INVESTIGATING A LARGE HUMAN PROTEIN ASSEMBLY BY NATIVE MASS SPECTROMETRY: THE SAGA COMPLEX

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Introduction

In recent years native MS has emerged as a remarkable tool for investigating non-covalent protein complexes. By native MS the mass of intact protein assemblies, their stoichiometry, interactions between subunits, the position of subunits within subcomplexes (core and peripheral subunits) and the pathway of assembly can be determined[1]. Native MS can be coupled with ion mobility (IM-MS), whereby the topology and shape of complexes and subcomplexes can also be studied. Overall, native MS and IM-MS are gaining more relevance as methods for studying the structure of protein complexes[2].

Spt–Ada–Gcn5–acetyltransferase (SAGA) is a protein assembly formed by 18 distinct subunits, whose total mass is of 1284.1 kDa[3]. It acetylates and deubiquitinates histone residues, thereby introducing modifications that are essential for a regulated gene expression. The SAGA complex plays crucial roles not only in the control of histone function, but also in transcriptional activation, transcriptional elongation, and mRNA export. Despite the importance of this multifunctional nanomachine, its structure and function are only poorly understood. Using native MS, IM-MS, and analytical ultracentrifugation we investigate the human SAGA complex to obtain a complete two-dimensional map of its inter-subunit interactions. We also aim to determine the shape and topology of human SAGA subcomplexes in solution and in the gas phase.

Preliminary data

We started our investigation of human SAGA complex by analyzing its histone acetyl transferase (HAT) submodule. This is a tetrameric subcomplex composed of the proteins GCN5 (93.9 kDa), ADA2b (48.5 kDa), ADA3 (48.9 kDa) and SGF29 (33.2 kDa). It was expressed in insect cells[4], purified by affinity and gel filtration chromatography and analyzed by native MS. Signals with masses corresponding to the intact tetramer (224.5 kDa), the ADA2b/ADA3/SGF29 trimer (130.6 kDa), the GCN5/ADA2b dimer (142.3 kDa) and the ADA3/SGF29 dimer (81.7 kDa) were detected. These findings confirmed not only the stoichiometry but also the interaction network. For instance, the data indicate that GCN5 interacts with ADA2b, and rule out a significant interaction with SGF29. To further verify the position of ADA2b and ADA3 which are isobaric, we carried out experiments using a truncated form of ADA3 (39.3 kDa). This preliminary study demonstrates that the analysis of SAGA subcomplexes by native MS is both feasible and highly informative.

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