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Effect of pH on the Formation of Acylated Octreotides by Poly(lactide-co-glycolide)

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ABSTRACT – The formation of acylated peptide impurities in poly(lactide-co-glycolide) (PLGA) formulations is one of the major challenges to the development of successful sustained-release product. Octreotide, synthetic analogue of somatostatin, has been identified to be acylated in PLGA microsphere formulations. The purpose of this study was to investigate the pH effect on the formation of acylated octreotides by PLGA. In the incubation with PLGA in 0.1 M phosphate buffer at pH 7.4, approximately 98% of octreotide adsorbed to PLGA through 14 days and 66.3% of acylated octreotides were produced after 42 days, whereas the interaction of octreotide with PLGA was significantly inhibited in the incubation at pH 4, in which the acylated octreotides were observed to be 9.2% after 42 days. In the interaction study at pH 4.1-7.4, the production of acylated octreotides was demonstrated to be dependent on environmental pH. Below pH 5.0, the acylation of octreotide was significantly inhibited. This study indicates that the pH is the major factor for the formation of acylated octreotide in PLGA formulations.

Key words – Octreotide, Stability, Peptide acylation, Poly(lactide-co-glycolide)

Biodegradable poly(D,L-lactide-co-glycolide) (PLGA), copolymer of lactic and glycolic acids, has been extensively used for the sustained-release drug delivery and tissue engineering because of its highly biocompatible and biodegradable characteristics (Gombotz and Pettit, 1995; Anderson and Shive, 1997). Injectable biodegradable microspheres and implants prepared using PLGA provide a distinct advantage over daily injections by controlling the release of the encapsulated peptides or proteins over the course of weeks to months (Sinha and Trehan, 2005). Sustained depot formulations of luteinizing hormone-releasing hormone and somatostatin analogues have been commercialized, such as the Lupron Depot® (leuprolide acetate), Zoladex® implant (goserelin acetate), and Sandostatin LAR® (octreotide acetate), and several additional products are under clinical investigation (Wischke and Schwendeman, 2008).

Although biodegradable PLGA has been successfully used for the long-term controlled drug release, the instability of peptides and proteins in the PLGA matrix during manufacture, storage and after administration remains one of the major challenges to successful product development (Bilati et al., 2005). Recently, the formation of peptide impurities inside or outside PLGA matrix as a result of peptide acylation with lactic and glycolic acid units was found in various peptides such as salmon calcitonin, atrial natriuretic peptide, human parathyroid hormone, growth hormone-releasing peptide-6 (Lucke et al., 2002; Na et al., 2003; Na and DeLuca, 2005; Murty et al., 2005a; Murty et al., 2005b; Houchin et al., 2006; Na et al., 2007; Ryu and Na, 2009). The formation of acylated peptide impurities in PLGA formulations is regarded as one of obstacles to be overcome for the successful drug delivery, because it may be the cause of the incomplete drug release, and can lead to changes in the biological properties of peptides, such as the loss of biological activity or a change in immunogenicity (Na et al., 2007).

Octreotide, which is a synthetic octapeptide analogue of the naturally occurring hormone somatostatin (H_2N-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-ol) and exerts pharmacologic actions similar to the endogenous somatostatin (Chen et al., 1993), has been commercially formulated in PLGA microsphere formulations (Sandostatin LAR depot, Novartis, Basel, Switzerland) as a monthly dosage form for the treatment of acromegaly and certain endocrine tumors. In several previous studies with octreotide, the peptide acylation has been shown to occur in PLGA microsphere formulations (Murty et al., 2005a; Murty et al., 2005b; Na and DeLuca, 2005; Ryu and Na, 2009). The primary amines of octreotide at N-terminus and lysine side chain are identified to be the major targets for peptide acylation by PLGA (Na and DeLuca, 2005). The acylation of octreotide with PLGA was proposed to involve an ionic interaction between a protonated amine (mainly ε-amine of lysine residue) and carboxylate of PLGA end-group, followed by a nucleo-

