*Transactions of the Materials Research Society of Japan* 28 [3] 1021-1024 (2003)

# Fluorescence Study on the Rapid Deswelling of Comb-type Poly(N-isopropylacrylamide) Hydrogel

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The shrinking mechanism of comb-type grafted poly(N-isopropylacrylamide) gel due to temperature jump across its volume transition temperature has been investigated. Gel networks were labeled by dansyl probe, and the temporal change in microenvironment of dansyl-labeled gel was investigated by means of fluorescence spectroscopy. The comb-type poly(N-isopropylacrylamide) gel exhibited a rapid shrinking compared to normal-type NIPA gel, and the change in its microenvironment was found to become hydrophobic more than 10 times faster than normal-type poly(N-isopropylacrylamide) gel by observation of temporal change in the maximum emission wave length, $\lambda_{em}$, of the dansyl group. The freely mobile characteristics of grafted chains are expected to show the rapid dehydration to make tightly packed globules with temperature, followed by the subsequent hydrophobic intermolecular aggregation of dehydrated graft chains. The dehydrated grafted chains created the hydrophobic cores, which enhance the hydrophobic aggregation of the networks.

Key Words: Poly(N-isopropylacrylamide) gel, Shrinking kinetics, Fluorescence spectroscopy

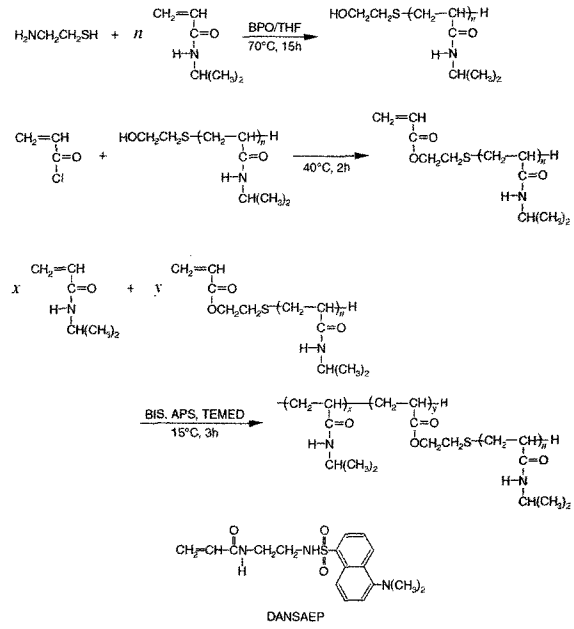
## 1. INTRODUCTION

In recent years, considerable research attention has been focused on hydrogels that are able to alter their volume and properties in response to environmental stimuli such as temperature, pH and ionic strength.[1-3] Because of their drastic swelling in response to environmental stimuli, these polymeric hydrogels have been investigated for many biomedical and pharmaceutical applications, including controlled drug delivery, molecular separation, tissue culture substrate and materials for improved biocompatibility.[4-6] Among these "intelligent" gels, temperature- and pH-sensitive hydrogels are most widely investigated. However, since the process is diffusion controlled, the rate of gel swelling and shrinking is strongly dependent of the size of gel. Based on the cooperative diffusion of polymer network in a medium, Tanaka-Fillmore theory indicates that the characteristic time of gel swelling and shrinking is describes the following equation.[7]

$$\tau \approx R^2/D \qquad (1)$$

where $R$ and $D$ are the size of gel and the cooperative diffusion coefficient of gel network, respectively. For typical polymer gels, $D$ is on the order of $10^{-7}$–$10^{-6}$ $cm^2/s$, depending on polymer concentration, cross-linking density, etc. Since it is not easy to increase the value of $D$ by a factor of $10^2$ or more, a reduction of gel size has been considered to be an only way to achieve quick response.Recent studies, however, have shown that the rate of gel shrinking could be accelerated by (i) introducing a comb-type grafted chains,[4,8] (ii) a microporous structure prepared by γ-irradiation,[9] (iii) a phase-separated structure prepared under the existence of diluents,[10] or (iv) having a pathway of water by incorporating hydrophilic chains.[6] With regard to (i), Okano et al developed a novel architecture of poly(N-isopropylacrylamide) gel (PNIPAM gel), which exhibited drastic acceleration of the shrinking rate compared to conventional PNIPAM gel. Dangling chains in a gel can easily collapse on an external stimulus because one side of the chain is free, which induces strong shrinking tendency in a gel.

