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PAPER

Conformational relaxation dynamics of a poly(*N*-isopropylacrylamide) aqueous solution measured using the laser temperature jump transient grating method

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We observed phase transition and phase relaxation processes of a poly(*N*-isopropylacrylamide) (PNIPAM) aqueous solution using the heterodyne transient grating (HD-TG) method combined with the laser temperature jump technique. The sample temperature was instantaneously raised by about 1.0 K after irradiation of a pump pulse to crystal violet (CV) molecules for heating, and the phase transition was induced for the sample with an initial temperature just below the lower critical solution temperature (LCST); the following phase relaxation dynamics was observed. Turbidity relaxation was observed in both the turbidity and HD-TG responses, while another relaxation process was observed only in the HD-TG response, namely *via* the refractive index change. It is suggested that this response is due to formation of globule molecules or their assemblies since they would have nothing to do with turbidity change but would affect the refractive index, which is dependent on the molar volume of a chemical species. Furthermore, the grating spacing dependence of the HD-TG responses suggests that the response was caused by the counter propagating diffusion of the coil molecules as a reactant species and the globule molecules as a product species and the lifetime of the globule molecules ranged from 1.5 to 5 seconds. Thus, we conclude that the turbidity reflects the dynamics of aggregate conditions, not molecular conditions. The coil and globule sizes were estimated from the obtained diffusion coefficient. The sizes of the coil molecules did not change at the initial temperatures below the LCST but increased sharply as it approaches LCST. We propose that the coil-state molecules associate due to hydrophobic interaction when the initial temperature was higher than LCST minus 0.5 K and that the globule-state molecules generated from the coil-state molecules showed a similar trend in temperature. The phase transition was also induced by heating under a microscope, and the relaxation process was followed using the fluorescence peak shift of a fluorescent molecule-labeled PNIPAM. The result also supports the existence of a globule molecule or its assembly remains for several seconds in the phase relaxation.

1. Introduction

Poly(*N*-isopropylacrylamide) (PNIPAM) is a well-known water soluble polymer with both hydrophobic isopropyl and hydrophilic amide groups. A PNIPAM aqueous solution changes from a homogeneous to a heterogeneous phase by temperature change and the phase transition temperature is called as the lower critical solution temperature (LCST $\approx$ 33.2 °C).[1] At room temperature, in the homogeneous phase, PNIPAM is dissolved in a random coil state in water and hydrogen bonds are formed between water molecules and the amide groups in PNIPAM. When it is heated to the temperature higher than LCST, a phase transition occurs because the hydrogen bonds are dissociated and hydrophobic interactions in the molecular chain become predominant; the coil state is changed into the globule state.[2] The globule molecules aggregate due to hydrophobic interactions and the transparent aqueous solution gets turbid. This phase separation has been applied widely and some examples are in drug delivery systems,[3] in separation of suspension solutions[4] and to modify the surface of gold nanoparticles.[5] In the phase transition, a hydrated random coil state is changed into a dehydrated globule state, and the balance between the hydrogen bonds and the hydrophobic

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interactions with the isopropyl group is thought to be important to the mechanism. There have been various studies to clarify the phase transition based on theoretical calculations,[6] light scattering,[7] differential scanning calorimetry,[8] ultrasonic spectroscopy,[9] calorimetry,[10] NMR,[11] Raman spectroscopy[12] and FTIR spectroscopy.[13] Relatively, only a few studies have been carried out on the phase transition dynamics and a few papers on the dynamics using fluorescence[14] and turbidity[15] were found. Still, it has not been clarified yet how the microscopic polymer conformational change leads to macroscopic phase separation and the following phase relaxation processes. The turbidity was measured to monitor the phase separation, where the scattering media with the size of the used wavelength can be observed. To monitor the change in the polymer conformation, a fluorescence probe technique has been utilized. The correlation of the size and property observed by both the techniques has recently received much attention.

We have recently developed the heterodyne transient grating (HD-TG) method, which features a simple optical setup and highly sensitive detection of the photochemical dynamics *via* refractive index change.[16–18] In this study, we applied the HD-TG technique combined with the laser temperature jump method,[19] where a solution is heated by a pulsed laser and the following dynamics are monitored. We investigate the phase transition and phase recovery dynamics of a PNIPAM solution by comparing the HD-TG response (refractive index change) with a turbidity response (transmittance change). In addition, we investigate changes in the molecular environment of a fluorescent molecule-labeled PNIPAM,[20,21] where the phase transition is induced by a focused laser under a microscope.[22,23] The temporal change in the peak shift of the fluorescence spectrum was observed during the phase relaxation processes and the results were compared with the HD-TG results.

