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Biodegradable nanoparticles of amphiphilic triblock copolymers based on poly(3-hydroxybutyrate) and poly(ethylene glycol) as drug carriers

Cheng Chen[a,b,*], Chung Him Yu[a], Yin Chung Cheng[a], Peter H.F. Yu[a], Man Ken Cheung[a]

[a]*State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, PR China*
[b]*Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China*

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Abstracts

New amorphous amphiphilic triblock copolymers of poly(3-hydroxybutyrate)–poly(ethylene glycol)–poly(3-hydroxybutyrate) (PHB–PEG–PHB) were synthesized using the ring-opening copolymerization of β-butyrolactone monomer. They were characterized by fluorescence, SEM and ^{1}H NMR. These triblock copolymers can form biodegradable nanoparticles with core–shell structure in aqueous solution. Comparing to the poly(ethylene oxide)–PHB—poly(ethylene oxide) (PEO–PHB–PEO) copolymers, these nanoparticles exhibited much smaller critical micelle concentrations and better drug loading properties, which indicated that the nanoparticles were very suitable for delivery carriers of hydrophobic drugs. The drug release profile monitored by fluorescence showed that the release of pyrene from the PHB–PEG–PHB nanoparticles exhibited the second-order exponential decay behavior. The initial biodegradation rate of the PHB–PEG–PHB nanoparticles was related to the enzyme amount, the initial concentrations of nanoparticle dispersions and the PHB block length. The biodegraded products detected by ^{1}H NMR contained 3 HB monomer, dimer and minor trimer, which were safe to the body.

Keywords: Atactic poly(3-hydroxybutyrate); Amphiphilic copolymers; Biodegradable nanoparticle; Biodegradation behavior; Drug carriers

1. Introduction

Microbial poly(3-hydroxybutyrate) (PHB) has been a new research focus lately as an important candidate of biodegradable aliphatic polyester. PHB is accumulated by a variety of bacteria as an energy and carbon storage material, and hence its remarkable characteristics are excellent biodegradability and biocompatibility in various environments [1]. Due to the relatively high crystallinity and completely chirality in molecular chains, microbial PHB is unsuitable for molecular design of specialty polymers such as amphiphilic block copolymers.

The amphiphilic diblock copolymers of PHB and monomethoxy poly(ethylene glycol) (mPEG) were self-assembled into sterically stabilized colloidal suspensions of PHB crystalline lamellae [2]. The triblock copolymers coupling two chains of poly(ethylene oxide) (PEO) with a low-molecular-weight isotactic PHB chain in the middle PEO–PHB–PEO were also investigated [3,4]. The crystallinity of the PHB block in the copolymers clearly increased compared with that of the pure PHB precursor. Moreover, their critical micelle concentrations (CMC) (13–1100 mg/L) were very higher relatively to that of poly(ε-carprolactone)–poly(ethylene glycol) (PCL–PEG) and poly(L-lactic acid)–PEG (PLLA–PEG) (2.5–35 mg/L) [5,6]. The result means that the PEO–PHB–PEO micelles were very easy to dissociate upon dilution in the blood stream after intravenous injection, which was also supported by the fact that all PEO–PHB–PEO copolymers were soluble in water [4]. Among above investigations, although the molecular weights of the PHB blocks in those copolymers

*Corresponding author. State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, PR China. Tel.: +852 3400 3173; fax: +852 2364 9932.
E-mail addresses: chencheng510@yahoo.com, bcchen92@polyu.edu.hk (C. Chen).

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were very lower (2000–5000), the copolymers still showed good crystallization because of completely chirality of the PHB segments. In addition, PHB has lower biodegradation rate in vivo, resulting in that its application in biomedical research is very limited. It is worthy to be noted that intracellular native PHB is mobile and amorphous elastomer and can be degraded by the intracellular depolymerases. During the recovery process, PHB is denatured and then becomes crystalline. Denatured PHB can be hydrolyzed by extracellular depolymerases but hardly degraded by intracellular depolymerases [7]. Therefore, PHB shows longer biodegradation period in vivo, especially compared with biodegradable PLLA, PCL and poly(lactide-*co*-glycolidel) (PLGA). For the PEO–PHB–PEO micelles, the segments of microbial PHB can cause the dense core because of good crystallization, whose architecture is disadvantageous to encapsulate drugs. According to previous results, atactic PHB was synthesized by the anionic polymerization of β-butyrolactone (BL), and it showed good solubility but lacked crystallinity [8]. Hence, it was proposed that atactic PHB instead of microbial PHB was a good alternative to obtain amphiphilic copolymers containing the PHB segments because it could avoid crystalline lamellae of the PHB segments in aqueous solution. Furthermore, amorphous PHB was also close to its original state in the cells.

