

Photoisomerization of Azobenzene Units Controls the Reversible Dispersion and Reorganization of Fibrous Self-Assembled Systems

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N-(L-valyl-L-valyl-L-valyl)azobenzene-4-carboxamide [Azo(LVal)₃] is a low molecular weight gelator that forms a photofunctional fibrous assembled system; this assembly undergoes dispersion/reorganization upon *trans*-to-*cis* photoisomerization, which, as a result of the breaking and reforming of hydrogen bonds, induces reversible sol–gel transitions. In this paper, we describe the mechanism by which azobenzene isomerization induces the breaking and reorganization of these assembled systems. We applied Fourier transform infrared spectroscopy to investigate the effect of the irradiation time on the change in absorption intensity in the amide I region. The lifetime of the *cis* isomer influences the photoinduced breaking and reforming of hydrogen bonds between trivalyl units. Because the *cis* isomer of Azo(LVal)₃ had a long lifetime, its assemblies underwent reversible phototriggered dispersion and organization. In contrast, the lifetime of the *cis* isomer of 4'-dimethylamino-*N*-(L-valyl-L-valyl-L-valyl)azobenzene-4-carboxamide [pMR(LVal)₃] was too short to disrupt the hydrogen bonds in its fibrous self-assembled system.

Introduction

Assembled nanostructures of proteins, lipids, and DNA are responsible for many of the unique functions of biological systems. ATP synthase, microtubules, and ion channels are typical examples of biological supramolecular systems. A major challenge in materials design is predicting the formation of self-assembled structures based on their local molecular-level interactions; a further challenge is then to impart such structures with the ability to respond to external stimuli (e.g., solvent, heat, electrons, photons). Light is a particularly useful and precise means of stimulus because it can be modified according to its wavelength, intensity, and polarization. Controlling self-assembly processes with light has led to the design of many photochromic supramolecular systems.

Low molecular weight organogelators (LMOGs) are attractive because of the reliability of forming these self-assembled fibrillar networks.¹ LMOGs being built from small molecules, which are held together by noncovalent interactions, exhibit reversible deformation and reassemble upon physical and chemical stimulus. Responsive self-assembled systems are interesting with many potential applications in areas such as sensor, drug delivery and catalysis. A wide variety of responsive assembled molecular systems that can be addressed by chemical and physical triggers have been developed.^{1–4}

Recently, we prepared a molecular system, featuring an azobenzene derivative linked to three valine units, that underwent organization and dispersion upon exposure to different wavelengths of light (Figure 1a).⁵ In this system, the component molecules formed a fibrous assembly stabilized via a β -sheet structure formed between the valyl units. Although many previous LMOGs have contained azobenzene moieties,^{6–18} only a few of them are truly photoresponsive LMOGs because the

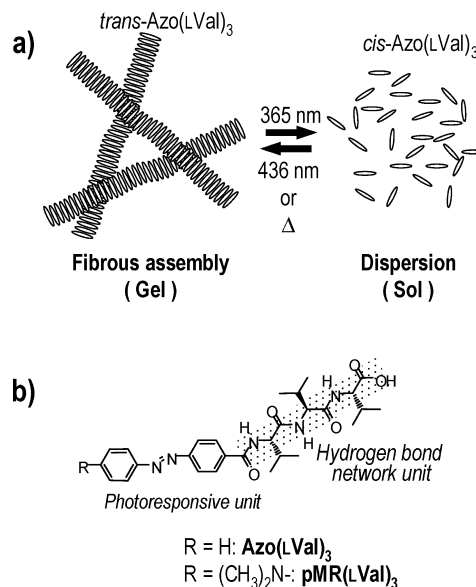


Figure 1. (a) Schematic representation of the photocontrolled dispersion and reorganization of the molecular system; (b) chemical structure of trivalyl derivatives incorporating azobenzene units.

photoisomerizations of azobenzene moieties are typically suppressed in the gel state.^{6,12–15,18} Correlation between photoisomerization of chromophores and sol–gel transition remain incompletely understood.

