CE-MS in bottom-up and top-down proteomic and glycomic profiling of ng- and sub-ng-level samples

Kendall Johnson, Anne-Lise Marie and Alexander R. IVANOV

Barnett Institute of Chemical and Biological Analysis, Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Ave., Boston, Massachusetts, 02115, United States

Abstract

Informative qualitative, quantitative and structural proteomic characterization of limited samples (such as, small populations of rare cells, microneedle biopsies, extracellular vesicles (EVs) isolated from minute volumes of physiological fluids or single cells) and especially, profiling of post-translational modifications (PTMs, for example, acetylation, glycosylation and phosphorylation) of such specimens have been a significant challenge because of very low abundance and high heterogeneity in biological matrices. Alterations of proteomes, glycomes, phosphoproteomes and acetylomes may be associated with several pathologies, including immune, cardiovascular, neurological and oncological diseases, as well as other biological phenomena.

In this study, we evaluated a combination of advanced sample preparation, ultra-low flow high-efficiency capillary electrophoresis coupled to mass spectrometry (CE-MS) with and/or without using a high-field asymmetric waveform ion mobility spectrometry (FAIMS) interface in proteomic (both bottom-up and top-down) and glycomic profiling of limited samples to evaluate the potential applicability for high sensitivity, robust and reproducible proteomic profiling of low ng- and sub-ng-level complex biological samples.

We explored several CE-MS methods to enhance the sensitivity and depth of glycomic and proteomic, with a specific interest in acetylomic and phosphoproteomic, profiling of several types of limited biological specimens. Glycomic, as well as bottom-up and top-down CE-MS-based proteomic, profiling strategies were evaluated using cultured human cells, blood isolates and commercial digest standards. The initial characterization of near 1 MDa non-covalent multimeric protein assemblies and related protein-protein and protein-ligand interactions with resulting conformational changes at high sensitivity was performed using native CE-MS of purified protein complexes.

The results acquired in the above-outlined experiments demonstrate the potential applicability of developed CE-MS-based techniques in proteomic, glycomic and native CE-MS studies of individual cells and minute amounts of biological and clinical samples to address unmet needs that answered pressing questions in biology and medicine.