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Photoresponsive Reversible Aggregation and Dissolution of Rod−Coil Polypeptide Diblock Copolymers

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Stimuli-responsive block copolymers are very appealing systems due to their polymorphism of morphologies in selective solvents (e.g., micelles, star micelles, vesicles, tubules, and complex supramolecular aggregates) which can be further engineered by the relative block lengths, the solvent composition, and their concentration.[1−4] The most frequently used stimuli are pH, temperature, redox potential, magnetic field, ultrasound, and light.[5,6] Light-responsiveness is usually provided by photochromic molecules attached to the polymers. Spiropyran (SP) is a well-known and well-investigated compound because of the spiropyran-to-merocyanine (SP-MC) light-induced isomerization.[7] SP compounds are colorless, nonpolar, and hydrophobic molecules when in their closed form. Under UV light irradiation, SP molecules isomerize to the MC form (open form), converting into colored, polar, and hydrophilic molecules. However, the MC form reverts back to the SP form under visible light or thermally.[8,9] Because of this reversible behavior, spiropyrans have been successfully used in applications such as data recording,[10] optical and electrical switching,[11] light-actuated nanovalves,[12] and reversible solubility control of enzymes.[13]

There are several reports on amphiphilic block copolymers decorated with spiropyran and azobenzenes.[14−21] Lee et al. reported the formation of photosensitive amphiphilic diblock copolymer micelles in aqueous solution, which could be partially disrupted and regenerated by UV and visible light irradiation, respectively.[22] Recently, Jin et al. reported a photo- and thermo-responsive diblock copolymer in aqueous solution which showed a reversible double-responsive micelle formation by exposing to UV light at 30 °C or at 15 °C under visible light.[23] However, to the best of our knowledge, there is no report available in the literature on photochromic block copolymer undergoing a complete reversible micellar transition.

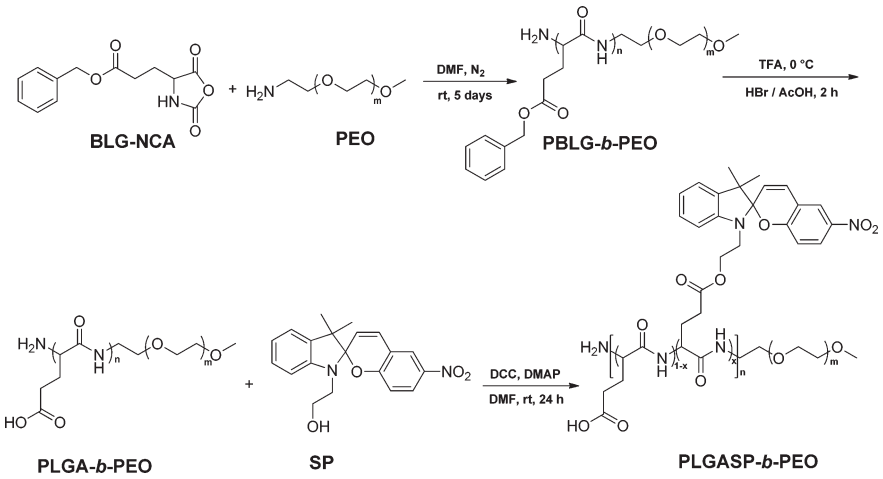
Polypeptides are attractive in the biomedical field due to their unique protein-mimetic properties.[24−27] Light-responsive polypeptide block copolymer systems capable to undergo reversible micellization could target unchallenged potential applications not only in the biomedical but also in the drug delivery area due to the desirable combined effect of noninvasive stimuli-responsiveness and biocompatibility. Available state of the art in the field is encouraging to pursue such an effort: it is well-known that poly(L-glutamic acid) solutions containing spiropyran as side-chain motif can undergo conformational changes (from α-helix to random coil and vice versa) with UV and visible light, respectively.[28−30] Ciardelli et al. described a dark-adapted polymer conformational change from the merocyanine open form (MC) to the spiropyran closed form (SP) due to irradiation with sunlight.[31] Cooper et al. described a systematic kinetic studies of the α-helix to coil dark reactions.[32]

In this work we report for the first time on SP-decorated amphiphilic polypeptide block copolymers and their light-responsive behavior. The chemical approach for the synthesis of the diblock copolymers used is illustrated in Scheme 1.