Azobenzenes are typically divided into three classes, azobenzenes, aminoazobenzenes, and push/pull azobenzenes, that are distinguished in terms of their spectral feature and photophysical responses, which are intimately tied to their substitution patterns.¹⁹ The lifetimes of the *cis* isomers of push/pull azobenzenes are typically very short relative to those of normal azobenzenes. Therefore, in this study we used Fourier transform infrared (FTIR) spectroscopy to investigate the effect of photoisomerizations on the dispersion, and organization of

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LMOG assemblies prepared from two different trivalyl derivatives, one possessing an azobenzene unit and the other a push/pull azobenzene unit (Figure 1b).

Experimental Section

Instrumentation. ^1H NMR spectra were recorded by using a Varian VXR-300 spectrometer. UV-vis absorption spectra were recorded by using a Jasco V-750 spectrometer. FTIR spectra (transmittance) were recorded using a Mattson Infinity Gold FTIR spectrometer equipped with an MCT detector; the spectrometer was purged with N_2 gas to minimize the amount of water present in the sample chamber; spectra were recorded at a 4 cm^{-1} resolution through the coaddition of 512 scans. Gels and solutions were injected in a sealed-demountable cell equipped with CaF_2 windows (GL Science) and 0.05 mm thick of spacer was used. Photoirradiation was performed using UV (365 nm) and visible (450 nm) light from a handmade light-emitting diode (LED) lamp; the intensity of the light was controlled using Neutral Density (ND) filters. Field emission scanning electron microscopy (FE-SEM) images were recorded using a TOPCON DS-720 instrument operated at 5 kV; specimens were prepared by placing a drop of the gel on an amorphous carbon-supporting film mounted on a standard electron microscopy grid; the drop was then blotted off with filter paper, followed by drying in vacuo; the specimens for SEM analyses were sputter-coated with Pt (ca. 2 nm thick).

Laser flash photolysis experiments were performed at room temperature using a Minilite II instrument (Unisoku). The second harmonic at 532 nm of a pulsed YAG laser, HOYA continuum was used for excitation (average power: ca. 375 mW; pulse width: 3–5 ns). The gels under study were placed in quartz cuvettes having an optical path of $4\text{ mm} \times 2\text{ mm}$. The monitoring light (480 nm) was aligned orthogonal to the excitation light and detected on the other side of the cuvette. Traces after 160 flashes made at 0.1 s intervals were integrated; the rate constants for thermal cis-trans isomerization were obtained through first-order kinetic analyses of the traces.

4'-Dimethylamino-*N*-(*L*-valyl-*L*-valyl-*L*-valyl)azobenzene-4-carboxamide [pMR(LVal)₃] was synthesized using a procedure similar to that described previously.³ Yield: 70%. Mp 247–249 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.86–0.95 [m, 18H, $\text{CH}(\text{CH}_3)_2$], 1.98–2.16 [m, 3H, $\text{CH}(\text{CH}_3)_2$], 2.50 [s, 6H, $\text{N}(\text{CH}_3)_2$], 4.11 [dd, $J = 8.0, 6.1\text{ Hz}$, 1H, NCHCO], 4.27–4.39 [m, 2H, NCHCO], 7.81–7.93 [m, 8H, ArH], 8.01 [d, $J = 8.5\text{ Hz}$, 1H, NH], 8.44 [d, $J = 8.3\text{ Hz}$, 1H, NH]. FTIR ($\text{DMSO}-d_6$, cm^{-1}): 1722 ($\nu_{\text{C=O}}$), 1655 ($\nu_{\text{C=O}}$, amide I), 1600 ($\nu_{\text{ArC-C}}$), 1522 ($\nu_{\text{N-C=O}}$, amide II).

