Aggregation-Induced Expansion of Poly-(N-isopropyl acrylamide) Solutions Observed Directly by the Transient Grating Imaging Technique

Daiki Kato,† Woon Yong Sohn,†‡ and Kenji Katayama*†‡

1Department of Applied Chemistry, Chuo University, Tokyo 112-8551, Japan
2PRESTO, Japan Science and Technology Agency (JST), Saitama 332-0012, Japan

Supporting Information

ABSTRACT: The anomalous volume expansion of poly-(N-isopropyl acrylamide) (PNIPAM) solutions was observed during the thermally induced polymer phase transition of aqueous solutions having concentrations in the 3−7 wt % range. The process occurred on a millisecond time scale, and a laser temperature-jump time-resolved technique was used to bring about the process. After heating a solution with a pulse laser exploiting light absorption by dyes added to the solution itself, a phase transition was observed to take place, and the temporal changes associated with it were visualized through the transient grating imaging technique, whereby the solution was heated with a stripe pattern. We found several processes occurring on a millisecond time scale, all of which clearly took place after each PNIPAM molecule had collapsed structurally from a coiled to a globular conformation. During the so-called demixing process, the globular polymers aggregated with each other within 10 ms, and suddenly the polymer phase expanded as aggregation progressed further. After this process, the individual globular polymers reverted to their coiled conformation via hydration during the remixing process. We proposed that solution expansion was caused by the mutual entangling of multiple globular PNIPAM molecules, instead each globule polymer was separated.

INTRODUCTION

Thermoresponsive polymers are regarded as promising materials for applications such as drug delivery1,2 and the collection of chemicals in solutions.3,4 Currently, one of the best-known thermoresponsive polymers is poly-(N-isopropyl acrylamide) (PNIPAM), which has attracted much interest because of the fact that its phase transition occurs at near-ambient temperature. PNIPAM has been utilized for various applications, such as the collection of precious metals5 and waste chemicals.6 Notably, it has also been utilized as a separation material in chromatographic procedures.7 Furthermore, much attention has been devoted to this polymer’s phase-transition process because it is assumed to have similarities with the protein folding.5,9

The detailed process of phase transition, typical of this material, is still controversial, even with respect to the temperature at which it occurs10 because of the nonequilibrium behavior of this chemical. It has been suggested that the phase-transition and the following recovery processes of this material include some unclear states that have a very long lifetime and are characterized by the presence of “mesoglobules”.11 In differential scanning calorimetry (DSC) and turbidity measurements, which are techniques that have been typically used for studying thermodynamic properties, the phase-transition and the recovery processes display a hysteresis behavior. In particular, as the temperature increases, the coiled molecules of PNIPAM go through a collapsed state12 and acquire a globular conformation that is typical of a dehydrated state. Moreover, many indications exist that PNIPAM may remain in a mesoglobular state even after the temperature decreases.

The kinetics/dynamics of PNIPAM’s phase change have been studied with various methods, such as measuring turbidity13 and utilizing fluorescent probes,14 Brillouin spectroscopy15, modulated temperature DSC,16 and small-angle neutron/X-ray scattering.15,17 Before the demixing process, where the globular polymers are separated with water, molecular contractions occur in the nanosecond to microsecond time scale,14 followed by the demixing process, which occurs in the millisecond scale.15 Van Mele et al. indicated the vitrification of polymer-rich phase caused by the retardation of remixing process,16 and Müller-Buschbaum et al. identified a multistep process involving the dehydration of the polymer and the occurrence of structural changes that take place on a time scale on the order of tens of minutes.17 Philipp et al. found that two states with different elastic constants are formed during the demixing process.15,16 During this process,
they also observed an anomalous increase of the volume expansion coefficient of the polymer solution at around the temperature of the phase transition, involving a change in the number of hydration water molecules attached to PNIPAM because of release.19