The BLG-NCA (γ-benzyl L-glutamate *N*-carboxyanhydride) monomer was synthesized according to a previously published method.[33] The two benzyl-protected diblock copolymers were synthesized via ROP of the BLG-NCA monomer using the corresponding PEO monoamino-terminated polymer (M_n = 20 300 g mol^{-1} and M_n = 10 400 g mol^{-1}, PDI = 1.13 and 1.24, respectively) as macroinitiator according to the method described in the literature.[34,35] The two resulting diblock copolymers ($PBLG_{20}$-*b*-PEO_{460} and $PBLG_{10}$-*b*-PEO_{235}) have a number-average molecular weight and polydispersity index of M_n = 24 600 g mol^{-1} and 12 500 g mol^{-1}, PDI = 1.21 and 1.35, respectively, as calculated from the corresponding ^{1}H NMR and GPC experiments (see Supporting Information, Figure SI-1). Deprotection of the glutamate residues was carried out as published previously.[36,37] ^{1}H NMR experiments showed that the resulting diblock copolymers ($PLGA_{20}$-*b*-PEO_{460} and $PLGA_{10}$-*b*-PEO_{235}) maintained the same degree of polymerization with the complete removal of the benzyl protecting groups and had their corresponding number-average molecular weight of M_n = 22 800 and 11 600 g mol^{-1}, respectively.

The final photosensitive diblock copolymers of PLGASP-*b*-PEO were obtained after esterification of the free carboxylic groups as described in the literature.[31,38] The number-average molecular weight were calculated by ^{1}H NMR ($PLGASP_{20}$-*b*-PEO_{460}, M_n = 26 200 g mol^{-1}; $PLGASP_{10}$-*b*-PEO_{235}, M_n = 13 300 g mol^{-1}). The mol % of SP attached to the polypeptide

Scheme 1. Synthetic Route for Obtaining Photoresponsive Diblock Copolymers ($PLGASP_{20}$-*b*- PEO_{460} and $PLGASP_{10}$-*b*-PEO_{235})



block in both diblock copolymers was calculated from the UV−vis calibration curve of the free SP compound, resulting in 50 mol % content for both block copolymers.

The two photoresponsive diblock copolymers formed flowerlike micelles and micellar aggregates, respectively, by adding H_2O dropwise to a solution in EtOH. The final copolymer concentration in 90:10 H_2O/EtOH was 1 wt % and the pH ∼ 7. A 0.01 wt % aqueous solution of the block copolymer was exposed to UV light (λ = 350 nm) until the photostationary state was reached. In Figure 1a, the photoisomerization process for the block copolymer $PLGASP_{20}$-*b*-PEO_{460} is shown. Before UV irradiation, no absorption peak was found at 544 nm and the solution was colorless—a sign of absence of the merocyanine open form. After UV irradiation, the peak at 544 nm was gradually increasing as a function of the UV irradiation time. The solution became pink and reached the photostationary state in 5 min. Figure 1b shows the back photoisomerization process upon subsequent exposure to visible light at λ = 590 nm. After 180 min of irradiation, a colorless solution and a new photostationary equilibrium appeared, in which the spiropyran closed form is the principal component. The isomerization kinetics was calculated by tacking the maximum absorption at 544 nm, and the isomerization ratio after UV irradiation for 5 min is calculated to be ∼85% (see Supporting Information, Figure SI-2). Similar photoisomerization behavior was observed for the other block copolymer $PLGASP_{10}$-*b*-PEO_{235} (see Supporting Information, Figure SI-3).

The flowerlike micelles and micellar morphologies of block copolymers were studied by cryogenic transmission electron microscopy. A solution of the block copolymer (0.1 wt %), after a specific light exposure (UV or visible light), was dropped onto a holey carbon copper grid (Quantifoil R2/1); the grid was blotted 1 s and vitrified into liquid ethane (−170 °C) using FEI vitrobot. Grids with the vitrified sample solutions were then cryo-transferred into a Philips CM 12 transmission electron microscope using a Gatan cryo-transfer holder. Samples were imaged using an acceleration voltage of 100 kV and bright field mode. Figure 2a−c shows the sequence of cryo-TEM micrographs corresponding to various steps of the irradiation process for the block copolymer $PLGASP_{20}$-*b*-PEO_{460}. Before UV irradiation, when the peptide block is mostly hydrophobic due to the closed form of the SP, the sample shows a complex morphology reminiscent of flowerlike micellar structures with diameter of about 70 nm. The higher

