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Poly(ethylene glycol)-poly(vinyl alcohol)-adamantanate: synthesis and stimuli-responsive micelle properties†

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Abstract

A pH-sensitive amphiphilic polymer based on poly(vinyl alcohol) (PVA), modified with poly(ethylene glycol) (PEG) and adamantane (Ad) pendant groups, has been synthesized and the self-assembly properties of this PEG-PVA-Ad construct investigated. PEG-PVA-Ad polymer forms micelles *via* self-assembly at concentrations as low as 26 mg L⁻¹. These polymer micelles can be destroyed by low pH or the addition of β -CD.

Stimuli-responsive materials, including liposomes, hydrogels, polymer micelles, and nanoparticles, that respond to various stimuli such as light, temperature, pH, and redox potential have been widely investigated as controlled release systems.^{1–18} Self-assembly of appropriately-designed amphiphilic polymers in aqueous media are known to form polymer micelles with hydrophobic cores whose dimensions depend on the hydrophilic/lipophilic balance (HLB) of the polymer surfactant.^{19–21} Many different classes of hydrophobic cargo can be loaded in the micellar core.^{21–24} Activation of appropriately designed micelles by an external stimulus can induce cargo release with a kinetic profile that depends on the intensity and duration of the stimulus. It is also strongly dependent on the intrinsic sensitivity and design of the polymer construct. During the past two decades, there have been numerous reports describing stimuli-sensitive polymer and block copolymer micelle systems that respond to single triggering stimuli.^{25,26}

This work describes a self-assembling, pH-sensitive poly(vinyl alcohol) (PVA) polymer main chain bearing adamantane (Ad) and poly(ethylene glycol) (PEG) branches. PVA was selected as the backbone due to its biocompatibility, chemical stability, high hydrophilicity, processability and common use as a hydrogel component.²⁷ PEG grafts were used to improve the HLB and steric stabilization properties of the modified PVA scaffold. Acid sensitivity was introduced into the PVA construct *via* benzylidene-adamantane grafts. These

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benzylidene-adamantane modifications also provide the driving force for hydrophobic condensation of the grafted PEG-PVA-Ad polymer into a polymer micelle. Acid-catalyzed cleavage of the acetal-linked Ad groups from the polymer results in destabilization of the polymer micelles and release of its hydrophobic cargo. An alternative strategy for activating cargo release from this polymer micelle system is the engagement of host–guest interactions between the Ad polymer micelle core and β -cyclodextrin (β -CD) as a solution additive. Strong host–guest interactions of this type²⁸ have been previously used for the construction of stimuli-responsive materials.^{11,29–31}

The synthesis sequence for PEG-PVA-Ad is shown in Scheme 1. Commercial PVA (27kD) was modified with Ad and PEG pendant groups in stepwise fashion. Several PVA substitution reactions were attempted to install the Ad pendant groups, including direct esterification with Ad-COC I in DMSO, however, these reactions were abandoned since the degree of modification was low. Thus, we elected to introduce the Ad group using a 4-hydroxybenzaldehyde spacer between the PVA backbone and the Ad pendant group. Acetal formation between PVA and Compound **1** in DMSO produced **2** in 85% yield. Methoxy-PEG pendant groups were then grafted onto the Compound **2** scaffold using a one-pot stepwise treatment with CDI and MeO-PEG₇₅₀-NH₂, prepared as described in Kulkarni *et al.*,³⁰ to give the PEG-PVA-Ad pendant polymer amphiphile.

¹H NMR spectra of Compound **1**, PVA, **2** and PEG-PVA-Ad appear in Supporting Information. The structure of PEG-PVA-Ad was confirmed by the presence of a single peak at 5.51 ppm, corresponding to the benzylidene acetal proton, peaks at 7.03 and 7.42 ppm, corresponding the benzylidene aryl protons, a large peak at 3.5 ppm due to the PEG methylene groups, and several peaks in the 1.57–2.1 ppm range due to the adamantyl group. The PEG-PVA-Ad composition was estimated by comparing the ¹H NMR peak integration ratios of the benzylidene protons, the CH₂ protons of PVA, and the CH₂ protons of PEG, indicating a composition of ~117 adamantyl grafts and 72 PEG₇₅₀-OMe grafts on the PVA core.

The presence of hydrophobic Ad and hydrophilic PEG pendant groups promotes self-assembly in aqueous media. Ultrasonic dispersion of a 600 mg L^{−1} suspension of PEG-PVA Ad in deionized water produced a clear solution after 15 min of insonification using a 1/8" microtip probe sonicator (pulsed mode, 50% duty cycle @ 50W). Dynamic light scattering (DLS) analysis of this solution revealed the presence of particles with a hydrodynamic radius of 103 ± 12 nm, consistent with the dimensions expected for a polymer micelle assembly. Interestingly, the DLS results in 1 : 1 EtOH:H₂O revealed the presence of much larger structures (194 nm) due to solvent swelling of the micelle structure in the mixed solvent system.

