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Thymine-functionalized amphiphilic biodegradable copolymers for high-efficiency loading and controlled release of methotrexate

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ABSTRACT

In this study, a novel thymine-functionalized six-membered cyclic carbonate monomer (TAC) was synthesized by the Michael-addition reaction between thymine and acryloyl carbonate (AC). The corresponding functional amphiphilic block copolymer mPEG-*b*-PTAC was further successfully synthesized by ring-opening polymerization using immobilized porcine pancreas lipase (IPPL) as the catalyst and mPEG as the macroinitiator. Meanwhile, mPEG-*b*-P(TAC-co-DTC) and mPEG-*b*-PDTC were also synthesized by the same enzymatic methods for comparison on different TAC contents. The structures of monomer and copolymers were characterized by ^{1}H-NMR, ^{13}C-NMR and FTIR. All the amphiphilic block copolymers could self-assemble to form nano-sized micelles in aqueous solution. Transmission electron microscopy (TEM) observation showed that the micelles dispersed in spherical shape with nano-size before and after MTX loading. ^{1}H-NMR and FTIR results confirmed the successful formation of multiple hydrogen-bonding interactions between exposed thymine groups of hydrophobic PTAC segments and 2,6-diaminopyridine (DAP) groups of MTX molecules, which resulting in the higher drug loading capacity and the pH-sensitive drug release behavior. MTT assays also indicated lower toxicity of copolymer but higher potent cytotoxic activity of MTX-loaded copolymer against HeLa cells.

1. Introduction

Over the past decades, polymeric micelles as drug delivery systems have received significant attention, due to many unique merits such as enhancing the water solubility of the hydrophobic drug, prolonging the circulation time in the bloodstream by evading reticuloendothelial system (RES), etc [1–3]. Polymeric micelles are formed by self-assembling of amphiphilic block copolymers in aqueous solution with a core and shell structure [4,5]. The hydrophilic polymeric shell, such as poly(ethylene glycol) (PEG), could protect the drug from the aqueous environment. The hydrophobic polymeric core could contain poorly water-soluble drugs such as paclitaxel (PTX), methotrexate (MTX) and doxorubicin (DOX). In most cases, drugs are entrapped into the hydrophobic core of micelles via hydrophobic interactions [6], while achieving high drug-loading capacity and a controllable drug-release property are still two main challenges for the design of efficient drug carriers [7,8]. A powerful strategy is the introduction of non-covalent interactions between drugs and carriers, such as hydrogen bond interactions, π–π interactions and electrostatic interactions. These non-covalent interactions could enhance the drug loading capacity [9] and also provide an effective approach for controlled drug-release due to their environmental sensitivities. For example, pH-selective and directional hydrogen-bonding interactions could become much stronger when multiple hydrogen bonds are formed together [10]. As it well known, the multiple hydrogen-bonding interactions between complementary nucleobase pairs play an important role in maintaining the structural stability of DNA. The nucleobase thymine is identified to be able to non-covalently bind with 2,6-diaminopyridine (DAP) through complementary multiple hydrogen-bonding interactions, which could provide an effective approach to designing reversible interactions between drug carriers and guest drug molecules [11,12].

Biodegradable aliphatic polycarbonates have been used as the hydrophobic core of polymeric micelles due to their low toxicity, favorable mechanical properties and biodegradability [13,14]. In addition, the properties of aliphatic polycarbonates could be conveniently modified and designed by introducing pendant functional groups, which can be used to not only adjust the properties

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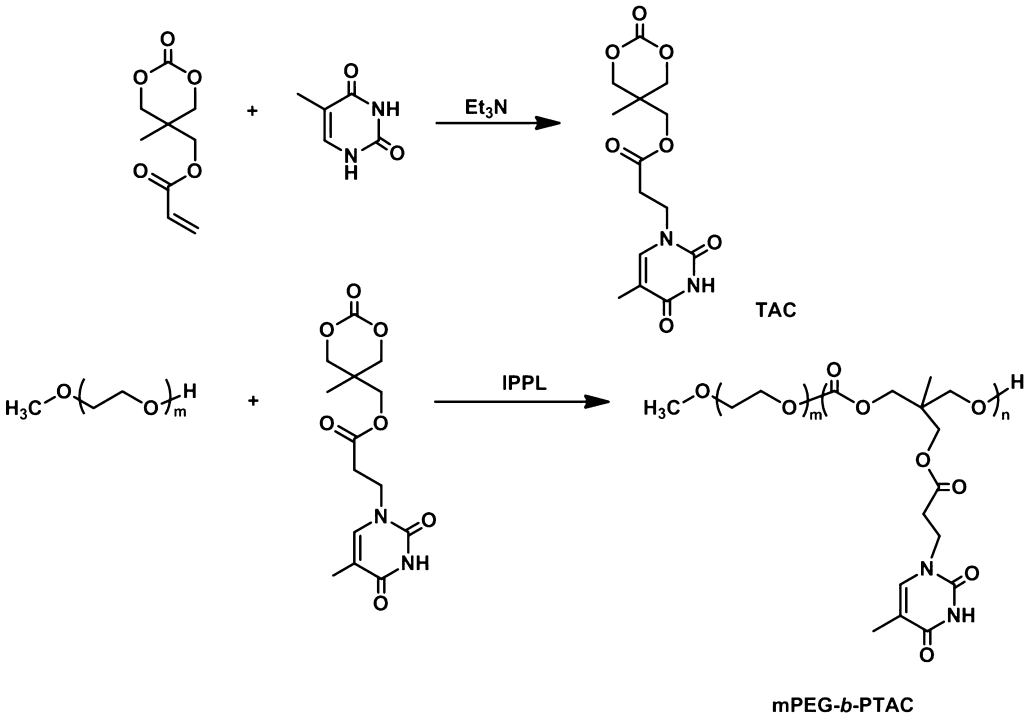

