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A new class of inhibitors of peptide sorption and acylation in PLGA

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ABSTRACT

Acylation of peptides occurring within controlled-release depots prepared from copolymers of lactic and glycolic acid (PLGA) is a degradation reaction that may compromise product safety and efficacy. As peptide sorption to PLGA is believed to be a common precursor to peptide acylation, a new method to inhibit acylation is presented involving disruptors of peptide sorption, namely, inorganic divalent cations. Kinetics of sorption of a model peptide, octreotide acetate, to free-acid end-group PLGA was monitored in the presence and absence of water-soluble inorganic divalent cationic salts in HEPES buffer solution (pH 7.4, 37 °C). Sorption of cations and octreotide attained pseudo-equilibrium by 24 h. From 24-h sorption isotherms, all cations studied inhibited octreotide sorption to PLGA—the inhibiting effect of the cations increased in the order: $Na^{+}<Mg^{2+}<Ca^{2+}$, $Sr^{2+}<Ni^{2+}<Mn^{2+}$. Long-term inhibition of octreotide sorption in the presence of 15 mM $CaCl_2$ and $MnCl_2$ translated to decreased acylated octreotide present in solution by greater than 50% at 21 days incubation, i.e., from 32% in the cation-free control to 14 and 13% for $CaCl_2$ and $MnCl_2$, respectively. Over one month in vitro release, PLGA implants encapsulating octreotide acetate and $CaCl_2$ or $MnCl_2$ also showed substantial inhibition of acylation relative to no-salt or NaCl controls, and similarly, strong inhibition of acylation upon divalent salt incorporation was observed during solvent extrusion of suspended peptide with polar organic carrier solvents. Hence, disrupting peptide sorption to PLGA with addition of inorganic divalent cations is a simple and viable strategy to inhibit acylation of peptides in PLGA delivery systems.

1. Introduction

Injectable biodegradable microspheres and implants control the release of peptide or protein drugs over the course of weeks to months, providing a distinct advantage over daily injections in terms of patient acceptability and compliance. The PLGA family of copolymers of lactic and glycolic acids is one of the few biodegradable polymers used in US FDA approved pharmaceutical products or medical devices [1], and is widely used in commercially available controlled-release peptide delivery systems, including the Lupron Depot® (leuprolide acetate), Sandostatin LAR® (octreotide acetate), and Zoladex® implant (goserelin acetate).

A very significant challenge in the development of controlled-release PLGA systems is the instability of peptides and proteins. For larger protein molecules numerous physical and chemical pathways of instability have been extensively reviewed [2–5]. Acylation was postulated [3] and later proven as a pathway of instability for peptides encapsulated in PLGA implants [6]. Nucleophilic primary amines, such as from the N-terminus and lysine side chain, can interact with solid PLGA and/or PLGA degradation products to form acylated peptide adducts [7]. Peptide acylation may potentially result in loss of activity [8], a change of receptor specificity, or immunogenicity (see [6] and citations therein). For the important, clinically used octreotide, acylation has been shown to occur in both linear PLGA and glucose-star PLGA copolymers [9,10], and a mechanism has been proposed to involve an ionic interaction between a protonated amine and the carboxylate PLGA end-group, followed by a nucleophilic attack of another nucleophilic amine on the lactate or glycolate carbonyl carbon and subsequent polymer hydrolysis [11].

Several methods to minimize acylation within PLGA microparticles have been proposed, including: (a) increasing the microclimate pH from 2 to 6 [6,9,12], (b) reducing polymer hydrolysis rate by encapsulating in PLGAs of high lactic:glycolic ratio [6,10], (c) facilitating the release of water-soluble oligomers (for example by using PEG as a porosigen) [6], and (d) shielding the reactive amino-group on the peptide by PEGylation [11]. Unfortunately, these methods either do not strongly inhibit acylation, limit formulation options, or involve chemically modifying the drug molecule. Additional approaches, particularly the use of additives, are needed to optimize PLGA delivery of peptides susceptible to this reaction.

Na and DeLuca [11] have shown that the interaction of octreotide with PLGA was attenuated when the polymer was end-capped and when octreotide was PEGylated, resulting in an inhibition of acylation. This important finding strongly suggests a critical precursor role of the ionic interaction between dicationic octreotide and the carboxylic acid

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end-groups of PLGA in acylation. This peptide–polymer interaction is the basis for our first effort to identify new acylation inhibitors.

PEGylating polypeptide therapeutics is an attractive option for molecules that would benefit from improved physical stability, resistance to proteases, reduced immunogenicity, or increased half-life. In some cases, PEGylation may be a useful strategy for extending an existing product's patent-life by applying for regulatory approval of the new chemical entity. Unfortunately, there are many documented cases where PEGylation has resulted in a substantial decrease in receptor affinity [13] or may not be a feasible strategy because of financial concerns with gaining approval of a new drug entity.

The interaction of cations in the diffuse side of electric double layers of negatively charged surfaces is well documented [14]. Similarly, the binding mechanism of divalent cations to surfaces such as emulsion droplets [15] and phospholipid membranes [16–19] has been the topic of extensive research. The specific interaction of divalent cations with biological membranes is essential for several cellular processes, including endo- and exo-cytosis, signal transduction, transport of molecules, and binding of proteins. This body of work motivated us to investigate the effect of water-soluble inorganic divalent cationic salts on the interaction between octreotide and PLGA. Indeed, we found for certain cations, such as Ca^{2+} and Mn^{2+}, which are significantly present in living systems, a very strong inhibition of both peptide sorption to PLGA and peptide acylation using octreotide as a model peptide.