2. Experimental

2.1 Laser temperature jump method

The laser temperature jump method is used to analyze reaction kinetics by observing relaxation to an equilibrium state after a thermal perturbation.[19] Since the heat is generated by the absorption of a pulsed laser with short time duration, the process can be observed with a high temporal resolution.[24] This technique has been used for the analysis of enzyme kinetics[25] and for understanding the folding–unfolding dynamics of proteins,[26] *etc*. A sample solution is indirectly heated by energy transfer from light-absorbing dyes dissolved into it, or is directly heated by absorption of light by the solvent molecules. If the pulse width is very short, we can assume that the solution temperature changes instantaneously. The heated solution temperature is relaxed to the initial temperature due to thermal diffusion, and the reaction process can be monitored in a time-resolved measurement.

2.2 Heterodyne transient grating method

The principle of the HD-TG method has been previously described in detail.[16,27] When a pump beam is incident on a transmission grating; an intensity pattern of an optical fringe is formed close to the grating. When a liquid sample is placed near the transmission grating, it can be excited by the fringe pattern of the pump light. The refractive index of the liquid changes with the same pattern as the optical fringe because of photochemical or photothermal processes; the pattern of refractive index change is called a transient grating. When another light beam (probe light) is incident on the transient grating, a part of the probe is transmitted (reference), and a part of the probe is once diffracted by the transmission grating and refracted by the transient grating in the same direction as the reference (signal). The two diffracted beams are directed along the same path, and they are mixed and detected. The intensity of the heterodyne component is expressed as

$$I \propto 2E_{\mathrm{ref}}E_{\mathrm{pr}}(\Delta n(t)\cos\phi + \Delta k(t)\sin\phi) \quad (1)$$

where E_{ref} and E_{pr} are the electric fields of the reference and probe; ϕ is the phase difference between the signal and the reference; and Δn and Δk are the real and imaginary parts of the refractive index change, respectively. The phase difference is determined by the optical path difference between the signal and the reference; it can be controlled by changing the distance between the transmission grating and the sample.[28] The $\Delta n(t)$ responses were measured throughout the following experiments because no species having absorption in the probe wavelength is generated.

In a TG experiment, the refractive index change mainly occurs because of thermal energy release (thermal grating) and subsequent transiently generated chemical species by the photoreaction (species grating).[29] The temporal change in the refractive index is expressed as

$$\Delta n(t) = \Delta n_{\mathrm{T}}(t) + (\Delta n_{\mathrm{R}}(t) + \Delta n_{\mathrm{P}}(t)) \quad (2)$$

where Δn_{T} is the thermal grating, which is a temperature rise profile with a stripe pattern whose spacing is equal to the optical fringe of the pump, and decays due to thermal diffusion. Δn_{T} is generally negative for most solvents. Δn_{R} and Δn_{P} are caused by the population change in the reactant and the product. The products are created and the reactants are depleted during the course of the photoreaction (species grating). The signal intensity of the species grating is given by the sum of the changes in the refractive indices due to the reactant and the product. The sign of Δn_{R} is opposite to that of Δn_{P} because the phase of the spatial concentration modulation is shifted by 180° with respect to that of the reactant. For typical chemical species that have an absorption in the UV region, Δn_{P} is positive and Δn_{R} is negative for a visible probe wavelength. The signal intensity of the species grating becomes weaker as the spatial modulations of the refractive index become uniform, and this is accomplished by translational diffusion in a direction perpendicular to the grating stripe. The reactant or product species can be assigned from the sign of the refractive index change based on the negative change of the thermal grating.[30]

The signal due to the thermal grating usually decays 2 to 3 orders of magnitude faster than that due to the species grating. The decay time (τ) of the species grating is expressed as

$$\frac{1}{\tau} = D\left(\frac{2\pi}{\Lambda}\right)^2 + \frac{1}{\tau_{\mathrm{lifetime}}} \quad (3)$$

where D is the diffusion coefficient of a chemical species, Λ is the grating spacing, and τ_{lifetime} is the lifetime of the chemical species.

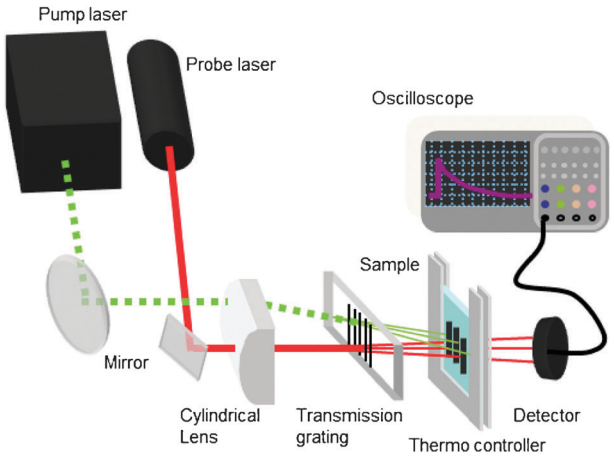

Fig. 1 The optical configuration of the heterodyne transient grating (HD-TG) experiment. Turbidity can only be measured by removing the grating.

