Journal of Chromatography A, 1429 (2016) 354–363

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A capillary electrophoretic–mass spectrometric method for the assessment of octreotide stability under stress conditions

Elnaz Tamizi[a,1], Yuanzhong Yang[a], Abolghasem Jouyban[b], Geoffrey F. Kelso[a], Reinhard I. Boysen[a], Milton T.W. Hearn[a,*]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, Monash University, Melbourne, Victoria 3800, Australia
[b] Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
14 December 2015
Accepted 14 December 2015
Available online 17 December 2015

Keywords:
Octreotide
Capillary electrophoresis
Mass spectrometry
Degradation pathways
Stress stability studies
Stability indicating methods

ABSTRACT

A capillary zone electrophoretic-electrospray ion trap mass spectrometric method has been developed to assess the stability and pathways of degradation of the cancer therapeutic octapeptide, octreotide. As a somatostatin analogue, octreotide contains a single disulphide bond linking Cys^2–Cys^7 with the structure of NH_2-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-OH. Resolution of octreotide from its degradation products was achieved using a capillary zone electrophoretic method with bare fused silica capillaries, a 10 mM ammonium formate buffer, pH 3.20, at 25 °C and an applied voltage of 25 kV. An ion trap low energy collision induced dissociation procedure was applied for the characterization of the chemical structures of the degradation products derived from an acidic, alkaline, neutral and thermal solution treatment of octreotide. The results so obtained indicated that linear octreotide degradation products were formed under acidic and alkaline conditions, due to the hydrolysis of a ring amide bond and a hitherto unknown desulfurization of the Cys–Cys disulfide bond, respectively. Degradation under neutral conditions occurred via cleavage of the exocyclic *N*-((2*R*,3*R*)-1,3-dihydroxybutan-2-yl) amide bond which also preceded the ring amide hydrolysis under acidic conditions. The developed method was further successfully applied to assess the kinetics of these octreotide degradations. Overall, this method is suitable for the rapid and precise assessment of the stability and quality control of octreotide as a synthetic peptide-based pharmaceutical product, and has led to the discovery of a new Cys–Cys disulfide degradation pathway.

1. Introduction

Advances in biomedical research and biotechnology have led to remarkable developments in the biopharmaceutical industry. More than 150 peptides and proteins are currently approved by the Food and Drug Administration of the USA as therapeutic agents for the prevention, diagnosis and/or treatment of different diseases and this number is rapidly increasing [1–4]. In this growing industry, there is a demand for advanced analytical techniques which can be applied as sensitive and reliable tools for the development of robust quality control procedures for these products [2]. Central to the development of these quality control procedures is the ability to establish and use multiple analytical methods that discriminate impurities which arise from the manufacturing processes *per se*, from the formulation procedures employed, and from the consequences of storage, transport/end of chain market outlet handling.

Studies that address the storage aspects and stability of highly purified products are a crucial part of the quality control of therapeutic peptides and proteins. In addition to real time—real condition stability studies that specify expiry date and the suitability of different storage and handling conditions of biopharmaceutical products, stability studies under stress conditions are equally important for the identification of the degradation products, investigation of the degradation pathways and validation of stability indicating methods (SIMs) [5,6]. SIMs are specific, accurate and precise analytical methods that identify and quantify therapeutic compounds in the presence of excipients, impurities and/or degradation products. These methods can detect the effect of

* Corresponding author.
E-mail address: milton.hearn@monash.edu (M.T.W. Hearn).
[1] Current address: Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

http://dx.doi.org/10.1016/j.chroma.2015.12.039
0021-9673/

environmental factors such as temperature, humidity, light or pH on the storage quality of active pharmaceutical ingredients/compounds (APIs) as a function of time and have become a cornerstone of stability studies [7–9]. One of the most powerful analytical techniques used in the biopharmaceutical industry is capillary electrophoresis (CE) [10]. CE can be coupled to mass spectrometry (MS) to provide sensitive and detailed information about the accurate molecular mass and structure of bio-molecules. Because of these attributes, CE–MS has emerged as a powerful tool for quality control of therapeutic peptides and proteins [11,12].

A synthetic analogue of the hormone somatostatin, the octapeptide, octreotide (OCT) (NH_2-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-OH) has a single disulfide bond linking the two Cys residues. In comparison to somatostatin, OCT is a more potent inhibitor of human growth hormone and has a longer half-life. These advantages have led to the use of octreotide in the treatment of somatostatin-related disorders, such as acromegaly and gigantism. Furthermore, it has an inhibitory effect on glucagon and insulin secretion, as well as on cell proliferation and tumor growth. Thus, it has been utilized in the treatment and control of certain endocrine and some carcinoid tumors [13–20].

