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Research paper

Tailored protein release from biodegradable poly(ε-caprolactone-PEG)-*b*-poly(ε-caprolactone) multiblock-copolymer implants

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ABSTRACT

In this study, the *in vitro* release of proteins from novel, biodegradable phase-separated poly(ε-caprolactone-PEG)-*block*-poly(ε-caprolactone), [PCL-PEG]-*b*-[PCL]) multiblock copolymers with different block ratios and with a low melting temperature (49–55 °C) was studied. The effect of block ratio and PEG content of the polymers (i.e. 22.5, 37.5 and 52.5 wt%) as well as the effect of protein molecular weight (1.2, 5.8, 14, 29 and 66 kDa being goserelin, insulin, lysozyme, carbonic anhydrase and albumin, respectively) on protein release was investigated. Proteins were spray-dried with inulin as stabilizer to obtain a powder of uniform particle size. Spray-dried inulin-stabilized proteins were incorporated into polymeric implants by hot melt extrusion. All incorporated proteins fully preserved their structural integrity as determined after extraction of these proteins from the polymeric implants. In general, it was found that the release rate of the protein increased with decreasing molecular weight of the protein and with increasing the PEG content of the polymer. Swelling and degradation rate of the copolymer increased with increasing PEG content. Hence, release of proteins of various molecular weights from [PCL-PEG]-*b*-[PCL] multi-block copolymers can be tailored by varying the PEG content of the polymer.

1. Introduction

The use of proteins and peptides as therapeutic agents has significantly increased in the last few decades. Since the 1980s, more than 200 biopharmaceuticals have been registered [1]. However, despite extensive research, the development of patient-friendly dosage forms capable of maintaining the concentration of the proteins in plasma at pharmacologically effective levels for extended periods of time is still a major challenge [2]. Oral delivery of proteins and peptides has so far been unsuccessful, since it results in a low bioavailability due to degradation caused by gastrointestinal enzymes and low permeability through the gastrointestinal membrane. Therapeutic proteins are preferably administered parenterally as an aqueous solution, but due to their short half-life most proteins require frequent injection. For these reasons, research on the development of parenteral sustained release (or depot) formulations has expanded enormously during the last few decades. Biodegradable polymers such as poly(DL-lactide-co-glycolide) (PLGA) or poly(DL-lactide) (PDLA) have been widely applied as release controlling polymers in microparticle and implant-based depot formulations for peptides and proteins [2–4]. These polymers degrade in the body due to hydrolysis and are finally metabolized into water and carbon dioxide [5]. Sustained release depot formulations are typically administered parenterally via subcutaneous or intra-muscular injection, with the use of customized applicators [6,7]. Due to incompatibility of both the polymers and the drug molecules with standard sterilization procedures such as heat, autoclavation or radiation, these depot formulations are usually manufactured aseptically.

Abbreviations: PEG, poly (ethylene glycol); PCL, poly (ε-caprolactone); PDLA, poly(DL-lactide); PLGA, poly(DL-lactide-co-glycolide); BSA, bovine serum albumin; Lys, lysozyme; Gos, goserelin; Ins, insulin; CA, carbonic anhydrase; TFA, trifluoroacetic acid; $CDCl_3$, deuterated chloroform; DMF, dimethylformamide; DMSO, dimethylsulfoxide; NaN_3, sodium azide; BDI, butanediisocyanate; HME, hot melt extrusion; DSC, differential scanning calorimetry; ΔH, heat of fusion; ^{1}H NMR, proton-nuclear magnetic resonance; GC-FID, gas chromatography-flame-ionization detection; SEC-HPLC, size exclusion-high performance liquid chromatography; RP-HPLC, reversed-phase-high performance liquid chromatography; UV–VIS, ultraviolet–visible spectroscopy; SDS–PAGE, sodium dodecyl Sulfate–polyacrylamide gel electrophoresis.