We have studied the phase-transition dynamics of PNIPAM solutions by using the temperature-jump transient grating (TG) method.20,21 In this technique, a polymer sample solution containing heat-generating dyes is subjected to instantaneous heating with a stripe pattern of pulsed light, and the resulting change in the refractive index of the solution is measured. We have determined that various processes occur during the phase transition.21 In particular, during the phase recovery from the globule state to the coil state. We have identified the presence of some intermediate states without any visible absorption characterized by the change in the refractive index of the PNIPAM solution20 that would correspond to the mesoglobular state. However, the phase-transition dynamics was very delicate for the experimental conditions of the temperature and the heating pump light intensity, and the dynamic processes were varied by a change of temperature on the order of 0.1°C.21 Furthermore, polymer aggregation caused the detection light to scatter, and this caused the TG response that was caused not only by the refractive index change but also by the transmittance change because of the turbidity change, which, in turn, increased the difficulty of interpreting the data. Kita and Wieand clarified the Soret effect involving the PNIPAM molecules using an optical configuration similar to that utilized in the TG technique. In this, a distribution of different temperatures across a solution is obtained using a continuous heating laser; subsequently, the molecular diffusion instigated by the built-up temperature distribution is measured. PNIPAM molecules have a positive Soret coefficient, indicating that these molecules diffuse from high-temperature to low-temperature regions.22,23 Orlishausen and Köhler identified two opposite regions formed in the laser-heated region: one is the expanded region of the solution because of the positive Soret coefficient, promoting the stretching of the molecules, and the other is the contraction region because of the aggregation of molecules to the phase-separated region.24

Recently, we have developed a TG imaging technique whereby changes in the refractive index of a solution can be imaged directly. This technique was applied to the study of the phase transition of liquid crystals, and its use enabled us to clarify the details of the process, particularly in the milliseconds to seconds time scale, when the phase-transition response of a PNIPAM solution is easily influenced by atmospheric conditions. Because in this approach images can be obtained even in the presence of light scattering, we applied this method to the study of remixing/demixing processes induced by phase transition in a PNIPAM solution. Surprisingly, we observed a peculiar phase-change behavior, which we termed “aggregation-induced expansion”. This phase change occurred when the polymer density reached a threshold value.

RESULTS AND DISCUSSION

Figure 1a consists of a graph in which are reported the TG responses versus time in logarithmic scale measured for a 7 wt % PNIPAM aqueous solution. The dynamics inferred from these data was consistent with our previous observations,21 although a degree of difference that is because of different concentrations of the PNIPAM solution is evident. According to the data from previous studies, the PNIPAM polymer solution’s response up to 10⁻³ s includes molecular dehydration (~20 ps), contraction (~200 ns), coil–globule transition (~100 μs), and thermal decay (~1 ms). Even after thermal decay is assumed to have already taken place, the signal intensity increased and reached a peak at 20–40 ms, followed by a decay. On the basis of the assignments made for the observations in the milliseconds-to-seconds time scale in previous studies,20,21 after the thermal decay, the aggregation/release of globular molecules (demixing/remixing, 1–100 ms) took place; subsequently, the globular molecules or the assembly of several globule molecules (mesoglobules11) decayed into a coil state on a time scale on the order of milliseconds to seconds, depending on the temperature setting. Because the peak/decay course in the graph reported in Figure 1a was similar to the response observed when the temperature setting was about 1 °C lower than the phase-transition temperature measured by DSC measurements (33.2 °C), it could be assumed that the peak/decay pattern observed in the millisecond to seconds time scale was because of the aggregation of globular molecules (demixing), the release of globular molecules from the aggregate (remixing), and the

Figure 1. (a) TG response and (b) corresponding TG image sequences from 2 ms to 10 s for a 7 wt % PNIPAM solution. The TG image sequences were divided into four time regions, and the original video is included in Movie S1 in the Supporting Information.
The Soret effect was studied with respect to the initial conditions. Scheme 1 depicts the process, which caused a loss in the refractive index in contrast to the original solvation of the polymer molecules (remixing) took place, leading to the coil structure and recovery of the process III. Finally, a gradual decay of the globular PNIPAM molecules proceeded and as water molecules were progressively released from the resulting globule aggregates. Because of the changes in their densities, both the water-rich and polymer-rich phases underwent additional changes in their refractive indexes, and the contrast of the TG images became stronger. Notably, the dark and bright areas of the images became even darker and brighter, respectively.

