Synthesis and pH-Dependent Micellization of Sulfonamide-Modified Diblock Copolymer

Ravindra R. Pal, Min Sang Kim, and Doo Sung Lee*
Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon, Gyeonggi 440-746, Korea

Received May 4, 2005; Revised November 30, 2005

Abstract: The main objective of this study was to develop and characterize pH-sensitive biodegradable polymeric materials. For pH-sensitivity, we employed three kinds of moieties: 2-amino-3-(1H-imidazol-4-yl)-propionic acid (H), N-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]succinamic acid (SM), and 2-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenylcarbamoyl]-propionylamino}-3-(3H-imidazol-4-yl)-propionic acid (SH). The pH-sensitive diblock copolymers were synthesized by ring opening polymerization and coupling reaction from poly(ethylene glycol) (MPEG), ε-caprolactone (CL), D,L-lactide (LA) and pH-sensitive moieties. The pH-sensitive SH molecule was synthesized in a two-step reaction. The first step involved the synthesis of SHM, a methyl ester derivative of SH, by coupling reaction of SM and L-histidine methyl ester dihydrochloride, whereas the second step involved the hydrolysis of the same. The synthesized SM, SHM and SH molecules were characterized by FTIR, 1H-NMR and 13C-NMR spectroscopy, whereas diblock copolymers and pH-sensitive diblock copolymer were characterized by 1H-NMR and GPC analysis. The critical micelle concentrations were determined at various pH conditions by fluorescence technique using pyrene as a probe. The micellization and demicellization studies of pH-sensitive diblock copolymers were also done at different pH conditions. The pH-sensitivity was further established by acid-based titration and DLS analysis.

Keywords: diblock copolymer, pH-sensitive moieties, critical micelle concentration, micellization/demicellization.

Introduction

Although some significant advances have been recently made in the field of intelligent polymers, the problem of optimum delivery at physiological pH remains a formidable challenge. The polymers that respond to a small change in pH may find a wide range of applications in pharmaceutical, biomedical, bioengineering, and other industrial areas. The micelles have been attractive as a carrier for poorly water-soluble drugs and due to small size (< 100 nm) and the evading capability from scavenges by mononuclear phagocyte system in the body. For these such micelles have been employed as an anticancer drug carrier, combining with tumor targeting capability by enhanced permeability and retention (EPR) effect. The pH-sensitivity is one of the most interesting properties of polymers used for drug delivery systems and have been extensively investigated. It becomes generally known that the extracellular pH of tumors is lower than that of normal tissues; pH value of about 7.0 in tumors and 7.4 in normal tissues. The small but clear difference in pH has been an interesting subject for tumor targeting and various efforts have been devoted to construct pH-sensitive micelles or liposomes. However, because conventional pH-sensitive functional groups (carboxylic groups) provide limited pH-sensitivity in polymers, their applications in biological and pharmaceutical systems, which often only small fluctuation in pH around 7.4, have severely been limited.

It is well documented in literature that certain pH-sensitive groups like sulfonamide and imidazole ring and imidazole ring have pH-activity, even when they are located in the polymeric chain. Sulfonamide, a generic name for the derivatives of para-amino benzene sulfonamide, shows weak acidic nature, whereas the imidazole ring has an electron pair on the unsaturated nitrogen that endows histidine with amphiteric nature by protonation-deprotonation, which leads to pKb value and pH-solubility properties.

In this study, we have synthesized pH-sensitive diblock copolymers, which is composed of methoxy poly(ethylene glycol)-poly(ε-caprolactone-co-D,L-lactide) (MPEG-PCLA) and pH-sensitive moieties such as 2-amino-3-(1H-imidazol-4-yl)-propionic acid (H), N-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]succinamic acid (SM), and 2-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenylcarbamoyl]-propionylamino}-3-(3H-imidazol-4-yl)-propionic acid (SH). The physiochemical properties of the micelles made from...
these copolymers were investigated in terms of size, critical micelle concentration (CMC) and pH-sensitivity.

