May post-translational succination be involved in cardiac arrhythmia? A joint study between (ion mobility) mass spectrometry and molecular dynamics

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Introduction:

In the Krebs cycle, the conversion of fumarate into malate by the addition of a water molecule is catalyzed by fumarate hydratase. But in some diseases, there is a germline mutation of the fumarase gene resulting in an enymatic deficiency and therefore an accumulation of fumarate (1). This molecule can undergo a Michael addition with proteins bearing free thiol functions (free cysteines). This physiologically irreversible reaction is called succination (Figure 1). The products, called 2-succinylcysteine (2SC) residues, are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed (2).

In this study, a Mass Spectrometry (MS)-based approach was selected as a potential alternative to current assays to highlight 2-succinocysteine. One of the targeted proteins of this study is SUMO1 (Small Ubiquitin-like modifier 1). This protein is involved in the stabilization of some proteins, including SERCA2A, a protein involved in Ca²⁺ regulation during cardiac contraction (Figure 2) (3). Thus, our main objective is to evaluate by Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) and molecular dynamics whether the succination of SUMO1 has an impact on its 3D structure and therefore, whether it has an impact on its function (SUMOylation of SERCA2A) and induces cardiac arrhythmia.

Is succination a spontaneous process?

The first step in this study was to prove that the succination reaction can be spontaneous. Indeed, although this has been suggested in the literature [4], there is no direct evidence. To do so, experiments between Glutathione (GSH) and diethyl fumarate (DEF) or fumaric acid were performed in vitro in ammonium acetate solution (pH 7 and 37°C). The reaction mixture was qualitatively studied by (LC)-mass spectrometry at different times to highlight the efficiency of the reaction and obtain kinetic data.

Kinetic studies

The kinetic study showed a Michael-type behavior of the reaction between GSH and DEF (Figure 5) with an order of reaction of 1 for DEF and 0.93 for GSH. The reaction reached its maximum conversion in 24h with DEF and 50% conversion in 5h with fumaric acid.

What about SUMO1?

The native mass spectrum of SUMO1 on SYNAPT G2-Si (positive mode) was investigated at 37°C (pH 7) for 24 hours with 100 mM concentrations of diethyl fumarate. The blue-shifted signals correspond to the SUMO1 protein, and the baseline-corrected peaks represent the succinated SUMO1. The mass difference between these signals corresponds to the mass of DEF divided by the charge.

Conclusions and perspectives

• The succination reaction is a spontaneous process (GSH and SUMO1).
• The difference in CCS between SUMO1 and SUMO1+DEF corresponds to 20 Å (theory and experiment).
• Good fit between experimental and theoretical CCS for ubiquitin 6+ (3% error).

What to do next?

• Improve the theoretical workflow for SUMO1 (10% error).
• Study structural modifications in solution by circular dichroism.
• Study the impact of the SUMO1 succination on the interaction with SERCA2a.

Theoretical studies

To get structural information, the theoretical methodology was optimized (on a well-known model: human ubiquitin 6+) to simulate as correctly as possible the protein ion mobility in the experiments and then to calculate theoretical CCS to compare to experimental results. The SUMO1 structure was uploaded from the protein data bank (pdb). Molecular dynamics (MD) simulations were performed to get structural information at the atomic level (1 μs in explicit solvent + 2 μs in vacuum) (Figure 7). Simulations were carried out with the AMBER suite of programs, using the ff15SB force-field for the protein, G4PF2 for the DEF moiety and the TI3P model for water. Conformations are extracted from MD and injected into the CollideScope program to compute theoretical CCS (CCSₜ) through the Trajectory Method (TM) (5). This method is currently the most accurate to compute CCS and compare them to the CCSₜ.

CCS measurement

CCS were measured on a Waters SYNAPT G2-Si (Travelling Wave Ion Mobility Cell, TWIMX) by using our CCS calibration based on polymer CCS measured on a linear drift tube in He (6).

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