Polarity Does Not Matter: Molecular Weight Reverses the Photoisomerization-Induced Phase Separation of an Azobenzene-Bearing Polymer

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We find the non-canonical photoisomerization-induced phase separation of an azobenzene-bearing polymer. The polymer composed of acrylate-based azobenzene (AzoAA) and *N*,*N*-dimethylacrylamide (DMA), namely poly(AzoAA-*r*-DMA), phase separates under visible light-induced *cis*-to-*trans* isomerization at high molecular weight, whereas the phase separation is realized under ultraviolet light-induced *trans*-to-*cis* isomerization at low molecular weight. Conventionally, the origin of photoisomerization-induced phase separation is believed to arise from the difference in polarity between the apolar *trans* and polar *cis* states; thereby the direction of phase changes, either to separate or dissolute, is uniquely determined by the polarity changes during the isomerization of azobenzene. Contrary to this common perception, the poly(AzoAA-*r*-DMA) in this study phase separates through both *trans* and *cis* isomerization,

depending on the molecular weight. The non-canonical phase separation of poly(AzoAA-*r*-DMA) reported herein suggest that molecular weight plays a significant role in determining the phase behavior of azobenzene-bearing polymers. This study provides a platform for the development of spatial temporally controlled delivery vehicles and microreactors.

1. Introduction

Azobenzene is a well-known photochromic molecule that has been extensively studied to understand its fundamental properties and application prospects.[1] *Trans*-azobenzene is thermodynamically more stable than the *cis* isomer. Under conditions of ultraviolet (UV) light irradiation, the *trans* state converts to the metastable *cis* state, and the *cis* state reverts to the most stable *trans* form when exposed to thermal conditions or under conditions of visible (vis) light irradiation. The *trans* state adopts a planar configuration, and the dipole moment of the *trans* isomer is recorded to be 0.5 D.[1d] The *cis* isomer adopts a bent configuration, and the dipole moment of this state is higher (3.0 D) than the dipole moment of the *trans* isomer.[1d] The reversible photoresponse of azobenzene has attracted the attention of researchers, and photomodulation of the phase behavior of thermoresponsive polymers in a solvent is being widely researched in the field of thermodynamics of polymer solution.[2] The temperature/light responsiveness of azobenzene-bearing thermoresponsive polymers has been a subject of intense research since the late 1970s. Azobenzene-bearing polymers are characterized not only in aqueous solutions[2f, 2h–o, 3] but also in organic solvents[2a, 2b] and ionic liquids.[2c–e] Polarity changes associated with the photoisomerization of azobenzene have been reported to control the solvatophilicity of the polymers, resulting in the alteration of the phase separation temperatures. Specifically, in polar solvents, such as water[2f, 2h, 2i, 2k–n] and ionic liquids,[2c] azobenzene-bearing polymers in the polar *cis* state are more solvatophilic than the molecules in the *trans* state, leading to the increase in the phase separation temperature (typically, the lower critical solution temperature; LCST) upon *trans*-to-*cis* photoisomerization. In contrast, in apolar organic solvents such as cyclohexane and decalin, the phase separation temperature (typically, the upper critical solution temperature (UCST)) decreases under conditions of *cis*-to-*trans* photoisomerization, and this can be attributed to the fact that the apolar *trans* state is more preferable for solvation.[2a] Numerous researchers have studied the phase behavior of azobenzene-bearing polymers and reported that the changes in solubility reflect the changes in polarity of azobenzene molecules under conditions of photoisomerization.[2]

Polarity has thus been primarily used to explain the light-induced solubility switching property of azobenzene-bearing polymers. However, some researchers have reported counterintuitive phase behavior of these molecules that cannot be explained only by the polarity difference between the *cis* and *trans* states. In other words, the temperature at which the LCST-type phase separation occurs in the *cis* state is lower than the temperature at which the phase separation occurs in the *trans* state.[4] Furthermore, even in the intuitive case where the LCST is higher in the *cis* state than the LCST corresponding to the *trans* state, small changes in the dipole moment (attributable to photoisomerization) result in significant differences in the phase separation temperatures between the *trans* and *cis* states. The difference in the LCSTs of the *trans*/*cis* states in the case of poly[(4-phenylazophenyl acrylate)-*r*-(*N*,*N*-dimethylacrylamide)] (poly(AzoAA-*r*-DMA)) was as large as 30 °C with 6 mol% of AzoAA.[2o] Such results led us to speculate that not simply polarity alone, but also other factors affect the phase behaviors of the *trans* and *cis* states of azobenzene-bearing polymers.