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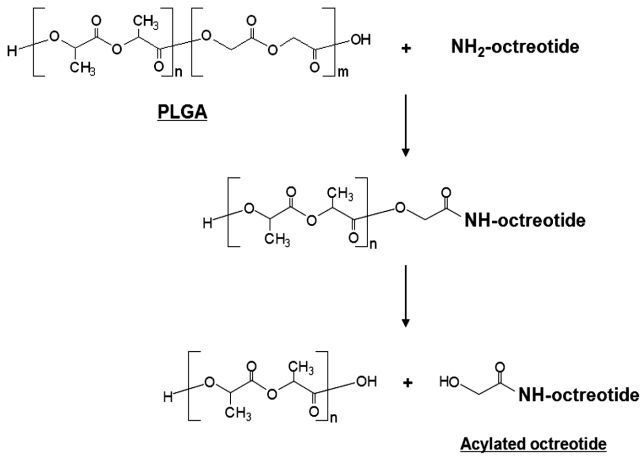

Figure 1. The formation of acylated octreotides by reaction with PLGA and the subsequent hydrolysis of polymer.

philic attack of another primary amine (mainly N-terminal amine) on the lactate or glycolate carbonyl carbon and subsequent hydrolysis of PLGA (Figure 1).

The purpose of this study was to investigate the effect of pH on the formation of acylated octreotides by interacting with PLGA. As indicated in the previous study (Na and DeLuca, 2005), the primary amines of N-terminus and lysine residue of octreotide play an important role in the peptide acylation by PLGA and their roles may be dependent on environmental pH. This study monitors the formation of acylated octreotides at different pH and elucidates the importance of pH control for inhibiting peptide acylation.

Materials and Methods

Materials

Octreotide acetate (molecular weight 1019.26) was obtained from Bachem (Torrance, CA, USA). Hydrophilic 50:50 PLGA polymer (molar ratio of D,L-lactide:glycolide=50:50, viscosity: 0.16-0.24 dL/g, product name: RESORMER® RG502H) was supplied by Boehringer Ingelheim (Ingelheim, Germany). DL-lactic acid, glycolic acid, and α-cyano-4-hydroxycinnamic acid (α-CHCA) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) and trifluoroacetic acid (TFA) were obtained from J.T. Baker (Philipsburg, NJ, USA) and Acros organic (Pittsburgh, PA, USA), respectively. All other chemicals were of analytical grade and were used as obtained commercially.

Interaction Study of Octreotide with PLGA

The formation of acylated octreotides by PLGA was investigated by adding 100 mg of RG502H PLGA polymer to 10 mL of octreotide (peptide concentration of 200 μg/mL) in 0.1 M acetate buffer (pH 4.0) or 0.1 M phosphate buffer (pH 7.4) at 37°C (n=3 per sample). Samples were collected at 1, 3, 7, 14, 21, 28, 35, and 42 days. The supernatants after centrifuge were analyzed by reversed phase-HPLC in triplicate per sample.

Effect of pH on Octreotide Acylation by PLGA

Effect of pH on the formation of acylated octreotides by PLGA was investigated at the range of pH 4.1-7.4 with 0.1 M phosphate buffers containing lactic acid or glycolic acid. 10 mg of RG502H PLGA polymer was added to 1 mL of octreotide (peptide concentration of 200 μg/mL) in 0.1 M phosphate buffers (pH 7.4) containing 0.2% (pH 6.9), 0.4% (pH 6.4), 0.6% (pH 5.8), or 1.0% (pH 4.1) of lactic acid or 0.6% of glycolic acid (pH 5.0) at 55°C (n=3 per sample). Samples were collected at 1, 3, and 10 days. The supernatants after centrifuge were analyzed by reversed phase-HPLC in triplicate per sample.

HPLC Analysis

HPLC analysis was performed with a Dionex HPLC system (Dionex Co., Sunnyvale, CA, USA) consisted of a quaternary gradient pump with an on-line vacuum degasser (Model P680A), an automated sample injector (Model ASI-100), thermostatted column compartment (Model TCC-100) and 4-channel multi UV-vis detector (Model 170U). Separations of octreotide and its acylated products were carried out on a Prosphere C-18 column (4.6 × 250 mm, 5 μm, Alltech, Deerfield, IL, USA) with a Prosphere C-18 guard column (4.6 × 7.5 mm, 5 μm, Alltech, Deerfield, IL, USA). A gradient elution was performed with mobile phase A (0.1% TFA in water) and mobile phase B (0.1% TFA in acetonitrile). Linear gradient elution was performed from 80:20 to 65:35 (mobile phase A:B) for 20 min at a flow rate of 1.0 mL/min. Total run time was 30 min and sample injection volume was 40 μL. UV absorbances were monitored at 215 nm.

MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis was performed on a Kratos Kompact SEQ time-of-flight mass spectrometer (Manchester, UK) as reported previously (Na and DeLuca, 2005). The α-CHCA in 50% of acetonitrile in water with 0.1% TFA was used as a matrix. Data for 2 ns pulses of the 337 nm nitrogen laser were averaged for each spectrum in a linear mode, and positive ion TOF detection was performed using an accelerating voltage of 20 kV.

Results and Discussion

The formation of acylated octreotides by interaction with PLGA was monitored in 0.1 M acetate buffer (pH 4.0) and 0.1 M phosphate buffer (pH 7.4) at 37°C for 6 weeks. In a previous study (Na and DeLuca, 2005), the interaction between octreotide and PLGA was observed with initial adsorption of octreotide to PLGA followed by chemical reaction and the release of native and acylated octreotides (Figure 1). Figure 2(A) shows the interaction profile of octreotide with hydrophilic RG502H PLGA in 0.1 M sodium phosphate buffer at pH 7.4. From day 1 through day 14, approximately 2.2% of octreotide remained in the supernatant and then the amount increased to 33.2% by day 42. The acylated octreotide was observed from day 14 (5.2%) and the amount increased to 66.3% by day 42. At day 42, the sum of amounts of the native and acylated octreotides reached 99.5%. In the MALDI-TOF MS analysis of samples at 42 days, several acylated octreotides with the additional mass of glycolic acid (GA, 58 Da) and/or lactic acid (LA, 72 Da) units were observed as follows: octreotide-1GA, *m/z* 1077; octreotide-1LA, *m/z* 1091; octreotide-Na (sodium)-1GA, *m/z* 1100; octreotide-1Na-LA, *m/z* 1114; octreotide-2GA, *m/z* 1135; octreotide-1GA-1LA, *m/z* 1149; octreotide-Na-2GA, *m/z* 1158; octreotide-Na-1GA-1LA, *m/z* 1172; octreotide-Na-3GA, *m/z* 1216; octreotide-Na-2GA-1LA, *m/z* 1230.

The interaction profile of octreotide with hydrophilic RG502H PLGA in 0.1 M sodium acetate buffer at pH 4 was shown in Figure 2(B). At pH 4, the interaction of octreotide with PLGA was significantly inhibited. At day 1, approximately 86.2% of octreotide remained in the supernatant and the acylation products of octreotide were observed to be 5.3-9.2% from day 14 to 42. This result indicates that the initial adsorption of octreotide to PLGA and the subsequent reaction of octreotide with PLGA are dependent on the environmental pH.

To further investigate the pH effect on the formation of acy-

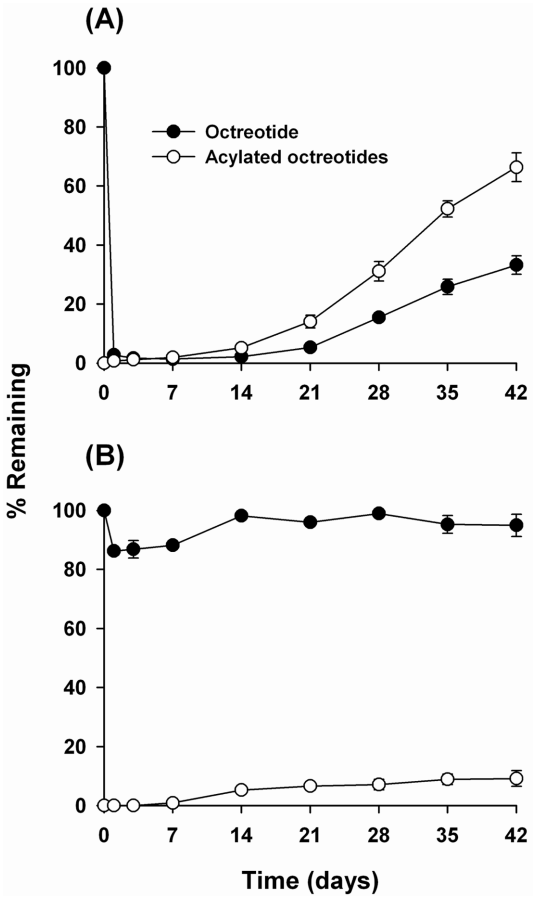

Figure 2. The production of acylated octreotides after incubating octreotide with PLGA in 0.1 M phosphate buffer (pH 7.4) (A) and 0.1 M acetate buffer (pH 4.0) (B) at 37°C for 42 days.

