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 enzymatic 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-succinocysteine (2SC) residues are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed [2].

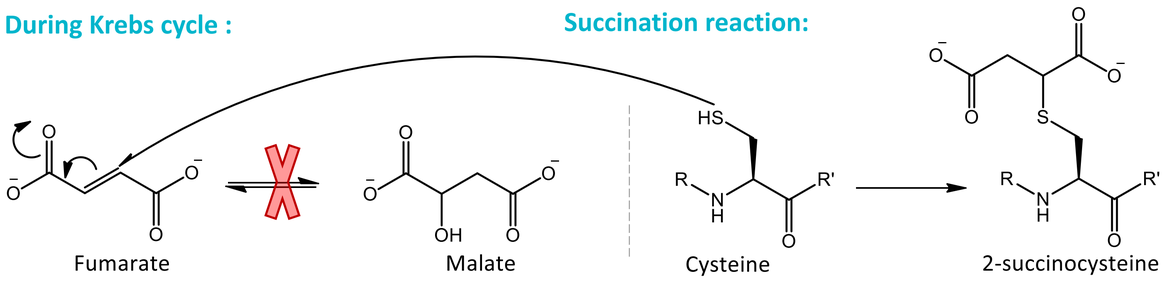

Figure 1: On the left, the deficiency of the hydration reaction catalyzed by the fumarate hydratase leads to an accumulation of fumarate. On the right the succination reaction between cysteine and the fumarate.

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 Ca^{2+} 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.

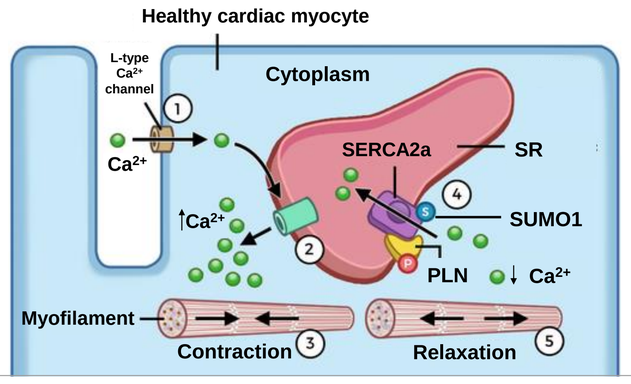

Figure 2: Scheme of SERCA2a function in the sarcoplasmic reticulum (SR) of cardiac myocytes. Upon a signal from a neurotransmitter, the SR releases Ca^{2+} ions into the cytoplasm, causing the heart to contract. These ions are then taken back by SERCA2a, a calcium-transporting ATPase, which allows the heart to relax. Figure adapted from Labster Theory website.

Is succination a spontaneous process?

Liquid chromatography coupled to mass spectrometry

(WATERS QToF Ultima API-US)

GOALS of the study

How evaluate the 3D structure of proteins?

Experimental : Ion mobility mass spectrometry
(WATERS SYNAPT G2-Si)

Theoretical studies

Comparison between theoretical and experimental Collisional Cross Section (CCS)

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.

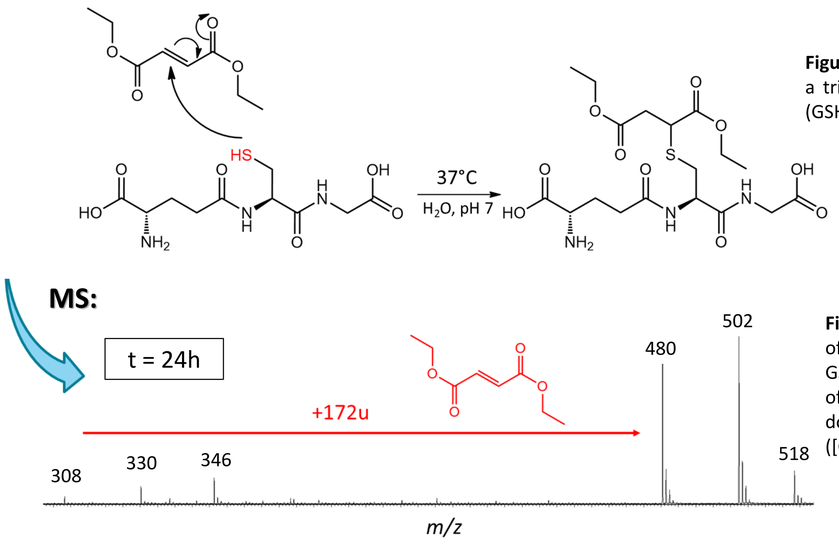

Figure 3: Succination reaction between a tripeptide containing a thiol function (GSH) and DEF.

