Polymer 52 (2011) 4928–4937

Contents lists available at SciVerse ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Core-shell structured nanoassemblies based on β-cyclodextrin containing block copolymer and poly(β-benzyl L-aspartate) via host-guest complexation

Jianxiang Zhang[a,b], Peter X. Ma[a,c,d,e,*]

[a] Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Pharmaceutics, College of Pharmacy, Third Military Medical University, Chongqing 400038, China
[c] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[d] Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, MI 48109, USA
[e] Department of Materials Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form
14 August 2011
Accepted 21 August 2011
Available online 26 August 2011

Keywords:
Host-guest interactions
Self-assembly
β-cyclodextrin

ABSTRACT

Double hydrophilic copolymers (PEG-*b*-PCDs) with one PEG block and another block containing β-cyclodextrin (β-CD) units were synthesized by macromolecular substitution reaction. Via a dialysis procedure, complex assemblies with a core–shell structure were prepared using PEG-*b*-PCDs in the presence of a hydrophobic homopolymer poly(β-benzyl L-aspartate) (PBLA). The hydrophobic PBLA resided preferably in the cores of assemblies, while the extending PEG chains acted as the outer shell. Host-guest interaction between β-CD and hydrophobic benzyl group was found to mediate the formation of the assemblies, where PEG-*b*-PCD and PBLA served as the host and guest macromolecules, respectively. The particle size of the assemblies could be modulated by the composition of the host PEG-*b*-PCD copolymer. The molecular weight of the guest polymer also had a significant effect on the size of the assemblies. The assemblies prepared from the host and guest polymer pair were stable during a long-term storage. These assemblies could also be successfully reconstituted after freeze-drying. The assemblies may therefore be used as novel nanocarriers for the delivery of hydrophobic drugs.

1. Introduction

Core-shell structured nanoparticles with different sizes, topologies, and material properties have been widely studied for applications in drug delivery, biomedical imaging, diagnostics, and biosensing [1–6]. These nanostructured systems can be constructed by polymers, nanocrystals, metals or their composites that possess distinctly different physicochemical characteristics. For nanoparticles with a core-shell architecture, multiple functionalities can be conveniently integrated to generate multi-functional nanoplatforms for better performances [1,7]. Among these core-shell nanostructures, polymer systems are the most attractive for biomedical applications, mainly due to their synthetic flexibility, structural diversity, versatile modulability, and excellent biocompatibility [2,3,8–10].

Self-assembly of macromolecular amphiphiles has long been recognized as a powerful strategy to fabricate core-shell architectured nanostructures [11]. Nanoassemblies thus constructed have found applications in a variety of fields such as cosmetics, materials science, pharmaceutics, bioengineering, biomedicine, gene therapy, and tissue engineering. For instance, polymer micelles with a hydrophobic core and a hydrophilic shell have been intensively investigated for the delivery of lipophilic therapeutics, especially for cancer therapy [2,3,10,12,13]. Several nano-sized micellar formulations of antitumor drugs are already in clinical trials, and their efficacy has been demonstrated [14]. In addition, core-shell particles with a polyelectrolyte complex core that are assembled via electrostatic force have been of great interest for gene therapy [8,15]. In these nanosystems, the core serves as nanocontainers for single/multiple therapeutics, imaging reagents or their combinations. The outer shell, however, can endow particles with colloidal stability and long circulation capability. Additional targeting units such as antibody and ligand can also be anchored onto the shell for selective delivery.

Until now, most of the polymeric core-shell assemblies are constructed via hydrophobic interactions, using amphiphilic copolymers with block, graft, comb, branch or dendritic architectures [16–23]. The driving forces for the assembling can also be other non-covalent forces such as electrostatic, hydrogen-bonding,

* Corresponding author. Department of Biologic and Materials Sciences, University of Michigan, 1011 North University Ave, Ann Arbor, MI 48109-1078, USA. Tel.: +1 734 764 2209; fax: +1 734 647 2110.
E-mail address: mapx@umich.edu (P.X. Ma).

0032-3861/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2011.08.030

stereocomplexation and charge transfer interactions [3]. Recently, cyclodextrin (CD) containing polymers have been widely used as building blocks to fabricate various networks and particulate assemblies, taking advantage of host-guest interactions [24,25]. For example, supramolecular hydrogels and discrete nano- or microparticles can be constructed by the inclusion interactions between CD units and guest molecules [26–34]. One of our research foci has been on the development of core-shell structured nanoassemblies using β-CD containing polymers as macromolecular hosts [25]. β-CD conjugated polymers with a block or branch architecture were found to be able to assemble in the presence of guest molecules including small-molecule drugs and polymers with hydrophobic groups [35–38]. Through this protocol, we were able to fabricate thermosensitive nanosystems for temperature-triggered payload release using a thermosensitive guest poly(N-isopropylacrylamide) (PNIPAm) [35], construct multifunctional assemblies for simultaneous drug and gene delivery using β-CD conjugated polyethyleneimine (PEI-CD) [37], and prepare chemical-responsive nanoassemblies for the delivery of lipophilic drugs [36]. The aim of this study was to systematically investigate the assembling behaviors of a β-CD containing hydrophilic–hydrophilic diblock copolymers (PEG-*b*-PCD) with poly(β-benzyl L-aspartate) (PBLA), and thoroughly examine the characteristics possessed by the assembled core-shell structures for potential drug delivery applications.