Results and Discussion

Organization and Characterization of an Assembly Composed of pMR(LVal)₃. We monitored the ability of pMR(LVal)₃ to undergo self-assembly in 10 different solvents; Table 1 summarizes the results. Clear gels were obtained in $\text{DMSO}/\text{H}_2\text{O}$ (1:1), $\text{DMS}/\text{H}_2\text{O}$ (1:1), 1,4-dioxane/ H_2O (1:1), $\text{MeOH}/\text{H}_2\text{O}$ (1:1), $\text{EtOH}/\text{H}_2\text{O}$ (1:1), $\text{iPA}/\text{H}_2\text{O}$ (1:1), and $\text{nPA}/\text{H}_2\text{O}$ (1:1). These stable gels exhibited fully thermo-reversible behavior.

To gain insight into the driving force for gelation, we examined UV-vis and FTIR spectra of these systems. We observed evidence for interactions between the azobenzene units in the UV-vis absorption spectra of pMR(LVal)₃ in solution and in the gel state (Figure 2a). The signal for the absorption of the azobenzene unit was broad in the gel state relative to that obtained from solution. Furthermore, we observed an absorption peak attributable to H-aggregation at 365 nm in the

TABLE 1: Gelation Properties in Various Solvent Systems (G, gel; P, Precipitate)

solvent (v/v)	pMR(LVal) ₃	Azo(LVal) ₃ ^a
DMSO/H ₂ O (1/1)	G	G
DMF/H ₂ O (1/1)	G	G
DMA/H ₂ O (1/1)	G	G
1,4-dioxane/H ₂ O (1/1)	G	P
MeOH/H ₂ O (1/1)	G	G
EtOH/H ₂ O (1/1)	G	G
iPA/H ₂ O (1/1)	G	P
nPA/H ₂ O (1/1)	G	
acetone/H ₂ O (1/1)	P	G
MeCN/H ₂ O (1/1)	P	G

^a Reference 5.

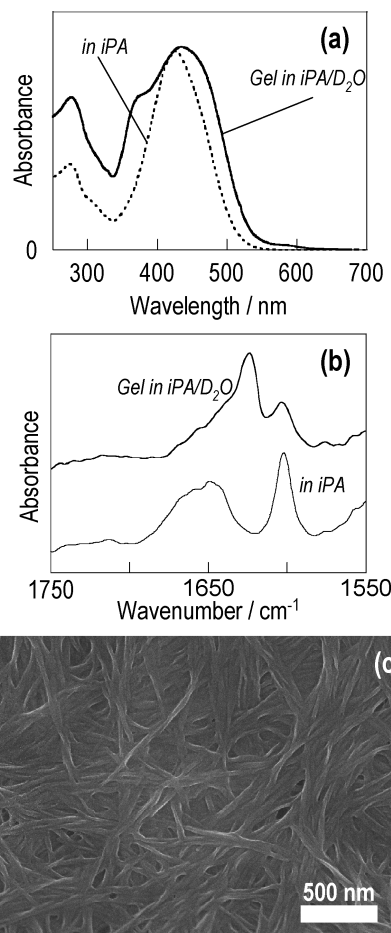


Figure 2. (a) UV-vis absorption spectra of pMR(LVal)₃ in the gel state (solid line, 1.8 mM) and in iPA solution (dotted line, 1.8 mM). (b) FTIR spectra of pMR(LVal)₃ in the gel state in iPA solution (1.0 mM). (c) FE-SEM image of pMR(LVal)₃ in the gel state.

gel state. Together, these findings suggest that stacking of the azobenzene units occurred in the gel state.^{20–22}

The amide region (1500–1750 cm^{-1}) of FTIR spectra provides highly detailed information regarding the hydrogen bonds formed by peptide units. Figure 2b displays partial FTIR spectra of pMR(LVal)₃ in solution and in the gel state. Amide I band in the gel state appeared as a sharp signal at 1623 cm^{-1} , characteristic of a β -sheet structure. No peaks attributable to the formation of antiparallel strand structures were present between 1680 and 1690 cm^{-1} , suggesting that pMR(LVal)₃ adopted a parallel β -sheet structure.^{23,24}