In this study, biodegradable amphiphilic PHB–PEG–PHB copolymers with different PHB compositions were synthesized with easier method than those previously reported [3,8]. In aqueous medium, these amphiphilic copolymers can form the nanoparticles. The properties of the nanoparticles as drug carriers were investigated, including CMC values, particle size, and drug loading content and loading efficiency. Moreover, the biodegradation behavior of the PHB–PEG–PHB nanoparticles was also evaluated in vitro.

2. Materials and methods

2.1. Materials

All chemicals, except otherwise stated, were purchased from Aldrich Chemical Co. BL monomer and dichloromethane (CH_2Cl_2) were refluxed and dried with calcium hydride prior to use. PEG [number-average molecular weight (M_n) = 4000] was used after purification by precipitation method. Other organic solvents were analytical grade and used as received.

2.2. Experimental procedures

2.2.1. Polymerization of the triblock copolymers

The purified BL monomer and PEG were placed in a dried polymerization flask with appropriate ratios, and stannous octoate [$Sn(Oct)_2$] in desired concentrations was added as a solution in dried CH_2Cl_2. The reactants were mixed and then dried under lower pressure at 40 °C for 1 h to completely remove CH_2Cl_2. The reactions were carried out under nitrogen atmosphere at desired temperatures. After specified reaction time, the reacted products were repeatedly dissolved in chloroform, and then precipitated in hexane to remove the unreacted BL monomer. The isolated products were dried in vacuum at 40 °C for 48 h.

2.2.2. Nanoparticle preparation

Aqueous dispersions of the nanoparticles were prepared by a precipitation/solvent evaporation technique without any surfactants for the investigation of their native ability of forming nanoparticles. A triblock copolymer solution in acetone was added dropwise to distilled and deionized water (DD H_2O) under ultrasonic situation. Acetone was removed under lower pressure and ultrasonic situation. The effect of varying the concentrations of nanoparticle dispersions on size was also investigated. The desired concentrations of nanoparticle dispersions were adjusted by the dilution of adding proper DD H_2O into the initial nanoparticle dispersions. All dispersions were filtered using disposable 0.45 μm Millipore filters, without significant effect on the particle yield or size distribution. For NMR experiments, deuterated water (D_2O) was used instead of DD H_2O.

2.2.3. CMC

The CMC was determined by a fluorescence probe technique using pyrene as the fluorescence probe. In order to prevent the formation of microcrystals, the pyrene concentration of 6×10^{-7} M was used in all experiments, which was lower than its saturated concentration in water (7×10^{-7} M). Nanoparticle dispersions were added into the vials in the presence of pyrene in DD H_2O, and then were placed into Incubator Shaker at 37 °C for 24 h. After sonicating 2 h, they were allowed to store overnight at room temperature.

2.2.4. Drug loading content and drug loading efficiency (DLC and DLE)

Pyrene was chosen as an imitative drug to determine DLC and DLE of the nanoparticles because of its unique fluorescence and hydrophobic characters. The pyrene solutions in DD H_2O were prepared with different concentrations of 0.1–10^{-4} mg/L. Its absorbance at 333.1 nm in the fluorescence excitation spectra was measured to generate a calibration curve for quantitative analysis. The nanoparticle dispersions were prepared in the presence of pyrene, and their initial concentrations were 1 mg/mL. Excess pyrene was removed by dialysis in DD H_2O for 2 days. Finally, the nanoparticle dispersions containing pyrene were centrifuged to separate the nanoparticles loaded pyrene. By comparing the change in the intensity at 333.1 nm, the amount of loaded pyrene was known. DLC and DLE were calculated as following:

$$\text{DLC} = \text{Weight of loaded pyrene in nanoparticles} / \text{Weight of nanoparticiles} \times 100\%,$$

$$\text{DLE} = \text{Weight of loaded pyrene in nanoparticles} / \text{Weight of pyrene added} \times 100\%.$$

2.2.5. Drug release from the nanoparticles in vitro

Extracellular PHB depolymerase was isolated from the culture medium of *Pseudomonas lemoignei*. It was prepared according to the previous method [9]. In a typical enzymatic biodegradation experiment, dust-free nanoparticle dispersions were prepared in the presence of pyrene, and then dialyzed in DD H_2O for 2 days to remove excess pyrene. Dust-free enzyme solution (0.005, 0.01 and 0.05 mL) was added into 1 mL nanoparticle dispersions at room temperature to start biodegradation. During enzymatic biodegradation processing, the fluorescence spectra of pyrene and the pH values of nanoparticle dispersions were recorded, respectively.

2.3. Measurements

The NMR analysis of the specimens was carried out on a Varian Inova 500-MHz NMR spectrometer. Chemical shifts were given in ppm using tetramethylsilane (TMS) in $CDCl_3$ as internal reference and sodium 3-trimethylsilylpropionate-d4 (TSP) in D_2O as external reference. The ^1H NMR spectra were recorded at room temperature and 50 °C, respectively. After measurements, the nanoparticle specimens were frozen and lyophilized, and then redissolved in $CDCl_3$ for quantitatively analysis. FTIR spectra were recorded on a Nicolet Avatar 360 Fourier transform