The values of τ^{-1} plotted as a function of q^2 give the diffusion coefficient and lifetime of the species as the slope and y-intercept, respectively ($q = (2\pi/\Lambda)$ is controllable by the grating spacing).[31] When the y-intercept was approximately zero, it suggests that intermediate species have longer lifetimes than the time for the species to diffuse the length of the grating spacing; it is likely to be a stable species, not an intermediate species.

The optical setup of the HD-TG method is shown in Fig. 1. The pump light was the second harmonic light of a Nd:YAG laser (GAIA, Rayture Systems) with a wavelength of 532 nm, a pulse width of 6 ns, a repetition rate of 10 mHz and laser intensity was between 10.0 and 12.0 mJ pulse^{-1}. The probe light was a continuous wave He–Ne laser (LHRP-0201, Thorlabs) with a wavelength of 633 nm. The pump and probe lights were set parallel, and then overlapped on the sample position with a cylindrical lens (f = 150 mm). The size of the pump beam at the sample position was 300 μm long and 5 mm wide. The transmission grating was fabricated on a Pyrex® glass plate and it was placed between the cylindrical lens and the sample cell. The grating spacing used was 60–80 μm. The optical glass cell has an internal thickness of 0.5 mm. The sample cell was placed in a handmade thermostatic chamber made of aluminium with an optical window. The chamber was contacted on a Peltier thermo-control device (VPC-20, VICS) with a precision of 0.1 K. The signal was detected with a photodiode detector (DET 110, Thorlabs) equipped with a spectral cut filter for the pump light. The detected signal was stored in a digital oscilloscope (WAVERUNNER 6KA, Lecroy). The signal was measured for various initial temperatures controlled by the Peltier device. Typically the signal was averaged 5 times. The temperature jump depends on the pump power, the dye concentration and the heating volume. The maximum temperature jump at the peak of an optical fringe was estimated to be about 1.0 K under our experimental conditions.

2.3 Turbidity measurements

Turbidity changes when the phase transition is induced by the pump light irradiation, that is, the PNIPAM molecules aggregate to form a phase separated region whose size is on the order of the wavelength used. The turbidity change was monitored by the transmitted light intensity of the probe light, namely turbidity dynamics can be measured only by removing the transmission grating from the optical setup of the HD-TG method and most of the equipment such as the pump light, the probe light, detector and oscilloscope were identical to those in the HD-TG experiment.

2.4 Sample preparation

PNIPAM with a number-averaged molecular weight of 20 000–25 000 g mol^{-1} was purchased from Sigma-Aldrich. The cloud point (CP; the temperature for 50% transmittance) of the solution was 33.2 °C. Crystal violet (CV) was used (Wako Chemicals) as a heater molecule, as it can be easily dissolved in water, and has a good stability for light. When a solution including CV is pumped by a pulsed laser with a wavelength of 532 nm (absorption maximum: 590 nm), most of the excited energy is transformed into heat within several picoseconds[32] due to the rotational relaxation of molecules, because CV has a high internal conversion ratio.[33] The dynamics observed using Rhodamine B as a heater molecule were the same as those observed using CV. Since the absorption spectrum of CV or Rhodamine B does not change in the presence or absence of PNIPAM, those heater molecules were dissolved uniformly in a solution and the absorbance spectra showed no aggregation or assembly formation between molecules. The concentration of PNIPAM and CV was 3.0 wt% and 0.3 mM, respectively. The viscosity was 2.93 mPa s as measured by using a viscometer (SV-10, AND).