Experimental

Materials and Methods. All the reagents and solvents have been used as received from Aldrich. Methoxy poly (ethylene glycol) (MPEG), \(M_n=750, 2,000\), D,L-lactide (LA), \(\varepsilon\)-caprolactone (CL), sulfamethazine ((4-amino-N-4,6-dimethyl-pyrimidin-2-yl)-benzenesulfonamide), anhydrous methylene chloride, dicyclohexyl carbodiimide (DCC), disopropyl carbodiimide (DIPC), succinimidy anhydride, 2,4-(dimethyl amino) pyridine, toluene-sulfonic acid, 1,4-dioxane (anhydrous), \(N,N\)-dimethyl formamide (DMF) and \(L\)-histidine (LH) were used as received from Aldrich. Whereas, \(N\)-acetyl-histidine [2-amino-3-(1H-imidazol-4-yl)-propionic acid] (H) was acquired from TCI (Tokyo Kasai Kogyo Co. Ltd., Japan). The coupling catalyst (DPTS) was a complex structure of 4-(dimethylamino) pyridine (DMAP) and \(p\)-toluene sulfonic acid (PTSA). DPTS was synthesized as the reported procedure.\(^{23}\) \(L\)-Histidine methyl ester hydrochloride was synthesized from \(L\)-histidine according to the reported procedure.\(^{24}\)

\(^{1}\)H-NMR and \(^{13}\)C-NMR spectra were recorded on a Varian-Unity Inova 500NB operated at 500 MHz. DMSO and CDCl\(_3\) were used as solvent. The FTIR spectra were recorded on Unicam-5000 spectrometer using KBr pellet technique. Molecular weights of diblock and synthesized pH-sensitive polymers were measured by GPC with two styragel columns (Shodex-KF802.5, KF-803L). CMC (critical micelle concentration) and CMP (critical micelle pH) of diblock copolymer were measured by fluorescence spectrometer (AMINCO-BOWMAN\(^{\circ}\) Series2). And micelle sizes were determined by DLS (dynamic light scattering).

Synthesis of 2-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfanoyl)phenyl] succinic acid (SM): SM was synthesized by carboxylation reaction of amino group of sulfamethazine. The detail synthetic route was outlined in the Scheme I.

The 250 mL round-bottom flask containing sulfamethazine (5 g) and succinic anhydride (2.69 g), equipped with reflux condenser and septum with nitrogen atmosphere was charged with 100 mL of 1,4-dioxane and 2-dimethylamino pyridine (10% w/w). The mixture was stirred with heating at reflux temperature for 10 hrs and then the reaction content was dried. After that the solid compound was well washed with water to remove unreacted succinic anhydride and 2-dimethyl amino pyridine and dried in vacuum. The synthesized compound was obtained in 95% yield.

![Scheme I](image)

Figure 1. FTIR spectra of (A) SM, (B) SHM, and (C) SH using a KBr pellet technique.
famoyl)-phenylcarbamoyl]-propionylamino}-3-(3H-imidazol-4-yl)-propionic acid (SH): SH compound was synthesized by multiple step reaction. First step involved the synthesis of SM, which have been explained above, while the second step employed the coupling reaction of SM with L-histidine methyl ester dihydrochloride to form compound (3), which can be further hydrolyzed resulting the desired molecule, SH.

Synthesis of 2-[3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenylcarbamoyl]-propionyl amino]-3-(3H-imidazol-4-yl)-propionic acid methyl ester (SHM): SHM was synthesized by coupling reaction of SM and L-histidine methyl ester hydrochloride in the presence of DCC and DMAP at room temperature. The detail synthetic route was outlined in the Scheme II.

The procedure for the synthesis is as follows: A suspension of 4.84 g (20 mmol) of finely powdered L-histidine methyl ester dihydrochloride in 75 mL of 9:1 chloroform/methanol (v/v) was treated at 0°C with dry ammonia gas for 30 min. The reaction mixture was filtered and concentrated in order to remove traces of ammonia. The resulting oil was dissolved in 120 mL of 3:1 acetonitrile/dimethylformamide (v/v), and 7.568 g (20 mmol) of SM was added. The solution was cooled in ice, 5.15 g (25 mmol) of DCC and 1.22 g (10 mmol) of DMAP were added, and the reaction mixture was stirred for 2 hrs at 0°C. After that, reaction content was dried under reduced pressure. The solid compound was well washed with water to remove urea and 4-dimethyl amino pyridine and dried in vacuum. The synthesized compound was obtained in 80% yield.