The fact that the stripe pattern disappeared during process II and reappeared during process III is surprising because in both processes, the observed phase pattern was induced by density changes brought about by PNIPAM globule aggregation. However, at the time scale in process II, we observed an increase in light scattering, which is assumed to result from the formation of aggregates of increased size, which, in turn, gave rise to Mie scattering. Furthermore, a flow was observed to be induced within the sample, which the video, included in the Supporting Information, can clearly confirm. On the basis of the evidence from previous studies, this flow is assumed to be a consequence of the release of water during phase separation, and the process of expelling water was discussed in relation with the elastic property of the PNIPAM polymer. Therefore, during process II, it is believed that light scattering was induced by an increase in size of PNIPAM globule aggregates, which caused the stripe pattern to become obscured.

After the flow had stopped, the stripe pattern reappeared in process III. Finally, a gradual decay of the globular PNIPAM structures to reform the coil structure and to recover the original solvation of the polymer molecules (remixing) took place, which caused a loss in the refractive index in contrast with respect to the initial conditions. Scheme 1 depicts the proposed dynamic scheme.

It is mentioned that the phenomena observed in the present study are different from the thermophoretic diffusion, namely the Soret effect, whereby a stationary temperature gradient causes the mass diffusion of molecules. In the experiments described above, the sample solution was heated by a pulse laser, and the temperature profile (thermal grating) decayed within a millisecond (a decay in $10^{-4}$ to $10^{-3}$ s in Figure 1a). Therefore, no temperature gradient existed in the sample after the decay of thermal grating.

In our previous reports, we had come to the conclusion that light scattering was induced by the aggregation of the globular PNIPAM molecules, which resulted in the polymer globule aggregates randomly distributing in the solution and acting as light scatterers. However, this conclusion was clearly mistaken because the stripe pattern was maintained even during processes II and III, when the globule aggregates had already formed.

Notably, the polymers suddenly started to expand after several milliseconds (processes I and II). During processes I–III, the globular-structure molecules should form aggregates; however, the relevant TG images are characterized by an expansion of the stripe interval, after the density increases because of the aggregation of globular molecules. Although the thermal expansion coefficient of PNIPAM has been reported to increase drastically around the phase-transition temperature, this is the first study in which the expansion was recorded in real time, and it was found that the expansion proceeded discontinuously for several milliseconds after the phase transition, namely after the aggregation of globular molecules proceeded for several milliseconds.

Philipp et al. investigated the anomalous enhancement of the thermal expansion coefficients around the temperature of phase transition. They reported that the thermal expansion coefficient is dependent on the PNIPAM concentration. We, on the other hand, studied the dependence of the demixing/remixing dynamics of a PNIPAM solution on the concentration of the polymer solution. Figure 2 reports the TG responses versus time ($10^{-3}$ to $10^{-2}$ s) of PNIPAM solutions at different concentrations. The intensity of the TG signal peak observed in correspondence with the $10^{-3}$ to $10^{-2}$ s time range depended on the PNIPAM concentration. Initially, this intensity gradually increased alongside the polymer concentration, with a maximum value observed for a solution with a 7 wt % concentration. However, at higher concentrations, the
peak intensity started to decrease progressively. Because the TG signal intensity qualitatively depends on the size and number of PNIPAM globule aggregates, given that the refractive index change is proportional to the medium’s density, the data reported in Figure 2 indicate that the aggregation proceeded effectively, peaking at a PNIPAM concentration of ∼7 wt%.

To investigate the demixing/remixing processes of a PNIPAM solution, TG image sequences were measured for PNIPAM solutions in the 3−15 wt % concentration range (relevant videos can be found in the Supporting Information). The expansion of the stripe was observed for solutions in the 5−10 wt % concentration range, whereas no expansion was observed for the 3 and 15 wt % solutions. These results are consistent with the observations on the thermal expansion coefficient.15 All of the same experiments were performed using SY as a dye, and the general trend was the same as that observed in experiments involving new coccine (NC) as the dye (Figures S1−S3 in the Supporting Information). No dependence of the results showed that the observation did not depend on guest dyes but on PNIPAM.

