Native capillary electrophoresis-mass spectrometry of near 1 MDa multimeric protein assemblies

Anne-Lise Marie, Florian Georgescu, Kendall Johnson, John R. Engen, Alexander R. Ivanov

Barnett Institute of Chemical and Biological Analysis, Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Avenue, Boston, MA, 02115, United States

Abstract

Robust and sensitive analytical techniques must be developed to effectively investigate protein-ligand and protein-protein interactions under native conditions, where non-covalent interactions are preserved. Here, we report a capillary electrophoresis-mass spectrometry (CE-MS) method developed to detect, confirm the identity and structurally characterize non-covalently bound multimeric protein assemblies under near-physiological conditions.

The native CE-MS method we developed for the analysis of high molecular mass protein complexes (~1 MDa), using a sheathless interface (CESI 8000 Plus ESI-MS-High-Performance System, SCIEX), positive electrospray ionization mode and high-resolution Orbitrap mass spectrometer (QE-UHMR, Thermo Fisher Scientific), is fast (< 30 min per run), highly sensitive (amole level) and reproducible (RSDs ≤ 0.7% and 6% for migration times and peak intensities, respectively). No buffer exchange of the samples with MS-friendly buffers is required, which preserves the native state of the proteins and protein complexes, minimizes sample consumption and sample loss and simplifies the analytical workflow. The separation performance of the method was demonstrated with successful detection and separation of the tetradecameric (~800 KDa) and heptameric (~400 kDa) forms of GroEL chaperone and oligomeric substrate proteins interacting with GroEL such as NanA (~130 kDa). Protein-ligand and protein-protein interaction studies were performed with GroEL and several binding partners (substrate proteins, GroES co-chaperone and nucleotides). The conformational changes of near 1 MDa GroEL 14-mer protein assembly upon complexation with adenosine tri- and diphosphates (ATPγS or ADP) and GroES induced a characteristic shift of the migration time, resulting in the successful separation of apo-GroEL, GroEL/nucleotide and GroEL/GroES complexes. CE-pseudo-MS3 fragmentation methods were also developed to structurally characterize near 1 MDa multimeric protein assemblies under native conditions and allowed us to confirm the sequence of GroEL wild-type and several in vitro cloned GroEL mutants. Significant differences were observed in the fragmentation patterns of the different types of analyzed GroEL variants in correlation with the introduced sequence alterations.

The CE-MS method we developed represents a new and sensitive approach to investigate interactions between multimeric protein assemblies, a large variety of interacting small molecules and high molecular mass species under near-physiological conditions. This method can provide much-needed information on the binding stoichiometry, kinetics, and structures of the analyzed protein-ligand and protein-protein complexes.