Among the various factors that can potentially affect the phase behavior of the molecules, we focused on the effects of molecular weight. Molecular weight effects on the phase behaviors of azobenzene-bearing polymers have not been systematically investigated to date; however, the molecular weight has profound effects on the phase separation temperature of polymers.[5] It has been observed that, in the case of poly(*N*-isopropylacrylamide) (PNIPAAm) functionalized with various end groups, increasing the molecular weight increased, decreased, or did not change the LCST. The change in the temperature depended on the functionalized end groups.[6] Based on such studies, we hypothesized that the molecular weight differentially affects the phase behavior of the azobenzene-bearing polymers in the *cis* and *trans* states. To this aim, we investigated the effects of molecular weight on the phase behavior of a statistical copolymer poly(AzoAA-*r*-DMA), which has been reported by Kröger *et al*.[2o] to have the largest difference in the phase separation temperature between the *trans* and *cis* states in an aqueous solution. Our results could not be explained solely by the dipole moment of azobenzene. The photoresponsive phase behaviors differed significantly depending on the molecular weights of the poly(AzoAA-*r*-DMA). Specifically, the molecular weight dependence of the phase separation temperature was only confirmed for the *trans* state, whereas the phase separation temperature was independent of the molecular weight of the poly(AzoAA-*r*-DMA) in the *cis* state. As a result, light-induced phase separation of poly(AzoAA-*r*-DMA) was realized in both directions of photoisomerization (*trans*-to-*cis* and *cis*-to-*trans*), depending on the molecular weight of the molecules. In the case of Kröger *et al*., phase separation was reported only during

cis-to-*trans* photoisomerization.[2o] Observation made using microscopy techniques revealed that light-induced phase separation was achieved through the formation of poly(AzoAA-*r*-DMA)-rich coacervates. Such coacervates are formed via liquid-liquid phase separation of biomacromolecules in living systems. There is a growing interest in the field of engineering liquid–liquid phase separation to mimic the biological functions of coacervates.[7] In this context, the system developed by us may not only serve as a reductionist model of the living counterpart[7a] but may also present a platform to realize spatial-temporally controlled loading/releasing of cargoes[7b] or initiation/termination of chemical reactions[7c] under conditions of UV or vis light irradiation.

2. Results and Discussion

AzoAA was synthesized by reacting acryloyl chloride with 4-hydroxyazobenzene to investigate the phase behavior of poly(AzoAA-*r*-DMA) (**Scheme 1**). Subsequently, the AzoAA monomer was copolymerized with the non-ionic DMA following the process of free-radical polymerization to obtain a series of poly(AzoAA-*r*-DMA) with comparable azobenzene content (4 mol%), but different molecular weights (**Table 1, Supporting Information Figure S1 and S2**). Based on the Fineman-Ross analysis,[8] the monomer reactivity ratios of DMA (r_1) and AzoAA (r_2) were determined to be 0.59 and 4.66, respectively (**Supporting Information Table S1 and Figure S3**). The Wall's method[9] was then used to obtain the triad sequence distribution of poly(AzoAA-*r*-DMA). The sum of the sequence distribution ratios of AzoAA adjacent triads in poly(AzoAA-*r*-DMA), namely (AzoAA-AzoAA-DMA) and (AzoAA-AzoAA-AzoAA), was calculated to be 3.8% (**Supporting Information Table S2**). The sum indicated that AzoAA and DMA are rather randomly distributed along the polymer chain. We have previously reported that the photoisomerization of AzoAA is reversible under conditions of UV (365 nm; *trans*-to-*cis* isomerization) and vis (436 nm; *cis*-to-*trans* isomerization) light irradiation.[10] The composition of the *cis* state after irradiation with UV (*trans*-to-*cis* isomerization) and vis (*cis*-to-*trans* isomerization) light were reported to be 90 and 10%, respectively.[10] *Cis*-to-*trans* isomerization can also proceed over time in the dark because the *trans* state is thermodynamically more stable than the *cis* state.[11] The effects of temperature on the process of thermal relaxation from the *cis* state to the *trans* state were investigated using poly($AzoAA_{4.0}$-*r*-$DMA_{96.0}$)$_{36kDa}$ in phosphate-buffered saline (PBS) (**Supporting Information Figure S4**). The thermal relaxation rates at 40, 50, and 60 °C were determined by analyzing the first-order plots generated with the absorbance data recorded at 323 nm (**Supporting**

Information Table S3).[11] The first-order rate constants at each temperature were comparable to the rate constants previously reported for the thermal relaxation of unsubstituted azobenzene in ionic liquids.[2e, 12] The half-life values corresponding to the thermal relaxation process were calculated using the rate constants, and the values ranged from 2.6 h (60 °C) to 24.8 h (40 °C). Therefore, under these conditions, the AzoAA monomer gradually reverted to the *trans* state in the dark. The samples in the *cis* state were continuously irradiated with UV (365 nm) light to avoid thermal relaxation during subsequent experiments.

Scheme 1. Synthesis route for the poly(AzoAA-*r*-DMA).

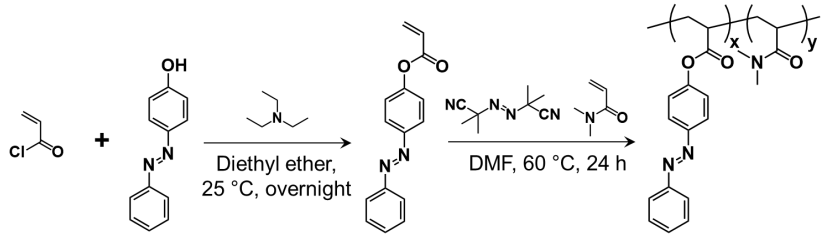

Table 1. Characterization of the poly(AzoAA-*r*-DMA) with similar compositions of azobenzene.

