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Stability of Octreotide Acetate in Aqueous Solutions and PLGA Films

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ABSTRACT – As a synthetic analog of the naturally occurring hormone somatostatin, octreotide has been commercially formulated in poly(lactide-co-glycolide) (PLGA) microspheres for the treatment of acromegaly. The purpose of this study was to evaluate stability of octreotide acetate in aqueous solutions at various pH values and PLGA films. Stability-indicating reversed-phase high-performance liquid chromatographic method was developed with good precision and accuracy, and it was applied to the stability studies. In aqueous solutions at pH 2.5-9.0, the degradation of octreotide followed approximately first order kinetics and the most favorable stability was found at pH 4. In PLGA films, the formation of acylated octreotides reached approximately 55% of the released octreotides. Various acylated octreotides was structurally identified by liquid chromatography-mass spectrometric analysis.

Key words – Octreotide, Stability, PLGA

For the sustained release of peptide and protein drugs, poly(D,L-lactide-co-glycolide) (PLGA) has been widely used in the formulations such as biodegradable microspheres.[1,2] However, stabilization of peptides and proteins in the PLGA matrix during manufacture, storage and after administration remains as one of the major challenges to successful product development.[3,4] Recently, the formation of peptide impurities in PLGA formulations 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.[5-9] The acylation of peptide drugs in PLGA formulations is regarded as one of obstacles to be overcome for the successful delivery of bioactive materials, because it may lead to changes in the biological properties of peptides, such as the loss of biological activity or a change in immunogenicity.[9]

Octreotide, a synthetic octapeptide analogue of the naturally occurring hormone somatostatin (H_2N-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-ol), exerts pharmacologic actions similar to the endogenous somatostatin.[10] It has been commercially formulated in poly(lactide-*co*-glycolide) (PLGA) microsphere formulations (Sandostatin LAR depot, Novartis pharma, Basel, Switzerland) as a monthly dosage form for the treatment of acromegaly and certain endocrine tumors.[11] In several previous studies, octreotide was also identified to be acylated in PLGA microsphere formulations.[12-16] The primary amines of octreotide at N-terminus and lysine side chain are shown to be the major targets for peptide acylation by PLGA.[16]

The first purpose of this study was to evaluate stability of octreotide acetate in aqueous solutions at various pH values. This study was aimed to investigate stability of octreotide in different pH environment, because PLGA formulations provide changing pH environment due to the release of glycolic and lactic acid by polymer hydrolysis. Stability-indicating HPLC method was developed and applied to the stability study to find the most optimal pH condition for octreotide stability. The formation of acylated octreotides in PLGA films was studied by the validated HPLC method and the structures of acylated octreotides were identified by LC-MS.

Materials and methods

Materials

Octreotide acetate (H_2N-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-ol, molecular weight 1019.26) from Bachem (Torrance, CA, USA) was a gift from Oakwood Laboratories, LLC (Oakwood, OH, USA). Hydrophilic 50:50 PLGA polymer (RG502H) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Acetone (HPLC grade) was obtained from Merck (Darmstadt, Germany). 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.

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HPLC Analysis of Octreotide Acetate

Chromatography was performed using 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 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). Octreotide was eluted with a linear gradient 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.

Validation of HPLC Method

Calibration plots were prepared by injecting specific concentration of octreotide acetate solutions (2, 5, 10, 20, 50, 100 and 200 μg/mL in water) to HPLC. Three injections of each concentration were performed. Calibration curves were constructed using average of peak area. Linear regression analysis of the data gave slope, intercept and correlation coefficient data, which were then used to calculate octreotide concentration in each sample. To validate the method for octreotide acetate, intra-day accuracy and precision were evaluated by analysis at various concentration levels on the same day. Three different concentrations (2, 50 and 200 μg/mL) levels were selected. Analysis was done by preparing three samples at each concentration level and the estimated concentrations were calculated based on calibration curves. To assess the inter-day accuracy and precision, the intra-day assay was repeated on three different days.

Stability Study of Octreotide Acetate at Different pH Values

Stability study of octreotide acetate was performed with 0.1 M glycine buffer (pH 2.5), 0.1 M acetate buffer (pH 4.0), 0.1 M phosphate buffer (pH 7.4), and 0.1 M borate buffer (pH 9.0). Octreotide acetate was dissolved in each buffer solution at a concentration of 100 μg/mL. The solutions were then placed into incubator at 37 and 55°C. Samples collected at specific time were analyzed by the validated HPLC method.