Figure 4: Evaluation of the spontaneity of the succination reaction between GSH and DEF [1:1]. After 24 h, signals of succinated GSH ($[GSH+DEF+X]^+$) dominate those of unsuccinated GSH ($[GSH+X]^+$), with X = H, Na and K.

Kinetic studies

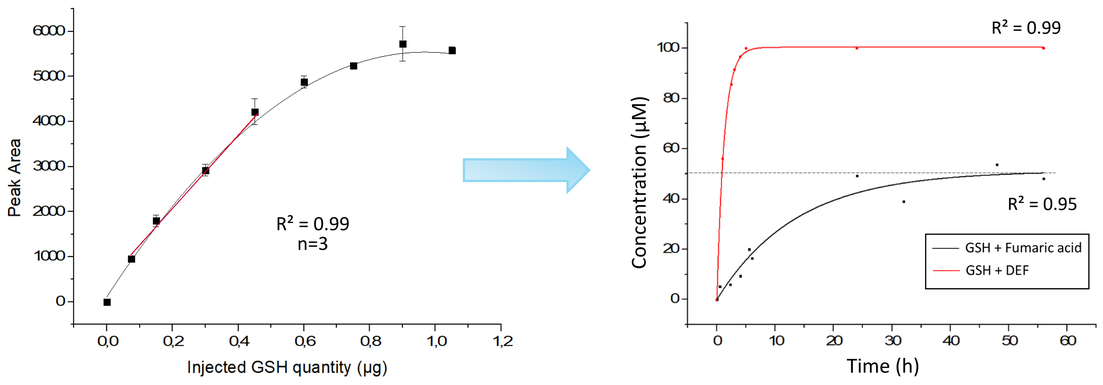

Figure 5: On the left: Evaluation of the Waters QToF API-US dynamic range for GSH. The linearity is preserved from 0 to 0.5 µg injected. On the right: Kinetic studies of the succination reaction with DEF and fumaric acid at a 1:5 ratio. The reaction reaches 100% conversion in 24h with DEF and 50% conversion in 50h with fumaric acid.

What about SUMO1?

Figure 6: Native mass spectrometry of SUMO1 on SYNAPT G2-*Si* (positive mode). SUMO1 was incubated at 37°C (pH 7) for 24 hours with 100 molar equivalents of diethyl fumarate. The black-labeled signals correspond to the **SUMO1 protein**, and the blue-labeled ones represent the **succinated SUMO1**. The mass difference between these signals corresponds to the mass of DEF divided by the charge.

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 structure in Ion Mobility 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 water solvent + 2 µs in vacuum) (**Figure 7**). Simulations were carried out with the AMBER suite of programs, using the ff14SB force-field for the protein, GAFF2 for the DEF moiety and the TIP3P model for water. Conformations are extracted from MD and injected into the Collidoscope program to compute theoretical CCS (CCS_{th}) through the Trajectory Method (TM) [5]. This method is currently the most accurate to compute CCS and compare them to the CCS_{exp}.

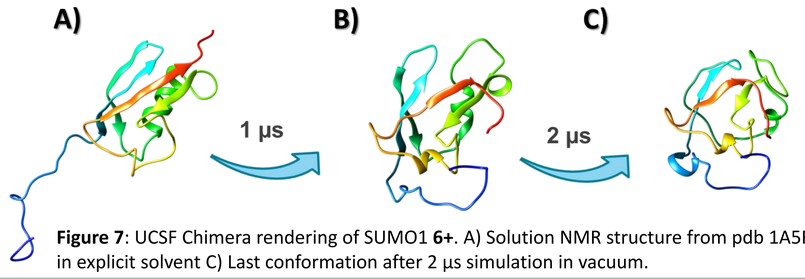

Figure 7: UCSF Chimera rendering of SUMO1 **6+**. A) Solution NMR structure from pdb 1A5R, B) Last conformation after 1 µs simulation in explicit solvent C) Last conformation after 2 µs simulation in vacuum.