On the basis of the TG images, we calculated the maximum expansion ratio for different polymer concentrations (Figure 3). At the bottom of Figure 3 is reported the ratio between the TG image of 5 ms and those for the maximum expansion for each concentration. The volume expansion ratio drastically increased in the 3−5 wt % polymer concentration range; however, it decreased gradually in the 5−10 wt % concentration range. Philipp et al.19 and Winnik et al.27,28 reported that the expansion is caused by the release of hydration water molecules. The TG image sequences indicated that the polymer region (the dark-colored region) increased in width (size) to become larger than the water region (the light-colored region) around 1 s after pump irradiation.

The maximum peak intensity of the TG responses reported in Figure 2 was observed at almost the same polymer concentration as the expansion peak. Because the TG signal intensity is dependent on the density of the aggregated molecules, the analogies between the concentration dependence of the parameters reported in the y-axes of the graphs in Figures 2 and 3 indicate that the aggregation was relevant to the expansion process. During the aggregation process, hydration water molecules were released from the aggregates, so that the globular polymers lost water molecules, whereas the water region acquired them.

Because the formation of the globular molecules via dehydration on the molecular level occurs on a time scale on the order of hundreds of nanoseconds21 and the following contraction (collapse of PNIPAM) takes place on a time scale on the order of hundreds of microseconds,13,14 the two processes just alluded to have already been completed before the millisecond region is reached. Therefore, at this stage, each polymer molecule is in the globular state, and the process taking place should be the diffusion-driven demixing between the globular molecules and the water molecules. Because only the change in the refractive index contrast, corresponding to the density change, was observed for <10 ms, we suppose that the globular polymer molecules aggregated with each other, whereas water molecules were expelled from the polymer region. If this process had continued exclusively, there would be no reason for the observed expansion; therefore a qualitatively different process must also have taken place. One possible explanation for the mentioned expansion would be the entanglement between globular molecules. Because a PNIPAM molecule has many hydrophilic and hydrophobic functional groups along its polymeric chain, each globular molecule could untangle and merge with another via globule−globule interaction.
To examine this possibility, the ratio of the number of PNIPAM-based globules to that of water molecules and the ratio of PNIPAM molecules to that of water molecules were calculated. These values were 1:25 000 and 1:120, respectively, for 5 wt % solution of PNIPAM (MW: 25 000), for which the largest expansion was observed. Because each monomer is hydrated with \( \sim 10 \) water molecules in the coiled state, a number reduced to \( \sim 2 \) in each of the globular monomer,19 8 water molecules are released per PNIPAM monomer during the phase transition, indicating that almost 10% of the solution’s water content was expelled during the demixing process. Furthermore, each globular polymer comprised \( \sim 200 \) monomer units, meaning that \( \sim 400 \) water molecules were still part of the polymer assembly to keep each globular molecules isolated. Because the number of the hydration water molecules is on the same order as the number of monomer units in each globule, it is possible that a globular PNIPAM molecule favors merging with another one by the interaction of the hydrophobic and hydrophilic groups in different globular PNIPAM molecules instead that each one remain isolated because of hydration by water. We assume that the globule aggregates expanded in size because of this tangling of globular molecules. To verify this assumption, we need to know the change in the enthalpy and entropy resulting from replacing hydration water molecules with a globular PNIPAM molecule, and further investigations of the thermodynamic properties of this process are necessary. Currently, such investigations are underway.

## CONCLUSIONS

Using a newly developed TG imaging technique, we were able to observe the expansion of a PNIPAM solution during the demixing process occurring at the time of polymer phase transition. The expansion was observed during the aggregation of globular molecules taking place within a specific polymer concentration range. After the PNIPAM molecules underwent the transition between the coiled and the globular conformation, the globular polymers aggregated with each other as part of the so-called demixing process, and we propose that there are two aggregation processes that took place. During the initial process, the density of the globule aggregates increased, whereas the volume of these aggregates expanded during the second aggregation process. This observation can be made only by imaging the refractive index change because no obvious color change is involved in the phase transition and the following aggregation processes, and the TG imaging technique would be a powerful tool for observing phase-transition dynamics. The results from the present study may pave the way for investigation of the nonequilibrium phase-transition thermodynamics of polymer solutions.