Scheme 1. Synthesis of thymine-functionalized cyclic carbonate TAC and amphiphilic block copolymer mPEG-*b*-PTAC.

of polycarbonates but also facilitate further modification [15,16]. Aliphatic polycarbonates with pendant functional groups could be synthesized by ring-opening polymerization (ROP). In our previous studies, immobilized *porcine pancreas* lipase on silica particles (IPPL), with promising stability and recyclability, has proven to be a powerful catalyst for the ROP of cyclic carbonates without or with PEG/mPEG as the macroinitiator [17–19]. The resulting amphiphilic block copolymers with well-defined functional polycarbonates as the hydrophobic segments have been investigated as attractive drug carriers [20,21].

In this article, a novel thymine-functionalized six-membered cyclic carbonate monomer (TAC), was synthesized by the Michael-addition reaction between thymine and acryloyl carbonate (AC). And the corresponding amphiphilic block copolymer mPEG-*b*-PTAC was further synthesized in bulk using IPPL as the catalyst and mPEG as the macroinitiator. As shown in Scheme 1, the hydrophilic long-circulating PEG block acted as the outer shell, while the biodegradable PTAC was employed as the hydrophobic core of the copolymer micelles. In comparison to mPEG-*b*-PTAC, mPEG-*b*-P(TAC-*co*-DTC) and mPEG-*b*-PDTC were also synthesized by enzymatic methods simultaneously. Methotrexate (MTX), containing 2,6-diaminopyridine (DAP) domain, is a widely used anticancer drug in the treatment of many types of cancer [22,23]. Based on the multiple hydrogen-bonding interactions between exposed thymine groups of hydrophobic PTAC segments with DAP groups of MTX molecules, mPEG-b-PTAC was proposed as a novel powerful controlled drug delivery system for high-efficiency loading and controlled release of MTX to cancer cells.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol) (mPEG, M_n = 2000) were purchased from Sigma–Aldrich. AC and DTC were synthesized according to the literatures [24,25]. IPPL was prepared according to He et al. [26]. Thymine and methotrexate (MTX) was obtained from Shanghai Kayon Biological Technology Co., Ltd. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's phosphate buffered saline (PBS), 3-Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corp. HeLa cells were incubated in DMEM containing 10% FBS and 1% antibiotics (penicillin-streptomycin, 10,000 U/mL) at 37 °C and a humidified atmosphere containing 5% CO_2. Other reagents were of analytical grade and purified by general methods.

2.2. Synthesis of mPEG-b-PTAC, mPEG-b-P(TAC-co-DTC) and mPEG-b-PDTC

2.2.1. Synthesis of thymine-functionalized cyclic carbonate TAC

Thymine-functionalized cyclic carbonate TAC was synthesized by the Michael-addition reaction between the imine groups of thymine and the acryloyl groups of AC. Briefly, AC (2.0 g, 0.01 mol) and 10 molar excess of thymine (12.6 g, 0.10 mol) were dissolved in DMF, and triethylamine (1.0 g, 0.01 mol) was added under stirring at 60 °C for 24 h. The reaction mixture was concentrated and dissolved in dichloromethane, while the excess of thymine was removed by filtration. Then the filtrate was condensed and the TAC was recrystallized from CH_2Cl_2:THF (1:1) mixture (Yield: 64%).

2.2.2. Synthesis of mPEG-b-PTAC, mPEG-b-P(TAC-co-DTC) and mPEG-b-PDTC

mPEG-*b*-PTAC, mPEG-*b*-P(TAC-*co*-DTC) and mPEG-*b*-PDTC were synthesized in bulk by IPPL-catalyzed ring-opening polymerization. The vessels containing mPEG, TAC (or/and DTC) (TAC:DTC molar feed ratio of 1.0:0, 1.0:1.0 and 0:1.0, respectively) and IPPL (3 wt‰ of monomer) with a magnetic stirring bar were dried in vacuo with anhydrous phosphorus pentoxide at room temperature for 24 h. Then the vessels were sealed and immersed into an oil bath at 120 °C for another 24 h. The reaction mixture was dissolved in dichloromethane and the insoluble IPPL was removed by filtration. Then the filtrate was concentrated and dissolved in THF, dialyzed (MWCO: 14,000 Da) for 48 h in distilled water refreshed