Polymer sample	[AzoAA]/[DMA] composition[a)]	M_n [kDa][b)]	M_w [kDa][b)]	M_w/M_n[b)]
Poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$	3.9/96.1	5.9	17	2.9
Poly(AzoAA$_{4.2}$-*r*-DMA$_{95.8}$)$_{16kDa}$	4.2/95.8	16	60	3.7
Poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$	4.0/96.0	36	170	4.7

a) Determined using the ^{1}H nuclear magnetic resonance (NMR) spectroscopy technique. b) Determined using the gel permeation chromatography technique using poly(methyl methacrylate) as the molecular weight standard.

Temperature-dependent transmittance data were recorded using the UV-vis spectroscopy technique to study the thermoresponsive phase separation of the synthesized poly(AzoAA-*r*-DMA) molecules in PBS. The transmittance of polymer solutions has been utilized conventionally to evaluate the macroscopic solubility of polymers. Drop in transmittance indicates the macroscopic phase separation of polymers in solvents. For all the synthesized poly(AzoAA-*r*-DMA) molecules, the transmittance in the PBS solution dropped at elevated temperatures, suggesting that poly(AzoAA-*r*-DMA) underwent LCST-type phase separation (**Figure 1**). The phase separation temperature was approximately 32 °C for the *trans* state for poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ (Figure 1 (a)). Photoisomerization to the *cis* state under

conditions of UV irradiation shifted the phase separation temperature to 42 °C. The increase in the phase separation temperature has been attributed to the fact that the polarity of *cis*-azobenzene (dipole moment 3.0 D) is higher than that of *trans*-azobenzene (0.5 D).[13] The high polarity of *cis*-azobenzene promoted the solvation of poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ in an aqueous solution, and the degree of solvation achieved in this case was higher than the degree of solvation achieved for *trans*-azobenzene. Interestingly, the phase separation temperatures of poly(AzoAA-*r*-DMA) in the *trans* and *cis* states overlapped for the lower molecular weight sample, namely poly(AzoAA$_{4.2}$-*r*-DMA$_{95.8}$)$_{16kDa}$ (Figure 1 (b)). For even lower molecular weight poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$ the phase separation temperature corresponding to the *trans* state was higher than the phase separation temperature corresponding to the *cis* state (Figure 1 (c)). Such counterintuitive phase behavior (in other words, the LCST in the *cis* state is lower than the LCST in the *trans* state) occurred irrespective of the AzoAA/DMA ratio for low molecular weight polymers (**Supporting information Table S4**). Paying closer attention to the three graphs in Figure 1, the molecular weight dependence of the phase separation temperature was confirmed only for the *trans* state. As the molecular weight decreased, the phase separation temperature increased for the *trans* state, while the phase separation temperature remained approximately constant for the *cis* state. The difference in the molecular weight-dependence of the phase separation behaviors of the *trans* and *cis* states was confirmed using the comparable amount of azobenzene (4 mol%).

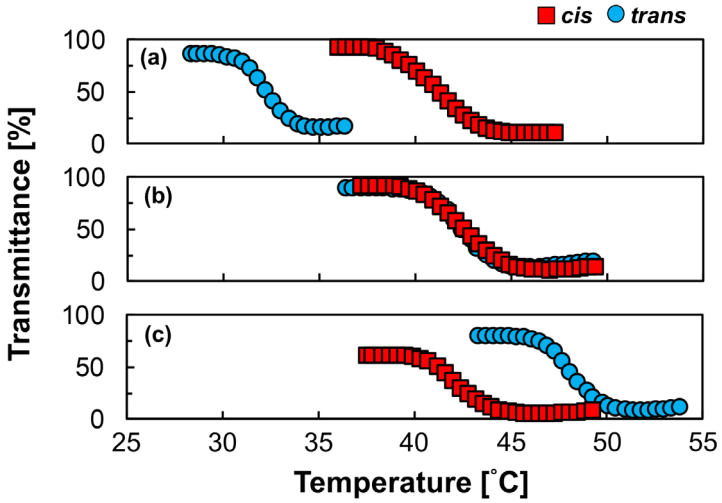

Figure 1. Temperature-dependent transmittance of 0.5 wt% poly(AzoAA-*r*-DMA) in PBS before/after UV irradiation (365 nm). (a) Poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$, (b) poly(AzoAA$_{4.2}$-*r*-DMA$_{95.8}$)$_{16kDa}$, and (c) poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$.