2.5 Microscopic observation

The phase transition and phase relaxation behavior of a fluorescent molecule-labeled PNIPAM was measured from the dynamics of the transmitted image and the fluorescent spectrum. A PNIPAM solution was heated by focusing an IR laser beam of a Nd:YVO4 laser (MATRIX 1064-10-CW, Coherent) with a wavelength of 1064 nm and water is directly heated through the vibrational overtone band of OH under an optical microscope.[22,23] An IR laser beam was introduced into the microscope and focused by an objective lens (UPlan FLN 60× NA: 0.9, Olympus). The laser power was 300 mW at the sample position. When the phase transition is induced by heating, a few molecules are assembled to grow till the size is large enough to be trapped, and the trapped particle is kept trapped. When the laser is stopped, the trapped particle is dissociable into smaller particles and finally they escaped from the trapping. The sample chamber was prepared by stacking two cover glasses with a Parafilm® spacer with a thickness of *ca.* 80 μm. The focused position was set at 40 μm from the bottom of the cover glass. NIPAM co-polymerized with 3-(2-propenyl)-9-(4-*N*,*N*-dimethyl-aminophenyl-phenanthrene) (VDP) was synthesized, and the synthesis method was previously described in detail.[34] The content of the VDP unit was 0.1 mol%. The concentration of VDP labeled PNIPAM was 3.5 wt%. The VDP unit has the property of intramolecular charge transfer and is sensitive to local polarity changes, and it was used for the observation of the microscopic polarity change during the phase transition.[35] A continuous wave

He–Cd laser (IK3410R-F, KIMMON) with a wavelength of 325 nm was used for excitation of florescence and it was once focused to the back focal plane of the objective lens and directed to irradiate the whole area in a microscopic view region. The fluorescence from the sample was detected with a polychrometer (SP2300i, Princeton Instruments), and the transmitted images were detected with an EMCCD (PIXIS 400, Princeton instruments).

3 Results and discussions

3.1 Turbidity and HD-TG measurements

The turbidity response and HD-TG response of a PNIPAM solution are shown in Fig 2(a) and (b) in the millisecond and second ranges, respectively. The initial temperature was 32.4 °C, the temperature jump was 1.5 K with the laser intensity of 10.5 mJ pulse^{-1} and the grating spacing was 80 μm. The time $t = 0$ indicates the timing of the excited laser irradiation. In the turbidity response, a negative signal corresponding to the reduction of the transmitted light was initially observed for several tens of milliseconds, and the signal returned to the initial intensity for less than a hundred milliseconds. When the initial temperature was kept low enough not to go beyond the phase transition temperature (<31 °C) by the pump irradiation, this response was not observed. As the initial temperature approached

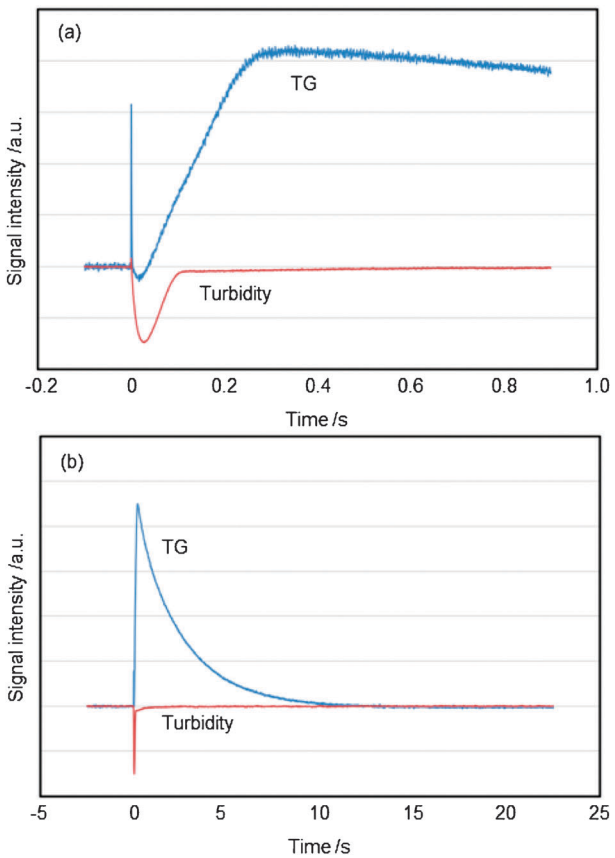

Fig. 2 A turbidity response and a transient grating response of a PNIPAM solution (3.0 wt%) (a) in the millisecond and (b) second ranges. The initial temperature was 32.4 °C, and the grating spacing was 80 μm.

the LCST, this response emerged. Thus, the behavior was observed due to the phase transition of the PNIPAM solution. Since the probe light was scattered by phase-separated PNIPAM and the light intensity was reduced, negative signal was observed. Since the thermal diffusion in the solution occurs over several milliseconds, the sample was cooled to a temperature lower than the LCST. Consequently the phase-separated PNIPAM returned to the homogeneous phase and became transparent. Similar turbidity responses were reported by Tsuboi *et al.*[15]

