Journal of Colloid and Interface Science 431 (2014) 216–222

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Synthesis of poly(*N*-isopropylacrylamide)-*co*-poly(phenylboronate ester) acrylate and study on their glucose-responsive behavior

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ARTICLE INFO

Article history:
Received 23 April 2014
Accepted 23 May 2014
Available online 6 June 2014

Keywords:
Glucose-responsive
Insulin release
PNIPAM
Triblock polymer
Random polymerization

ABSTRACT

We introduced thermo-sensitive poly(*N*-isopropylacrylamide) (PNIPAM) into the polymer structure of poly(ethylene glycol)-*block*-poly(phenylboronate ester) acrylate (MPEG-*block*-PPBDEMA) by block and random polymerization pathways in order to investigate the effect of polymer architecture on the glucose-responsiveness and enhance their insulin release controllability. By following the structure, the continuous PNIPAM shell of the triblock polymer MPEG-*block*-PNIPAM-*block*-PPBDEMA collapsing on the glucose-responsive PPBDEMA core formed the polymeric micelles with a core-shell-corona structure, and MPEG-*block*-(PNIPAM-*rand*-PPBDEMA) exhibited core-corona micelles in which the hydrophobic core consisted of PNIPAM and PPBDEMA segments when the environmental temperature was increased above low critical solution temperature (LCST) of PNIPAM. The micellar morphologies can be precisely controlled by temperature change between 15 and 37 °C. As a result, the introduction of PNIPAM greatly enhanced the overall stability of insulin encapsulated in the polymeric micelles in the absence of glucose over incubation 80 h at 37 °C. Comparing to MPEG-*block*-PNIPAM-*block*-PPBDEMA, the nanocarriers from MPEG-*block*-(PNIPAM-*rand*-PPBDEMA) showed great insulin release behavior which is zero insulin release without glucose, low release at normal blood glucose concentration (1.0 mg/mL). Therefore, these nanocarriers may be served as promising self-regulated insulin delivery system for diabetes treatment.

1. Introduction

Diabetes is a chronic life-threatening disease with an increasing tendency around the world. Considerable impetus has been given to the research on glucose-responsive controlled insulin delivery systems due to its potential application in maintaining normal blood glucose levels [1–4]. Several self-regulated insulin delivery systems have been developed in recent years. Among, boronic acid derivatives have been extensively explored as a promising candidate for the design of glucose detection system in view of its well-known properties of binding to diol moieties with high affinity through reversible boronate ester formation [5–8]. In particular, the introduction of phenylboronic acid moieties into the polymers and block polymers structure sheds light on realizing the modulated insulin delivery, due to their selective glucose-responsiveness in aqueous milieu and their advantages of greater stability, long term storability and lower cytotoxicity, comparing to protein-based components such as glucose oxidase and Concanavalin A [9–14]. Recently, significant efforts to decrease the pK_a value of phenylboronic acid residues and enhance their sensitivity to glucose at physiological pH further broaden the application region of these intelligent glucose-responsive delivery systems [8,15–25].

It is well-known that the rigid *cis*-diols found in many saccharides generally exhibit higher affinities with organoboronic acids than acyclic diols like ethylene glycol, and boronate ester architecture has a relatively lower pK_a than boronic acid itself [26]. Based on such structural properties, our group has previously envisioned one type of amphiphilic block polymers with hydrophobic block-containing pinacolboronate esters units generated via the phenylboronic acid-acyclic diol complexation [27]. Due to saccharide molecules having predominant association force with organoboron, the presented pinacol phenylboronate moieties on the polymer were prone to combine with sugar molecules and detach from the polymer architecture as phenylboronate-saccharide complex. Thus, the disengagement of phenylboronate groups would trigger the polarity transfer of poly(phenylboronate ester)acrylate block from hydrophobic to hydrophilic, leading to disruption of the initial polymeric micelles formed via self-assembly of the amphiphilic polymer, and releasing the loaded molecules. Our group

