Reversible Temperature-dependent Dispersion–Aggregation Transition of Poly(*N*-isopropylacrylamide)–[60]Fullerene Conjugates

Atsushi Tamura, Katsumi Uchida, and Hirofumi Yajima*
Department of Applied Chemistry, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601

(Received November 28, 2005; CL-051470; E-mail: yajima@rs.kagu.tus.ac.jp)

Poly(*N*-isopropylacrylamide) (PIPAAm) exhibits a reversible temperature-dependent soluble/insoluble transition at its lower critical solution temperature (LCST) in water. The [60]-fullerene–PIPAAm monoadduct (PIPAAm–C_{60}) was synthesized by the azide addition using azido-terminated PIPAAm. Below LCST, the PIPAAm–C_{60} was dispersed and formed a micelle-like structure in water. Above LCST, the aggregation of the micelles was observed by turbidity measurements and dynamic light scattering (DLS). The PIPAAm–C_{60} exhibits rapid and reversible dispersion–aggregation changes in response to narrow range temperature alternation across LCST.

Fullerene possesses a promising utility in the biomedical field due to its unique chemical and physical properties such as antioxidants, enzyme inhibition, and photo-driven DNA cleavage.[1,2] However, the insolubility of fullerene in water has hampered many of its potential applications. One strategy to overcome this problem is the introduction of charged functional groups to fullerene molecules[2] or modification with hydrophilic polymer chains.[3] Many different types of water-soluble [60]fullerene C_{60} derivatives have been so far reported. Among them, the C_{60} end-capped polymers exhibit self-assembly properties such as micelle-like formation because hydrophobic C_{60} core self-assembles on a nanometer scale.[4] Additionaly, the self-assembly behavior of the C_{60} end-capped cationic polymer such as poly[2-(dimethylamino)ethyl methacrylate] was changed by pH and temperature.[5]

Poly(*N*-isopropylacrylamide) (PIPAAm) is soluble in aqueous media at a solution temperature below its lower critical solution temperature (LCST). Above this point, it undergoes a discontinuous phase transition, precipitating from solution suddenly and reversibly over a narrow temperature range.[6,7] Polymer chains of PIPAAm are hydrated and expanded in water below LCST, and change to compact forms above the LCST by a sudden dehydration and inter- and intramolecular hydrophobic interactions.[8] For example, by exploiting the thermoresponsive conformational changes of PIPAAm chains, thermally responsive block copolymer micelles comprising poly(*N*-isopropylacrylamide-*b*-D,L-lactide), which were dispersed below LCST and aggregated above LCST in water, were produced for active targeting as drug carriers.[9]

In this paper, in order to develop a functionalized C_{60} derivative possessing the property to change the water-solubility in response to temperature for a new biomedical material, the C_{60} with poly(*N*-isopropylacrylamide) (PIPAAm–C_{60}) monoadduct was synthesized and the dispersion behavior of the PIPAAm–C_{60} having thermally sensitive property in aqueous solution was examined by turbidity measurements and dynamic light scattering (DLS) measurements.

According to the previous study,[7,10] PIPAAm with a terminal hydroxy group at one end (PIPAAm-OH) was synthesized by telomerization using 2-mercaptoethanol as a chain-transfer agent. The molecular weight and the molecular weight distribution of the PIPAAm-OH were determined to be 4280 and 1.58, respectively, by gel permeation chromatography (GPC) in THF at 40 °C (against polystyrene standards). The terminal hydroxy group of the PIPAAm was converted to a tosyl group using *p*-toluenesulfonyl chloride, followed by installation of an azide group using sodium azide. The introduced azide group of the PIPAAm was confirmed by IR measurements. The azido-terminated PIPAAm (PIPAAm-N_3; 170 mg) and C_{60} (100 mg; 0.14 mmol) were dissolved in 100 mL of chlorobenzene, and the solution was refluxed at 120 °C for 48 h.[11] After filtration and dialysis against water to remove unreacted C_{60} and PIPAAm-N_3, the brown-colored powder of PIPAAm–C_{60} was obtained (see Figure S1 in Supporting Information). The molecular weight and the molecular weight distribution of the synthesized PIPAAm–C_{60} were determined to be 5150 and 1.65, respectively. It was estimated from the GPC results of PIPAAm-OH and PIPAAm–C_{60} indicated that approximately one PIPAAm chain was introduced to one C_{60} molecule. In addition, no peaks for multiaddends of PIPAAm were observed from GPC curve of PIPAAm–C_{60}, confirming the monoadduct of PIPAAm to C_{60} molecules was obtained (see Figure S2 in Supporting Information). At 20 °C (below LCST), the PIPAAm–C_{60} showed a high solubility in water confirmed by the existence of the characteristic C_{60} peaks in the UV–vis absorption spectra.[12]

Figure 1 shows the optical transmittance of PIPAAm–C_{60} in water (2.0 mg/mL) at various temperatures, which was measured at 600 nm with a UV–vis spectrometer. As control, 2.0 mg/mL PIPAAm-OH solution was used. The LCST of the solution was determined as the temperature at which the onset of turbidity took place. Transmittance of the PIPAAm–C_{60} solution changed in response to temperature, conforming the behavior of PIPAAm-OH. Below 32 °C, transmittance of PIPAAm–C_{60} was only 72% owing to the brown color of the PIPAAm–C_{60} solution, but the solution was not turbid, indicating that the PIPAAm–C_{60} in water was completely dispersed. This transmittance was rapidly reduced near 33 °C, and was almost zero above 35 °C, indicating that the PIPAAm–C_{60} was aggregated analogously to PIPAAm-OH above 35 °C. LCST values of PIPAAm-OH and PIPAAm–C_{60} were estimated to be approximately 31 and 32 °C, respectively. It's considered that slight increase of the LCST value of PIPAAm on C_{60} moiety was induced by a restricted conformation[13] of PIPAAm chain owing to binding to C_{60} molecules.