Pyrene solubilization, as detected by fluorescence intensity changes as a function of amphiphile concentration, was used as an additional probe for the formation of PEG-PVA-Ad micelles. Pyrene has been widely used as a hydrophobic probe for monitoring micellar systems.^{32,33} The fluorescence emission intensity ratio, $I_{\text{I}}/I_{\text{III}}$, where Band I = 372–374 nm and Band III = 382–384 nm, is an effective measure of the medium polarity where the probe resides. The ratios decrease, and fluorescence intensities increase, due to pyrene

solubilization within the hydrophobic micelle core. Previous reports have shown that the critical aggregation concentration (CAC) value can be determined by a plot of the pyrene emission intensity as a function of the polymer concentration.^{34,35} Fig. 1A shows the fluorescence emission spectra of pyrene in the presence of PEG-PVA-Ad across a concentration range from 2.3 to 600 mg L⁻¹. These data show that fluorescence intensity changes were modest between 2.3–37.5 mg L⁻¹, however, it increases substantially at PEG-PVA-Ad concentrations of 75 mg L⁻¹. The emission intensity of pyrene at 400 nm *versus* PEG-PVA-Ad concentration is shown in the inset of Fig. 1. A sudden increase in emission intensity was observed at a PEG-PVA-Ad concentration of about 26 mg L⁻¹, suggesting this value as the CAC for this PEG-PVA-Ad polymer construct. These structures appear to be stable at pH 7.4 in 20 mM phosphate buffer containing 120 mM NaCl for more than 12 h at 37 °C.

An acid-labile acetal linkage was used to connect the hydrophobic Ad group to the PVA polymer backbone so that the PEG-PLA-Ad micelles would display pH-responsiveness. ¹H NMR was used to monitor the rate of benzylidene acetal cleavage in our PVA-Ad polymer precursor, Compound **2**, at pH 4.0 (Fig. S1, ESI[†]). In order to clearly discern the aryl and acetal starting material peaks, as well as the aryl and aldehyde peaks of the degradation products, we chose to study the acid-catalyzed transformation of **2** in D₂O with native β-CD present to boost the aqueous solubility of this compound. At pH = 7, the aryl (6.8–7.6 ppm) and benzylidene acetal (4.7 ppm) peaks were clearly observed. After incubation of this solution in pH = 4.0 buffer for 2 d, the ¹H NMR spectrum exhibited three new peaks at 9.9, 8.1, and 7.0 ppm; these new resonances displayed higher intensity than the PVA-Ad starting material peaks. Comparison of these new signals with Compound **1** starting material indicated that the degradation product of **2** was Compound **1**.

In order to determine the degradation rate of PEG-PVA-Ad polymer micelles, we used pyrene as a probe for monitoring the time-dependent stability of the PEG-PVA-Ad dispersion. Fig. 2 shows the pyrene fluorescence emission intensity changes as a function of time in the presence of PEG-PVA-Ad (above the CAC) in pH 1.0 solution with a fixed excitation wavelength of 339 nm. This data shows that the fluorescence intensity decreases sharply during the first hour of reaction time before reaching a stable pyrene emission intensity of approximately 50 au. We infer from these findings that 60% of the polymer micelle-solubilized pyrene was released from the core of the micelle within 1 h at pH 1.0. Upon hydrolysis of the benzylidene acetal Ad pendant groups, the amphiphilic PEG-PVA-Ad polymer becomes increasingly hydrophilic, thus creating an imbalance in the HLB of the initial PEG-PVA-Ad self-assembly that ultimately causes disruption of the polymer micelle and release of solubilized pyrene from its hydrophobic core.

Ad and pyrene are both known to serve as effective β-CD guest molecules in water *via* host–guest complexation (e.g. log K_b for these compounds have been reported as 4.60 and 2.69, respectively).²⁸ This property suggests that the presence of β-CD could change the pyrene solubilization characteristics of PEG-PVA-Ad through host–guest complexation of the

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pendant Ad units. We tested this hypothesis *via* NMR titration, AFM imaging and pyrene fluorescence titration experiments.

^1H NMR spectra showed dramatic shifts in all peaks of aryl and Ad protons upon addition of β -CD to the sample, indicative of complex formation between β -CD and the pendant Ad groups of **2** (Figure S2). AFM images of $6 \times 10^{-5} \text{ mg L}^{-1}$ PEG-PVA-Ad on mica were obtained after slow evaporation of the deionized water solvent at room temperature overnight. These data clearly indicate the presence of many $17 \pm 3 \text{ nm}$ diameter spherical micelle particles (Fig. S3, ESI †). Although the micelle sizes determined in solution by DLS are much larger than that observed on dry mica surfaces by AFM, we attribute these differences to the fully hydrated, hydrophilic PEG arms in the aqueous samples that produce the larger particles observed by DLS as opposed to the condensed, compact micellar structures that result from sample dehydration prior to AFM analysis. The spherical micelle structure is clearly disrupted by the addition of β -CD in equimolar quantities relative to Ad in the PEG-PVA-Ad polymer micelle dispersions. As suggested by our ^1H NMR experiments, host–guest interaction between β -CD host molecules and the pendant Ad guest ligands produces a transformation from spherical particles to elongated structures ($10 \pm 3 \text{ nm}$ diameter rod-like structures in AFM images) due to dispersion of the hydrophobic Ad segments responsible for micelle core formation and steric exclusion by the PEG grafts to maximize their separation along the flexible PVA backbone. This outcome is conceptually similar to the acid-catalyzed hydrolysis experiments that resulted in micellar collapse due to hydrolytic removal of the hydrophobic domains.