Several CE-based methods have been reported for the analysis of OCT. Xin et al. [21] have described the application of a custom-built CE–ESI–Fourier transform ion cyclotron resonance mass spectrometer for the identification and separation of OCT in a mixture of three peptides. An emphasis of these earlier studies has been placed on the detection and quantitation of impurities, such as deletion, truncated, oxidation, deamidation and other types of side products that are generated during peptide synthesis procedures and the manufacture of the target peptide [1]. For example, Jaworska and coworkers [22] have described a two dimensional HPLC–CE method with UV monitoring for the analysis of OCT and its impurities, which were mainly the side products from the chemical solid-phase peptide synthesis (SPPS) of OCT. These investigators observed that the degradation of OCT to [des-Thr-ol]^8-OCT was the main degradation product formed at elevated temperature in a pharmaceutical formulation after 12 weeks at 37 °C under neutral pH conditions. Due to the orthogonality of the reversed-phase chromatographic and CE methods, a number of unidentified compounds were detected but not characterized. Liu and colleagues [23] have applied a capillary electro-chromatographic method with a temperature-responsive polymer coated capillary for the separation of OCT in a mixture of thymopentin and OCT. Also, in earlier work [24], a CE method was employed with UV detection for the analysis of OCT in pharmaceutical formulations subjected to stress conditions. However, this earlier study was based on UV/Vis detection and could not provide any information on the molecular structures of degradation products or insights into the pathways of OCT degradation under stress conditions, which require the application of mass spectrometric detection. Therefore, the main objective of the present investigation was to develop and validate a rapid CE–MS method that was applicable to such stability studies of OCT, including the identification of its degradation products and the pathways responsible for the formation of these products under stress conditions.

2. Materials and methods

2.1. Materials

OCT as a freeze-dried powder (98% wt/wt) and trypsin (from bovine pancreas) were obtained from Sigma-Aldrich (St. Louis, USA). Sodium hydroxide, hydrochloric acid, ammonium formate, ammonium acetate, acetic acid, formic acid, acetonitrile, methanol and isopropanol were obtained from E. Merck (Darmstadt, Germany). D,L-dithiothreitol (DTT) was obtained from Fluka (Seelze, Germany). All solutions were prepared from double distilled Milli-Q water (Pall Corporation, Australia) and filtered through a 0.2 μm pore size filter (Pall Corporation, Australia) before use.

2.2. Instrumentation

CE–UV analyses were performed using a 3D CE G1600AX instrument from Agilent Technologies (Waldbronn, Germany), equipped with a diode array detector (DAD) with detection range from 190 to 600 nm. In CE–UV analyses instrument control, peak integrations and peak purity calculations were performed with an Agilent Technologies ChemStation® software. Bare fused silica capillaries, with an inner diameter of 50 μm and outer diameter of 360 μm, were purchased from Polymicro Technologies (Phoenix, USA). The total length of the capillary was 86 cm and the detection window was located at 26 cm from the capillary inlet with the UV detection at 195 nm. Prior to analysis the capillary outer coating at the capillary tips was removed to about 5 mm from the tip. At the beginning of the each working day, the capillary was flushed with 0.1 M sodium hydroxide for 10 min and water for 5 min, and was then conditioned by the background electrolyte (BGE) for 20 min. Between each run the capillary was flushed for 3 min with BGE, and samples were injected hydrodynamically for 5 s at 50 mbar.

The ion trap mass spectrometer (Agilent Technologies LC/MSD trap) was coupled to the CE system *via* the Agilent Technologies coaxial sheath liquid sprayer interface. After preliminary experiments, performed to optimise the MS detection of OCT and its degradation products, the instrument parameters were selected as follows: nebulizer gas (N_2) pressure was set at 10 psi, drying gas (N_2) flow rate at 5 L/min, and drying gas temperature at 200 °C; positive ionization mode was selected by employing 3600 V on the inlet of the MS; cone voltage was 40 V and voltage for low-energy collision induced dissociation (CID) analysis was set at 2 V. In all experiments sheath liquid consisted of methanol and water (50:50% v/v) with 0.1% formic acid (v/v). Sheath liquid was supplied by an Agilent Technologies isocratic G1310A LC pump (Waldbronn, Germany) at a flow rate of 6 μL/min. Mass spectra were obtained with a speed of 13,000 per second in the mass range of m/z = 100–1100 and processed by Agilent Technologies Data Analysis software. All reported masses are monoisotopic (MH^+). The MS fragment species were labeled based on the nomenclature commonly applied in tandem mass spectrometry [25]. The mass and fragmentation pattern of each degradation product were calculated using the Spectrum Mill MS Proteomics Workbench software (Agilent Technologies, Waldbronn, Germany).

The pH adjustments of BGEs were carried out using a pH meter (Radiometer Analytical, France) and an oven was used to provide thermal stress (SEM, Australia).

2.3. Preparation of the background electrolyte (BGE)

A stock solution of ammonium formate buffer with a concentration of 100 mM was prepared by dissolving ammonium formate in double distilled water. For the preparation of the BGE, the appropriate volume of the stock solution was diluted to obtain a concentration of 10 mM with double distilled water and its pH was adjusted with 5 M formic acid or 5 M sodium hydroxide.

2.4. Treatment of the OCT samples with DTT and trypsin

Since OCT is a cyclic peptide, it is not completely fragmented through a low-energy CID procedure. To obtain information on the linear amino acid sequences, OCT was cleaved with trypsin or the disulphide bond ruptured with DTT. A solution of OCT (200 μg/mL)