To obtain visual insight into the aggregated structure, we used electron microscopy to observe the morphology of the assembly

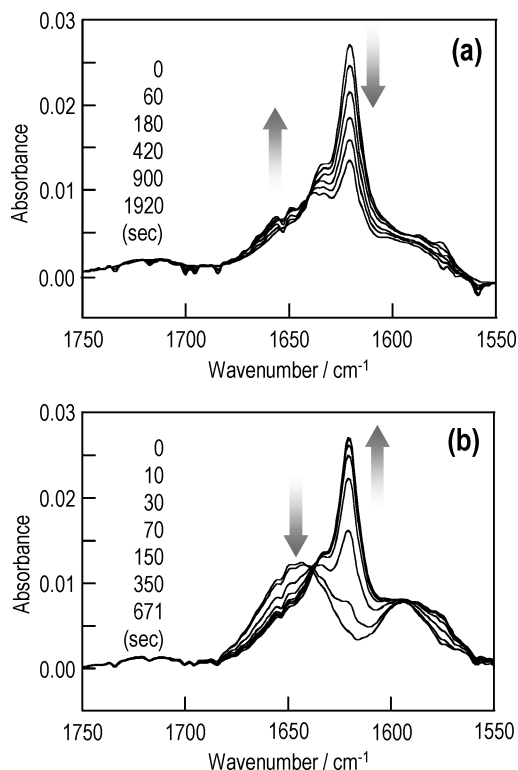


Figure 3. Amide I region of the FTIR spectra of in the gel state upon irradiation with (a) UV and (b) visible light.

(Figure 2c). We detected fibrous assemblies having a minimum width of ca. 10–50 nm.

These findings are consistent with molecular organization of pMR(LVal)₃ in the fibrous assemblies. In analogy with the assembly of Azo(LVal)₃,⁵ hydrogen bonds between oligopeptide units led to the formation of β -sheets, which gathered together to form fibrous structures.

Photoirradiation Effect on Hydrogen Bonds in Assemblies.

To examine whether photoisomerizations of the azobenzene units influenced the breaking and reforming of hydrogen bonds between the peptide units, we measured the effect of the irradiation time on the appearance of the FTIR spectra. Upon irradiation with UV light (365 nm, ca. 2 mW), trans-to-cis photoisomerizations of the azobenzene unit occurred, leading to disruption of hydrogen bonds (Figure 3a), as evidence by the decrease in the intensity of the narrow absorption peak (1620 cm⁻¹) assigned to the β -sheet structure. Concomitantly, the intensity of the absorbance at ca. 1650 cm⁻¹, which we attribute to the monomeric state amide I signal, increased, suggesting that the peptide units existed in the monomeric state after exposure to UV light cleaved the hydrogen bonds. We suspect that the change in solubility that resulted from the increased molecular polarity of the *cis*-azobenzene unit was a driving force for the dissociation of the assemblies. The presence of an isosbestic point (1635 cm⁻¹) indicates that β -sheet structure and the monomeric state were the only two species present in this photoinduced morphological change.

The reverse reaction occurred after applying visible light. Figure 3b displays the change in the FTIR spectra of *cis*-Azo(LVal)₃ in DMSO-*d*₆/D₂O (3/2) after irradiation with visible light (450 nm, ca. 20 mW). Prior to this photoirradiation, *cis*-Azo(LVal)₃ was dispersed in solution; it did not form fibrous assemblies because of its high affinity with the solvent. We attributed the absorption signal at 1640 cm⁻¹ to the monomeric state amide I. Upon irradiation with visible light, *cis*-to-trans