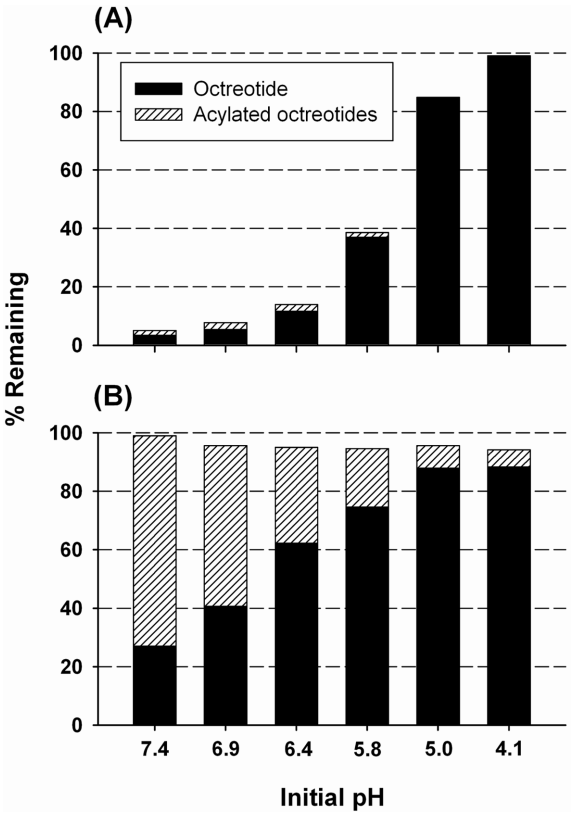

Figure 3. Effect of pH on the formation of acylated octreotides by PLGA after 1 day (A) and 10 days (B) of incubation in different pH buffers at 55°C. Initial pH 7.4: 0.1 M phosphate buffer, pH 6.9: 0.1 M phosphate buffer containing 0.2% lactic acid, pH 6.4: 0.1 M phosphate buffer containing 0.4% lactic acid, pH 5.8: 0.1 M phosphate buffer containing 0.6% lactic acid, pH 5.0: 0.1 M phosphate buffer containing 0.6% glycolic acid, pH 4.1: 0.1 M phosphate buffer containing 1.0% lactic acid.

lated octreotides by PLGA, octreotide was incubated with hydrophilic RG502H PLGA polymer in various buffers ranged from pH 4.1 to 7.4 at 55°C. The solutions ranged from pH 6.9 to 4.1 were prepared by the addition of 0.2-1.0% (v/v) lactic acid or glycolic acid. After incubation for 10 days at 55°C, the pH values of every solution were significantly lowered to pH 3.5-3.2. Figure 3(A) shows initial adsorption of octreotide to PLGA polymer at day 1. The adsorption was significant in the solutions at the initial pH 7.4-5.8, whereas the solutions at the initial pH 5.0 and 4.1 showed substantially lower adsorption. The initial adsorption was closely related to the formation of acylated octreotides. As shown in Figure 3(B), the acylated octreotides were substantially formed in the solutions at the initial pH ranged from 7.4 to 5.8, whereas the octreotide acylation was inhibited in the solutions at the initial pH below 5.0. With initial pH 5.8, approximately 20% of octreotide was acylated, whereas 7.7% of octreotide were acylated at initial pH 5.0. This result indicates that the acylation of octreotide occurs at initial drug release stage in the microspheres prior to the formation of acidic microclimate within the PLGA matrix owing to polymer degradation.

Conclusion

The pH is major factor for the formation of acylated octreotides in PLGA formulations. At neutral pH, several acylated octreotides were produced via initial adsorption to PLGA followed by acylation reaction and hydrolysis of polymer. The formation of acylated octreotides by PLGA could be significantly inhibited at acidic pH. Therefore, the control of initial pH inside PLGA matrix may be one of strategies for preventing from the acylation of octreotide in PLGA formulations.

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