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SHORT COMMUNICATION

Reduction-induced micellization of a diblock copolymer containing stable nitroxyl radicals

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Abstract A novel micelle formation induced by reduction was attained using a diblock copolymer supporting 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). Poly(4-vinylbenzyloxy-TEMPO)-*block*-polystyrene (PVTEMPO-*b*-PSt) showed ultraviolet (UV) absorption at 467 nm as $\lambda_{\max}$ based on the TEMPO radicals. As the phenylhydrazine was added to the copolymer solution in benzene, the UV absorbance decreased. The decrease in the absorbance suggested that the TEMPO radicals were reduced to the colorless hydroxylamine by phenylhydrazine. The PVTEMPO-*b*-PSt copolymer showed no self-assembly in benzene due to the nonselective solvent. A light scattering study demonstrated that the scattering intensity of the copolymer increased with a decrease in the UV absorbance. The hydrodynamic diameter of the copolymer rapidly increased with the addition of phenylhydrazine and became almost steady over the molar ratio of phenylhydrazine to the VTEMPO unit of 0.2. It was found that the hydroxylamine in the micelles reverted to the TEMPO radicals by oxidation with oxygen.

Keywords Poly(4-vinylbenzyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl)-*block*-polystyrene (PVTEMPO-*b*-PSt) · Reduction · Hydroxylamine · TEMPO · Phenylhydrazine · Micellization · Micelles

Introduction

Electron transport systems perform important functions concerning respiration and energy metabolism in eukaryotes [1, 2]. The electron transport reactions occur at the mitochondria inner membrane formed by electron transport proteins [3] and the lipid bilayer built up by the self-assembly of phospholipids as vital surfactants [4, 5]. The electron transport proteins include redox catalysts such as nicotinamide, iron [6, 7], and quinones [8]. The electrons produced by these redox reactions transfer through the lipid bilayer. While the relationship between the electron transport mechanisms and the molecular self-assembly in vivo has been clarified, control of the self-assembly by electron transport has been determined for an artificial polymeric surfactant [9, 10]. The control of the self-assembly is based on the redox system of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). TEMPO is a stable nitroxyl radical known as a spin trapping reagent [11], a spin label reagent [12], and a mediator for living radical polymerization [13, 14]. TEMPO forms a redox system in which the radical is converted into the oxoaminium cation (OAC) by one-electron oxidation and is converted into the hydroxylamine (HA) by one-electron reduction [15] (Fig. 1). The oxidation of TEMPO into the OAC is caused by chlorine [16], bromine [17], copper (II), and iron (III) [18], while the reduction into HA is brought about by hydrazobenzene [19], quinones [20], and ascorbic acid [21, 22]. We reported the novel micelle formation induced by oxidation [9] and disproportionation [10] using the redox system of TEMPO supported on a diblock copolymer. While the oxidation-induced micellization was based on the OAC–TEMPO system using chlorine as the oxidizing agent, the disproportionation-induced micellization occurred both for the OAC–TEMPO and TEMPO–HA

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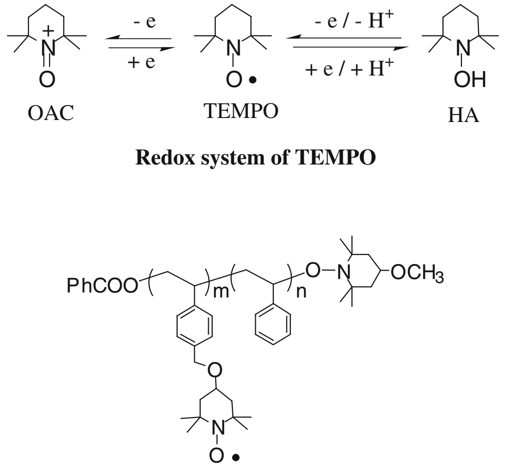

PVTEMPO-*b*-PSt

Fig. 1 Redox system of TEMPO and the structure of a PVTEMPO-*b*-PSt diblock copolymer

systems. We found a novel micelle formation induced by reduction based only on the TEMPO–HA system using phenylhydrazine as the reducing agent. The series of micellizations using the TEMPO redox systems indicate that the electron transport becomes a trigger that causes self-assembly of molecules, in addition to external triggers such as temperature [23, 24], pressure [25, 26], pH [27, 28], salt formation [29, 30], and noncovalent bond cross-linking [31, 32]. This short communication describes the reduction-induced micellization of a TEMPO-supported diblock copolymer by phenylhydrazine.

Experimental

Instrumentation The ultraviolet (UV) spectra were obtained using a Shimadzu UV-160A UV-Vis recording spectrophotometer. Light scattering measurements were performed with a Photal Otsuka Electronics ELS-8000 electrophoretic light scattering (ELS) spectrophotometer equipped with a system controller, an ELS controller, and a He–Ne laser operating at λ=632.8 nm.

