pH/TEMPERATURE SENSITIVE PENTABLOCK BASED ON D,L-SERINE

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Received: 15 July 2016; Accepted for publication: 3 December 2016

ABSTRACT

For recent decades, researchers have been increasing their attention to pH/temperature-sensitive polymers as drug/protein delivery systems. In this research, we aim to synthesize a new pH/temperature sensitive pentablock based on D,L-Serine. Amino groups of D,L-Serine were modified with benzenesulfonyl chloride in order to create sulfonamide groups as desired pH-sensitive groups. Pentablocks were then synthesized by conjugation reaction between carboxylic, hydroxyl groups of D,L-Serine and hydroxyl-terminal groups of temperature sensitive triblock PCL-PEG-PCL. The synthesized products were analyzed by 1H-NMR and their molecular weights were measured by gel permeation chromatography (GPC). The sol-gel transition of pentablock was also investigated by the inverting test method. The pH dependent property of the pH sensitive moiety could be very useful for preparing drug carriers.

Keywords: pH/temperature sensitive, D,L-Serine, benzenesulfonyl chloride, pentablock.

1. INTRODUCTION

Smart hydrogels have been applied in wide range of scientific applications such as medicine, agriculture, and biotechnology because they are classified as one of smart polymers that not only can exist in both liquid form and solid form but also have many functional properties (i.e. swelling, mechanical, permeation, surface and optical) [1 - 8]. Various stimuli-sensitive hydrogels that respond to pH [9], temperature [10, 11], electric fields [12, 13], and other stimuli have been studied both experimentally and theoretically [14, 15]. Among them, pentablock polymers which possess an ability of sol-to-gel transition with temperature/pH trigger have a great potential for drug delivery systems [16 - 18]. For instance, pH/temperature responsive hydrogels poly(b-amo

The copolymer solution could be injected with no surgical procedure, no clogging during injection, straightforward drug loading to the polymer solution,
no initial burst of release, and long sustained release. The PAE degradation rate is the main factor that influences the release of anionic drugs or proteins. Shim et al have synthesized pH/temperature-sensitive pentablock copolymer from temperature-sensitive of poly (ε-caprolactone-co-lactide) (PCLA) and PEG block copolymer (PCLA-PEG-PCLA), conjugated either side with pH-sensitive sulfamethazine oligomers (OSMs) to form (OSM-PCLA-PEG-PCLA-OSM). This block copolymer solution showed a reversible sol-gel transition as a result of both a small pH change in the range of pH 7.4 - 8.0, and a temperature change around body temperature [19 - 22]. These properties made this polymer a potential candidate for use as a carrier in the loading and releasing of drugs or proteins, in particular for the delivery of cationic proteins. Aside from these advantages, these pentablock have revealed one main disadvantage. The degradation period of them is too long while the effective release period of drug in body is around 7 - 10 days. As a result, the residue of pentablock gel remaining in patience body makes them uncomfortable.

In this study, D,L-Serine was used as a novel pH sensitive moiety and an cationic pH/temperature-sensitive pentablock copolymer hydrogel was created from D,L-Serine and PCL-PEG-PCL triblock via a conjugating reaction.

2. MATERIALS AND METHODS

2.1. Materials

Poly(ethylene glycol)s (PEGs) were purchased from Sigma–Aldrich Co. (Mn 1750). The chemical ε-caprolactone (CL) and phosphate-buffered saline (PBS) were obtained from Sigma–Aldrich. Stannous octoate [Sn(Oct)2] was obtained from Sigma–Aldrich and was dried for 24h under vacuum at ambient temperature prior to use. Dicyclohexyl carbodiimide (DCC) and 4-(dimethyl amino) pyridine (DMAP) were used as received from Sigma-Aldrich. D,L-Serine and Benzenesulfonyl chloride were purchased from Merck. Dimethylformamide (DMF) and 1,4-Dioxane were all obtained from Prolabo. All other reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Modification of D,L-Serine with Benzenesulfonyl chloride