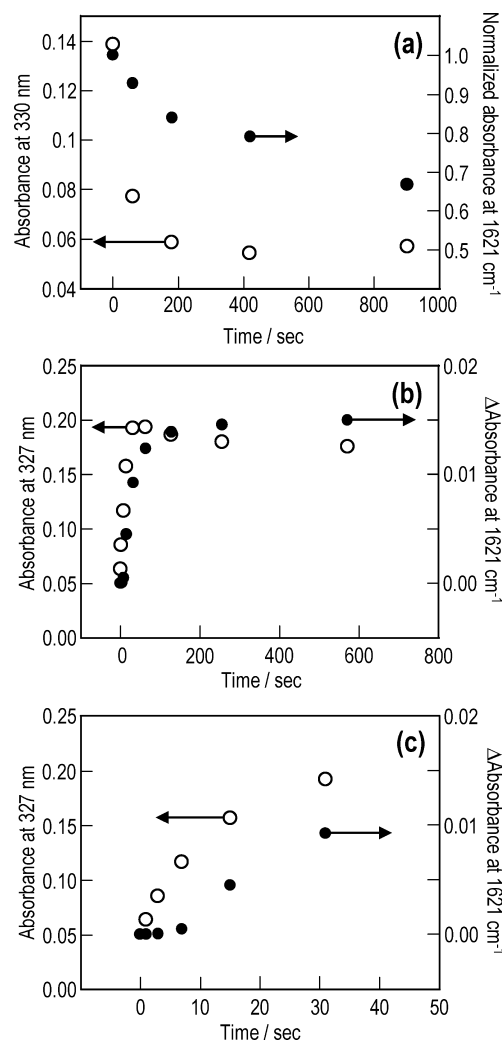


Figure 4. Intensities of UV-vis absorbances and the FTIR spectroscopic signal at 1621 cm⁻¹ as a function of time during the irradiation of Azo(LVal)₃ with (a) UV and (b,c) visible light.

photoisomerizations occurred, causing the absorption intensity of 1640 cm⁻¹ to decrease and that at 1621 cm⁻¹, attributable to the β -sheet structure, to increase. The isosbestic point at 1635 cm⁻¹ indicates that the photoinduced reformation of hydrogen bonds occurred between only two species, the free state and β -sheet structures.

To determine the correlation between the photoisomerizations of the azobenzene unit and the breaking and reforming of hydrogen bonds, we plotted (Figure 4) the absorption intensities of the signals for the π - π^* transition ($\lambda_{\text{max}} = 330$ nm) and the amide I in the β -sheet structure (1621 cm⁻¹) against the irradiation time. Figure 4a reveals that the trans-to-cis photoisomerizations occurred rapidly relative to the breaking of the β -sheet structure; after sufficient formation of the *cis* isomer, which exhibited high affinity for the solvent, the hydrogen bonds disrupted gradually.

Figure 4b,c displays the effect of visible light irradiation on the photoisomerizations and reformation of the β -sheet structure over time. The β -sheet structure formed rapidly upon *cis*-to-trans photoisomerization. Figure 4c reveals the change in the absorption intensity within a short-range of irradiation time; photoisomerizations occurred first, followed by the formation of the β -sheet structure.

Azobenzenes are employed extensively in photocontrolled assembled systems,^{25–27} photofunctional supramolecular sys-