## EXPERIMENTAL SECTION

**Materials.** PNIPAM was purchased from Sigma-Aldrich (molecular weight: 19 000–30 000 g/mol). Deionized water was used for the preparation of aqueous PNIPAM solutions in the 1–15 wt % concentration range. NC was one of the photothermal dyes25 dissolved in the PNIPAM solutions. To confirm that the presence of NC had no effect on the dynamics of PNIPAM, another photothermal dye, sunset yellow (SY), was used to study the dye dependence of PNIPAM dynamics (Figures S1–S3 in the Supporting Information). The concentration of NC was 1.0 mM in each PNIPAM solution.

The sample solution was placed in a handmade, 500 μm thick quartz cell, and the absorbance of samples was set about 1.0 for the pump light wavelength. The quartz cell was covered with an aluminum frame, whose temperature was controlled by a heater controller (TC200, Thorlabs). The initial temperature of the samples was set to 1 °C below the demixing temperature, which was determined by DSC. Figure S4 in the Supporting Information shows the DSC data for PNIPAM solutions at different concentrations. The temperature jump because of the pump pulse irradiation was about 2.0 °C, which was confirmed by the observation of the temperature at which the turbidity was increased because of the coil–globule transition \( (T') \) with an aid of the pump pulse irradiation. \( T' \) could be confirmed by monitoring the turbidity as the heater temperature was gradually increased. Because we had determined the phase-transition temperature \( (T) \) through DSC measurements, the temperature rise because of the pump pulse could be estimated from the difference between \( T \) and \( T' \).

**Measurements.** A detailed principle on the laser temperature-jump method is provided elsewhere.20 Although in previous studies we utilized the heterodyne TG method,21 in which the refractive index change was measured via the heterodyne signal, the homodyne TG method was used here;26 Figure S5a in the Supporting Information reports a schematic representation of the setup. In detail, an excitation pulse was made to pass through a transmission grating and, thus, the grating pattern produced was projected onto the sample using a 4f configuration of two lenses with different focal lengths, and the fringe space on a sample was reduced to half of the original one. The pump pulse was absorbed by the photothermal dye molecules present in the sample solution, and the pump energy was converted to heat, causing the temperature to rise locally in a pattern determined by the grating pattern mentioned above. Refractive index changes were induced within the sample according to the same fringe pattern, which is referred to as TG. Normally, the first-order light diffraction is detected in these kinds of experiments. However, because of the volume expansion of a PNIPAM solution in the observations, the grating spacing was changed during the measurements, causing the diffraction angles varied during the measurements; therefore, we detected the zeroth-order diffraction light, which is characterized by a diffraction angle that never changes during the expansion process. For imaging purposes (see Figure S5b in the Supporting Information), an additional 4f optical setup was constructed, and a CMOS camera (MV1-D1024E-160, Photon Focus) was placed on the image plane. After rejecting the pump pulse with a notch filter, the interferometric technique was used to retrieve the phase image of the sample. The image sequence was recorded continuously after the pump pulse irradiation had been initiated. Typically, 100 images were recorded for each trigger, and the interval between frames was 1–10 ms. Moreover, each image was processed by the subtraction of the original image without the pump irradiation from it, and only the photoinduced refractive index change was extracted.

Because the TG phase image is induced by the differences between the refractive indexes of the various locations within the sample, the intensity of each pixel of the image is proportional to the change in refractive index of a particular spot. The refractive index change for a polymer solution is given by the following equation
where the first term, called the temperature grating, corresponds to the refractive index change because of the temperature change, and the second term, the density grating, corresponds to the change in density of the sample. Because thermal grating decays a few milliseconds after the pump irradiation, past this time, the change in density of the sample induces the refractive index change.

The pump light was a Nd:YAG pulsed laser (pulse width: 5 ns, wavelength: 532 nm, and irradiation intensity: 36.8 mJ/cm² (K.K.).

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**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: kkata@kc.chuo-u.ac.jp. Phone: +81-3-3817-1913 (K.K.).

**ORCID**

Woon Yong Sohn: 0000-0003-4881-2350

Kenji Katayama: 0000-0003-3278-6485

**Notes**

The authors declare no competing financial interest.

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