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Synthesis of PHB-based carrier for drug delivery systems with pH-controlled release

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ABSTRACT

Synthetic route to prepare model PHB-amine conjugate containing hydrolysable imine bond is reported. Short-chain PHB crotonate is converted into PHB glyoxylate *via* clean and efficient ozonolysis followed by reductive decomposition of peroxidic products with dimethylsulfide. Aldehyde-functionalized PHB is obtained quantitatively without polymer backbone degradation while PHB-amine conjugate is synthesized with very high yield. Release properties of such-prepared conjugate is confirmed in hydrolysis experiment revealing pH-dependent kinetics of amine release. Simplicity of the protocol in conjunction with unique properties of PHB carrier are believed to be powerful tool for development of novel drug conjugates.

1. Introduction

Drug delivery systems (DDSs) represent one of the most rapidly advancing areas of research contributing to human health care. DDS offer numerous advantages compared to conventional drug administration such as improved efficacy, reduced toxicity and improvement of the targeting to tissue or cells [1]. Such systems are classified as a function of the structure and the release mechanism into: membrane, matrix, hydrophilic matrices based systems or stimuli responsive systems where drug release is triggered by change of pH, temperature, etc. [2]. One of the pH-sensitive DDS are systems comprising imine bond [3,4]. Such bond is easily cleaved *in vivo* and its degradation rate depends on pH of the environment therefore conjugates with imine bonds are desired in the development of DDS [3,4]. Moreover, except for pH-dependent release of bioactive compound, amine group of the compound is recovered after imine hydrolysis. As it was shown by Gao et al. [3] nanoparticles loaded with doxorubicin were used as a drug delivery system with drug release controlled by rate of imine hydrolysis which took place in the cell endosome. On the other hand, the most common method of imine formation is reaction of primary amines with carbonyl compounds [5]. Noteworthy, numerous drugs possess primary amine groups, therefore polymeric carriers containing carbonyl function will be of high interest to prepare imine bond containing DDS. In addition, polymeric carrier applied in DDSs must fulfill several crucial requirements, such as degradability, biocompatibility as well as products of their degradation must be non-toxic and the carrier should improve drug delivery [6,7].

Recently it was demonstrated that short-chain poly[(*R*,*S*)-3-hydroxybutyrate] (*a*PHB) is very efficiently internalized by living cells [8] which is consistent with improved transportation of ibuprofen-PHB conjugate *via* cell membrane [9] while previous work describes lack of the carrier toxicity [10]. In fact *a*PHB is a synthetic isomer of naturally occurring poly[(*R*)-3-hydroxybutyrate] (*n*PHB) which is one of the simplest members of the polyhydroxyalkanoates family. *n*PHB is a biodegradable aliphatic polyester produced by a wide variety of organisms and microorganisms, *e.g.*, bacteria synthesize high-molar-mass *n*PHB as a carbon and energy source [11,12]. Moreover,

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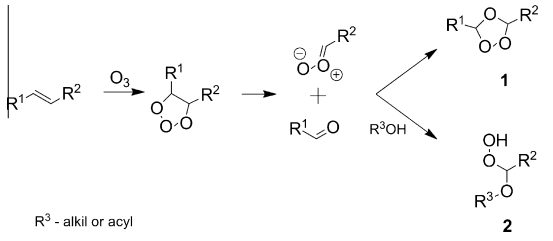
short-chain *n*PHB present in eukaryotic cells forms complexes with polyphosphates which assemble into ion channels in the cell membrane [13]. Natural one PHB is synthesized on an industrial scale employing biotechnological processes [14,15]. Synthetic analogs of *n*PHB can be obtained by ring-opening polymerization (ROP) of *β*-butyrolactone (2-methyl-2-oxetanone, BL) with formation of the isotactic (*i*PHB) [16–19], atactic (*a*PHB) [20–26], and syndiotactic [27–29] poly(3-hydroxybutyrate). In addition to ROP of BL, low-molar-mass PHB can also be synthesized by polycondensation of 3-hydroxybutyric acid [30,31]. Low-molar-mass *n*PHB may be also obtained *via* high-temperature thermal degradation [32–35] or base catalyzed moderate-temperature degradation [36,37]. Regardless tacticity of the precursor, both methods lead to formation of short-chain poly(3-hydroxybutyrate)s containing crotonate end groups. However, for PHB conjugate preparation with pH-sensitive imine bond it is required to introduce the carbonyl functionality into oligoester molecule. Recently it was demonstrated that selective oxidation of PHB crotonate with *m*-chloroperbenzoic acid proceeded into reactive oxirane-functionalized PHB almost quantitatively without affecting the polymer main chain [38]. Ozonolysis is cleaner, more atom efficient and also a more effective method for the oxidative cleavage of carbon–carbon double bonds leading among others to formation of carbonyl compounds. In fact, ozonolysis is a concerted cycloaddition of ozone to C=C bond with formation of molozonide followed by its rearrangement to a peroxidic product. However, to avoid the formation of unstable and explosive 1,2,4-trioxolane (secondary ozonide) (1), it is recommended to use solvents participating in ozonolysis, *e.g.*, alcohols (methanol or ethanol) or acids (acetic acid mainly) to form more stable and less volatile α-alkoxyhydroperoxides or α-acyloxyhydroperoxide (2), respectively (Scheme 1) [39,40]. Next, the peroxidic products can be converted into non-peroxidic ones by reductive or oxidative decomposition. Reductive route with water, sulfide ion, bisulfide ion, iodide, dimethylsulfide, phosphines, tertiary amines and zinc/or magnesium/acetic acid [39,40] results usually in the formation of aldehydes or ketones. Oxidative decomposition usually employs: peroxyacids, silver oxide, chromic acid, oxygen, permanganate or hydrogen peroxide [40] and it leads to ketones or acids as the resulting products. Moreover, products of ozonolysis may be also decomposed by hydrolytic, pyrolytic or photolytic methods [39].