Fluorescence methods, such as steady state spectroscopy, fluorescence anisotropy, and fluorescence




**Scheme.** Preparation of PNIPAM macromonomer and Comb-type gel.

decay measurement have been shown to be quite effective in the investigation for the microscopic environment around a chromophore.[11] The dansyl probe has been widely used as a fluorescence probe to study conformational transition in protein and synthetic polymers. This group has a photophysical property that gives information about the local polarity and mobility of the microenvironment, as well as the binding behavior of the group.[12-14]

The shrinking behavior in PNIPAM gel has so far been studied by simple swelling ratio measurements as a function of time. Although these studies demonstrated essential roles of the hydrophobic interactions for triggering volume phase transitions in gels, it has not disclosed any microscopic view of the PNIPAM gel. In this study, therefore, we investigated the temporal change in microenvironment of dansyl-labeled conventional normal-type gel (NG) and comb-type gel (GG) by means of fluorescence spectroscopy in order to elucidate the shrinking mechanism of the gels.

## 2. EXPERIMENTAL

**Materials.** N-isopropylacrylamide (NIPAM; Kohjin) was recrystallized form the mixture of toluene and n-hexane. Tetrahydrofurane (THF, Kanto), diethyl ether (Kanto), cyclohexane (Kanto) and acryloyl chloride (Aldrich) were purified just before use according to standard procedure. 2-Hydroxyethanethiol (HESH, Tokyo Kasei), benzoyl peroxide (Nakarai), N, N'-methylene-bis-acrylamide (BIS, Kanto), N, N, N', N'-tetramethyl-ethylenediamine (TEMED, Kanto), and ammonium peroxide (APS, Kanto) were used as received.

**Macromonomer Synthesis.** A NIPAM polymer with a terminal hydroxyl end group (PNIPAM-OH) was prepared by radical telomerization of NIPAM monomer using HESH as a chain transfer agent (Scheme).

**Preparation of PNIPAM gel.** Comb-type and normal-type PNIPAM gel were prepared by radical polymerization in micropipettes with inner diameter $d_o$ (Scheme). The feed composition is listed in Table I.

**Shrinking kinetics of gels.** The gel sample was placed in a thermostated cell filled with distilled deionized water, the temperature of which was controlled to within 0.1°C of the desired temperature. The temperature-jump (T-jump) experiments were carried out by exchanging the circulating water which temperatures were set to desired temperatures. The time required for T-jump was about 30sec. The swelling degree of gel, $d/d_o$, was obtained by measuring the diameter of the cylindrical gel, $d$, under a microscope. The measurement was repeated at least three times and its average was used as the value of $d$.

**Measurements of photophysical properties.** The fluorescence emission spectra were recorded on a Hitachi F-4010 fluorescence spectrophotometer with a thermostated cell filled with distilled deionized water, the temperature of which was controlled to within 0.1°C of the desired temperature. The excitation wavelength was set at 345 nm. The measurement of the temporal shift in $\lambda_{em}$ was carried out within the wavelength range of $\lambda_{em}$ of the initial temperature and that of destination temperature. The scanning rate was set to 10 nm/sec, and the period for scanning the whole range was about 25 sec. The measurement was repeated at least three times and its average was used as the value of $\lambda_{em}$. Fluorescence anisotropy ratio, $r$, was calculated from four polarized fluorescence spectra by using

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH}) \quad (2)$$

$$G = I_{HV}/I_{HH} \quad (3)$$

where $I$ is the fluorescence intensity and the subscripts represent the orientation of polarizers ($V$ is vertical, and $H$ is horizontal), which are located for incident light (the first subscript) and for emitted light (the second subscript). The $G$ value was used for the correcting the depolarization characteristics of the grating-type monochrometer. The anisotropy, $r$, were averaged for emission from 400 to 650 nm.

**Table 1.** Feed composition for preparation of normal-type NIPAM gel (NG) and comb-type NIPAM gel (GG).

| Sample code | NG | GG |
|---|---|---|
| NIPAM monomer (g) | 15.60 | 10.92 |
| NIPAM macromonomer* (g) | 0 | 4.68 |
| DANSAEP (g) | 0.043 | 0.043 |
| BIS (g) | 0.266 | 0.266 |
| TEMED (μL) | 48 | 48 |
| APS (g) | 0.008 | 0.008 |
| DMF/water (20/80 in vol.) | 100 | 100 |