Materials Poly(4-vinylbenzyloxy-TEMPO)-*block*-polystyrene (PVTEMPO-*b*-PSt) was prepared as reported previously [9]. The number average molecular weight of the copolymer was Mn(PVTEMPO-*b*-PSt)=31,200-*b*-49,400 by ^{1}H nuclear magnetic resonance (NMR) and the molecular weight distribution was Mw/Mn=1.88 by size exclusion chromatography. Benzene was purified by refluxing on sodium for several hours and distilled over sodium. Phenylhydrazine was distilled over calcium hydride under reduced pressure.

Light scattering measurements PVTEMPO-*b*-PSt (24 mg, 3.22×10^{-2} mmol of the VTEMPO unit) was dissolved in 3.5 mL of benzene degassed with nitrogen, and using a syringe, the solution was injected through a microporous filter into a cell. Phenylhydrazine (320 μL) was dissolved in 2 mL of benzene. The phenylhydrazine solution (2 μL, 0.1 eq of phenylhydrazine to VTEMPO unit) was added to the copolymer solution with a syringe at room temperature. The solution was allowed to stand at room temperature for 5 min, then subjected to light scattering at θ=90° at 20 °C.

Oxidation of the micelles by oxygen The micellar solution was prepared from PVTEMPO-*b*-PSt (24 mg) in 3.5 mL of benzene and phenylhydrazine (10 μL of a benzene solution (2 mL) of phenylhydrazine (320 μL)), following the procedure mentioned above. Sodium hydride (2 mg) was added to this micellar solution. Oxygen was bubbled into the solution for 1 min at room temperature. The solution was passed through a microporous filter and subjected to UV analysis at 20 °C.

Results and discussion

TEMPO is red in color and has a UV absorption around 470 nm. This radical is reduced by phenylhydrazine to the colorless hydroxylamine [33]. The PVTEMPO-*b*-PSt diblock copolymer showed an absorption with a $\lambda_{\max}$ value at 467 nm based on the TEMPO radical. It was found that the red color of the copolymer solution in benzene faded out as phenylhydrazine was added to the solution. Figure 2 shows the variation in the UV absorption of the copolymer with the addition of the phenylhydrazine. The absorbance based on TEMPO decreased with an increase in the molar ratio of the phenylhydrazine to the VTEMPO unit (PH–TEMPO). The absorbance plotted at 500 nm versus the PH-to-TEMPO ratio is shown in Fig. 3a. The absorbance continued to decrease up to 0.8 and was almost constant above it. The absorbance did not reach 0 even at 1.0 due to the long foot of the large absorption peak observed at 240–390 nm. The formation of the hydroxylamine was also confirmed by the ^{1}H NMR measurement of the copolymer in $CDCl_3$ in the presence of phenylhydrazine. Signals based on the tetramethyl protons of

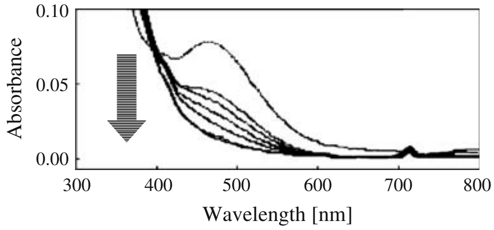

Fig. 2 Variation in the UV absorbance as phenylhydrazine was added to the copolymer solution in benzene. The PH-to-TEMPO ratios were 0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 from the top

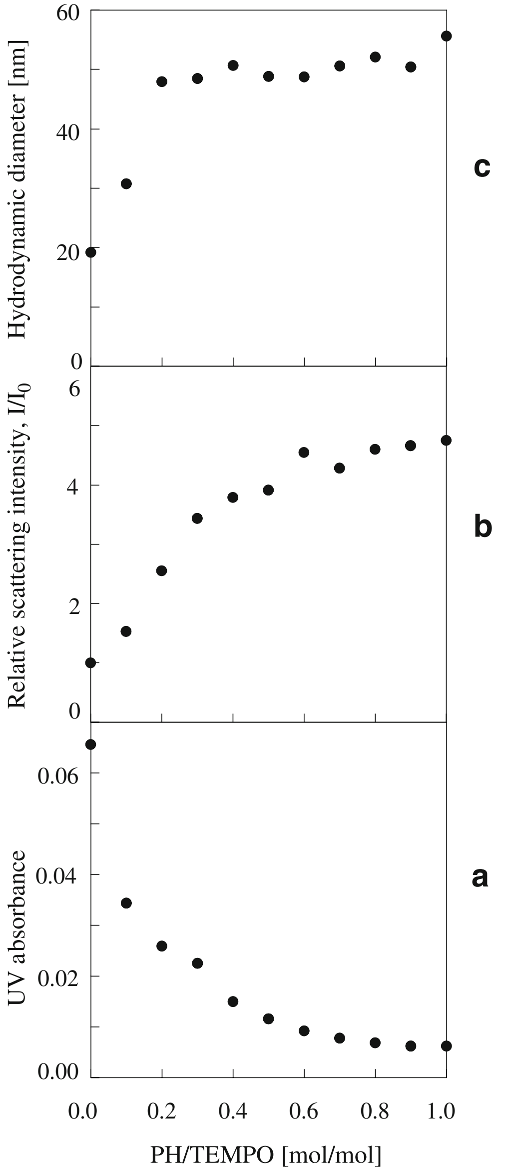

Fig. 3 Plots of the UV absorbance at 500 nm (**a**), relative scattering intensity (**b**), and hydrodynamic diameter (**c**) vs the PH-to-TEMPO ratio

the hydroxylamine derivative of TEMPO were observed at 1.18 and 1.26 ppm.