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Microparticle-based sustained release depot formulations are typically manufactured via process routes that require the use of organic solvents to dissolve the polymers and aqueous media to extract the organic solvents from the microparticles. It is well known that the resulting organic solvent–water interfaces can have a devastating effect on the structural integrity and bioactivity of protein therapeutics. Processes such as molding, melt compression and hot melt extrusion offer the possibility for solvent free processing, which make them attractive alternatives for the manufacturing of sustained release depot formulations for proteins. However, these production processes often expose the proteins to heat and shear stresses. Hot melt extrusion of polymers like PLGA and PDLA requires high processing temperatures, which in combination with the shear stresses may lead to protein degradation [8].

In a previous study, we have synthesized novel hydrophilic multiblock co-polymers composed of semi-crystalline poly(ε-caprolactone) [PCL] blocks and amorphous blocks consisting of PCL and poly(ethylene glycol) (PEG). We have shown that the combination of this type of hydrophilic [PCL-PEG]-*b*-[PCL] polymers and low temperature HME allows incorporation of proteins into the implants without protein degradation and controlled release of fully intact protein [9].

When formulating proteins and peptides into polymeric implants, one needs to take into account the various factors that affect the release kinetics. Protein release from polymeric matrices is governed by the physico-chemical properties of both polymer and drug, as well as by the conditions at the site of release. Among others, protein release is affected by protein charge [10], protein loading [9] and protein molecular weight [11]. Essential polymer properties include the degradation rate and swellability [12], which depend on composition and molecular weight of the polymer [13]. Although several studies have already been conducted on the importance of protein molecular weight on the release from polymeric depots [14–16], the aim of this study was to investigate in more detail the correlation between protein molecular weight and protein release from this new type of hydrophilic [PCL-PEG]-*b*-[PCL] polymers with different PEG contents. We synthesized [PCL-PEG]-*b*-[PCL] multiblock co-polymers with different [PCL-PEG]/[PCL] ratios and prepared protein-loaded implants thereof by HME. The release of five proteins with different molecular weight, i.e. goserelin (1.2 kDa), recombinant human insulin (5.8 kDa), lysozyme (14 kDa), carbonic anhydrase (29 kDa) and bovine serum albumin (66 kDa) from [PCL-PEG]-*b*-[PCL] polymers with varying PEG content was evaluated. Additionally, the degradation behavior of the polymers was studied to obtain more insight into the release mechanism of the proteins from these polymeric implants.

2. Materials and methods

2.1. Materials

Goserelin acetate (Gos) was purchased from BCN (Barcelona, Spain). Lyophilized human recombinant insulin (Ins) (26.9 units/mg, ~5.8 kDa), lyophilized Lys (Lys) from chicken egg white (70,000 units/mg, ~14 kDa), lyophilized carbonic anhydrase (CA) from bovine erythrocytes (protein >3500 W-A units/mg, ~29 kDa) and lyophilized albumin from bovine serum (BSA), (protein >96%, ~66 kDa), Micrococcus Lysodeikticus, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride and trifluoroacetic acid (TFA) were all purchased from Sigma (St. Louis, Missouri, USA). Inulin 4000 g/mol was a gift from Sensus (Rosendaal, The Netherlands). Sodium azide, ε-caprolactone, 1,4-butanediol and 1,4-dioxane were purchased from Acros Organics (Geel, Belgium). Acetonitrile (HPLC gradient grade) was purchased from Biosolve® (Valkenswaard, The Netherlands). Hydrochloric acid 37% (reagent grade) was purchased from VWR International Ltd. (Leicestershire, UK). Tri (hydroxymethyl) aminomethane was purchased from Merck (Darmstadt, Germany). Stannous octoate was obtained from Sigma. 1,4-Butanediisocyanate was purchased from Bayer. Deuterated chloroform and lithium bromide were obtained from Fisher; dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and PEG standards (FLUKA, Buchs, Switzerland) were purchased from Sigma.