*$M_n$ = 5170, $M_w$ = 7340, $M_w/M_n$ = 1.42

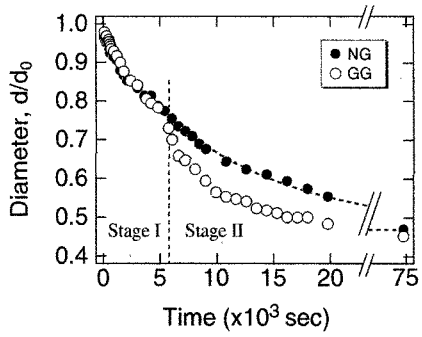



**Figure 1.** Variation of $d/d_0$ of the NG and GG after T-jump from 10°C to 35°C. The line is the fit with eq. 4.

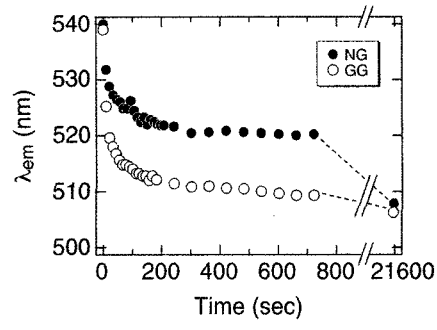



**Figure 2.** Temporal change in $\lambda_{em}$ of dansyl probe for NG and GG after T-jump from 10°C to 35°C.

## 3. RESULT AND DISCUSSION

**Shrinking Kinetics:** The kinetics of swelling of gel was treated by Tanaka and Fillmore.[7] The time variation of gel size, e.g., the gel diameter, could be described by a single-exponential function

$$\frac{d(t)-d(\infty)}{d(0)-d(\infty)} \approx \frac{6}{\pi^2}\exp\left(-\frac{t}{\tau}\right) \qquad (4)$$

where $d(t)$ is the gel diameter at time $t$ and $\tau$ is the characteristic time for swelling. It has been confirmed Eq. 4 was also valid for the shrinking kinetics as far as the shrinking did not accompany phase separation.[15] However, the shrinking kinetics is completely different from that predicted by Tanaka and Fillmore when the size of gel changes drastically or a phase separation is accompanied. This is due to the fact that the theory is limited to the linear regime and the collective diffusion constant is assumed to be invariant upon shrinking/swelling.[7] Fig. 1 shows the variation of the degrees of swelling, $d/d_0$ ($\equiv d(t)/d_0$), of the gels after T-jump from 10°C to 35°C. In the case of NG, the change in the swelling ratio is satisfactory reproduced by Eq. 3, which indicates the shrinking process is diffusion-controlled one with the collective diffusion constant 2.7 x $10^{-7}$ $cm^2/s$. In the case of GG, however, the shrinking process is characterized by two stages. In the stage I, the process seems to be diffusion controlled and a diffusion constant of the gel network is 2.6 x $10^{-7}$ $cm^2/s$. In the stage II, however, the shrinking rate is accelerated than that of the stage I. Since analysis with a single-exponential function (Eq. 4) became no more applicable to whole shrinking process, the time required for half-shrinking, $t_{1/2}$, was defined as follows[15]:

$$\frac{d(t_{1/2})-d(\infty)}{d(0)-d(\infty)} = \frac{1}{2} \qquad (5)$$

The values of the time required for half-shrinking, $t_{1/2}$, for for NG and GG T-jumped form 10°C to 35°C are 6988 s and 3880 s, respectively. $t_{1/2}$ for GG is approximately one half of that for NG. The NG became opaque immediately after the T-jump from 10°C to 35°C, suggesting that heterogeneous structure was formed in the gel networks. Since a dense, collapsed polymer layer impermeable to water is formed near the gel surface, the shrinking rate of NG is limited by suppressed water permeation from the gel interior through the collapsed polymer skin. In contrast to NG, introducing 30% (w/w) of NIPA macromonomer (GG) made the gels shrink faster. Above transition temperature, grafted chains are dehydrated, and then hydrophobic aggregation force forms between dehydrated grafted chains. In stage II, therefore, these aggregations of the NIPA chains contribute to an increase in void volume, which allow gel having a pathway of water molecules through the gel by phase separation.

**Temporal changes in $\lambda_{em}$ for T-jump process.** The temporal change in microenvironment of dansyl-labeled NG and GG gel were investigated by means of fluorescence spectroscopy. Fig. 2 shows the temporal changes in $\lambda_{em}$ for gels after T-jump from 10°C to 35°C. Observed time-variation behavior of $\lambda_{em}$ are similar as that of gel size and analysis with a single-exponential function is not applicable. We defined the time required for half- shift, $t_{1/2}$, of $\lambda_{em}$ as follows:

$$\frac{\lambda_{em}(t_{1/2})-\lambda_{em}(\infty)}{\lambda_{em}(0)-\lambda_{em}(\infty)} = \frac{1}{2} \qquad (6)$$

The values of the time required for half-shift, $t_{1/2}$, for NG and GG T-jumped form 10°C to 35°C are 150 s and 14.3 s, respectively. The change in the microenvironment for GG is accelerated by an order of magnitude comparing with that for NG. The grafted chains maintain their mobility, while NIPA networks are anchored at several points by cross-linking on each chain restricting the mobility.

