Selective injection and fractionation of phosphopeptides by capillary electrophoresis for MALDI and ESI mass spectrometry

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Methods

Instrumentation
• Agilent 2100CE Capillary Electrophoresis System, Micromass Q-TOF Global Ultima mass spectrometer, and Bruker Daltonics Reflex IV MALDI TOF mass spectrometer.

Materials
• Fused silica capillaries with an OD 360µm and an ID 50 µm were used for all experiments. Capillaries for ESI experiments were 48.5 cm in length whereas capillaries for the MALDI experiments were 39 cm long.
• For MALDI, capillaries were treated with a phospholipid, 0.1mM 1,2-Diaryl-3-sn-glycero-phosphate (DLPC) in 20mM Tris-HCl pH 7.4, to eliminate electroosmotic flow (EOF) and prevent sample adsorption.
• For ESI experiments EOF suppression and prevention of sample adsorption were achieved by derivatizing the capillaries with cross-linked polyacrylamide as described previously.

Experimental Challenges

Adaptation of selective sampling technique for MALDI MS to ESI MS
• The phospholipid capillary coating was found to disassemble in the presence of acetonitrile and methanol, which are commonly used in ESI to enhance sample ionization.
• Our first attempt to solve this was to introduce the organic solvent subsequent to the selective injection step, directly upstream of the ESI emitter tip using a sheath flow or a 3-way junction.
• Unfortunately, trace amounts of phospholipids still desorbed from the coating during the run. Although the phospholipid did not cause any problem previously with MALDI MS, it was detected by ESI MS and caused significant suppression of the phosphopeptide signals.
• This problem was finally solved by the use of a covalently bonded cross-linked polyacrylamide coating.
• This coating was stable in the presence of organic solvent so the need for a sheath flow or a 3-way junction was abolished and 25% methanol was incorporated into the running buffer.

Salt adducts
• Throughout this work sodium and potassium adducts on the phosphopeptides of α-casein have caused a major decrease in signal.
• This salt contamination is always most prevalent on the multiply phosphorylated peptides, one can raise the sampling pH, for example to pH 5, to include interactions.

Discussion and Conclusion

• With this technique we have shown how multiply phosphorylated peptides can be selectively extracted and separated from a mixture, like an α-casein digest.
• This allows for the detection of multiply phosphorylated peptides which would otherwise be suppressed due to their poor ionization efficiencies.
• Although ESI MS data is only shown here for multiply phosphorylated peptides, one can raise the sampling pH, for example to pH 5, to include the singly phosphorylated peptides in the analysis.
• Peptides c352 and d652 were detected in low amounts most likely owing to the lower abundance of S2.

References & Acknowledgements


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