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http://dx.doi.org/10.1016/j.jcis.2014.05.051
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reported the preliminary in vitro studies, and established insulin release characteristic and good biocompatibility of these nanocarriers formed from the amphiphilic polymers with poly(ethylene glycol) (MPEG) as hydrophilic block and poly(phenylboronate ester) acrylate (PPBDEMA) as hydrophobic block [28]. Although insulin release rate of these reported nanocarriers can be tuned by glucose concentration, unfortunately, we find that the loaded insulin was almost completely released from these nanocarriers after 70-h incubation even at the normal blood glucose concentration such as 1.0 mg/mL. This motivates us to explore smart glucose monitoring for insulin intelligent administration to better match physiological need of patients.

Poly(*N*-isopropylacrylamide) (PNIPAM) is one well-known temperature-responsive polymer which can undergo a phase transition from a soluble state to an insoluble state when heated above its lower critical solution temperature (LCST) of about 32 °C in aqueous solution [29–31]. Moreover, the copolymerization of other monomers prepared amphiphilic polymers that can self-assemble to be core-shell-corona structure when PNIPAM blocks become hydrophobic and collapse on the core above its LCST [32,33]. PNIPAM participation into the hydrophobic core/shell not only increases the overall robust mechanical strength and reversible swellability of the polymeric micelles, but also significantly influences their stimuli-responsive behavior.

To further adjust the glucose-sensitivity of our studied poly(phenylboronate ester)acrylate series, which is wished to be low or no insulin release at normal blood glucose level, high and rapid release at the pathologic environment, herein, we design two kinds of amphiphilic copolymers by block and random polymerization pathways, which are poly(ethylene glycol)-*block*-poly(*N*-isopropyl acrylamide)-*block*-poly(phenylboronate ester) acrylate (MPEG-*block*-PNIPAM-*block*-PPBDEMA) and poly(ethylene glycol)-*block*-[poly(*N*-isopropylacryl amide)-*random*-poly(phenylboronate ester) acrylate] [MPEG-*block*-(PNIPAM-*rand*-PPBDEMA)]. For such triblock polymer MPEG-*block*-PNIPAM-*block*-PPBDEMA, the thermo-responsive PNIPAM block collapses to form the continuous shell embedded with MPEG corona to protect the glucose-responsive PPBDEMA core at 37 °C. While, for MPEG-*block*-(PNIPAM-*rand*-PPBDEMA), one complex hydrophobic core composed of PNIPAM-*rand*-PPBDEMA blocks will be formed above LCST of PNIPAM. Comparing to the triblock polymer, the nanocarriers formed from MPEG-*block*-(PNIPAM-*rand*-PPBDEMA) exhibit effective and controllable glucose-responsiveness which is zero insulin release without glucose and low release at 1.0 mg/mL of glucose concentration.

2. Materials and methods

2.1. Materials

Monomethoxy poly(ethylene glycol) MPEG5000 purchased from Fluka, was dried by azeotropic distillation with toluene, and residual toluene was removed under high vacuum prior to use. CuBr purchased from Aldrich, was purified by stirring in acetic acid overnight, followed by washing with ethanol and diethyl ether, and dried in vacuum. Triethylamine (TEA) and methylene dichloride (CH_2Cl_2) were dehydrated with KOH and $CaCl_2$ overnight and distilled, respectively. Anisole was dried using sodium with benzophenone as color indicator. All of the above purified solvent and reagents were stored in solvent storage flasks prior to use. Macroinitiator monomethoxy poly(ethylene glycol)-2-bromo-propyl ester (MPEG5000Br) and monomer phenylboronate ester acrylate (PBDEMA) were synthesized by a similar procedure as reported previously [27,34]. All other reagents such as 2-bromopropionyl bromide, *N*-isopropylacrylamide (NIPAM), pentamethyldiethylenetriamine (PMDETA) purchased from Aldrich, acryloyl chloride, 1,1,1-trimethylolpropane, $CuBr_2$, phenylboronic acid were used as received without further treatment.