The aim of the presented work was development of synthetic route to prepare model PHB-amine conjugate containing hydrolysable imine bond illustrating perspective application of PHB carrier in delivery systems of amine functionalized drugs. The work is focused on the clean synthesis, *via* ozonolysis, of low-molar-mass PHB bearing aldehyde (glyoxylate) end group and further anchoring of primary amine onto the functionalized PHB. Amine release properties of such-prepared conjugate were also examined.



Scheme 1. Criegee mechanism of ozonolysis [39,40].

2. Experimental

2.1. Materials

β-Butyrolactone (BL) (+98%, Aldrich) was purified as described previously [26]. *n*PHB (M_w = 430,000 $Đ_M$ = 2.99; ICI product – PHB G08), was used as received. KOH, $KHCO_3$, $NaHCO_3$, HCl_{aq} (35–38%), *n*-hexane (95%), ethanol (99.8%), CH_2Cl_2 (p.a.), $CHCl_3$ (p.a.), methanol (MeOH) (p.a.), acetone (99.5%) (all from POCH), aniline (99%, Aldrich) and dimethylsulfide (DMS, 98%, Aldrich) were all used as received. Crotonic acid (Aldrich) was recrystallized from *n*-hexane. Potassium crotonate was prepared by potentiometric titration of recrystallized crotonic acid in ethanol solution with KOH ethanol solution. Then the alcohol was stripped off from the reaction mixture and the obtained salt was dried under vacuum at rt. to constant weight. 18-Crown-6 ether (18C6) (Aldrich) was dried under vacuum at 50 °C for 72 h. THF (POCH) was distilled over potassium-sodium alloy just before use. Ozone was produced using an Ekotech Korona L (output stream: 60 L/h O_2, 3 g/h,) or OzoneLab™ OL80F/DST (output stream: 15 L/h O_2, 292 mg/h O_3) generator.

2.2. Polymers preparation

2.2.1. Poly[(R,S)-3-hydroxybutyrate] crotonate

β-Butyrolactone anionic polymerization was conducted as previously reported [38]. The BL conversion progress was measured by FTIR spectroscopy based on the carbonyl carbon signals of BL and the poly(3-hydroxybutyrate) at 1815 and 1735 cm^{-1}, respectively, as described previously [41]. After the polymerization had been completed the polymer was precipitated in *n*-hexane, re-dissolved in CH_2Cl_2. The solution was washed thoroughly three times with diluted HCl_{aq} (*ca.* 1%) and ten times with distilled water. The product was recovered by precipitation in cold *n*-hexane followed by drying under vacuum at rt. till constant weight. The final product was characterized using ^{1}H NMR, ^{13}C NMR and SEC techniques.

SEC ($CHCl_3$, PS standards): M_n = 1600; $Đ_M$ = 1.2. See Supplementary Information for NMR data.

2.2.2. Poly[(R)-3-hydroxybutyrate] crotonate

The *n*PHB polymer (20 g, 0.14 mmol) was mixed with $KHCO_3$ in weight ratio 10:1 and treated as described earlier [37,38]. Obtained material was dissolved in $CHCl_3$, and washed three times with diluted HCl_{aq} (*ca.* 1%) followed by ten washings with distilled water. Next, the polymer was precipitated in *n*-hexane and dried under vacuum at rt. The final product was characterized using ^{1}H NMR, ^{13}C NMR and SEC techniques. SEC (chloroform, PS standards):