In the TG response, a spike-like response corresponding to the thermal grating was observed with a time constant of a few milliseconds[16] and then a small negative signal and its decay for about 100 milliseconds were observed, same as the turbidity response. However, additional response with a rise for about hundreds of milliseconds and decay for about ten seconds were observed. This response was not observed for temperatures low enough not to reach the LCST (Fig. 4(a)). The small negative response and its relaxation correspond due to a similar process to the turbidity. In the turbidity response, the source of the signal should scatter light, and the size of the scattering objects which macroscopically reduce the light transmission is at least on the order of several hundreds of nanometres. Since the effective diameter of a single PNIPAM molecule is on the order of a few nanometres, the source of the turbidity response is assumed to be PNIPAM aggregates, considering that the scattering intensity is inversely proportional to the square of the diameter of the scattering medium. As such, we may conclude that the initial negative response corresponds to the appearance or disappearance of those aggregates. Considering the additional component in the HD-TG response, the responsible object is not large enough to scatter light but it does change the refractive index, so that its size must be on a molecular level. Therefore, the most probable candidate is a globule molecule released from the aggregate but not changed into a coil state. It is supposed that the globule molecule released from the aggregate does not influence the turbidity, but gives the refractive index change due to the difference from the coil molecule. Then it is considered that the generated globule molecule as a product species and the coil molecule as a reactant species counter-propagate due to diffusion, which would be the origin of the HD-TG response. It is known that the refractive index is sensitive to the molecular volume changes due to changes in polarizability,[36,37] this effect has been utilized for detection of protein folding and unfolding processes, *etc.*[38,39] So far, it has been believed that the turbidity relaxation corresponded due to the process of the globule-to-coil transition,[15] but we consider that it corresponds to the process from aggregates to globule molecules.

Ishikawa *et al.*[22] and Hofkens *et al.*[23] observed that the time for the PNIPAM phase transition becomes shorter if the solution is repeatedly exposed to light. It was suggested that the solution may pass through an intermediate state that cannot be observed under a microscope; the formation time is affected by the intermediate state and the solution does not relax completely to the original state. Ding *et al.*[8] reported that two peaks were observed around the LCST in the cooling scan of the differential scanning calorimetry measurement. This result suggests that there may be an intermediate state in the phase recovery process. Considering the results of these papers

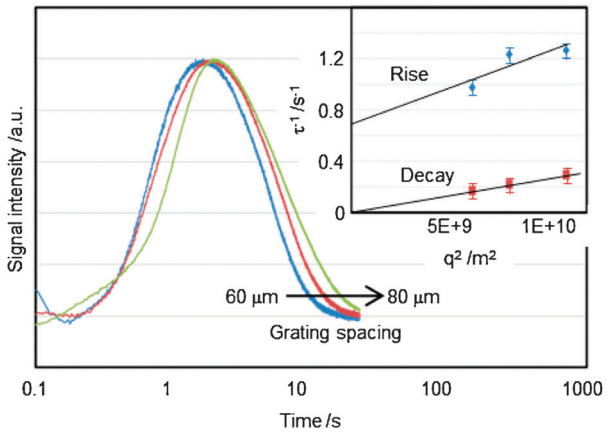

Fig. 3 Transient grating responses for grating spacings in the range 60–80 μm. The relation between the inverse of the decay times (τ^{-1}) and q^2 are shown in the inset. The initial temperature was 32.8 °C and the temperature jump was 1.0 K.

and our results, we propose that the PNIPAM aggregate formed due to the phase transition does not immediately return to the coil state in the relaxation process, but the aggregates of the globules first relax to molecularly disperse and then they change into the coil state.

To confirm our assumption on the signal origin, the grating spacing dependence was investigated. An example of the HD-TG responses on a logarithmic time scale is shown in Fig. 3 at an initial temperature of 32.8 °C. The temperature jump was 1.0 K. Rise-and-decay responses and their dependence on the grating spacing were clearly confirmed. Based on the refractive index of the thermal grating, the refractive index changes for the rise and decay components are positive and negative, respectively, and they can be assigned to the diffusion processes of the globule molecules as a product species, and that of the coil molecules as a reactant species, respectively. The inset shows the corresponding τ^{-1} *vs.* q^2 plot for the rise and decay components. The plot for the decay component intersects the origin, which indicates that the response is caused by a steady-state molecule, and it supports that this component corresponds to the coil molecules. The plot for the rise has *y*-intersect, and the lifetime was estimated to be 1.5 seconds. This result can be interpreted that the globule molecules diffuse with their recovery to the coil state. The estimated lifetime is on the same order as that obtained in NMR.[11]