D,L-Serine (4.0 g) dissolved in a solution of NaOH (3.04g in 60 ml H2O) at 0 °C and stirred for 2 h before Benzenesulfonyl chloride (4 ml in 40 ml 1,4-Dioxane) was added dropwise. The reaction was then conducted at 25 °C for the next 10 h and an excess amount of diethyl ether was used to wash this reaction solution as soon as the reaction stopped. After that, the aqueous layer was retrieved, acidified with 10%HCl to a pH 1-2 and then extracted with ethyl acetate, while the combined organic layers was stirred in the presence of MgSO4 as a drying agent. As soon as MgSO4 were filtered out of these layers by paper filters (0.2 mm), the organic solution was evaporated under vacuum to give Benzenesulfonyl-D,L-Serine (mSerine) as a bright yellow compound.

2.2.2. Synthesis of Temperature-Sensitive PCL-PEG-PCL Tri-block Copolymers

PCL-PEG-PCL triblock copolymer was synthesized by ring opening polymerization of ε-caprolactone (CL) initiated by hydroxyl groups at the end of PEG (Mn = 1750) in the presence...
of stannous octoate as a catalyst. The compositions and molecular weights of triblock copolymers which control the balance of hydrophobic/hydrophilic of PEG/PCL were adjusted by the feed ratios of PEG and PCL. The synthesis process of triblock copolymer which had PCL/PEG = 1.8/1 (weight ratio); PEG 1750 could be described as below: 4 g PEG (1750) and 0.04 g stannous octoate in a dried two-neck round-bottom flask were dried at 110 °C for 2 h under vacuum. Then, the temperature decreased under 60 °C, 7.6 g CL was added and a dry nitrogen was supplied into the flask. The reaction mixture was dried for 1 h under vacuum at 60 °C. After drying step, the reaction temperature was fixed at 130 °C. The ring opening reaction then took place in 18 h. The reactants were cooled to room temperature before being dissolved in chloroform, and then the solution was precipitated in excess diethyl ether. The precipitated product was dried under vacuum at room temperature for 48 h. The overall yield of this triblock copolymer is over 85 % after drying.

2.2.3. Synthesis of Temperature-Sensitive OS-PCL-PEG-PCL-OS pentablock

To start with, 1 mmol triblock copolymer PCL-PEG-PCL, 0.4 mmol DMAP and 4 mmol DCC were dissolved in 40 ml DMF at room temperature. The feed ratio of triblock copolymer/mSerine/DCC/DMAP was 1.0/4.0/4.0/0.4 mol.

The reaction system was carried out in Argon environment. After that, 4 mmol mSerine in 20 ml DMF was injected dropwise into the system within 3 h. After 2 days’ reaction, DMF was
completely removed from the reaction solution via a rotary evaporation at 80 °C and the dried residue was dissolved in THF. In the next step, PTFE filter (0.45 μm) was used to filter the THF solution. Next, the crude product was purified by precipitation into excess diethyl ether. This method was used to remove pH-sensitive homopolymers [17]. The OS-PCL-PEG-PCL-OS was dried under vacuum at 60 °C for 48 h.

2.2.4 1H NMR Analysis

The 1H-NMR spectra were obtained from Bruker Avance machine at 500 MHz and used to determine the molecular structures of D,L-Serine, triblock and pentablock. Their spectrums were analyzed in D2O, CDCl3 and DMSO solvent containing 0.03 % (v/v) Tetramethylsilane (TMS).

2.2.5. GPC Analysis

The molecular weight and molecular weight distribution of triblock and pentablock were measured by gel permeation chromatography (GPC) Agilent 1100 Series, with a PLgel MIXED column, at 50 °C. DMF was used as an eluent at a flow rate of 1 mL/min. Calibration was carried out using poly(ethylene glycol) (Polymer laboratories Inc.) with the molecular weight ranging from 420 to 22100.