2.2. Measurement

The chemical structures of the copolymers were characterized by ^1H and ^{13}C NMR carrying out on a 400 MHz NMR instrument (Bruker Corporation, Germany) at room temperature using $CDCl_3$ as solvent. The chemical shifts were measured against the solvent signal of $CDCl_3$ as internal standard. The molecular weights and polydispersity index of the polymers were determined with Waters 515-2410 gel permeation chromatograph (GPC) instrument equipped with Styragel HT6E-HT5-HT3 chromatographic column following a guard column and a differential refractive-index detector. The measurements were taken using THF as eluent (flow rate of 1.0 mL/min at 30 °C) and a series of narrow polystyrene standards for the calibration. The fluorescence spectra were recorded by a Hitachi F-4600 Fluorescence instrument (Hitachi High-Technologies Corporation, Tokyo Japan) at 37 °C. Particle sizes were measured with “Zetaplus” Zeta Potential Analyzer (Brookhaven Instrument) equipped with ZetaPlus Particle Sizing software with 35 mW solid state laser operated at a laser light wavelength of 660 nm. The size measurements were taken at 37 °C at a scattering angle of 90°. The polymeric micelles were imaged on a Hitachi H800 transmission electron microscopy (TEM) (Hitachi High-Technologies Corporation, Tokyo, Japan) operated with 100 KV.

2.3. Synthesis of MPEG-block-PNIPAM-block-PPBDEMA

The mixture of MPEG5000Br (0.201 g, 0.038 mmol), PMDETA (21 mg, 0.12 mmol), CuBr (14.4 mg, 0.1 mmol) and the given mass of NIPAM (in Table 1) in degassed anisole (1.0 mL) via three freeze-pump-thaw cycles was reacted in 25-mL Schlenk flask for 24 h at 110 °C. The additional degassed PBDEMA in 0.5 mL anisole was transferred into the above mentioned glass vial by stainless steel tube and continued to polymerize for additional 48 h at 110 °C. The cooled down reaction solution was diluted with THF (10 mL) and passed through a neutral alumina column for the catalyst removal. The concentrated reaction solution was dropwise added into the mixed solvent of hexane/diethyl ether (v/v = 4/1) to obtain polymers, followed by drying in vacuum at ambient temperature for 4 h to afford solid polymers in 19.4% yield. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.79 (d, $J = 8$ Hz, 2nH, $o\text{-}C_6H_5$), 7.42 (br, nH, $p\text{-}C_6H_5$), 7.32 (br, 2nH, $m\text{-}C_6H_5$), 3.80–4.09 (m, 6nH, CH_2OOC, CH_2OBOCH_2), 2.28 (br, nH, $CHCH_2$), 1.60–1.91 (br, 2nH, $CHCH_2$), 1.42 (m, 2nH, CH_2CH_3), 1.08 (br, 6nH, CH_3CHCH_3), 0.89 (m, 3nH, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 174.5, 164.7, 133.9, 131.2, 128.3, 127.6, 126.0, 70.6, 66.8, 63.2, 41.4, 37.8, 23.6, 22.7, 7.3.

2.4. Synthesis of MPEG-block-(PNIPAM-rand-PPBDEMA)

One 25 mL Schlenk flask with a magnetic stir bar was charged with the given molar ratio of NIPAM and PBDEMA (details in Table 1), MPEG5000Br (0.20 g, 0.038 mmol), PMDETA (21.2 mg, 0.12 mmol), CuBr (14.4 mg, 0.1 mmol) and 1.0 mL anisole was degassed by three vacuo-nitrogen cycles. The glass vial was immersed in an oil bath thermostated at 110 °C for 48 h. The cooled down reaction solution was diluted with CH_2Cl_2 (10 mL) and passed through a neutral alumina column for the catalyst removal. The concentrated reaction solution was dropwise added into the mixed solvent with hexane/diethyl ether (v/v = 4/1), and the collected polymers were dried in vacuum at room temperature for 8 h to obtain solid polymer in 29.4% yield. ^1H NMR (400 MHz, $CDCl_3$) δ