Resolving Protein Conformational States With Hybrid Trapped Ion Mobility Spectrometry-High Resolution Mass Spectrometry

Robert L.C. Voeten[1,2], Rob Haselberg[1], Govert W. Somsen[1]

[1] Division of BioAnalytical Chemistry, Amsterdam Institute of Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands,
[2] TI-COAST, Science Park 904, 1098 XH Amsterdam, The Netherlands

Presentation type: Oral/poster
Position: PhD candidate

Abstract

Like most macromolecules, proteins exhibit complex structural dynamics. Activity, binding and other biological properties of proteins heavily depend on their conformational state. Information on (changes in) protein conformation is essential to understand biological function. In this work, we exploited the feasibility of gas-phase trapped ion-mobility spectrometry (TIMS) in combination with high-resolution time-of-flight mass spectrometry (TOFMS) for the detection and resolution of protein conformational states.
Bovine carbonic anhydrase II (BCA) was selected as a model protein; it exhibits acid-induced unfolding. BCA was dissolved (8 µM) in solutions of different pH, buffer composition, ionic strength and organic solvent percentage and were analyzed by ESI-MS and the obtained charge-state distributions (CSDs) were studied. A trimodal CSD marked the transition point of pH-induced unfolding, and ammonium formate and increasing ionic strength appeared to stabilize the holo-state of BCA. Introducing TIMS prior to TOFMS allowed ion-mobility analysis of single charge states of BCA throughout the CSDs obtained under the different conditions. The trapping time, electric potentials and the electric field gradient were optimized to maintain protein integrity. From the TIMS data, collision cross sections (CCSs) of the BCA species could be calculated, which indicated coulombic-induced stretching of BCA with increasing charge. Interestingly, EIMs of single charge states showed multiple bands, revealing the coexistence of different conformational states of BCA under certain conditions. By comparing the EIMs of the same BCA charge state obtained under conditions of decreasing pH, a conformational transition reflecting protein unfolding in solution was disclosed. Furthermore, TIMS-TOFMS showed that the BCA holo-apo transition is accompanied by an equilibrium shift of the observed populations. It also indicates the existence of native-like conformations at increased charge states.
In conclusion, TIMS allows probing of protein conformational states as well as separation and evaluation of these species, providing detailed insights into the pH-induced unfolding of BCA.

Acknowledgement
This research was carried out within the HOSAna project which is funded by the Netherlands Organization for Scientific Research (NWO) in the framework of the Programmatic Technology Area PTA-COAST4 and is supported by Bruker, DSM and Postnova Analytics (Project number 053.21.117).