2. Materials and methods

2.1. Materials

Octreotide acetate was obtained from Novartis (Basel, Switzerland). PLGA 50:50 (Resomer 502H, I.V. 0.2 dl/g) was purchased from Boehringer Ingelheim GmbH (Ingelheim, Germany). (Hydroxyethyl)-piperazine-(ethanesulfonic acid) (HEPES), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), manganese chloride ($MnCl_2$), and sodium chloride (NaCl) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Nickel chloride ($NiCl_2$) and strontium chloride ($SrCl_2$) were purchased from Fisher Scientific. Standard solutions for inductively coupled plasma-optical emission spectroscopy (ICP-OES) were purchased from GFS Chemicals (Columbus, OH). All other reagents used were of analytical grade or purer and purchased from commercial suppliers.

2.2. Analysis of octreotide by HPLC and HPLC-MS

The concentration of native and acylated octreotide was determined by HPLC and the octreotide degradation products were identified as acylated octreotide by HPLC-MS, similarly as described by Murty et al. [10]. See Supplementary Information for details regarding the LC-MS analysis, including reference chromatograms and table of peak identifications. Injection volumes of 20 μL were loaded onto a Nova Pak C-18 column (3.9×150 mm, Waters) for RP-HPLC (Waters Alliance®) analysis using UV detection (280 nm). Solvent A was 0.1% TFA in acetonitrile and Solvent B was 0.1% TFA in water. A linear gradient of 25 to 35% A in 10 min, with a flowrate of 1.0 mL/min was used. The formation of acylated octreotide was verified by HPLC-MS, where a linear gradient of 15 to 45% A in 30 min was used, with a flowrate of 1.0 mL/min, followed by infusion into an electrospray ionization mass spectrometer with ion-trap detection in positive ion mode (ThermoFinnigan Surveyor HPLC and LCQ MS, San Jose, CA). Solutions of octreotide (0.9 mM) before and after the addition of $CaCl_2$ and $MnCl_2$ (50 mM) were analyzed by HPLC after 24 h incubation to validate that the divalent cations do not interfere with the HPLC analysis or degrade octreotide. The addition of $CaCl_2$ or $MnCl_2$ did not affect the concentration of octreotide detected (data not shown).

2.3. Analysis of divalent cations by ICP-OES

The concentration of divalent cations was analyzed by ICP-OES (Perkin-Elmer Optima 2000 DV with Winlab software). Solutions containing divalent cations were diluted with water prior to analysis. Ca^{2+} was detected at 396.85 nm in the radial plasma mode; Mn^{2+} was detected at 257.61 nm in the axial plasma mode. Each measurement was an average of three scans.

2.4. Octreotide sorption studies

Solutions of octreotide (0.2–3.0 mM, 1 mL) in HEPES buffer (0.1 M, pH 7.4) were added to PLGA particles (10 mg, as received) and incubated (37 °C) on a rotary shaker (320 rpm) (IKA KS 130 basic). For sorption inhibition studies, chloride salts of divalent cations (1–50 mM) or NaCl (50 mM) were added to octreotide solutions prior to incubation. HEPES buffer was necessary to solubilize the divalent cations, which can precipitate with conventionally used phosphate buffer ions. The amount of octreotide sorbed was determined by the loss of octreotide from solution. Samples were removed from the incubator, centrifuged (2 min at 9.0 rcf) (Eppendorf 5415 D), and the supernatant was analyzed by HPLC. To validate this method of sorption quantification, an octreotide mass balance was performed at 1 and 24 h during the sorption experiment by recovering the sorbed octreotide from the polymer via two-phase extraction (see Section 2.6 below). The total amount of octreotide recoverable at 1 and 24 h was 99 ± 1% and 92 ± 1%, respectively. Therefore, during sorption studies, virtually all sorbed octreotide was non-covalently bound to PLGA, with the likelihood that a small fraction had become covalently bound to the polymer or otherwise decomposed.

2.5. Preparation of PLGA millicylinders

Sieved octreotide acetate powder, or octreotide acetate and salt powder (sodium chloride, calcium chloride or manganese chloride, <90 μm), both at 5% theoretical loading (see Table 1), were suspended in 62.5% (w/w) PLGA/acetone or PLGA/methylene chloride solution. The suspension was then loaded into a 3 ml syringe and extruded into a silicone rubber tubing (0.8 mm) by a syringe pump (Harvard Apparatus, Holliston, MA) at approximately 0.01 mL/min. The silicone

Table 1
Summary of encapsulation of octreotide and salt in PLGA millicylinder formulations.

Formulation	Theoretical salt loading (wt.%)	Actual salt loading (wt.%)	Salt encapsulation efficiency (%)	Theoretical octreotide loading (wt.%)	Actual octreotide loading (wt.%)	Octreotide encapsulation efficiency (%)
No salt	–	–	–	5.07	3.1 ± 0.2[a]	61 ± 3
NaCl	5.2	3.1 ± 0.1	59.7 ± 0.1	5.20	3.6 ± 0.2	69 ± 4
$CaCl_2$	5.1	3.4 ± 1.0	67.5 ± 19	5.08	4.1 ± 0.2	81 ± 4
$MnCl_2$	5.1	3.8 ± 0.1	74.7 ± 0.9	4.94	4.0 ± 0.1	81 ± 2

[a] All values represent mean ± standard error of the mean (SEM, $n = 3$).