Fig. 4(a) shows the HD-TG responses on a logarithmic time scale at the different initial temperatures with a grating spacing of 80 μm, where the initial temperature was varied (25, 32.6–33.0 °C) but the temperature jump being kept to 1.0 K with the laser intensity of 11.0 mJ pulse^{-1}. Clearly, the response was not observed when the initial temperature was 25 °C lower than LCST, since the phase transition does not take place. With an increase in the initial temperature, the rise and decay response due to the diffusion of the coil and globule molecules shifted to the longer time range, which indicates the change in the diffusion coefficient, affected by temperature. By measuring the grating dependence at each initial temperature, the diffusion coefficients and lifetimes were examined, and the size for each state was estimated using the Einstein–Stokes equation (Fig. 4(b)).[40] The phase transition was not observed when the initial temperature was lower than 32.1 °C. For the

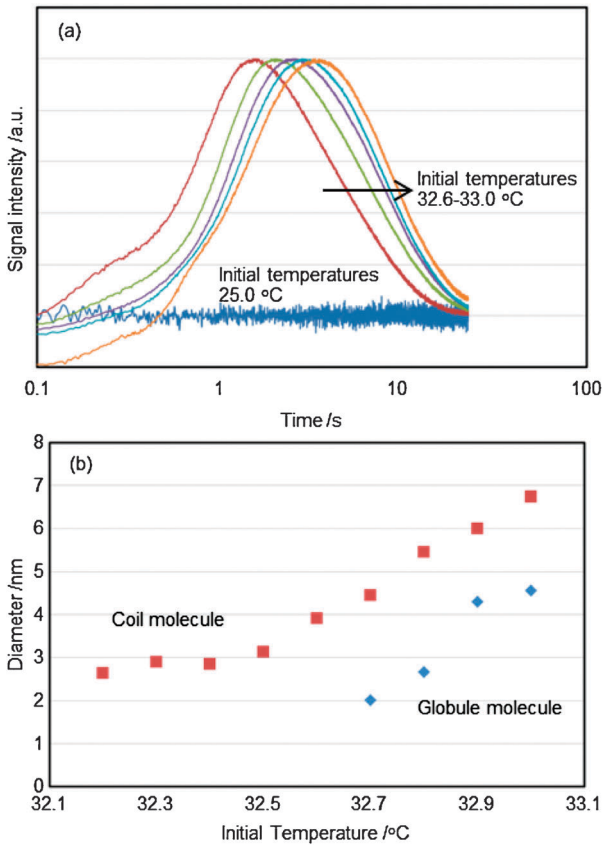

Fig. 4 (a) Transient grating responses in the logarithmic time scale of various initial temperatures: 25.0, and 32.6, 32.7, 32.8, 32.9, 33.0 °C. The temperature jump was 1.0 K, and the grating spacing was 80 μm. (b) The relation between the initial temperature and the diameter of the species, as calculated using the Einstein–Stokes equation.

initial temperature lower than 32.6 °C, the τ^{-1} *vs.* q^2 plot for the rise component was ambiguous to deduce the diffusion coefficient of the globule molecules, namely the size because of the non-negligible overlap between the rise component and the turbidity response.

The size of the coil molecules did not depend on temperature and was constant, approximately 3 nm, for the initial temperatures lower than LCST minus 0.5 K and the size increased sharply for the larger temperatures. The size of the globule molecules is always smaller than that of the coil molecules, and it is reasonable because the globule molecule is a packed state due to dehydration of a coil state. The size markedly increased in a similar manner to that for the coil molecules. From the result, the initial state of the coil state is varied according to the temperature above 32.6 °C, while it remained the same below the temperature.[8] Accordingly, the globule state generated from the coil state would also change its size. The lifetime of the globule state ranged from 1.5 to 5 s.

It has been reported that there are several different hydrated states of PNIPAM in water.[41,42] Corkhill *et al.*[42] reported that there are two kinds of water molecules hydrated with a coil state of PNIPAM; one is hydrated with the amide group, and the other is free waters just faced with the isopropyl group or located around the main chain. Sun *et al.*[13] reported that the hydrated coil state was dehydrated through two stages, namely the release of two different kinds of water molecules, after

which globule aggregates are generated. The size of the coil state may depend on different hydration and association conditions. It has been considered that the coil molecules are hydrated at low temperatures and gradually dehydrated when the sample temperature approaches the LCST. We suggest that the coil state consists of a single molecule at temperatures lower than 32.6 °C and that a few molecules make an assembly at the higher temperatures. Accordingly the generated globule state, which comes from the coil state, also has the similar size dependence on the temperature. Although the size of a single coil or a globule molecule cannot be estimated accurately, it is on the order of 2–3 nm considering a number average molecular weight of 20 000–25 000 g mol^{-1}, based on the fact that the size of β-lactoglobulin is 2.7 nm with a molecular weight of 18 kDa[43] and the size of bovine serum albumin (BSA) is 3.6 nm with a molecular weight of 65 kDa.[44] It is reasonable that the size of a PNIPAM molecule at the lower temperatures corresponds to a single coil molecule, and a few molecules are assembled at higher temperatures.