Film Preparation and Peptide Stability Study in PLGA Films

PLGA films were prepared as described in a previous publication[7] by dissolving 100 or 200 μg of octreotide acetate with 20 mg of polymer in 0.2 mL of acetone and sonicating for 30 min. The solution was then pipetted into eppendorf tubes and allowed to dry for 2 days. The dried PLGA films containing octreotide acetate were placed into eppendorf tube containing 1 mL of 0.1 M phosphate buffer (pH 7.4) and incubated at 55°C. Samples were collected at specific time after vortex for 1 min and centrifuged for 10 min at 5000 rpm. All samples were analyzed by RP-HPLC and LC-MS.

LC-MS of Octreotide Acetate and its Degradation Products

LC-MS analysis was performed using an Agilent Technologies Series 1100 LC/MSD VL system (Agilent Technologies, Palo Alto, CA, USA). HPLC conditions were the same as described above. The mass spectra obtained in the positive-ion mode. The following conditions were used: drying gas (N_2) flow of 10 L/min, drying gas temperature at 350°C, nebulizer pressure of 40 psi, and capillary voltage of 3.5 kV. The fragmentor voltage was 100 V. Ions are detected by scan mode with a mass range from *m/z* 400 to 1400.

Results and Discussion

HPLC Analysis of Octreotide Acetate

Reversed-phase HPLC method using gradient elution was validated for the analysis of octreotide acetate. Under the HPLC conditions, the standard of octreotide acetate in deionized water was detected as a single peak at a retention time of 14.0 min (Figure 1A). The HPLC method was stability-indicating, which could resolve intact octreotide acetate from the degradation compounds produced in sodium borate buffer (pH 9.0) at 55°C for 14 days (Figure 1B). The calibration curve was plotted from 2 to 200 μg/mL with a correlation coefficient greater than 0.9998. Intra-and inter-day precision values were determined as the coefficient of variations (each n=3). The intra-day precision ranged from 0.3 to 3.4%, and the inter-day precision was 0.7 to 2.5%. The accuracy ranged from 92.1 to 100.4% (Table I). These result indicated that the HPLC method was reliable and reproducible.

Stability of Octreotide Acetate in Aqueous Solutions

Stability of octreotide acetate in aqueous solutions was investigated by the validated HPLC method. Figure 2 shows a semilogarithmic plot of the residual percentage amount of octreotide acetate versus time in various pH buffers at 37 and 55°C. It was found that the pH affected the degradation rate of octreotide acetate with the observed degradation reaction rates

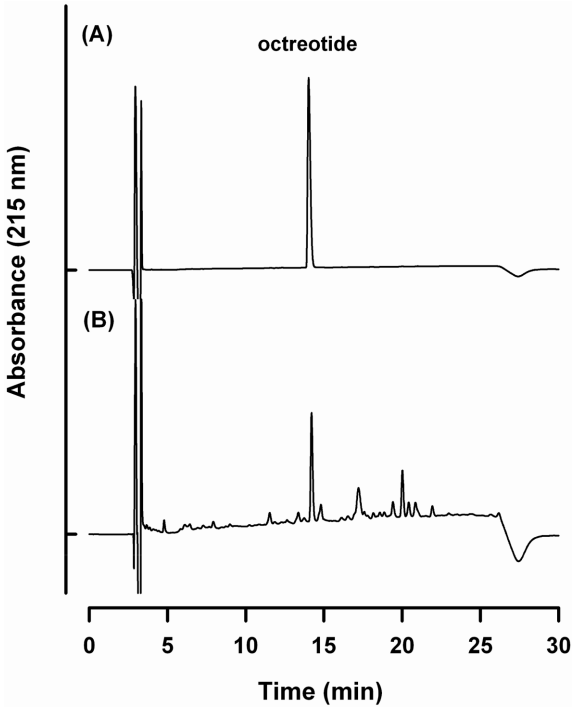

Figure 1–HPLC chromatograms of octreotide acetate standard in water (A) and octreotide incubated in 0.1 M borate buffer (pH 9) at 55°C for 14 days (B).