1H-NMR (500 MHz, DMSO-d6): 10.5 (2H, d, amide); 8.30 (1H, d, -NH-); 8.15 (1H, d, H 17); 6.82 (1H, s, H 3); 5.65 (1H, s, H16); 3.95 (1H, m, H13); 3.40 (1H, s, -NH-); 3.2 (2H, d, H14); 2.22 (6H, d, H15).

Figure 2. 1H-NMR spectra of (A) SM, (B) SH, and (C) SHM in DMSO-d6.
Figure 3. $^{13}$C-NMR spectra of (A) SM, (B) SH, and (C) SHM in DMSO-$d_6$.

Scheme II
Hydrolysis of methyl ester of SHM: 5 gram of methyl ester of SH was subjected to hydrolysis using 100 mL of 0.5 M NaOH for two days at room temperature, following the reported procedure. After this period the pH of solution was adjusted to neutral pH using 0.1 M HCl. The solid compound was filtered and dry in vacuum. The detail synthetic route was outlined in the Scheme III.

Biodegradable Diblock Copolymer Synthesis: Synthesis of various MPEG-PCLA diblock copolymers was carried out through a one step ring opening polymerization and the synthetic pathway is shown in Scheme IV. A typical example of ring opening polymerization for MPEG (M, of 750)-PCLA (CL:LA=1:1) is as follows: A 100 mL round-bottom flask was charged with MPEG (10 g) and Sn(Oct)2 (0.05% w/w) and vacuum dried at 80°C for 2 hrs. The solution was then charged with nitrogen gas and cooled. The CL (4.557 mL) and LA (3.87 g) were added and the reaction mixture was stirred for 30 min at 80°C. Then the temperature was gradually increased to 135°C. The reaction mixture was heated at the same temperature for 16 hrs. The remaining amount of CL (1.139 mL) was added and the reaction mixture was stirred for another 6 hrs at 135°C. Finally the contents was cooled to room temperature and diluted with methylene chloride. The reaction content was poured into hexane (500 mL) to precipitate the diblock copolymer. The hexane was decanted and the polymer was vacuum dried for 12 hrs.

To determine the composition of the diblock copolymer, 500 MHz 1H-NMR measurements were carried out. The molecular weight of the PCLA segment in the block copolymer was estimated from 1H-NMR spectrum by calculating the peak intensity ratio of the PLA (-COCH(CH3)O: 5.2 ppm), PCL (-COCH2(CH2)4O: 2.2 ppm) and the methylene protons of the PEG segments (OCH2CH2: 3.6 ppm) based on the number averaged molecular weight (Mn) of PEG determined from GPC measurements. MPEG-PCLA diblock copolymers with different molecular weight were synthesized by similar procedure as listed in Table I.

Synthesis of pH-Sensitive Diblock Copolymer: The syntheses of different pH-sensitive diblock copolymer were achieved by coupling reaction of different molecular weight diblock copolymer and pH-sensitive moieties such as SM.
SH and 2-acetamido-3-(1H-imidazol-5-yl)propanoic acid (H) (Scheme IV). The general outline for the synthesized is given as follows: MPEG-PCLA ($M_n$ of MPEG: 2,000, CL: LA=1:0.5) diblock copolymer (5 g), DPTS (0.27 g, 40% w/w of DIPC) and SH (0.9127 g) were put in two-neck flask and then dried under vacuum at 80°C for 30 min. Then mixture was charged with nitrogen and cooled to room temperature, thereafter methylene chloride (60 mL) was added and stirred. The DIPC (0.548 mL) was added to reaction mixture and stirred at room temperature for another 24 hrs. The resulting product was filtered and evaporated under vacuum. The product was dissolved in THF, then filtered to eliminate urea and then precipitated in hexane (500 mL). Precipitated product was dried under vacuum at 40°C for 12 hrs. All the other pH-sensitive diblock copolymers were synthesized by similar procedure.

The functionality of pH-sensitive groups was confirmed by measuring $^1$H-NMR and the molecular weight of final product was confirmed by gel permeation chromatography.

**Fluorescence Measurements and CMC Determination.** The micellization-demicellization behavior of pH-sensitive triblock copolymers was investigated by fluorescence spectroscopy using pyrene as a probe. A buffer solution of 0.1 N Borax and potassium phosphate was made. A stock solution of pyrene in THF is poured in a buffer solution and heated at 40°C for 2 hrs for evaporation of THF. The final concentration of pyrene was 1.0×$10^{-6}$ M. Emission spectra of pyrene were recorded at 350 to 440 nm (Figure 5). Micelles were prepared from dissolving polymer in a buffer solution directly. The intensity ratio of $I_3(\lambda_{384})$ to $I_1(\lambda_{374})$ of emission
molecules were ascertained by FTIR, $^1$H-NMR, and $^{13}$C-NMR spectroscopy. All the spectral data are given in experimental section.