Temperature profiles should be taken care for the HD-TG measurement because the average temperature within a beam area is raised even after the thermal grating decay. For example, when a 1.0 K is raised at the grating maxima, the temperature is still 0.5 K higher within the beam area than the surrounding after the thermal grating decay. However, since the beam is linearly focused down to 300 μm in length, it is assumed that the temperature within the beam area is cooled down to the ambient temperature after the thermal diffusion for a distance of 300 μm, which takes about 150 ms. Thus the effect of the temperature rise can be safely neglected for the time region of the species gratings of the coil and globule molecules.

3.2 Microscopic observation

To investigate this intermediate state in the phase relaxation using a different technique, the fluorescence was monitored for a VDP labeled PNIPAM solution under a microscope. IR irradiation of the VDP labeled PNIPAM solution led to the formation of a particle that was trapped at the focused position. The trapping behavior of these fluorescent PNIPAMs was similar to the result obtained for PNIPAM,[45] the details of this experiment will be published elsewhere. The phase relaxation process was initiated by turning off the laser (Fig. 5(a), $t = 0$ s). The successive images are shown in Fig. 5(a) and the temporal changes in the corresponding fluorescence spectra and the peak wavelength are shown in Fig. 5(b) and (c). The phase relaxation process was confirmed by the microscopic view, showing that the solution turns transparent 4 seconds after turning off the light. The fluorescence peak shifted from 502 to 456 nm by taking the probe molecule into the phase transition region, namely the hydrophobic environment ($t = 0$ s), and it gradually recovered to 502 nm in the phase relaxation process, because the probe molecule became gradually exposed to a polar solvent. The fluorescence peak wavelength did not change for the initial 5 seconds, and then gradually red-shifted for about 10 seconds as shown in Fig. 5(c). This temporal behavior of the fluorescence peak is quite different compared to that for the microscopic images. It is reminded that the time for the heat dissipation from the focused area is on the order of several milliseconds after turning off the light, obtained from the thermal diffusion theory.[46]