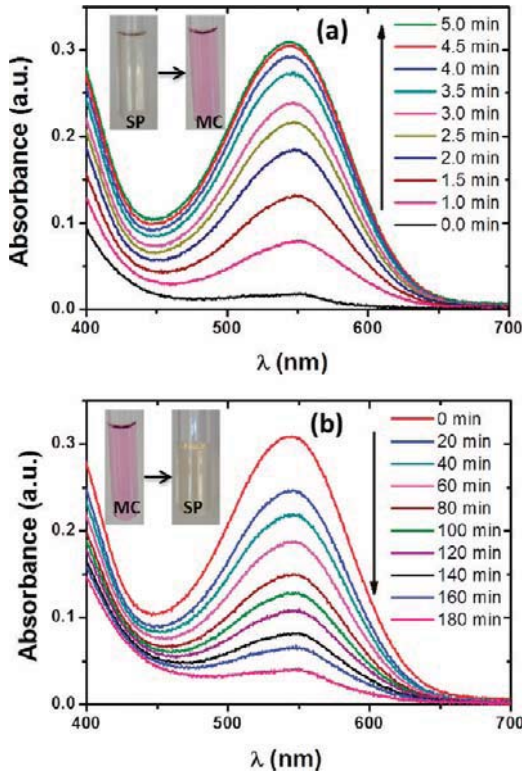


Figure 1. (a) Photoisomerization process for a 0.01 wt % of $PLGASP_{20}$-*b*-PEO_{460} in solution when irradiated with UV light (λ = 350 nm). (b) Back-photoisomerization process during irradiation with visible light (λ = 590 nm).

electron energy contrast species, e.g., the PLGASP, appears dark, whereas the highly swollen PEO cannot be resolved and thus merges with the continuous background. The flowerlike micellar morphology is understood to arise from the restrictions imposed by the highly rigid peptide block and by its length, and it is confirmed by the relatively high micellar aggregation number of 326 calculated by the Debye plot (see Supporting Information, Figure SI-4). After 5 min of UV exposure, the aggregates disrupted entirely—some residual ones with a reduced 50 nm diameter being still possible to be imaged—indicating that the block

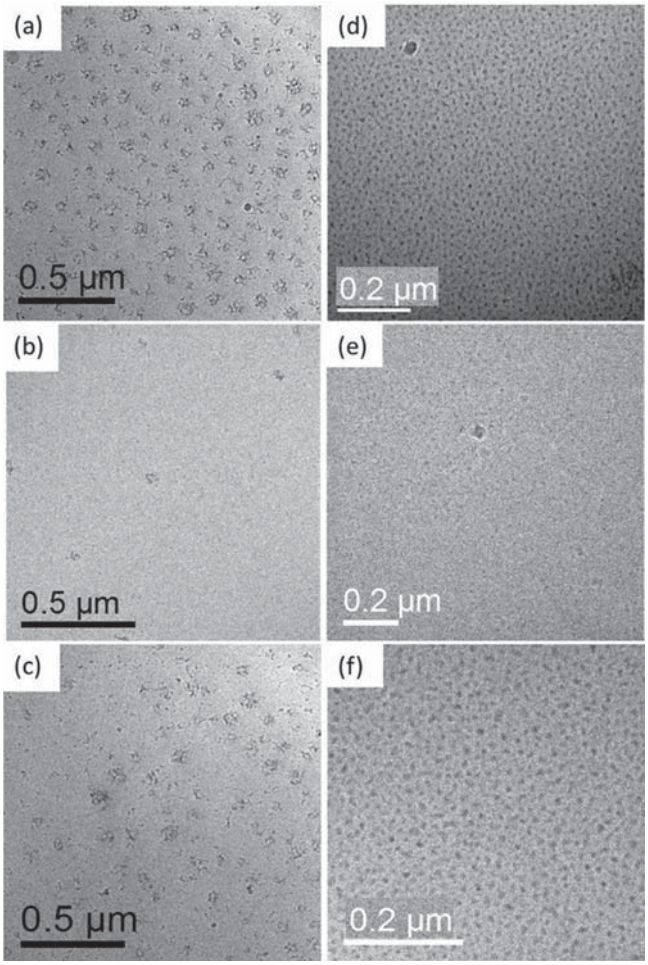


Figure 2. Left column: cryo-TEM images of the sample $PLGASP_{20}$-*b*-PEO_{460} (a) before UV irradiation, (b) after 5 min of UV irradiation, and (c) after 180 min of visible irradiation. Right column: cryo-TEM images of the sample $PLGASP_{10}$-*b*-PEO_{235} (d) before UV irradiation, (e) after 5 min of UV irradiation, and (f) after 180 min of visible irradiation.

