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 spectrometric immunoassay-selective reaction monitoring (MSIA-SRM) method to measure intact and truncated parathyroid hormone (PTH) isoforms in plasma, with a stable-isotope-labeled (heavy) protein standard for control of immunocapture and digestion efficiency.

Methods: Recombinant heavy PTH was expressed using a novel human cell-free, in vitro translation (IVT) system. Sample preparation and MSIA-SRM targeted quantification were as previously described.

Results: Heavy PTH with >95% incorporation was expressed and then used to assess MSIA capture and digestion efficiency.

Introduction

Parathyroid hormone (PTH) assays for monitoring PTH and PTH variants are important for the accurate diagnosis of endocrine and osteological diseases. The heterogeneity of PTH has traditionally been an impediment to the development of assays that distinguish full-length PTH (PTH-1-84) from N-terminally truncated PTH (PTH 7-84 and others). Because intact and truncated forms of PTH vary in biological activity, assays that can accurately quantify the ratio of intact hormone to its fragments are needed to determine the amount of biologically active PTH. To date, most immunoassays used to monitor PTH levels are based on traditional sandwich enzyme-linked immunosorbent assay (ELISA) methods. They cannot accurately discriminate intact PTH from truncated PTH. In addition, these methods typically employ primary antibodies to the N-terminus of the hormone, thereby preventing quantification of any fragments. To monitor the numerous isoforms of PTH, we used a mass spectrometric immunoassay (MSIA) for targeted enrichment and quantification of PTH and PTH variants using selected reaction monitoring (SRM) (Figure 1). In this study, we also expressed and characterized a stable isotope-labeled PTH protein for use as an internal standard for immunocapture and digestion efficiency.

Methods

Heavy PTH expression and purification: Full-length PTH (1-115 AA) was expressed as a C-terminal 6xHis fusion using the Thermo Scientific 1-Step Heavy Protein IVT Kit. Expressed protein was purified using a Thermo Scientific HiPrep Cobalt Purification Kit under denaturing conditions (8M urea in binding buffer) and refolded on the column before imidazole elution.

Heavy PTH MS analysis: For stable isotope incorporation determination, proteins were denatured, reduced, alkylated, digested with trypsin, and desalted. A Thermo Scientific LTQ Orbitrap XL hybrid ion trap-mass spectrometer with electron transfer dissociation (ETD) was used for peptide analysis. MS spectra were searched using Thermo Scientific Proteome Discoverer software version 1.3 with SEQUEST® against a custom PTH FASTA database. Static modifications included carbamidomethylation, lysine-b, arginine-b, and methionine oxidation used as dynamic modifications.

Results

The MSIA-SRM workflow for enrichment and quantification of low abundance proteins is shown in Figure 1. The wide dynamic range of proteins in blood presents a technical hurdle for the development of low-abundance analytes. For PTH, the reference range for PTH is 1.1–6.9 pg/mL or 10–65 pmol/L, making it one of the least abundant clinically important analytes. Anti-PTH antibodies immobilized on pipette tip microcolumns were used for PTH immunocapture and enrichment in conjunction with a Thermo Scientific Versette automated liquid handling (ALH) platform for high-throughput sample processing. Immunocaptured PTH was quantified by SRM mass spectrometry using a Thermo Scientific TSQ Vantage mass spectrometer. Using this workflow for protein-based immunoenrichment, both full-length PTH and PTH isoform variants can be monitored using different targeted peptides (Figure 2).

Conclusion

- The MSIA-SRM assay can be used to measure physiological PTH concentrations.
- Heavy PTH with >95% isotope incorporation was expressed using a human IVT system.
- Heavy PTH was used to assess MSIA capture efficiency and can be used to measure digestion efficiency.
- Research continues on immunocapture and digestion efficiency of heavy and light PTH in neat and serum samples.

References


FIGURE 1. MSIA-SRM workflow for enrichment and quantification of low abundance proteins.

FIGURE 2. (A) PTH protein variant map and (B) peptides chosen for multiplexed SRM assay

FIGURE 3. Recombinant PTH expression, purification, and MS analysis

FIGURE 4. (A) Calibration curve and (B) SRM quantitation of light and heavy PTH peptides