The temperature-dependent transmittance data were also recorded at different poly(AzoAA-*r*-DMA) concentrations (**Figure 2, Supporting Information Figure S5**) for the case of all three polymers. The concentration dependence of the phase-separation temperature for each polymer are summarized in **Figure 2**. Regardless of molecular weight, the phase separation temperatures decreased significantly as the polymer concentration increased from 0.1 to 1.0 wt%. The phase separation temperature remained unchanged in the concentration range of 1.0–3.0 wt%. Such concentration dependence of the phase separation temperature is typical for thermoresponsive polymers.[14] The phase separation temperatures for the *trans* states decreased as the molecular weight increased (Figure 2 (a)). It should be noted that the molecular weight dependence of the phase separation temperature was not observed for the *cis* state (Figure 2 (b)). In general, the phase separation temperature of a thermoresponsive polymer decreases as the molecular weight increases. This can be explained by the Flory–Huggins solution theory, which states that as the molecular weight increases, the number of possible configurations of the polymer–solvent mixture decreases, resulting in a decrease in the change in entropy of mixing.[15] The phase separation temperature is governed by the change in the Gibbs energy of mixing (ΔG_m), which is related to the change in the enthalpy of mixing (ΔH_m) and the change in the entropy of mixing (ΔS_m) by the equation $\Delta G_m = \Delta H_m - T\Delta S_m$ (T: temperature). In an LCST-type polymer, both ΔH_m and ΔS_m are negative. As ΔS_m decreases due to the molecular weight increase, the phase separation temperature (the temperature at which $\Delta G_m = 0$) decreases. However, the results obtained were different from the expected results: the phase separation temperature was found to be independent of the molecular weight in the *cis* state. The molecular weight independence of the phase separation temperature has been widely reported for the well-known thermoresponsive PNIPAAm system.[16] Tanaka *et al.* theoretically explained the molecular weight independence of the phase separation temperature based on a “cooperative solvation” mechanism.[17] In cooperative solvation, the presence of a bulky isopropyl group allows the progress of a chain of hydration events along the NIPAAm units, resulting in efficient hydration. Hydrophobic collapse is realized following a chain of dehydration events along the PNIPAAm chain and not through the independent dehydration of water molecules. A similar solvation mechanism might have taken place for the poly(*cis*-AzoAA-*r*-DMA), but not for the poly(*trans*-AzoAA-*r*-DMA). The polar *cis*-azobenzene allows cooperative hydration to proceed along the polymer chain, whereas the apolar *trans* azobenzene inhibits the process of cooperative hydration along the polymer chain. This phenomenon can partially account for the molecular weight independence of the phase behavior of the poly(*cis*-AzoAA-*r*-DMA). Furthermore, the

Flory–Huggins theory[5b] reveals that the phase separation temperature can be either dependent or independent of the degree of polymerization (**Supporting Information; "theoretical considerations"**). Although further studies must be conducted to investigate the factors governing the application of one expression or the other (in the Supporting Information; "theoretical considerations"), it can be hypothesized that the different expressions apply to the two photoisomerization states. The mechanism behind the interesting phase behavior of the two isomeric states of poly(AzoAA-*r*-DMA) will be revealed in the future in a different paper in this series.

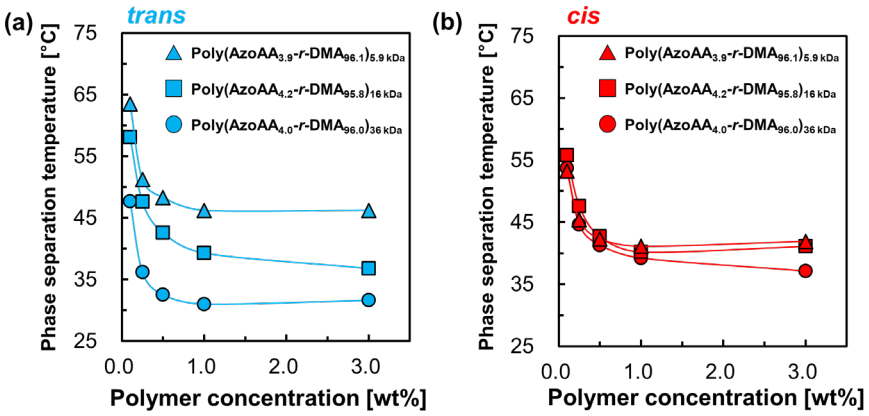

Figure 2. Concentration/molecular weight dependence of phase separation temperature. The plots in (a) and (b) present the phase separation temperatures before/after UV irradiation, respectively. In both (a) and (b), different symbols are used to represent different polymer samples: triangle, poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$, square, poly(AzoAA$_{4.2}$-*r*-DMA$_{95.8}$)$_{16kDa}$, circle, poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$.

We recorded micrographs of the polymer solutions in the temperature range of 30–50 °C to investigate the phase separation behavior in more detail (**Figure 3**, **Supporting Information Figure S6**). Microscopic structures were not observed in the micrographs recorded at low-temperature conditions for the *trans* and *cis* states of poly(AzoAA$_{4.0}$-*r*-DMA$_{96.1}$)$_{36kDa}$ (Figure 3). In contrast, the coacervates started to form at elevated temperatures (*trans* state: ≥35 °C; *cis* state: ≥45 °C). The size of the coacervate droplets increased with an increase in temperature, and this can be attributed to the coalescence of multiple coacervates.[17] The formation of coacervates was also observed for the other two polymers, and the coacervates formed at a temperature that was approximately the same as the phase separation temperatures shown in

Figure 1 (Supporting Information Figure S6 (a,b)). We speculate that in Figure 1, the poly(AzoAA-*r*-DMA)-rich coacervates scattered light, resulting in decreased transmittance.