copolymer molecularly dissolved in water, as a result of the hydrophobic−hydrophilic switch associated with the SP → MC conversion. After 180 min of visible light irradiation, flower-like micellar aggregates were recovered again, in identical concentration and shape observed prior to UV exposure. Similar light exposure history on a 0.1 wt % solution of the other block copolymer $PLGASP_{10}$-*b*-PEO_{235} resulted in a comparable light-responsive dissolution−micellization process, with two main differences: (i) A smaller size of the micelles was found, with a diameter of around 10 nm before UV irradiation. The micelles were recovered upon visible light irradiation in sizes comparable as prior UV exposure (Figure 2d−f). (ii) The micelles adopted a typical, classical core−shell morphology in this case, as a result of the twice smaller size of the hydrophobic peptide block, which released some of the constraints observed for the longer case.

In order to shed additional insight on the role of the secondary structure in the aggregation behavior, circular dichroism (CD) experiments were performed using a Jasco-815 spectrometer on a 1 mm cell containing 0.1 wt % polymer solutions. Samples were measured both before and after UV irradiation in order to track changes in the secondary structure of the polypeptide block. Before UV irradiation, the sample $PLGASP_{20}$-*b*-PEO_{460} clearly

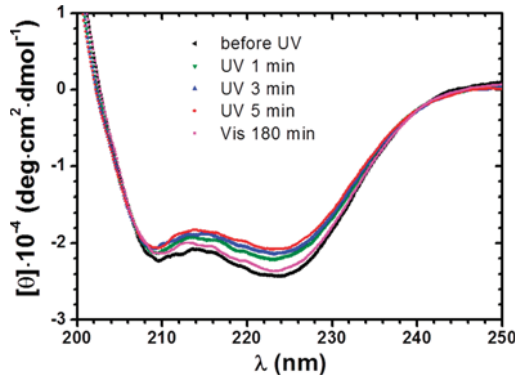


Figure 3. CD spectra for the 0.1 wt % solution of the block copolymer $PLGASP_{20}$-*b*-PEO_{460} before, during UV, and after visible light exposure.

showed the typical pattern of an α-helix conformation with two minima peaks at 209 and 223 nm corresponds to the $\pi-\pi^*$ and $n-\pi^*$ transitions, respectively.[39] Then the solution was irradiated with UV light (λ = 350 nm) at different exposition times, and the relative ellipticity changes were calculated by measuring the variation of the intensity of the peak at 223 nm. A systematic decrease in the α-helix content with respect to the initial state was observed, with a relative reduction of 9% at 1 min UV, 12% at 3 min UV, and 14% at 5 min UV exposure. The same solution was afterward exposed to visible light (λ = 590 nm) for 180 min and recovered 98% of the initial α-helix content, suggesting that the α-helix is nearly completely regenerated (Figure 3).

Similar behavior was observed for the block copolymer $PLGASP_{10}$-*b*-PEO_{235} (see Supporting Information, Figure SI-5), with a reduction of the relative amount of α-helix content of 7% after 1 min of UV irradiation, 11% after 3 min of UV, and 13% after 5 min of UV, with a recover of 97% of the initial α-helix content after subsequent irradiation of visible light during 180 min. These results confirm that both hydrophobicity and secondary structure contribute to the shape of the final aggregates observed.

The high ellipticity content emerging from the CD analysis and the relative small reduction upon UV irradiation are unexpected at first thought for a poly(glutamic acid) in neutral conditions which is only 50% substituted. A possible explanation for this trend is the presence of both acid (pK_a of glutamic acid = 4.25, spiropyran pK_a = 5.00) and basic (4-nitrophenol, pK_a = 7.15) groups on the modified polypeptide block. Before irradiation with UV light, the exchange of the acidic proton from the carboxylic group to the tertiary amino group of the spiropyran moiety leaves the glutamic acid negatively charged and the SP positively charged. During the photoisomerization process, the open form merocyanine structure develops to a conjugated phenolate group, which accepts the proton from the tertiary amino group, while the polypeptide rod remains negatively charged. Thus, both before and after UV irradiation, the polypeptide backbone has negative charges and the SP has positive charges, with the main changes upon irradiation arising only from the position in the lateral photochromic group in which the proton is transferred. This would result in the formation of a more polar phenol group in the photochromic unit upon UV irradiation, without, however a change in the overall charge of the block copolymer. In support of this scenario, the zeta-potential (see Supporting Information, Figure SI-6) undergoes negligible changes between the irradiated and non-UV-irradiated states. Furthermore, the UV−vis spectrum after UV irradiation of the spiropyran molecule in 90:10 H_2O/EtOH