Fluorescence spectroscopy was used as an additional line of evidence to demonstrate micelle destruction *via* host–guest complexation. β -CD addition to a fixed concentration of PEG-PVA-Ad micelles bearing pyrene produced a sequence of fluorescence intensity changes as a function of β -CD concentration (Fig. 3). At β -CD concentrations lower than 0.15 mM , the pyrene fluorescence intensity was stable and low due to weak host–guest complexation in this concentration regime and retention of pyrene within the hydrophobic core of the micelle (Fig. 3A). Within the 0.15 to 1.25 mM β -CD concentration range, however, the pyrene fluorescence spectra showed a gradual decrease (Fig. 3B) due to displacement of some Ad groups from the micelle core with accompanying loss of pyrene from the hydrophobic microenvironment. Since the observed pyrene intensity changes are modest, the micellar shape change is expected to be modest within this regime of PEG-PVA-Ad: β -CD stoichiometries. Paradoxically, when the β -CD concentrations exceeded 1.25 mM , the pyrene intensity progressively increased with increasing β -CD content. Moreover, the pyrene spectral shape changed significantly, with the 383 nm peak now exceeding that of the 400 nm peak (Fig. 3B). As a control, the fluorescence of pyrene mixed with only β -CD was measured. This control experiment showed that the pyrene spectra in Fig. 3B & S4D were similar. The decreasing value of I/I_{III} in Fig. 3B at high β -CD concentration indicates that pyrene is included within the CD cavity under these conditions. Therefore, the change in pyrene fluorescence as increasing amounts of β -CD are added to the PEG-PVA-Ad polymer micelle dispersion reveals the involvement of two processes: host–guest complexation between β -CD and Ad to release pyrene from the hydrophobic micelle core at low β -CD concentrations and host–guest complexation between β -CD and pyrene at β -CD

concentrations that are sufficiently high to exceed the available Ad units in the dispersion (Fig. 4). Since Ad has a higher β -CD binding constant than pyrene,²⁸ the initial process is micelle core disruption via β -CD $\leftrightarrow$ Ad interactions as suggested by AFM imaging (Fig. S3B, ESI†).³⁰ As the β -CD concentration increases to exceed the available Ad binding sites, the weaker binding ligand, pyrene, begins to occupy the excess β -CD cavities. This produces an increase in pyrene fluorescence and a change in pyrene peak shape due to the different solubilization properties of β -CD relative to the hydrophobic core of PEG-PVA-Ad polymer micelles. This interpretation is also supported by DLS measurements of PEG-PVA-Ad size changes as a function of added β -CD (Supplementary Information).

Conclusions

An amphiphilic PEG-PVA-Ad polymer was synthesized by grafting Ad and PEG pendant groups onto 27 K PVA. This construct forms micelles *via* self-assembly at concentrations as low as 26 mg L⁻¹. These PEG-PVA-Ad polymer micelles can solubilize hydrophobic cargo, yet be destroyed by low pH or the addition of β -CD to induce the release of their cargo. Utilization of this novel system for controlled release applications is currently under investigation.^{30,31}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Pyrene fluorescence emission spectra as a function of PEG-PVA-Ad concentration (2.3–600 mg L⁻¹), measured at a fixed excitation wavelength ($\lambda_{\text{ex}} = 339$ nm). The inset shows the emission intensity of pyrene at 400 nm (in arbitrary units) as a function of log [PEG-PVA-Ad].

Fig. 2. Time-dependent pyrene fluorescence emission spectra as a 150 mg L⁻¹ PEG-PVA-Ad dispersion at pH = 1.0 ($\lambda_{\text{ex}} = 339$ nm); time 0 $\rightarrow$ 90 min. The inset shows pyrene emission intensity changes as a function of reaction time ($\lambda_{\text{em}} = 400$ nm).

Fig. 3. Pyrene fluorescence emission spectra in the presence of PEG-PVA-Ad upon addition of β -CD at concentrations ranging from 0.001 to 1.25 mM (A) and 1.25 to 20 mM (B).

Fig. 4. Conceptual illustration of pH- and β -CD-induced structural transformation of PEG-PVA-Ad polymer micelles with pyrene in water.

Scheme 1.
Synthesis of PEG-PVA-Ad amphiphilic polymer.