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# The study of intact casein as a model system for the separation of intact phosphorylated proteins by CESI-MS

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CESI is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device. CESI-MS operates at low nL/min flow rates offering several advantages over nano LC including increased ionization efficiency and a reduction in ion suppression as well as low sample consumption. In this work, we describe the use of CESI in the study of intact casein in its various forms (alpha, beta and kappa). The objective of this short study was to investigate the use of CESI-MS for the separation of intact phosphoproteins with a view to its application for the top down analysis of this class of intact proteins. To this end, proteins were obtained from Sigma Aldrich, prepared in a variety of different solvents, and injected by pressure onto a positively coated capillary. The CE separation used reverse polarity with a background electrolyte (BGE) consisting of a mixture of volatile acids with water/organic solvent mixtures and the proteins were detected using a QTOF system fitted with a nanospray source. The CESI results were then compared with a standard C4 reverse phase HPLC separation of the same sample on the same MS system fitted with a standard source. The results show that CESI-MS was capable of separating alpha, beta and kappa casein as well as the separation of phosphorylated isoforms of alpha casein. All CESI separations were done at room temperature, produced sharper peaks than the corresponding LC separation, and provided an orthogonal separation to LC. This study will highlight the complementary nature of CESI compared to LCMS and provide a brief insight into the possibilities of using this technology for the study of intact proteins.

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