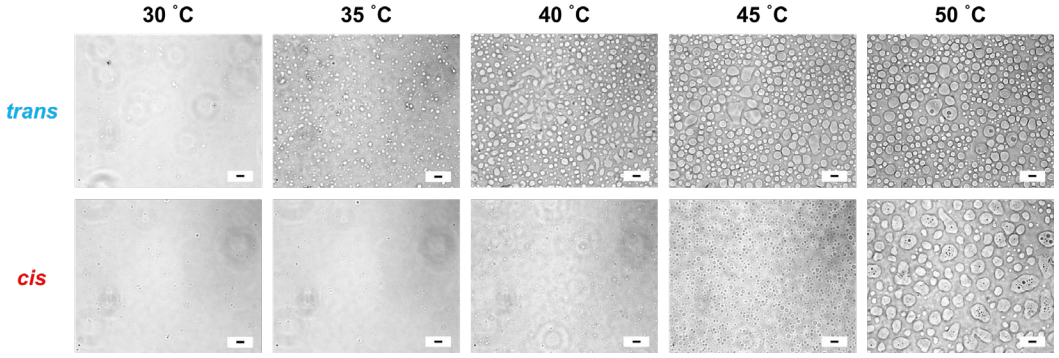

Figure 3. Micrographs for 0.5 wt% poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ in PBS at different temperatures. Scale bar: 10 μm.

The formation of coacervates via liquid–liquid phase separation is ubiquitous in living cells, and the process plays a crucial role in compartmentalizing biomolecules in the cytoplasm.[18] The ability of coacervates to segregate components has been exploited to engineer drug delivery vehicles,[7b] microreactors,[7c] and others.[19] The results prompted us to reversibly control the formation of coacervates, to precisely control chemical reactions and payload transport using light. *In situ* experiments were conducted with poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ (at 35 °C) and poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$ (45 °C) in PBS under conditions of UV or vis light irradiation using the bright-field microscopy technique (**Movies 1 and 2**). Consistent with the differences in the phase separation temperatures in Figure 1, coacervates formed when the irradiation light was switched from UV to vis (for poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ in Movie 1) or from vis to UV (for poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$ in Movie 2). The coacervates flowed in specific directions, and this could be attributed to the convective flow within the polymer solution. The polymer solution was loaded inside a 500 μm-thick, closed cell and heated from the bottom. This potentially resulted in the generation of a temperature gradient within the sample. When the irradiation lights were switched back to the original lights, rapid dissociation of the coacervates was observed. Furthermore, we attempted to observe the macroscopic transmittance changes corresponding to the polymer solutions during the process of photo-induced phase separation (**Figure 4**). For the poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ system, a high degree of transmittance was recorded during the initial stages under conditions of UV irradiation. This reflects the higher phase separation temperature for the *cis* state than the temperature at which the data are recorded (Figure 4 (a)). The transmittance dropped

significantly when UV light was switched to vis light, and this could be attributed to the phase separation of poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ in the *trans* state. Once the transmittance reached approximately 20%, it gradually increased to approximately 50%, following which a plateau was reached. We hypothesized that the gradual increase in transmittance could be attributed to the coalescence of the poly(AzoAA-*r*-DMA) coacervates. As described in Figure 3, coacervates can fuse over time, resulting in an increase in size. The fusion of the coacervates results in a decrease in the scattering ability of each coacervate. This can result in an increase in transmittance.[20] Cirulis *et al*. reported that the absorbance gradually decreased (therefore, the transmittance gradually increased) as the coacervates of rich elastin-like polypeptides coalesced.[21] The transmittance increased and reverted to the initial value when vis light was switched to UV light. The process of reversible photoinduced phase separation could be repeated for at least another cycle. In the case of poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$, a decrease in transmittance was observed under conditions of UV light irradiation, whereas the transmittance increased under conditions of vis light irradiation (Figure 4 (b)). The light-induced phase separation behaviors of the systems contradicted each other and depended on the molecular weight of the poly(AzoAA-*r*-DMA) system. This agreed well with the temperature-dependent transmittance data presented in Figure 1. The non-canonical, photoresponsive phase separation realized under these conditions could be potentially exploited to realize the spatiotemporal regulation of the loading/releasing of molecules or to control reactions under conditions of UV or vis light irradiation.[17b, 19b]

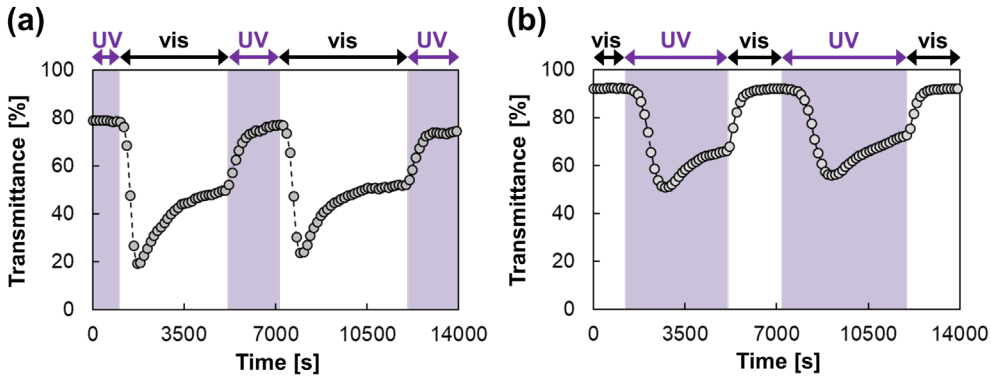

Figure 4. The transmittance of 1.0 wt% poly(AzoAA-*r*-DMA) in PBS under cyclic UV/vis irradiation at constant temperatures. (a) Poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ at 35 °C and (b) poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$ at 45 °C.