**Synthesis of Diblock Copolymer:** Ring opening polymerization of D,L-lactide and $\varepsilon$-caprolactone using MPEG in the presence of Sn(oct)$_2$ has been reported in a number of literatures. Ring opening polymerization is primarily initiated by coordinate ionic initiator. The mechanism of this reaction varies according to monomer and initiator. In this study, different molecular weight MPEG-PCLA diblock copolymer with hydroxyl group were synthesized by adjusting the feed ratio of MPEG, D,L-lactide, and $\varepsilon$-caprolactone as shown in Table I. While synthesis of diblock copolymer, CL (20 wt%) was added afterward to achieve the primary hydroxyl end group. The synthesized diblock copolymers were characterized by $^1$H-NMR and GPC analysis. The characteristic peak of methyl protons (-OCH$_3$) of MPEG in $^1$H-NMR of MPEG-PCLA is appeared at 3.5 ppm, whereas the peaks at 5.1 and 2.2 are assigned for (-CH) of DLLA and (-CH$_2$) of CL, respectively.

**Synthesis of pH-Sensitive Diblock Copolymer** The different pH-sensitive diblock copolymers were synthesized by coupling reaction using different pH-sensitive moieties. The diblock copolymer is degradable by moisture and heat due to the presence of ester linkage, and hence the coupling reaction should be done at room temperature. To overcome this problem we have selected coupling reagents, diisopropyl carbodiimide (DIPC) and DPTS were used as a reagent and a catalyst respectively. After the coupling reaction, the peaks in aromatic region were detected in $^1$H-NMR spectrum of the synthesized block copolymer, which indicates the formation of pH-sensitive block copolymer (Figure 4). The further characterization of pH-sensitive diblock copolymer was achieved by GPC analysis (Tables II and III).

**The CMC Determination and Effect on CMC.** The CMC determination and the pH effect on the CMC of the synthesized diblock copolymers with different pH-sensitive groups were carried out by fluorescence spectroscopic studies using pyrene as a probe. The pattern of emission spectra of pH-sensitive diblock copolymers in different pH conditions with various concentrations were studied in the presence of 1.0×10$^{-6}$M pyrene. Amongst all, the representative emission spectra at pH of 6.5, 7.4, and 8.5 are shown in Figures 6, 7, and 8. These figures show the change in intensity ratio $I_3/I_1$ in the emission spectra against polymer concentration.

The CMC value was determined from crossover points at lower concentrations and has been summarized in Tables II and III. The polymeric micelles formed from diblock copolymer incorporating with SH show relatively broad phase transition around 7.2, whereas micelles of block copolymers containing SM and H shows a very narrow pH changes. The pH-sensitive SM and H groups could not show clear CMC value in pH 8.5, where as SH molecule showed a very stable micelle in this pH solution. This fact was originated from...
The synthesized pH-sensitive diblock copolymers have got sulfonamide and imidazole secondary amino group in the main unit, which are responsible for basic and acidic changes due to ionization. The imidazole ring has an electron pair on the unsaturated nitrogen that endows histidine with amphoteric nature by protonation-deprotonation, which leads to pKb value and pH-solubility properties of it. The effect of the pH condition on the CMC was examined and

### Table II. Critical Micelle Concentration (CMC) of pH-Sensitive Diblock Copolymer Synthesized from MPEG (750) in Different pH Solution

<table>
<thead>
<tr>
<th>No.</th>
<th>MPEG/PCLA Ratio</th>
<th>Molecular Weight</th>
<th>pH-Sensitive Group</th>
<th>CMC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 6.5</td>
</tr>
<tr>
<td>1</td>
<td>1/0.5</td>
<td>2582</td>
<td>SM</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>1/1</td>
<td>3082</td>
<td>SM</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>1/0.5</td>
<td>6210</td>
<td>H</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>1/1</td>
<td>2645</td>
<td>H</td>
<td>0.29</td>
</tr>
<tr>
<td>5</td>
<td>1/0.5</td>
<td>3168</td>
<td>SH</td>
<td>-1.35</td>
</tr>
<tr>
<td>6</td>
<td>1/1</td>
<td>6345</td>
<td>SH</td>
<td>-1.47</td>
</tr>
</tbody>
</table>

* Determined from emission spectra. † Determined from emission curve. ‡ Unclear CMC value.

