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Method for analyzing posttranslational modifications using GEL IEF and mass spectrometry

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Invention Summary

Method for analyzing a sample possibly containing peptides or modified peptides; useful for biomarker discovery or validation of biomarkers. The method uses isoelectric focusing and mass spectrometry (MS) and enables identification of modified peptides with high resolution and predictability.

Background

The isolation and separation of biomolecules, such as proteins and peptides, has become of an increased interest during the past years. Some biomolecules need to be isolated as a last step of a biotechnological method for the production thereof, for example in the preparation of protein or peptide-based pharmaceutical compounds. Similarly, there is also a need to separate biomolecules for analytical purposes in order to be able to quantify and identify the proteins and/or peptides present in a sample. A wide variety of methods are used for the detection and quantification of the separated proteins and/or peptides. For identification and characterization of separated proteins, mass spectrometry (MS) methods are commonly used as these methods are fast and require very small amounts of proteins and/or peptides.

Posttranslational modifications (PTM) of proteins are very common regulators of their activity, and selectively sorting-out modified proteins from a non-modified protein population may offer means to a deeper understanding of their roles or may be used diagnostically. There are many PTMs; e.g. phosphorylation, glycosylation, alkylation, methylation, prenylation, or ubiquitination. Phosphorylation is the most studied modification and there are estimations that one third of the mammalian proteins may be phosphorylated some time in their life cycle. Capturing of phosphorylated proteins can be achieved using phospho-specific antibodies, or, more commonly, using immobilized metal affinity chromatography with chelated titanium ions (IMAC-Ti4+).

In isoelectric focusing (IEF), the separation takes place in a pH gradient that occupies the whole separation distance and is arranged so that the pH in the gradient increases from anode towards the cathode. While other alternatives also exist, the pH gradients required in isoelectric focusing are in practice generated in two different ways: with the aid of a solution of carrier ampholytes or with an immobilized pH gradient. IEF with carrier ampholytes usually gives less good resolution as there is a pH-drift in the generated pH field, in particular when the electrophoresis proceeds over long time.

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A common problem with isoelectric focusing of proteins and/or peptides is that the focused proteins/peptides are unevenly distributed in the gel with poor resolution and there is a need for improved methods and gels or strips for isoelectric focusing to enable reproducible isolation and enrichment of PTM peptides.

Technology

The invention relates to a novel method to separate and enrich post translationally modified (PTM) peptides with both high resolution and predictability. The method for assaying a digested protein sample, consists of:

a) running sample on an isoelectric focusing gel with a pH gradient to separate peptides in the sample;

b) fractionating said gel into smaller pieces;

c) extracting peptides from the fractionated gel pieces;

d) running mass spectroscopy (MS) on the extracted peptides from selected fractions; where the method comprises a step of:

f) identifying peptides and any possible post translational modification (PTM) of the peptides from step d) by identifying the modification degree of the peptides, i.e. the number of modifications per peptide), and/or the position of the modification on the peptide.

Depending on the PTM to be analyzed, a defined pH-range is selected and used in combination with a focusing gel of suitable length to give sufficient resolution and fractionation into an appropriate number of fractions.