3. Conclusion

In conclusion, a series of LCST-type poly(AzoAA-*r*-DMA) polymers with comparable azobenzene contents but different molecular weights were synthesized, and their thermoresponsive phase properties were studied. The phase separation temperature of poly(*trans*-AzoAA-*r*-DMA) decreased as the molecular weight increased. In contrast, the phase separation temperature of poly(*cis*-AzoAA-*r*-DMA) was independent of the molecular weight. As a result, the phase separation temperature increased under UV irradiation (*trans*-to-*cis* isomerization) for poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$, whereas vis irradiation (*cis*-to-*trans* isomerization) increased the phase separation temperature of poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$. Opposite phase behaviors were observed between the two polymers under UV-vis irradiation. Herein, we highlight the importance of molecular weight in engineering the photoresponsive phase behavior of azobenzene-bearing polymers. The results reported herein may fundamentally contribute to understanding the phase separation of functional polymers. Investigations are underway to question if the non-canonical phase behavior also occurs for azobenzene-bearing polymers with other comonomer combinations. Further studies may pave the way for the development of synthetic cell models, light-regulated drug delivery systems, and microreactors.

4. Experimental Section/Methods

Materials: *N,N*-Dimethylacrylamide (DMA) was kindly provided by KJ Chemicals (Tokyo, Japan), and the compound was passed through a column of aluminum oxide (90 active basic 0.063–0.200 mm, Merck, Germany) before use. 4-Hydroxyazobenzene, acryloyl chloride, and lithium bromide were purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan), and these compounds were used as received. All other reagents were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

Synthesis of 4-phenylazophenyl acrylate (AzoAA): The photoresponsive monomer 4-phenylazophenyl acrylate (AzoAA) was synthesized following previously reported protocols.[10] 4-Hydroxyazobenzene (12.9 g, 6.5×10^{-2} mmol) and triethylamine (13.6 mL, 9.8×10^{-2} mmol) were dissolved in diethyl ether (150 mL). A solution of acryloyl chloride (6.1 mL, 7.5×10^{-2} mmol) in diethyl ether (30 mL) was added dropwise to the previously prepared solution using a Pasteur pipette in an atmosphere of flowing nitrogen gas. The reaction was allowed to proceed overnight at 25 °C under an atmosphere of nitrogen. Triethylammonium

chloride was produced during the reaction, and this by-product was removed from the reaction mixture following the process of vacuum filtration. The filtered solution was washed several times using deionized water using a separation funnel, and the organic layer was collected in an eggplant flask. The solution containing the target monomer was concentrated using a rotary evaporator (Eyela, Tokyo, Japan), after which AzoAA was recrystallized in ethanol. The ^{1}H NMR spectroscopy technique (400 MHz, JEOL, Tokyo, Japan) was used to validate the formation of AzoAA. The spectra were recorded using deuterated acetonitrile as the solvent (Figure S1).

Synthesis of poly(AzoAA-r-DMA) with comparable azobenzene contents but different molecular weights: Photoresponsive poly(AzoAA-*r*-DMA)s were synthesized following the process of free-radical polymerization under three different conditions. The amounts of monomers (AzoAA and DMA) used for each polymerization reaction are listed in Table 2. The monomers AzoAA and DMA were dissolved in 4 mL of *N*,*N*-dimethylformamide (DMF) in a 5 mL glass vial. In another 5 mL glass vial, 49.3 mg (7.50×10^{-2} mmol) of 2,2'-azobis(isobutyronitrile) (AIBN) was dissolved in DMF (4 mL). The solutions of monomers and AIBN were placed inside a bell jar, which was then deoxygenated using a diaphragm pump (N820.3FT.40.18, KNF, Germany). The process of deoxygenation was carried out for 30 min. After deoxygenation, 1 mL of the AIBN solution was added to the monomer solution taken in the glass vial. The vial was tightly sealed, and the reaction mixture was allowed to polymerize at 60 °C over 24 h. After 24 h, the solution was exposed to the atmosphere to terminate the reaction. Subsequently, the reaction solution was poured into a dialysis tube (molecular weight cut-off: 3.5 kDa, Thermo Fisher Scientific, PA, USA), which was then immersed in deionized water to remove unreacted monomers from the reaction solution. Poly(AzoAA-*r*-DMA) was obtained following the process of freeze-drying (FDU-1110; Eyela, Tokyo, Japan). The AzoAA to DMA molar ratio for each poly(AzoAA-*r*-DMA) sample was determined using the ^{1}H NMR spectroscopy technique using a spectrometer (400 MHz, JEOL, Tokyo, Japan). The spectral profiles were recorded by dissolving the samples in deuterated chloroform (Figure S2). Relative molecular weights and polydispersity indices were determined using the gel permeation chromatography (GPC) technique. DMF containing lithium bromide (10 mM) was used as the eluent, and the GPC column (Tosoh, Osaka, Japan) was calibrated using poly(methyl methacrylate) as the molecular weight standard.