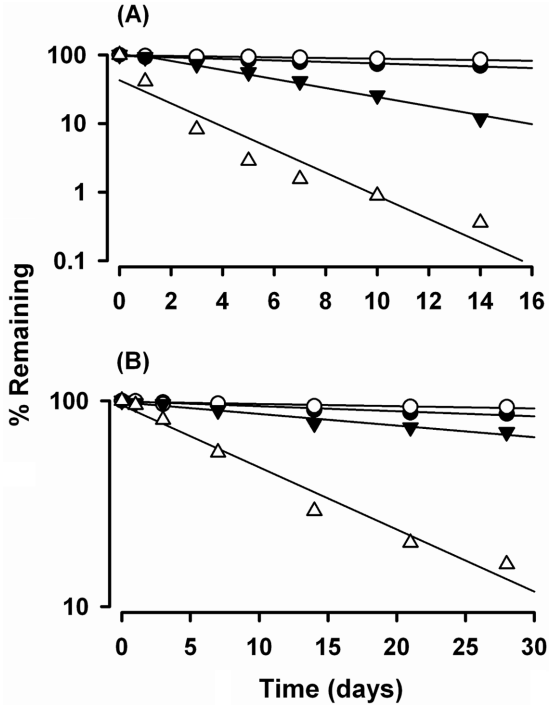

Figure 2–Stability of octreotide acetate in the different pH buffers at 55°C (A) and 37°C (B). ●: 0.1 M glycine buffer (pH 2.5), ○ : 0.1 M acetate buffer (pH 4.0), ▼: 0.1 M phosphate buffer (pH 7.5), and △: 0.1 M borate buffer (pH 9.0).

Table I–*Validation of HPLC method for the analysis of octreotide acetate*

Concentration (μg/mL)	Precision (%)		Accuracy (%)
	Intra-day	Inter-day	
2	3.4	2.4	92.1
50	0.4	2.5	94.0
200	0.3	0.7	100.4

approximately following first-order kinetics. Degradation rate constants were obtained from the slope of the semilog plots of the concentration versus time data by regression analysis. The observed reaction first-order rate constants of octreotide acetate are listed in Table II. Although the optimum pH for stability of octreotide acetate was not defined, the most favorable stability appeared to be in acetate buffer at pH 4. The half-life for degradation of octreotide acetate at 55°C was 27.1 days at pH 2.5, 60.3 days at pH 4, 4.6 days at pH 7.4, and 1.2 days at pH 9.

Table II–*Observed rate constants and half-lives for the degradation of octreotide acetate*

Buffer solution	pH	Temperature (°C)	Rate constant (1/day)	Half-life (day)
0.1 M glycine	2.5	37	0.0056	123.8
		55	0.0256	27.1
0.1 M acetate	4.0	37	0.0024	288.8
		55	0.0115	60.3
0.1 M phosphate	7.4	37	0.0131	52.9
		55	0.1519	4.6
0.1 M borate	9.0	37	0.0721	9.6
		55	0.5632	1.2

Stability of Octreotide Acetate in PLGA Films

Stability of octreotide acetate in PLGA matrix was studied in PLGA films prepared with hydrophilic PLGA (RG502H) polymer. Figure 3 shows the HPLC chromatograms of the PLGA film containing octreotide acetate incubated in 0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days. When compared with the chromatogram of octreotide standard solution incubated in the same condition, the film showed the several additional peaks, which are thought to be produced by the interaction of octreotide acetate with PLGA polymers.

The chemical structures of the additional peaks produced from PLGA film containing octreotide acetate were analyzed by LC-MS. In the LC-MS analysis of PLGA film incubated in