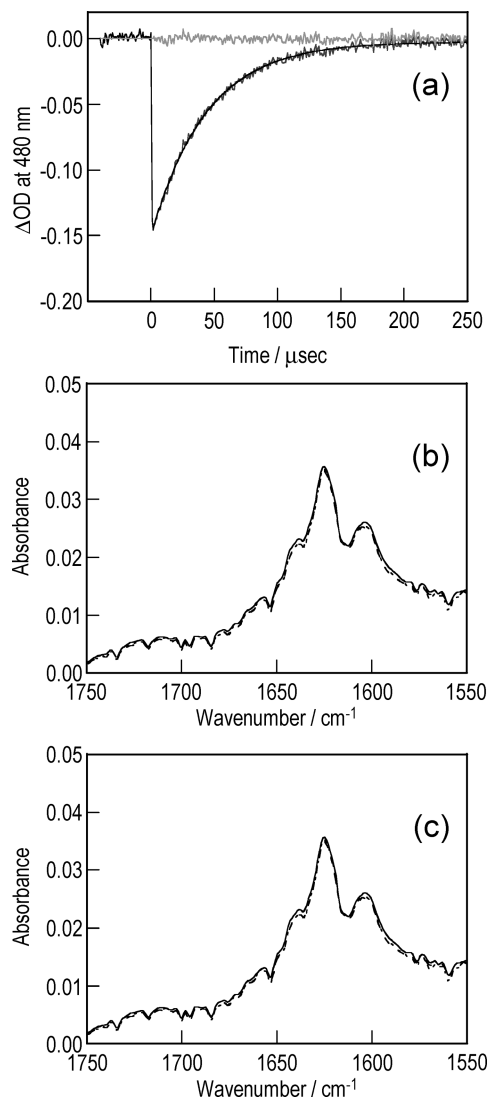


Figure 5. (a) Profiles of the absorbance changes at 480 nm for pMR(LVal)₃ in the gel state after irradiation with a laser photoflash. (b,c) Amide I region of the FTIR spectra of the gel of pMR(LVal)₃ (b) after and (c) during photoirradiation (dotted line). Solid lines indicate spectral features prior to photoirradiation.

tems,^{28–30} and in the study of photoinduced conformational changes of polypeptides,^{31,32} because of their reversible trans-to-cis photoisomerizations, which led to large changes in molecular geometry.³³ In our molecular system, however, it was not the case that the dispersion and reformation of the assemblies were controlled by the photoinduced changes in the molecular geometry of the azobenzene moiety. It should be noted that there was sufficient free volume in our fibrous assemblies featuring β -sheet structures^{34,35} for the photoisomerization of the azobenzene moieties to take place. Our UV-vis and IR measurements indicated that hydrogen bonds between the peptide units were slowly broken after photoisomerization of the azobenzene units. Thus, the change in affinity between Azo(LVal)₃ and the solvent, caused by the photoisomerization of the azobenzene units,³⁶ was the trigger for the breaking (reforming) of hydrogen bonds between the peptide units, leading to dispersion (reorganization) of the assembled system. The response of our photofunctional molecular gel was much more rapid compared to that of other photofunctional molecular gels triggered by changes in molecular geometry.^{14,15} Molecular systems that take advantage of changes in affinity between their component molecules and the solvent are “smart” photoresponsive molecular systems.

We also investigated the possibility of measuring the trans-to-cis photoisomerization of pMR(LVal)₃ spectroscopically, but we were unable to record this process at room temperature in aqueous solution. Because the cis isomer of methyl red (pMR), which has an electron push/pull substituents, is too labile to observe in aqueous solution at room temperature,^{37,38} we attempted to detect the trans-to-cis isomerization of pMR(LVal)₃ using laser flash photolysis. Figure 5a displays a typical curve for the recovery of absorbance at 480 nm following flash photobleaching ($\lambda_{\text{irr}} = 532$ nm), along with a plot from which we obtained the rate constant $k_{c \rightarrow t}$. After being triggered by the flash, the absorbance decreased instantly, indicating photoinduced trans-to-cis isomerization. This decrease in absorbance was followed by recovery within 250 μs , namely rapid cis-to-trans thermal isomerization. Fitting to a first-order exponential equation yielded a time of 43 μs and a rate constant ($k_{c \rightarrow t}$) of 23 000 s^{-1} . The contribution that diffusion made to the exponential recovery was negligible. Thus value of $k_{c \rightarrow t}$ is larger than that for pMR dissolved in iPA/H₂O (1:1).³⁹ Presumably the lability of the cis isomer was enhanced as a result of steric hindrance in the assembled systems.