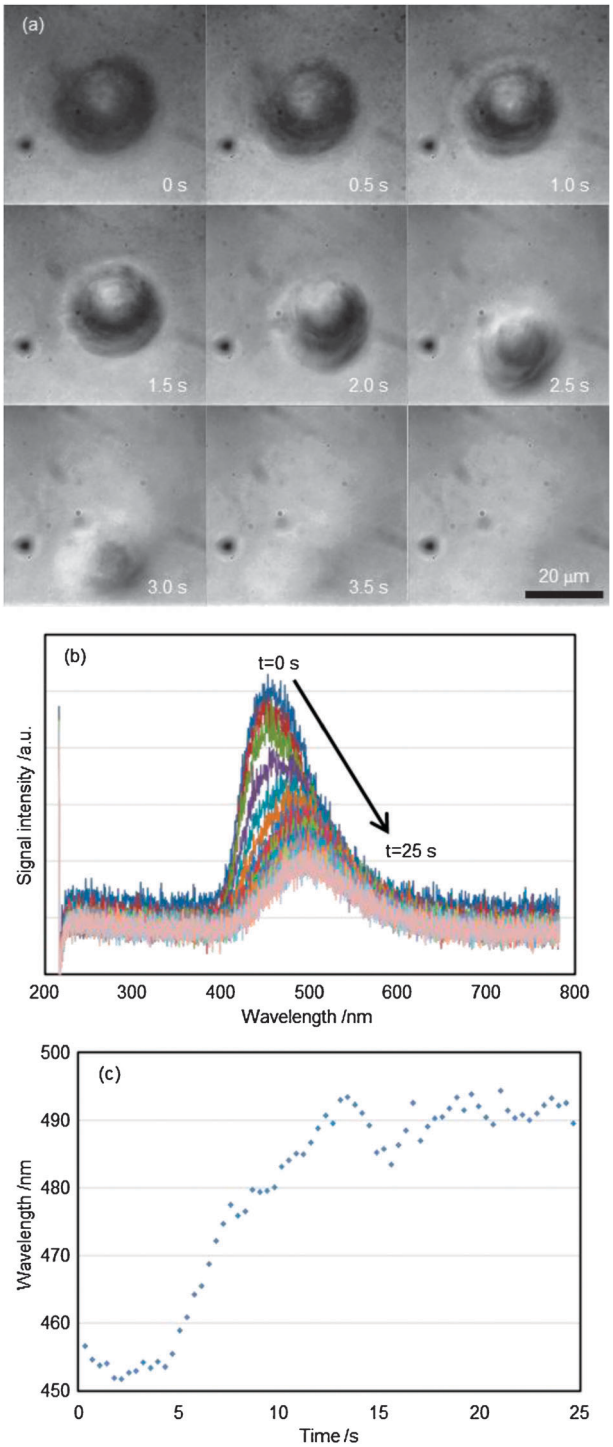

Fig. 5 (a) Successive pictures of the phase relaxation process of the VDP labeled PNIPAM solution (3.5 wt% in H_2O) after turning off the laser ($t = 0$ s). (b) Fluorescence spectra change of the phase transition region corresponding to pictures in (a). (c) The temporal change in the peak wavelength corresponding to the results in (b).

Comparing the results of the HD-TG, it is suggested that the transmitted image reflects the loosening of the aggregated globules, corresponding to the turbidity results, and the fluorescence reflects

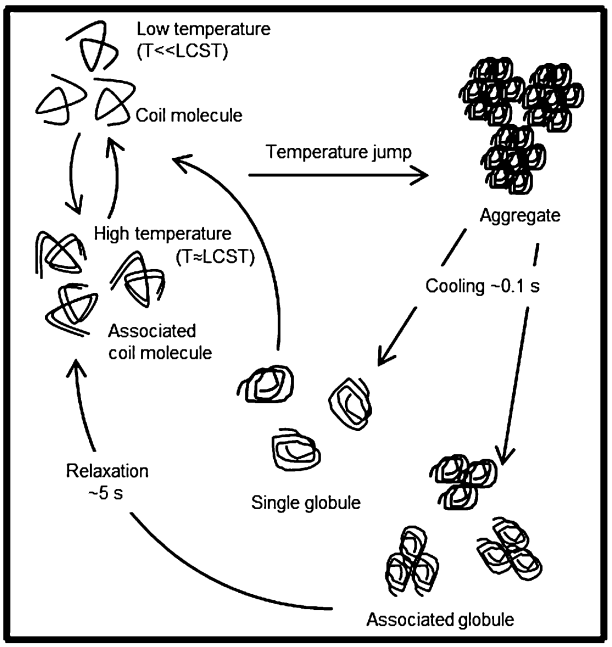

Fig. 6 Schematic diagram of the proposed coil–globule transition and relaxation of PNIPAM.

the microscopic environment of the probe molecule which cannot be observed with optical microscope images. The fluorescence spectrum and its change reflect the microscopic environment of the probe molecule, which corresponds to the results on the HD-TG response. Additionally the lifetime of the globule state obtained by the HD-TG measurement roughly agreed with the time constant of the fluorescence shift considering that the fluorescent probe is attached to the PNIPAM chain in the microscopic experiment, which supports that the fluorescence wavelength reflects a similar environment obtained by the HD-TG measurements.

On the basis of those results and considerations, the schematic diagram of the phase transition and phase relaxation processes is summarized in Fig. 6. The initial state of the coil molecules depends on the temperature; each molecule is dispersed at the temperature lower than 32.5 °C, a few molecules make an assembly at the higher temperature. The dehydration of such a coil state was induced by heating, and the state changed into the globule one, resulting in aggregation due to hydrophobic interactions of molecules. After the temperature lowering due to thermal diffusion, the aggregates are loosened to a single globule molecule or assembly of a few globule molecules less than 0.1 s. Then, they relax to the initial coil state depending on the temperature on the order of several seconds. Whether the coil or globule molecules stay as single molecules or as an assembly of a few molecules is determined by the initial temperature. This diagram is made clear by introducing the HD-TG method and by combing the results by beam heating fluorescence microscopy.

4. Conclusions

We observed the phase transition and phase relaxation processes of a PNIPAM aqueous solution by using the HD-TG method combined with the laser temperature jump technique. The sample temperature was raised by about 1.0 K instantaneously by absorption of a short light pulse by the heater molecules. The phase transition of a preheated sample close to the LCST was induced and the following phase relaxation dynamics were observed. We succeeded in observing directly the intermediate state in the phase relaxation, which is not observed by the turbidity measurements. Comparing the results with the turbidity dynamics, two relaxation processes were clarified; one is the relaxation corresponding to the loosening of globule aggregates and the subsequent dynamics corresponding to the globule to coil transition which occurs during several seconds. From the temperature dependence of the size of the coil and globule state, it is suggested that an assembly of coil or globule molecules is involved in the phase relaxation.

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