2.2.6. Sol-Gel Phase Transition Measurement

The transition behavior (the sol (flow)-gel (non-flow) phase) of the copolymers in aqueous medium was investigated by the inverting test method with a 4mL (10 mm diameter) vial test tube. The pentablock copolymer was dissolving in PBS buffer at a given concentration at 0 °C for 2 days. Its pH was then adjusted with NaOH 5 M and HCl 5 M, and stabilized at 0 °C for 1 day. The sample tubes were placed in a water-bath, where these tubes was then heated slowly from 0 to 60 °C at 0.2°C/min with a 5min hold at each temperature. The sol-gel transition at each temperature was determined by angling the vial horizontally after keeping it at a constant temperature for 5min, as described in our previous paper [19, 20].

3. RESULTS AND DISCUSSION

3.1. Sulfonamide group synthesis

The 1H-NMR spectrum of D,L-Serine in Figure 1a. The chemical shift of methylene protons appeared at 3.955 ppm (signal “a”), and a peak at 3.831 ppm (signal “b”) was assigned to the methine proton of D,L-Serine. In addition, D2O removed peaks of –NH2,-COOH in 1H-NMR of D,L-Serine as these groups have interchangeable protons.

The 1H-NMR spectrum of mSerine (D,L-Serine modified with benzenesulfonyl chloride) in Figure 1b: the presence of aromatic protons of benzenesulfonyl chloride were exhibited at 7.604 ppm and 7.295 ppm (signal “1,2”) and the characteristic signal assigned to the sulfonamide protons of the mSerine appeared at 7.972 ppm (signal “3”).

According to the above results, D,L-Serine was successfully modified by the reaction between 2 functional groups of amino and sulfonyl chloride.
3.2. Synthesis of PCL-PEG-PCL and OS-PCL-PEG-PCL-OS

Figure 1. $^1$H-NMR spectra of D,L-Serine (a) in D2O and mSerine (b) in DMSO.

Figure 2. $^1$H-NMR spectra of mSerine (a) in DMSO, PCL-PEG-PCL (a) and OS-PCL-PEG-PCL-OS (b) in CDCl$_3$. 
As can be seen in Figure 2, the chemical shift at $\delta = 3.647$ ppm (signals “a,a’,f”) represented the methylene hydrogen of EO unit (a, a’) and at the end of CL unit (f), while the methylene protons at the end of PEG unit were shown through a peak at 4.2 ppm (signal “b”). The signals at 1.33, 1.66, 2.35 ppm (signals “e,d,c”, respectively) were assigned to the other methylene hydrogens of CL. The corresponding peak areas were calculated in order to obtain the compositions [20].

In Figure 2c, besides these chemical shifts of PCL-PEG-PCL structure, there were the methylene hydrogen resonance of mSerine unit at 3.14 ppm (signals “5”). In addition, chemical shifts appearing at 7 - 8 ppm (signal “Ar-H”) corresponded to the aromatic protons of mSerine.

On the other hand, the molecular structure and molecular weight of PCL-PEG-PCL and OS-PCL-PEG-PCL-OS compositions obtained by integration of the corresponding peak area and GPC were shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PCL-PEG-PCL (Mn)$^a$</th>
<th>PEG/PC L (w/w)$^a$</th>
<th>Mole ratio of mSerine/triblock</th>
<th>OS-PCL-PEG-PCL-OS$^b$</th>
<th>Mw/Mn$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1750-4</td>
<td>1575-1575-1575</td>
<td>1/1.8</td>
<td>4/1</td>
<td>348-1575-1575-1575-348</td>
<td>1.14</td>
</tr>
<tr>
<td>1750-8</td>
<td>1575-1575-1575</td>
<td>1/1.8</td>
<td>8/1</td>
<td>877-1575-1575-1575-877</td>
<td>1.31</td>
</tr>
<tr>
<td>1750-12</td>
<td>1575-1575-1575</td>
<td>1/1.8</td>
<td>12/1</td>
<td>1344-1575-1575-1575-1344</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Molecular weights of PEG (1750) were provided by Sigma-Aldrich.