### Table III. Critical Micelle Concentration (CMC) of pH-Sensitive Diblock Copolymer Synthesized from MPEG (2k) in Different pH Solution

<table>
<thead>
<tr>
<th>No.</th>
<th>MPEG/PCLA Ratio</th>
<th>Molecular Weight</th>
<th>pH-Sensitive Group</th>
<th>Micelle Size (nm)</th>
<th>CMC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 6.5</td>
</tr>
<tr>
<td>1</td>
<td>1/0.5</td>
<td>2582</td>
<td>SM</td>
<td>20.4</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>1/1</td>
<td>3082</td>
<td>SM</td>
<td>64.5</td>
<td>0.24</td>
</tr>
<tr>
<td>3</td>
<td>1/0.5</td>
<td>6210</td>
<td>H</td>
<td>135</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>1/1</td>
<td>2645</td>
<td>H</td>
<td>150</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>1/0.5</td>
<td>3168</td>
<td>SH</td>
<td>85</td>
<td>-1.41</td>
</tr>
<tr>
<td>6</td>
<td>1/1</td>
<td>6345</td>
<td>SH</td>
<td>92</td>
<td>-1.62</td>
</tr>
</tbody>
</table>

* Determined from GPC analysis. † Determined from emission spectra at different pH. ‡ Mean diameter based on number average from DLS. § Unclear CMC value.

**Figure 6.** The intensity ratio of $I_3/I_1$ in the emission spectra as a function of the block copolymer concentration at pH 6.50.

**Figure 7.** The intensity ratio of $I_3/I_1$ in the emission spectra as a function of the block copolymer concentration at pH 7.40.
the results are presented in Figure 9.

A noticeable difference between all these pH-sensitive groups can be evaluated from this figure. The pH-sensitive SH molecule showed a very sharp and prominent change for CMC value with respect to pH, whereas the other two, SM and H molecules could not provide such a pH change effect. The CMC of MPEG-PCLA-SH shows a very broad range of micellization as compared to MPEG-PCLA-SM or MPEG-PCLA-H. However below pH 4.8 or higher to 8.5, demicellization starts to begin. The fact can be evidence due to the presence of both sulfonamide and amino group together in the main chain. The evident result says that, these pH sensitive groups showing their resultant value and responsible for collapse of micelle below pH 4.8 or higher to 8.5.

**Titration of pH-Sensitive Diblock Copolymer.** It has been documented in literature that histidine residues in protein and sulfamethazine derivatives in block copolymer significantly contribute towards physiological pH level. The acid-base titration profiles of diblock copolymer with pH-sensitive moiety are presented in Figure 10. All the polymer solution exhibited a buffering pH region of 4-9. The titration curve confirmed that the polymer with SH as a pH-sensitive moiety had a higher buffer capacity in the physiological pH range of pH 6.0-8.0 compared with other pH-sensitive moiety.

**DLS.** To investigate the pH effect on the size of micelles in aqueous media, DLS analysis was carried out. The micelles solutions in different pH conditions were prepared and its DLS studies were done(Table III and Figure 11). DLS results explained that, micelles obtained form SH shows regular size with good stability compared to SM and H molecules.
Conclusions

In this study, we suggested a novel pH-sensitive block copolymer prepared by coupling reaction with pH-sensitive molecules: 2-[(3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenylcarbamoyl]-propionylamino]-3-(3H-imidazol-4-yl)-propionic acid (SH), 2-amino-3-(1H-imidazol-4-yl)-propionic acid (H) and N-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]succinamic acid (SM). The diblock copolymer end capped with SH molecule as pH-sensitive group showed phase transition around 7.20. The sensitivity was further established by acid-base titration and dynamic light scattering. The facile synthesis of pH-sensitive moieties and end-capped polymer would make an interesting example for application in the filed of drug delivery systems.

Acknowledgements. This research was financially supported by the Ministry of Science and Technology(M10414030002-05N1403-00220) in Korea.

References