; Moerkerke, R.; Berghmans, H.; Koningsveld, R.; Dušek, K.; Šolc, K. *Macromolecules* **1997**, *30*, 410.
- (71) Moerkerke, R.; Meeussen, F.; Koningsveld, R.; Berghmans, H.; Mondelaers, W.; Schacht, E.; Dušek, K.; Šolc, K. *Macromolecules* **1998**, *31*, 2223.
- (72) Erman, B.; Flory, P. J. *Macromolecules* **1986**, *19*, 2342.
- (73) *Physical Properties of Polymers Handbook*; American Institute of Physics: Woodbury, NY, 1995.
- (74) *CRC Handbook of Chemistry and Physics*, 74th ed.; CRC Press: Boca Raton, FL, 1993.
- (75) Makhatadze, G. I.; Clore, G. M.; Gronenborn, A. M. *Nat. Struct. Biol.* **1995**, *2*, 852.
- (76) Guzzi, R.; Sportelli, L.; Rosa, C. L.; Milardi, D.; Grasso, D. *J. Phys. Chem. B* **1998**, *102*, 1021.
- (77) Luan, C.; Urry, D. W. *J. Phys. Chem.* **1991**, *95*, 7896.
- (78) Winnik, F. W. *Macromolecules* **1991**, *20*, 2745.
- (79) Winnik, F. W. *J. Phys. Chem.* **1989**, *93*, 7452.
- (80) Jancso, G.; Rebelo, L. P. N.; Van Hook, W. A. *Chem. Rev.* **1993**, *93*, 2645.
- (81) Szydlowski, J.; Van Hook, W. A. *Macromolecules* **1991**, *24*, 4883.
- (82) Luszczyk, M.; Rebelo, L. P. N.; Van Hook, W. A. *Macromolecules* **1995**, *28*, 745.
- (83) Luszczyk, M.; Van Hook, W. A. *Macromolecules* **1996**, *29*, 6612.
- (84) Bigeleisen, J. *J. Chem. Phys.* **1961**, *34*, 1485.
- (85) Singh, S. S.; Van Hook, W. A. *J. Chem. Phys.* **1987**, *87*, 6097.
- (86) In fact, the bulk change and the microenvironment change in a polymer gel are not always correlated. Polyacrylamide shows a different feature with PNIPAM. The preferential solvation in a probe gives dominant information. Shirota, H.; Ohkawa, K.; Kuwabara, N.; Endo, N.; Horie, K. Manuscript in preparation.
- (87) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *J. Chem. Phys.* **1989**, *91*, 1345.
- (88) Sasaki, S.; Maeda, H. *Phys. Rev. E* **1996**, *54*, 2751.
- (89) Sasaki, S.; Kawasaki, H.; Maeda, H. *Macromolecules* **1997**, *30*, 1847.