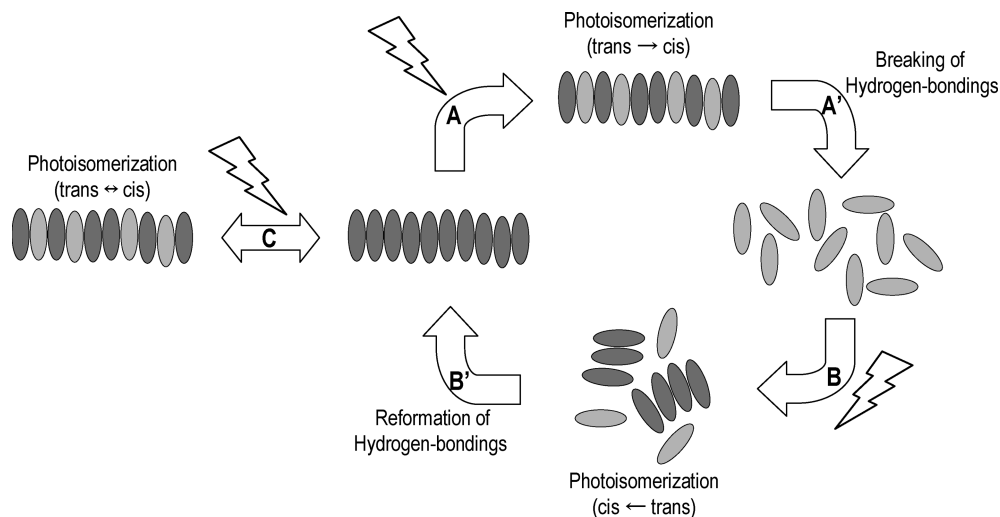


Figure 6. Schematic representation of the effect of photoisomerization on the dispersion and organization of the fibrous assemblies.

When we used FTIR spectroscopy to examine the photo-induced breaking and reforming of the hydrogen bonds in the fibrous assembly formed from pMR(LVal)₃, we found that the spectra did not change during photoisomerization (cf. Figure 5b,c). The lifetime of the cis isomer was too short for its formation to lead to the breaking of hydrogen bonds. Thus, the lifetime of the cis isomer is a key parameter affecting the breaking and reforming of hydrogen bonds in these assembled systems.

Figure 6 summarized the effects of photoisomerizations on the dispersion and organization of these assemblies. The cis form of Azo(LVal)₃, possessing an azobenzene unit, had a sufficiently long lifetime to undergo photoinduced dispersion and organization of the assemblies. These phenomena proceeded in a stepwise manner; trans-to-cis photoisomerizations (A) occurred first, leading to an increase in the polarity of the azobenzene unit, resulting in increased stability and hence, breaking of the hydrogen bonds between the tripeptide units (A'). Reorganization was also a stepwise process; after cis-to-trans photoisomerizations (B), the molecules that precipitated from the solvent assembled through the formation of hydrogen bonds, resulting in reorganization of the fibrous structure (B'). In contrast, the assembly formed from pMR(LVal)₃, which features a push/pull azobenzene unit, underwent photoisomerizations only (C).

Conclusion

UV-vis absorption and FTIR spectroscopy revealed a correlation between the photoisomerizations of azobenzene units in trivalyl derivatives and the breaking and reformation of hydrogen bonds between tripeptide units. Because photoisomerizations of azobenzene moieties occurs with large changes in molecular geometries, these processes can occur only if there is sufficient space present around the azobenzene units. We found that the β -sheet structures of fibrous assemblies can accommodate this photoisomerization process. The lifetime of the cis isomer is a key parameter triggering the breaking and reforming of hydrogen bonds in these assembled systems. For Azo(LVal)₃, the change in solvent affinity, resulting from the change in polarity after photoisomerization, induced the breaking or reforming of the fibrous system. For pMR(LVal)₃, however, the lifetime of the cis isomer was too short to disrupt the hydrogen bonds in the assembly. The formation of structures through molecular assembly is an essential process leading toward the characteristic functions of biological systems, particularly those that respond reversibly to external stimuli. For example, the assembly and disassembly of protein subunits are important factors affecting the movement of amoebas on surfaces. Improving our ability to control rapid self-assembly processes through the application of external signals, such as light, should lead to further realization of photochromic "smart" molecular systems.

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