DLS measurements of PIPAAm–C_{60} in water (0.5 mg/mL) were carried out using He–Ne laser (632.8 nm) at the scattering angle of 90° from 25 to 37 °C. Figure 1 shows cumulative average diameter in solution as a function of temperature. The cumulative diameter of PIPAAm–C_{60} in aqueous solution was approx-

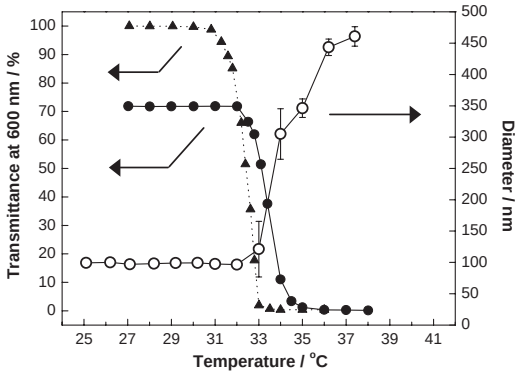

Figure 1. Temperature dependence of optical transmittance at 600 nm of PIPAAm (▲) and PIPAAm–C_{60} (●) and the cumulative diameter of PIPAAm–C_{60} (○) in water as a function of temperature. The optical transmittance of both PIPAAm and PIPAAm–C_{60} were measured at a concentration of 2.0 mg/mL. The cumulative diameter of PIPAAm–C_{60} was measured by DLS, at a concentration of 0.5 mg/mL.

imately 100 nm below its LCST. This result indicated that the PIPAAm–C_{60} formed a self-assembled structure in water, namely, a core–shell micelle structure composed of inner core of C_{60} molecules and outer shell layer of PIPAAm chains. Further confirmation about the structure was carried out by equilibrium surface tension measurements of PIPAAm–C_{60} aqueous solution below LCST. PIPAAm–C_{60} possessed the critical micelle concentration (CMC) of 1.0 mg/L ($\gamma_{CMC} = 44.7$ mN/m) in water, indicating that PIPAAm–C_{60} in water formed a self-assembled structure like an amphiphilic block copolymer micelle.[14] Okano et al.[15] reported that the alkyl-terminated PIPAAm (PIPAAm-$C_{18}H_{35}$) in water formed the micelle structure below LCST above its CMC (80 mg/L). The CMC of PIPAAm–C_{60} (1.0 mg/L) was significantly lower than that of PIPAAm–$C_{18}H_{35}$, indicating that PIPAAm–C_{60} in water formed a very stable micelle structure induced by hydrophobic interaction and strong π–π stacking effects between C_{60} molecules. A dramatic change in micelle diameter of PIPAAm–C_{60} occurred at LCST. Above LCST, PIPAAm chain collapses from its expanded (hydrated) form to compact (dehydrated) form due to the fluctuation of hydrophobic interactions and hydrogen bonding.[16] Thus, this result indicated that as the collapse drove the surface of PIPAAm–C_{60} micelles to switch from hydrophilic to hydrophobic, the aggregation between PIPAAm–C_{60} micelles was induced by hydrophobic interactions.

Figure 2 shows the transmittance changes of PIPAAm–C_{60} solution in response to reversible temperature changes between 20 and 40 °C across the LCST measured by UV–vis spectroscopy. As a result, PIPAAm–C_{60} exhibited the rapid and reversible changes for thermally responsive transmittance without hysteresis. Below LCST, the transmittance remained unchanged to be about 70%. PIPAAm–C_{60} was highly dispersed in water, in spite of reversible temperature changes across LCST. On the other hand, when the temperature was above LCST, the PIPAAm–C_{60} solution was rapidly turbid at every turnover, and therefore, PIPAAm–C_{60} in water were aggregated. Correspondingly, the cumulative average micelle diameter in PIPAAm–C_{60} solution, which was measured by DLS, was rapidly and reversibly varied

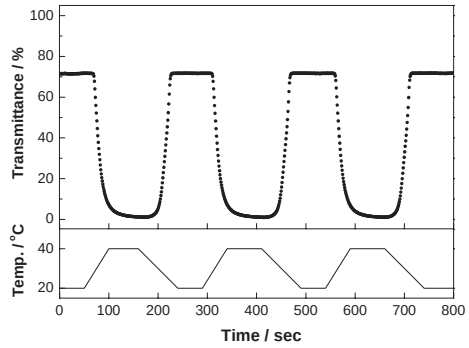

Figure 2. Time course of transmittance (600 nm) changes for the PIPAAm–C_{60} solution (2.0 mg/mL) in response to heating–cooling cycles between 20 and 40 °C across its LCST.

in response to a reversible temperature changes across LCST. This result indicated that the dispersion–aggregation transition of the PIPAAm–C_{60} micelles was reversible in response to temperature changes.

In summary, we have synthesized new water-soluble C_{60} derivatives with PIPAAm, possessing a unique property of the reversible dispersion–aggregation transition of the conjugate micelle in water in heating/cooling thermal cycles through the LCST. The conjugate of multifunctional C_{60} with stimuli-responsive polymers could be exploited for the development of an intelligent material in biomedical fields.

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