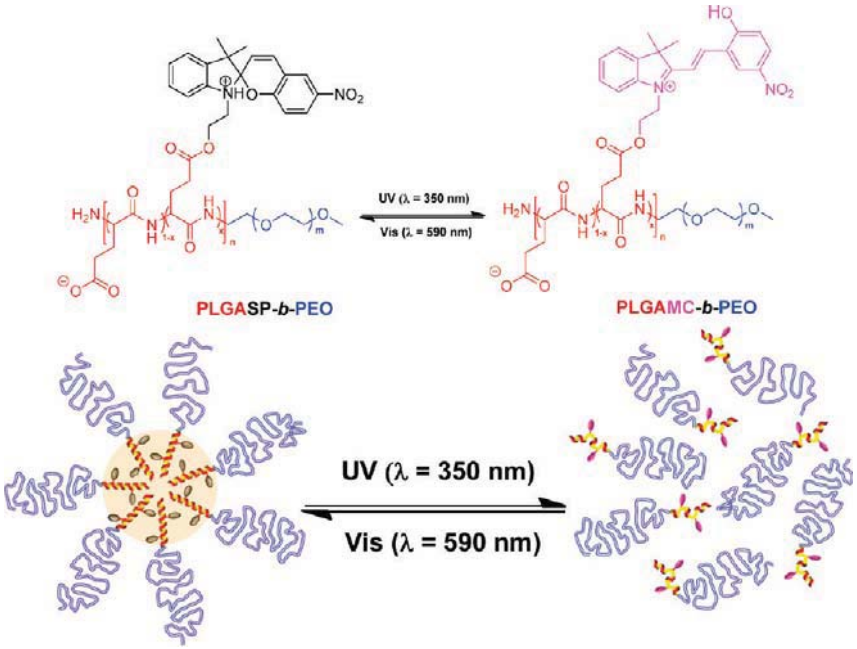


Figure 4. Schematic illustration of photoresponsive micellization/dissolution process for the PLGASP-*b*-PEO block copolymer studied in this work.

shows a clear red shift in the absorption peak when acetic acid is added (see Supporting Information, Figure SI-7). Thus, this mechanism bears similarities with be the counterion condensation observed in polyelectrolytes and leads to partial screening of charges, consequently stabilizing the α-helix secondary structure.

On the basis of the UV/vis, cryo-TEM, CD, and zeta-potential results, we propose the schematic picture for the photoinduced aggregation process highlighted in Figure 4. Prior to UV exposure, the diblock copolymer forms aggregates with either a flowerlike micelle or micellar structure in aqueous solution, with the core constituted by the hydrophobic PLGASP block. The shape and size of the aggregates are controlled by both the secondary structure and the length of the hydrophobic peptide block. When exposed to UV light (λ = 350 nm), the resulting PLGAMC block becomes water-soluble due to the presence of a phenol group in the merocyanine unit, which results in the dissolution of the aggregates. The amount of α-helix along the polypeptide block, as measured by CD, shows a small decrease upon UV exposure, which is expected to arise as a result of increasing numbers of defects presumably associated with the MC open form of the photochromic units. This process is shown to be reversible when the MC open form of the photochromic units is converted back to the SP form upon further exposure to visible light (λ = 590 nm).

In summary, we have shown a new pathway to the use of polypeptide block copolymers as robust light-responsive systems, capable to undergo a reversible aggregation−dissolution−aggregation process in water solutions in response to a suitable light exposure history. Because the stimulus used here is a noninvasive highly penetrating UV source and the block copolymers have a bioinspired molecular architecture, these photoresponsive rod−coil block polypeptide can be used as viable model systems to study photoinduced drug release processes or light-controlled biomedical applications.

Supporting Information. Detailed experimental procedures, ^{1}H NMR, UV/vis spectra, pH measurements, zeta-potential, kinetics, Debye plot, CD spectra and GPC traces.

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