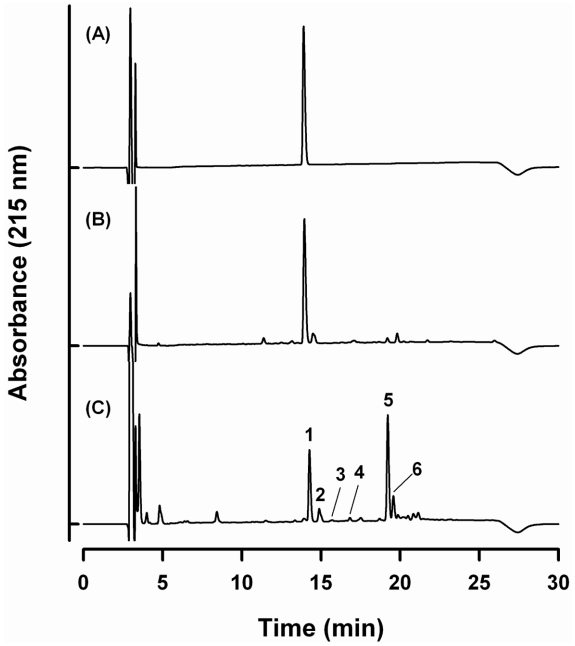

Figure 3–HPLC chromatograms of octreotide standard (A), octreotides after incubation in 0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days (B) and octreotides-PLGA film after incubation in 0.1 M phosphate buffer (pH 7.4) at 55 °C for 7 days (C).

Table III–*LC-MS identification of acylated octreotides from PLGA films after incubation in 0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days*

Peak number [a]	Mass (*m/z*)	Product
1	1019	Octreotide (Oct)
2	1099	Oct-Na^++GA[b]
3	1113	Oct-Na^++LA[c]
4	1099	Oct-Na^++GA
5	1077, 1099	Oct+GA, Oct-Na^++GA
6	1135, 1157	Oct+2GA, Oct-Na^++2GA

[a]Peak number in Figure 3(C), [b]GA: glycolic acid, [c]LA: lactic acid.

0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days, several acylated octreotide mass peaks were found by identifying mass difference due to the addition of lactic (+72 Da) or glycolic acid (+58 Da) unit. Major LC-MS peaks and their *m/z* values are listed in Table III.

Figure 4 shows peptide release profile of 0.5% and 1.0% octreotide-loading PLGA films after incubation in 0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days. Following initial adsorption of octreotide for 1 day, intact and acylated octreotides were released through 7 days. Total peptide releases were 36.0% for the 0.5% octreotide-loading film and 72.0% for the 1.0% octreotide-loading film through 7 days. Among

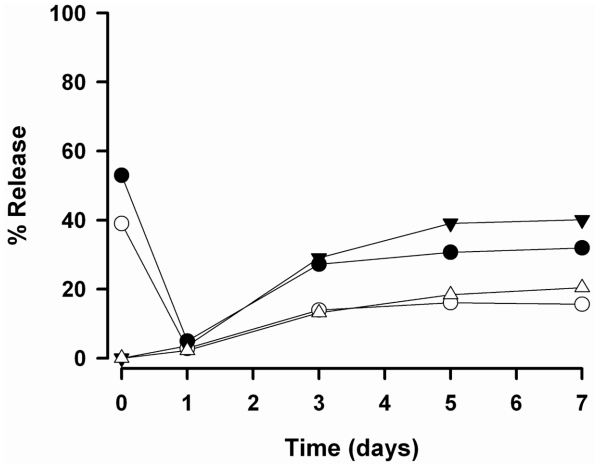

Figure 4–Formation of acylated octreotides from PLGA film incubated in 0.1 M phosphate buffer (pH 7.4) at 55°C for 7 days (● : intact octreotide from 1.0% octreotide-loading film, ○: intact octreotide from 0.5% octreotide-loading film, ▼: acylated octreotide from 1.0% octreotide-loading film, △: acylated octreotide from 0.5% octreotide-loading film).

the peptides released from PLGA films, the formation of acylated octreotide was 56.6% in the 0.5% octreotide-loading film and 55.6% in the 1.0% octreotide-loading film through 7 days. Consequently, octreotide was more released from the higher peptide-loaded films and the formation ratio of acylated octreotide was similar between 0.5 and 1.0% peptide-loaded films.

Conclusions

Stability-indicating HPLC method of octreotide acetate was validated and used for stability study in various buffer solutions and PLGA matrix. In buffer solutions at pH 2.5-9.0, the degradation of octreotide followed approximately first order kinetics and the most favorable stability was found at pH 4. In PLGA films containing octreotide acetate, the formation of several acylated octreotides was observed by LC-MS analysis and approximately 55% of acylated octreotides were found among the released octreotides.

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