2.2. Polymer synthesis

Low molecular weight poly(ε-caprolactone) [PCL] (M_w 4000 g/mol) and poly(ε-caprolactone)-PEG_{1500}-poly(ε-caprolactone) [PCL-PEG_{1500}] (M_w 2000 g/mol) prepolymers were synthesized by standard stannous octoate catalyzed ring-opening polymerization, as described previously [9]. Shortly, ε-caprolactone was dried over CaH_2 and distilled under reduced pressure in a nitrogen atmosphere prior to polymerization. PEG with a molecular weight of 1500 g/mol (PEG_{1500}) was dried for 17 h at 90 °C under vacuum. 1,4-Butanediol and 1,4-butanediisocianate were distilled under reduced pressure. The purity of distilled ε-caprolactone, 1,4-butanediol and 1,4-butanediisocyanate was confirmed by ^{1}H NMR ($CDCl_3$). PCL and PCL-PEG_{1500} prepolymers were then chain-extended with 1,4-butanediisocyanate to prepare [poly(ε-caprolactone)-PEG_{1500}-poly(ε-caprolactone)$_{2000}$]-*b*-[poly (ε-caprolactone)$_{4000}$] [PCL-PEG_{1500}]$_{2000}$-*b*-[PCL]$_{4000}$ multiblock co-polymers with different [PCL-PEG_{1500}]/[PCL] block ratios (30/70, 50/50 and 70/30 w/w abbreviated as 30[PCL-PEG_{1500}]-70[PCL], 50[PCL-PEG_{1500}]-50[PCL] and 70[PCL-PEG_{1500}]-30[PCL]) as described in [9]. Shortly, the prepolymers PCL and PCL-PEG_{1500} were introduced into a three-necked bottle under nitrogen atmosphere. Dry 1,4-dioxane (distilled over sodium wire) was added to a polymer concentration of 30 wt% and the mixture was heated to 80 °C to dissolve the prepolymers. 1,4-Butanediisocyanate (BDI) was added and the reaction mixture was stirred mechanically overnight for 20 h. After cooling to room temperature, the reaction mixture was transferred into a tray, frozen and vacuum-dried at 30 °C to remove 1,4-dioxane. The residual 1,4-dioxane content, as measured by GC headspace, was less than 200 ppm for all three multiblock co-polymers, showing effective removal of 1,4-dioxane by vacuum drying.

2.3. Polymer characterization

^{1}H NMR was used to verify the overall ε-caprolactate/PEG (CL/PEG) monomer ratio of the multiblock co-polymers after synthesis and during degradation. ^{1}H NMR was performed on a VXR Unity Plus NMR spectrometer (Varian, California, USA) operating at 300 MHz. The d_1 waiting time was set to 20 s, and the number of scans was 16–32. Spectra were recorded from 0 to 14 ppm. ^{1}H NMR samples were prepared by dissolving 10 mg of multiblock co-polymer into 1 mL of deuterated chloroform ($CDCl_3$), and the spectrum was determined from 0 to 8 ppm using $CHCl_3$ present as trace element in $CDCl_3$ as reference. The CL/PEG molar ratio was calculated from the O–$CH_2CH_2CH_2CH_2\mathbf{CH_2}$C (O)– methylene group of PCL at δ 2.2–2.4 and the –$\mathbf{CH_2CH_2}$–O methylene groups of PEG at δ 3.6–3.7.

The intrinsic viscosity of multiblock co-polymers dissolved in chloroform was determined by measuring at three different polymer concentrations at a temperature of 25 °C using an Ubbelohde viscometer (DIN, type 0C Schott Geräte supplied with a Schott AVS-450 Viscometer equipped with a water bath).

The residual 1,4-dioxane content of the multiblock copolymers was determined using a GC-FID headspace method. Measurements were performed on a GC-FID Combi Sampler supplied with an