$^a$Calculated from 1H-NMR.

$^b$Measured by GPC relative to poly(ethylene glycol) standards.

### 3.3. Sol-Gel Phase Transition Diagram

OS-PCL-PEG-PCL-OS pentablock copolymer showed a sol-gel transition responding to both temperature and pH. It produced a critical gel pH (CGpH) and two critical gel temperature (CGT) in the curve (a lower CGT is the transition from sol to gel, and an upper CGT is the transition from gel to sol) (Figure 3). At low temperature ($10 \, ^\circ\text{C}$) and high pH (8.5), OS was ionized and the solubility of the pentablock chain was increased [19]. Furthermore, the hydrophilic/hydrophobic balance of OS/PCL shifted to a more hydrophilic character at low temperature, as a result, OS-PCL-PEG-PCL-OS solution stayed as a sol state. With the decrease in pH from 8.5 to 7.4, the OS blocks were de-ionized and became hydrophobic. In addition, the hydrophilic/hydrophobic equilibrium changed to being more hydrophobic at high temperature. These reasons caused OS-PCL to play a role of hydrophobic block at high pH and high temperature (37 $^\circ\text{C}$ and pH 7.4). The pentablock copolymer chains then formed micelle structures in aqueous solution with PAE-PCLs as the core and PEGs as the shell. At a high concentration (higher than critical gel concentration), many bridges along micelles were constituted by OS-PCL-PEG-PCL-OS. The result of the bridge creating effect made the structure change to matrix and the pentablock copolymer solution stayed as a gel state.
Figure 3. Sol-gel phase transition diagrams of OS-PCL-PEG-PCL-OS (left-hand side); photographs of the sol/gel transition (right-hand side): a) solution at 5 °C and pH = 10, b) gel at 37°C and pH = 7.4, c) sedimentation at 50 °C and pH = 7.4.

Figure 4. Sol-gel phase transition diagrams of OS-PCL-PEG-PCL-OS block copolymer solutions with different mSerine/triblock mole ratio.

Effect of hydrophobic/hydrophilic ratio: Figures 4 presents the changes in gelation regions with the changes in mSerine/triblock mole ratio. As the hydrophobic/hydrophilic chain length ratio is increased from 4/1 (sample 1750-4) to 12/1 (sample 1750-12) (details in Table 1), the gelation regions expanded accordingly, and the pentablock copolymers transformed to gel stage at a higher pH value. That is because when the pH-sensitive chainlength becomes longer, the hydrophilic/hydrophobic balance turns to be more hydrophilic, such that the resulting micelles are generally formed with fewer associated chains. As more bridging connections are formed between the micelles, more grouped micelles are produced, and hence the sol to gel and gel to sol phase transitions occur at lower and higher CGTs, respectively.
4. CONCLUSIONS

In this research, D,L-Serine was successfully conjugated to temperature triblock PCL-PEG-PCL through carboxylic and hydroxyl groups of D,L-Serine and hydroxyl-terminal groups of triblock. The pentablock OS-PCL-PEG-PCL-OS solution transformed into solid in aqueous solution. This synthesized pentablock could dissolve in aqueous solution at low temperature (2-100°C) and high pH (9-10); existed in solid form at 30-400C and pH=7.4. The sol–gel transition diagram of OS-PCL-PEG-PCL-OS showed a CGpH and two CGTs.

Acknowledgements. This research is funded by Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number B2015 -20a- 01.

REFERENCES


TÓM TÁT

PENTABLOCK NHẤY CÁM PH/NHIỆT ĐỘ TRÊN CƠ SỞ D,L-SERINE

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Từ khóa: nhạy cảm pH/nhiệt độ, D,L-Serine, benzenesulfonyl chloride, pentablock.