The $\lambda_{em}$ of dansyl probe decreases initially very rapidly with time while the diameter of the microgel changes only slightly. Dansyl probe is expected to act as a molecular probe sensing the microscopic environment. The position, shape and intensity of the emission are sensitive to molecular mobility, solvation, and polarity of the microscopic environment around the chromospheres. The temporal change in the diameter and $\lambda_{em}$, is related to the several factors; variation of dehydration of chains, local viscosity, structural relaxation, and so forth. To understand the microscopic

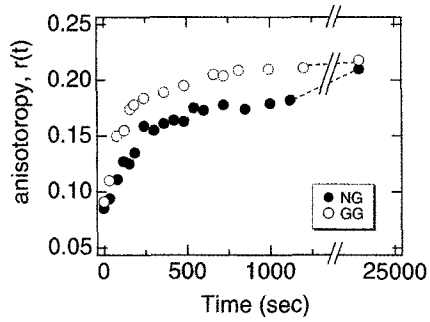



**Figure 3.** Temporal change in $r$ of dansyl probe for NG and GG after T-jump from 10°C to 35°C.

factors that occur during the gel shrinkage, we carried out the fluorescence anisotropy measurements. Fig. 3 shows the temporal changes in anisotropy, $r$ for gels after T-jump from 10°C to 35°C. Observed time-variation behavior of $r$ are similar to that of $\lambda_{em}$. We defined the time required for half-change, $t_{1/2}$, of $r$ as follows:

$$\frac{r(t_{1/2})-r(\infty)}{r(0)-r(\infty)}=\frac{1}{2} \qquad (7)$$

The values of the time required for half-change, $t_{1/2}$, for NG and GG T-jumped form 10°C to 35°C are 240 s and 108 s, respectively. The values of the time required for half-change in the microviscosity show following order; GG < NG. GG exhibits smaller $t_{1/2}$ value for $\lambda_{em}$ than that for $r$. These observations suggest that the rapid thermal response of the comb-type grafted NIPA gels microscopically attributes to the following mechanism; the rapid dehydration of the freely mobile NIPA graft chains due to the increase in hydrophobicity, and the subsequent hydrophobic intermolecular aggregation of dehydrated graft chains enhances the hydrophobic aggregation of the NIPA networks because of the increase in microviscosity. In the case of NG, however, the temporal change in $r$ for NG almost coincides with that in $\lambda_{em}$. This is due to the fact that the shrinking of NG is controlled by the collective diffusion of network, therefore dansyl locally probes both viscosity and hydrophobicity.

It should be mentioned that the characteristic time, $t_{1/2}$ values of $\lambda_{em}$ and $r$ are much smaller than that of diameter change, which are by a factor of 10 for NG, and by a factor of 100 for GG. The microscopic change is complete faster than macroscopic one. This is likely related to the structural relaxation of gel network and the change in the microscopic structure of gels during the shrinking process, though we cannot give clear interpretation here. We are investigating to elucidate the temporal change in microscopic structure of NG and GG after a T-jump using SAXS, which may provide powerful tools for studying these phenomena at the molecular level. A further investigation of the correlation between macroscopic behavior and microscopic structure will be reported in a future publication.

## 4. CONCLUSION

The shrinking mechanism of comb-type grafted NIPA gel due to temperature jump across its volume transition temperature has been investigated. Grafted chains or gel networks were labeled by dansyl probe, and the temporal change in microenvironment of dansyl-labeled gel was investigated using fluorescence spectroscopy. The change in microenvironment of the comb-type NIPA gel due to T-jump was found to become hydrophobic more than 10 times faster than normal-type NIPA gel by observing of the change in the maximum emission wavelength, $\lambda_{em}$ of the dansyl group as a function of time. The temporal change in anisotropy indicates that the freely mobile characteristics of grafted chains, which expected to show the rapid dehydration to make tightly packed globules with temperature, followed by the subsequent hydrophobic intermolecular aggregation of dehydrated graft chains. The dehydrated grafted chains created the hydrophobic cores, which enhance the hydrophobic aggregation of the networks.

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(Received April 18, 2003; Accepted April 29, 2003)