Stable-Isotope-Labeled Parathyroid Hormone as an Immunocapture and Digestion-Efficiency Internal Standard for MS-Based Immunoassays

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Overview

Purpose: Apply a mass spectrometry immunocapture reactive monitoring (MSIA-SRM) assay to measure physiological parathyroid hormone (PTH) levels in plasma using a stable-isotopically labeled (heavy) protein standard for correction of immunocapture and digestion efficiency.

Methods: Recombinant heavy PTH was expressed using a human cell-free, in vitro translation (IVT) system. Tryptic digestion and MS analysis were conducted using recombinant heavy and light PTH standards.

Results: Heavy PTH with heavy isotope incorporation was expressed using a human IVT system and used to assess MSIA capture and digestion efficiency.

Introduction

Recombinant human PTH assays for measuring PTH and PTH variants are important for hormone diagnostics of a wide range of diseases. The expression of PTH has traditionally been carried out as a supplement to the development of assays that distinguish full-length PTH (f-PTH) from biologically active PTH-like peptides (f-PTH-like peptides). Commercially available f-PTH-like peptides that can accurately quantify the ratio of full-length to f-PTH in f-PTH assays are needed to accurately determine the amount of biologically active f-PTH. To date, most immunoassays utilize early PTH levels based on traditional solubilized ELISA methods that cannot accurately discriminate intact f-PTH from truncated PTH. In addition, the MSIA-SRM assay can be used to measure the abundance of proteins in plasma on the basis of heavy PTH expression using an ion trap mass spectrometer.

Methods

Heavy PTH expression and purification: Full-length (f-PTH) f-PTH was expressed as described previously (1). The PTH expression of the human IVT system was conducted using a Thermo Scientific HiPEP Catalyst Purification Kit under denaturing conditions in a micro-column format and validated in the context of in vivo application.

Heavy PTH Ms analysis: For stable-isotope-labeled protein quantification, proteins were digested with trypsin and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Thermo Scientific LTQ Orbitrap XL mass spectrometer equipped with an ESI linear ion-trap (LTQ) MS and a Q-Exactive HUH MS spectrometer equipped with a hybrid linear ion-trap (LTQ-Orbitrap) MS. Stable isotope-labeled peptides were identified in the context of in vivo application.

Results

Previously, we developed the stable-isotope-labeled MSIA-SRM assay for PTH that allows for quantification of f-PTH and f-PTH-like peptides by measuring the stable isotope incorporation of common proteins. In this work, we used a novel protocol involving trypsin and the heavy PTH peptide SVSEIQLMHNLGK (z=3) for protein digestion. To quantify the protein expression of PTH, we used a stable-isotope-labeled MSIA-SRM assay with high sensitivity.

Conclusion

• The MSIA-SRM assay can be used to measure physiological PTH concentrations.
• Heavy PTH with heavy isotope incorporation was expressed using a human IVT system.
• Heavy PTH assay allowed for accurate MSIA capture and digestion efficiency and can be used to measure digestion efficiency.

References