Table 2. Amount of monomers used for the free-radical polymerization of each poly(AzoAA-*r*-DMA).

Sample	AzoAA	DMA
Poly(AzoAA$_{3.9}$-*r*-DMA$_{96.1}$)$_{5.9kDa}$	44.1 mg, 0.175 mmol	0.240 mL, 2.33 mmol
Poly(AzoAA$_{4.2}$-*r*-DMA$_{95.8}$)$_{16kDa}$	114 mg, 0.450 mmol	0.726 mL, 7.05 mmol
Poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$	189 mg, 0.750 mmol	1.47 mL, 14.3 mmol

Calculation of the monomer reactivity ratios: The monomer reactivity ratios of AzoAA and DMA were calculated from the Fineman-Ross analysis.[8] At first, various poly(AzoAA-*r*-DMA)s were synthesized under different AzoAA/DMA feed ratios (Table S1) by the free-radical polymerization. After 3 hours of polymerization, the polymers were collected in the same methods as explained in the previous section. The actual AzoAA/DMA ratios in the polymers were analyzed by the ^{1}H NMR spectroscopy technique. Subsequently, Figure S3 was fabricated from the feed and actual AzoAA/DMA ratios, after which the slope and the y-intercept of the fitted line plots were calculated to represent the monomer reactivity ratios r_1 and r_2. Furthermore, the triad sequence distribution of AzoAA and DMA in poly(AzoAA-*r*-DMA) with 4 mol% AzoAA was estimated based on the Wall's method (Table S2).[9]

Cis-to-trans thermal relaxation of AzoAA as evaluated by analyzing the time-dependent absorbance data: The *cis*-to-*trans* thermal relaxation of AzoAA was tracked by monitoring the changes in the absorbance properties of the solution containing poly(AzoAA-*r*-DMA) over time. Poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ was dissolved in phosphate buffered saline (PBS) to reach the final concentration of 0.01 wt%. Due to the high molar extinction coefficient of azobenzene,[1b] the polymer concentration was kept lower than that during the transmittance measurements. UV (light intensity:11 mW cm^{-2}, 365 nm) light was irradiated through a band-pass filter (Edmund Optics, NJ, USA) on a solution of poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ for 1 h using a mercury lamp (SX-UI 251HQ, USHIO, Tokyo, Japan). Following this, the solution of poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ was analyzed using the UV-vis spectroscopy technique using a UV-vis spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). The absorbance spectral profiles (250–650 nm) of the poly(AzoAA$_{4.0}$-*r*-DMA$_{96.0}$)$_{36kDa}$ solution were recorded every 4 h at a constant temperature.

As the process of thermal relaxation of azobenzene follows irreversible first-order kinetics,[10] the process of thermal relaxation from the *cis* to the *trans* state can be represented with a rate constant *k* as follows:

$$cis \xrightarrow{k} trans$$

Therefore, the rates of formation of the *cis* and *trans* states can be written, based on the rate constants, as follows:

$$-\frac{d[cis]_t}{dt} = \frac{d[trans]_t}{dt} = k[cis]_t \qquad (1)$$

The equation is solved, and the rate constant *k* is obtained as follows:

$$-kt = \ln \frac{A_t - A_\infty}{A_0 - A_\infty} \qquad (2)$$

where t is the duration of the thermal relaxation, A_t is the absorbance at time t, A_0 is the initial absorbance (*cis*-rich photostationary state), and A_∞ is the absorbance corresponding to the 100% *trans* state (after infinite time). The equation was analyzed, and the rate constant at each temperature was determined from the slope of the plots presented in **Figure S3**. The relation $A_{t_{1/2}} = (A_0 + A_\infty)/2$ was used to calculate the half-life ($t_{1/2}$) corresponding to the thermal relaxation process at each temperature point as follows:

$$t_{1/2} = k^{-1} \ln 2 \qquad (3)$$

Phase-separation behavior of poly(AzoAA-r-DMA) evaluated by analyzing the temperature-dependent transmittance data: The lower critical solution temperature-type phase separation behavior of the synthesized poly(AzoAA-*r*-DMA) was evaluated by recording the transmittance data using the UV-vis spectroscopy technique (UV-2600, Shimadzu, Kyoto, Japan). Poly(AzoAA-*r*-DMA) was dissolved in PBS at a specific polymer concentration. After adding the polymer solution to the measurement cell, the transmittance of the polymer solution was recorded at 600 nm, while the temperature of the polymer solution was raised at a rate of 1 °C min^{-1} while stirring. The temperature of the polymer solution was recorded simultaneously during the experiments using a temperature logger (LR5021; Hioki, Nagano, Japan). The polymer solution was not irradiated with light during the analysis of the *trans* state of azobenzene. During the analysis of the *cis* state, UV light (365 nm) was irradiated on the polymer solution for 1 h before recording the data using a mercury lamp (light intensity:11 mW

cm^{-2}, SX-UI 251HQ, USHIO, Tokyo, Japan) equipped with a band-pass filter (Edmund Optics, NJ, USA). The polymer solution was constantly irradiated while recording the transmittance data to minimize thermal relaxation during the *cis* to *trans* isomerization process. The phase-separation temperature was determined as the temperature at which 50% of the initial transmittance was recorded.