Theoretical CCS:

Ubiquitin 6+ : **1018 ± 3 Å²**
SUMO1 6+: **1248 ± 20 Å²**
SUMO1 + DEF 6+: **1267 ± 29 Å²**

Experimental : Conformational studies (CIU)

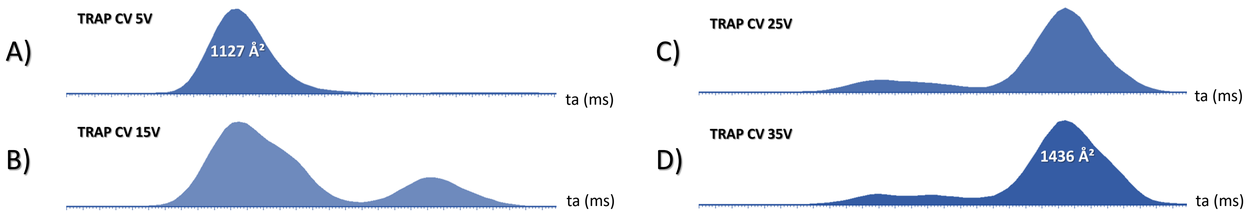

Figure 8: CIU (collisional induced unfolding) study on SUMO1 (6+). A) The mobilogram corresponds to the native structure. B),C) When we raise the TRAP CV we activate the protein and produce new conformations corresponding to CIU. D) The conformation obtained corresponds to the unfold protein.

CCS measurement

CCS were measured on a Waters Synapt G2-S*i* (Travelling Wave Ion Mobility Cell, TWIMS) by using our CCS calibration based on polymer CCS measured on a linear drift tube in He [6]:

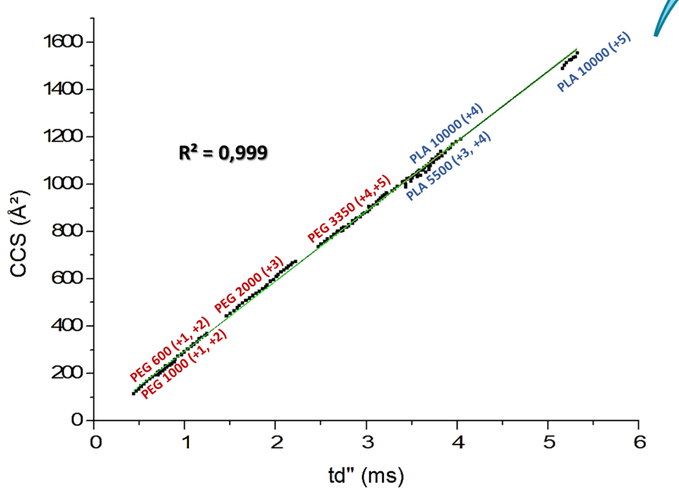

Figure 9: Calibration curve realized with different polymers at different charge states based on the works of Duez *et al.* [6] This curve allows us to interpolate a CCS based on a measured/calculated t_d'' for SUMO1.

Mason – Schamp equation adapted for TWIMS:

$$\Omega = \frac{\sqrt{18\pi}}{16}\frac{ze}{\sqrt{k_B T}}\sqrt{\frac{1}{m_I}+\frac{1}{m_N}}\frac{760}{P}\frac{T}{273{,}2}\frac{1}{N}\,A\,t_D^B$$

Ω_{ref} = A' td'' = **295,4 t_d''**

Experimental CCS:

Ubiquitin 6+: **983 ± 4 Å²**
SUMO1 6+: **1127 Å²**
SUMO1 + DEF 6+: **1147 Å²**

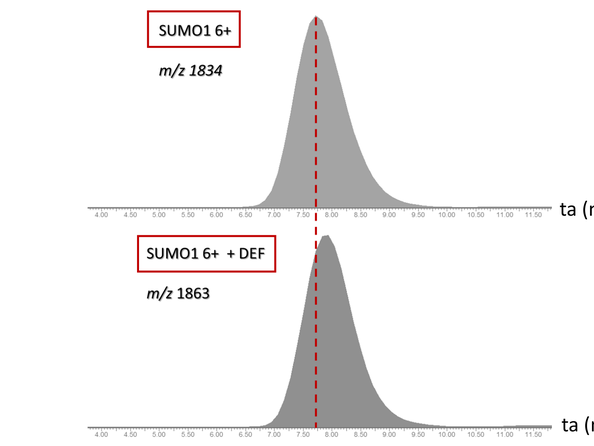

Figure 10: Arrival times corresponding to SUMO1 (6+) and SUMO+DEF (6+) under native conditions (previously tested on ubiquitin to confirm the validity of the calibration method). We observe a slight difference between these ATD (around 0,2 ms) which corresponds to a difference of 20 Å².

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 Å²*** (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

Acknowledgments

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