PMM - 111 Nanoelectrospray Differential Ion Mobility Spectrometry for Protein Sizing and Molecular Mass Determination: Method Development and Validation

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Introduction

The characterization of large biomolecules, including the determination of their size and molecular mass (M_r), is important for understanding fundamental aspects of their biochemical behavior. Even though MS has dominated this type of analysis, another similar technique based on nanoelectrospray ion mobility spectrometry, referred to as gas phase electrophoretic mobility molecule analysis (GEMMA) has received less attention, even though it has shown several benefits. The objective of this study was to evaluate GEMMA for its capability to determine the size and M_r of proteins and protein complexes, and to compare it with several established techniques such as quasi elastic light scattering (QELS), multiangle laser light scattering (MALLS), MALDI-MS and nES-MS, in terms of sensitivity, accuracy, reproducibility and other measurement characteristics.

Method

nES-IMS with condensation particle counter (CPC) detection was used in this study. This consisted of a nES source equipped with a neutralizing chamber, the differential ion mobility analyzer which is commercially named macroIMS, and a butanol-based CPC. MALLS and QELS were performed on instruments from Wyatt coupled on line to an HPLC system running size exclusion chromatography. nES-MS measurements were performed on an LTQ-Orbitrap instrument. Several commercially available proteins were analysed, along with several proteins that have been previously isolated and purified by the Economou group. These involve proteins of various sizes that are monomeric or comprise non covalent oligomers of various shapes and with sizes, ranging from 25,000 - 450,000 Da.

Preliminary Data

GEMMA was used to determine the size and M_r for approximately 15 proteins. This data was compared with relevant literature data and data obtained by using the previously mentioned complimentary techniques (QELS, MALLS, MALDI-MS and nES-MS). The purpose of this was to evaluate GEMMA's detection sensitivity, mass and size accuracy, and reproducibility. Analyte concentration is a critical parameter for achieving good size and mass accuracy when using GEMMA, and thus its affect on protein aggregation was studied in detail. In addition, GEMMA's capacity to accommodate a diverse range of solvents and buffers for protein analyses was also evaluated. Other analytical figures of merit including measurement time, total analysis time, amount of protein required for the measurement, amount of protein consumed during the measurement will also be reported on. Emphasis has been given to the development of an approach that allows for distinguishing between the presence of protein multimers/aggregates in solution and their formation as artifacts during the GEMMA measurement. The mechanism of artifact formation is discussed.

Novel Aspects

Sizing and M_r determination of proteins and their complexes. Comparison of their structural behaviour in the gas-phase with hydrodynamic analysis.