Photo-induced phase separation of poly(AzoAA-r-DMA) analyzed by transmittance data recorded at a constant temperature: The photo-induced phase separation of poly($AzoAA_{4.0}$-*r*-$DMA_{96.0})_{36kDa}$ and poly($AzoAA_{3.9}$-*r*-$DMA_{96.1})_{5.9kDa}$ was studied by recording the transmittance data using the UV-vis spectroscopy technique using a UV-vis spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). Poly($AzoAA_{4.0}$-*r*-$DMA_{96.0})_{36kDa}$ or poly($AzoAA_{3.9}$-*r*-$DMA_{96.1})_{5.9kDa}$ was dissolved in PBS to achieve the final concentration of 1.0 wt%. The transmittance of the polymer solution was recorded at 600 nm at a constant temperature (poly($AzoAA_{4.0}$-*r*-$DMA_{96.0})_{36kDa}$, 35 °C; poly($AzoAA_{3.9}$-*r*-$DMA_{96.1})_{5.9kDa}$, 45 °C). During the experiment, the photoisomerization of AzoAA was controlled by irradiating the system with either UV (light intensity:11 mW cm^{-2}, 365 nm) or vis (light intensity: 9 mW cm^{-2}, 436 nm) light. The temperature was monitored with a temperature logger (LR5021; Hioki, Nagano, Japan). The top of the measurement cell was irradiated using a mercury lamp (SX-UI 251HQ, USHIO, Tokyo, Japan) equipped with a band-pass filter (Edmund Optics, NJ, USA).

Analysis of the poly(AzoAA-r-DMA) system in PBS at different temperatures using the microscopy technique: Aqueous solutions of poly(AzoAA-*r*-DMA) were analyzed using the microscopy technique under conditions of heating to confirm the formation of microscopic structures during the phase separation of poly(AzoAA-*r*-DMA) in PBS. Poly(AzoAA-*r*-DMA) was dissolved in PBS to reach the final concentration of 0.5 wt%. A solution of poly(AzoAA-*r*-DMA) (20 μL) was cast on a coverslip of dimensions 30 mm × 30 mm (Matsunami, Osaka, Japan). To avoid the evaporation of the sample during the experiment, another coverslip of dimensions 30 mm × 30 mm was placed on top of the poly(AzoAA-*r*-DMA) solution. A 500 μm-thick silicone spacer was present between the two coverslips. The sample was placed on a stage-top heater (Thermo Plate, Tokai Hit, Shizuoka, Japan), and heated to a specific temperature. During the process of heating, the *trans* system was not irradiated with light, while UV (light intensity:11 mW cm^{-2}, 365 nm) light was irradiated on the *cis* system using a mercury lamp (SX-UI 251HQ, USHIO, Tokyo, Japan) through a band-pass filter (Edmund Optics, NJ,

USA) during the experiments. Thirty minutes after reaching the set temperature, the poly(AzoAA-*r*-DMA) solution was observed using an Olympus BX51 microscope (Olympus, Tokyo, Japan) controlled by MetaMorph.

Analysis of the photo-induced phase separation of poly(AzoAA-r-DMA) at a constant temperature using the microscopy technique: The photo-induced phase separation of poly(AzoAA-*r*-DMA) in PBS was observed under a microscope. As described previously, poly(AzoAA-*r*-DMA) was first dissolved in PBS at a concentration of 0.5 wt%. A solution of poly(AzoAA-*r*-DMA) (20 μL) was cast between two coverslips (30 × 30 mm, Matsunami, Osaka, Japan), and a 500 μm-thick silicone spacer was placed in the middle. The temperature of the poly(AzoAA-*r*-DMA) solution was regulated using a stage-top heater (Thermo Plate, Tokai Hit, Shizuoka, Japan), and the sample was analyzed using an inverted microscope (Primovert, Carl Zeiss AG, Germany). The temperature was kept constant during the experiments. During the experiments, the photoisomerization of AzoAA was controlled by irradiating the samples with either vis (light intensity: 9 mW cm^{-2}, 436 nm) or UV (light intensity:11 mW cm^{-2}, 365 nm) light using a mercury lamp (SX-UI 251HQ, USHIO, Tokyo, Japan) equipped with a band-pass filter (Edmund Optics, NJ, USA). The UV and vis lights were switched during the experiments by switching the band-pass filter.

Supporting Information

Supporting Information is available from the Wiley Online Library.

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It is discovered that the molecular weight reverses the photo-induced dissolution/phase separation of azobenzene-bearing polymers. Specifically, visible light (vis) irradiation phase separates high molecular weight (M.W.) polymers, while ultra-violet (UV) irradiation phase separates low molecular weight polymers. The findings can potentially pave the way for the development of engineered drug delivery systems and microreactors.

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Polarity Does Not Matter: Molecular Weight Reverses the Photoisomerization-Induced Phase Separation of an Azobenzene-Bearing Polymer