Light scattering studies demonstrated that the scattering intensity of the copolymer solution was inversely correlative to the variability in the UV absorbance. Figure 3b shows the variation in the relative scattering intensity versus the PH-to-TEMPO ratio. The scattering intensity increased with an increase in the ratio and became almost constant over 0.8. The increase in the scattering intensity suggests the formation of micelles. The hydrodynamic diameter of the copolymer also increased with the increase in the PH-to-TEMPO ratio (Fig. 3c). The diameter of the copolymer rapidly increased with the addition of a small amount of phenylhydrazine and became steady over 0.2. Whereas the scattering intensity became constant over the PH-to-VTEMPO ratio of 0.8, the hydrodynamic diameter was constant over 0.2. This difference in the variability between the hydrodynamic diameter and scattering intensity can be accounted for by the fact that the increase in the hydrodynamic diameter indicates the formation of micelles, while the increase in the scattering intensity exactly means an increase in the number of the micelles. The formation of the micelles by the addition of only a 0.2 ratio of the PH–TEMPO suggests that the micellization occurred before all the TEMPO radicals were converted into the hydroxylamine. The hydrogen bonding among the hydroxyl groups should have effectively caused the micellization in the nonpolar solvent.

The Marquadte analysis of the scattering intensity distribution of the copolymer also revealed the micelle formation. The PVTEMPO-*b*-PSt copolymer showed no self-assembly in benzene because both the blocks of PVTEMPO and PSt were solvophilic to benzene. Therefore, PVTEMPO-*b*-PSt existed as isolated copolymers, that is, unimers in the absence of phenylhydrazine. The hydrodynamic diameter of the unimers was estimated to be 19.2 nm based on the Marquadte analysis. Figure 4 shows the scattering intensity distributions obtained at the PH-to-VTEMPO ratios of 0.1 and 1.0. The scattering intensity distribution in the absence of phenylhydrazine could not be obtained due to the very low scattering intensity. It is obvious that the distribution of the unimers was shifted to the higher side of the hydrodynamic diameter at the 1.0 ratio, although part of the distributions overlapped. The micellar size was estimated to be 55.6 nm.

It has been reported that hydroxylamine is oxidized to TEMPO by oxygen [34]. To the micellar solution containing the hydroxylamine was added oxygen by bubbling after the hydroxylamine was converted into the aminoxy anions by sodium hydride in order to facilitate the oxidation by

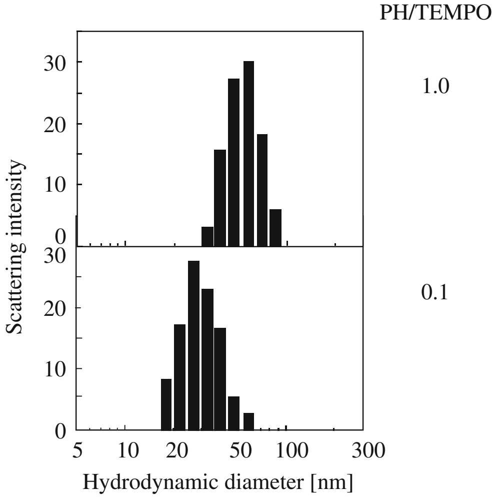

Fig. 4 Scattering intensity distribution of the hydrodynamic diameter of the copolymer at 0.1 and 1.0 of the PH-to-TEMPO ratio

oxygen. As oxygen was added to the micellar solution, the UV absorbance due to the TEMPO radicals increased. The hydroxylamine in the copolymer was converted into TEMPO by the oxygen, although the radicals immediately reverted to the hydroxylamine again due to the presence of phenylhydrazine in the solution. In addition, this experiment was performed at the PH-to-TEMPO ratio of 0.5 in order to minimize the influence of the phenylhydrazine.

Conclusions

This is the first attempt demonstrating that the micelle formation of the diblock copolymer was induced by the reduction. The reduction-induced micellization was attained using the TEMPO–HA system. The radicals supported on the copolymer were converted into the hydroxylamine by phenylhydrazine, resulting in the formation of the micelles. This was confirmed based on the fact that the scattering intensity of the copolymer solution increased with an increase in the PH-to-TEMPO ratio and that the UV absorbance of TEMPO attached to the copolymer decreased with it. The hydrodynamic diameter also increased with the ratio increasing; however, it became constant at the PH-to-TEMPO ratio of 0.2. This stable hydrodynamic diameter over this low ratio implied that the micellization proceeded before the TEMPO radicals were not completely converted into the hydroxylamine due to the strong hydrogen bonding in the nonpolar solvent. The hydroxylamine in the copolymer reverted to TEMPO by oxidation with oxygen. This suggests that the self-assembly of the copolymer is reversibly controlled by the redox system between TEMPO and the hydroxylamine.

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