2. Materials and methods

2.1. Materials

L-Aspartic acid β-benzyl ester was purchased from Sigma (St. Louis, USA). Triphosgene was obtained from Fisher (USA). β-Benzyl-L-aspartate N-carboxyanhydride (BLA-NCA) was synthesized according to the literature [39]. α-Methoxy-ω-amino-polyethylene glycol (MPEG-NH2) with an average molecular weight (MW) of 5000 was purchased from Laysan Bio, Inc. (Alabama, USA), and used without further purification. Ethylenediamine (EDA) was purchased from Sigma (St. Louis, USA) and distilled over CaH_2 under reduced pressure. Coumarin 102 (C102), pyrene (≥99%), β-cyclodextrin (β-CD, ≥98%), and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma (St. Louis, USA) and used as received. The method established by Baussanne et al. was employed to synthesize 6-monotosyl β-CD [40].

2.2. Synthesis of PEG-b-PCD

PEG-*b*-polyaspartamide containing EDA units (PEG-*b*-PEDA) was firstly synthesized using MPEG-NH2 according to the previously reported method [41]. PEG-*b*-PCD copolymers were then synthesized using a slightly modified method established in our previous study [38]. Briefly, lyophilized PEG-*b*-PEDA (600 mg) and 5-fold excess amount of 6-monotosyl β-CD were reacted in 30 ml anhydrous DMSO at 60 °C. This reaction was traced by ^{1}H NMR and FTIR measurements. At the predetermined time points, a certain amount of the reaction mixture was collected and dialyzed against 0.1 N NaOH for 2 days to remove unreacted 6-monotosyl β-CD, and then dialyzed against distilled water for another 2 days. After being filtered through a 0.22 μm syringe filter, the resultant aqueous solutions were lyophilized. The optimized reaction conditions were employed to synthesize copolymers used in the following studies (Table 1). For the synthesis of PEG-*b*-PCD-1, PEG-*b*-PEDA with the degree of polymerization (DP) of 5 for PEDA block was reacted with 5 M excess of 6-monotosyl β-CD in anhydrous DMSO at 60 °C for 7 days. Purification was performed following the aforementioned procedures. In the case of PEG-*b*-PCD-2 and PEG-*b*-PCD-3, PEG-*b*-PEDA copolymers with DP of 10 and 15 were used, respectively. The same purification procedure as that for PEG-*b*-PCD-1 was followed.

Table 1
Physicochemical properties of the polymers employed in this study.

Polymer	M_n	DP of the second block[a]
PEG-*b*-PCD-1	11000[a]	4
PEG-*b*-PCD-2	15000[a]	7
PEG-*b*-PCD-3	24000[a]	14
PBLA	2200[b]	–
HMw-PBLA	20000[b]	–

[a] Calculated based on ^{1}H NMR spectra.
[b] Molecular weight calculated based on MALDI-TOF results.

2.3. Synthesis of poly(β-benzyl L-aspartate) (PBLA)

PBLA was synthesized according to a previously described technique [42]. Briefly, 1.5 g BLA-NCA was dissolved in 30 ml anhydrous dioxane, into which an appropriate amount of n-hexylamine was added to achieve a molar ratio of monomer to initiator of 20:1. Polymerization was performed at room temperature (22 °C) for 5 days. After being precipitated from the diethyl ether, the polymer was dissolved in dichloromethane and was again precipitated from the diethyl ether. The resultant powder was dried under vacuum. The number-average molecular weight was determined by a matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer to be about 2000.

2.4. Preparation of host-guest assemblies based on PEG-b-PCD and PBLA

Assemblies based on PEG-*b*-PCDs and PBLA were prepared by a dialysis procedure. In brief, PEG-*b*-PCD and PBLA were dissolved separately in DMSO at 50 °C. A mixture solution with the appropriate weight ratio of PBLA/PEG-*b*-PCD was then dialyzed against deionized water for one day. Further characterization was performed after the dialysis solution was filtered through a 0.45 μm syringe filter. For the preparation of C102 containing assemblies, after C102, PBLA, and PEG-*b*-PCD-3 were dissolved into DMSO, a similar dialysis procedure was adopted.

2.5. Characterization of polymers and assemblies

^{1}H, ^{1}H-^{1}H Noesy, and ^{1}H-^{1}H Roesy spectra were recorded on a Varian INOVA-400 spectrometer operating at 400 MHz. The MALDI-TOF measurements were performed using a Waters Micromass TofSpec-2E operated in linear mode. Dithranol (purchased from Aldrich Chemical) was used as a matrix. The dried-droplet method was employed for sample preparation. Using pyrene as a fluorophore, the steady-state fluorescence spectra were measured on a JASCO FP-6200 fluorescence spectrophotometer with a slit width of 5 nm for both excitation and emission. All spectra were acquired from air-equilibrated solutions. For the fluorescence emission spectra, the excitation wavelength was set at 339 nm, while for the excitation spectra, the emission wavelength was 390 nm. The scanning rate was set at 125 nm/min. All tests were carried out at 25 °C. Sample solutions were prepared as described previously [43]. In brief, the aqueous solutions of copolymers or assemblies containing pyrene (6.0×10^{-7} M) were incubated at 50 °C for 12 h and subsequently allowed to cool overnight to room temperature.

The fluorescence anisotropy (r) was determined using a Fluoromax-2 fluorimeter equipped with an auto-polarizer accessory. The monochromator slits were set at 5 nm, and DPH was used as the fluorescence probe. The excitation wavelength was 360 nm, while the emission wavelength was 430 nm. The fluorescence