On the Impact of Post-Translational Succination on SUMO1 Structure: A Dual Approach Combining Gas-Phase and Solution Analysis

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Overview

- ***Purpose*:** SUMO1 is a key modifier of cardiac proteins and may undergo succination via its reactive cysteine residue under conditions of metabolic stress. This study explores the structural and functional implications of this post-translational modification using a multidisciplinary analytical approach.
- ***Methods:*** ESI-(IMS)MS, HDX-MS, Circular dichroism, and Molecular Dynamics (MD) simulations.
- ***Results:*** SUMO1 undergoes succination *in vitro*, leading to significant conformational changes. These findings provide insights into the susceptibility of SUMO1 to metabolic alterations and its potential role in cardiac dysfunction.

Introduction

SUMOylation is a post-translational modification (PTM) where Small Ubiquitin-like MOdifier (SUMO) proteins are covalently attached to lysine residues on target proteins. [1] It regulates a wide range of cellular processes and affects over a thousand known proteins. [2] In the heart, **SUMO1** plays a key role by modulating SERCA2a, a calcium pump crucial for proper muscle relaxation. In failing hearts, both SUMO1 levels and SERCA2a SUMOylation are reduced, leading to impaired cardiac function. Restoring SUMO1 expression has been shown to improve SERCA2a activity and overall heart performance. [3] Another important PTM is **succination**, occurring when **fumarate**, a metabolite that can accumulate under metabolic stress or due to fumarase deficiency (TCA cycle), reacts irreversibly with cysteine residues to form 2-(S-succino)cysteine (**2SC**) (see ***Figure 1***). [4] SUMO1, which contains a reactive cysteine in its α-helix, becomes a potential target for succination. If SUMO1 is succinated, its ability to support SERCA2a SUMOylation may be greatly reduced, thereby disrupting calcium regulation in cardiomyocytes. This raises the hypothesis that metabolic stress-induced succination of SUMO1 may contribute to cardiac dysfunction, linking metabolism, protein modification, and heart health.

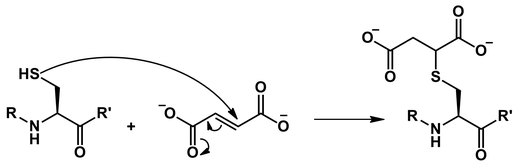


*Figure 1: Irreversible succination reaction between cysteine residue and fumarate through a Michael addition leading to 2-(S-succino)cysteine (**2SC**).*

Methods

Native IMMS experiments were performed using a Synapt G2-S*i* (ESI-TWIMS-MS). 50 mM Ammonium acetate solutions containing 5 µM SUMO1 or 2SC-SUMO1 (~11,000 g.mol^{-1}) were electrosprayed with a flow of 5 µL.min^{-1}. CCS ($^{TW}CCS_{N2\rightarrow He}$) were determined using the polymer calibration. [5]

MD simulations were performed using the AMBER suite with ff19SB (protein), GAFF2 (DEF moiety), and the OPC water model. The final 500 MD conformations were analyzed with Collidoscope using the Trajectory Method to calculate theoretical CCS ($^{TM}CCS_{He}$). [6] Solvent-accessible surface area (SASA) and molecular volume (V_{SASA}) were averaged over the final 10 frames using Materials Studio 2022.

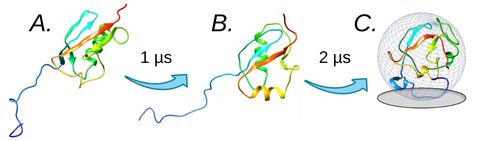


Figure 2: (A) Solution NMR structure from PDB 1A5R, (B) Last conformation after 1 µs simulation in explicit water solvent, (C) Last conformation after 2 µs of simulation in vacuum (µ = 1). CCS were calculated on these gas-phase conformations.

Results

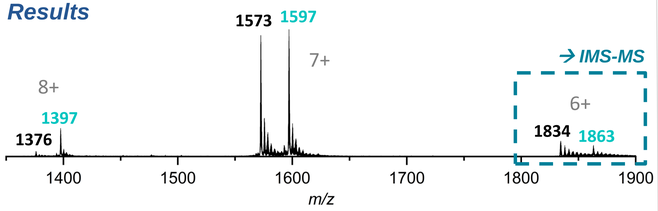
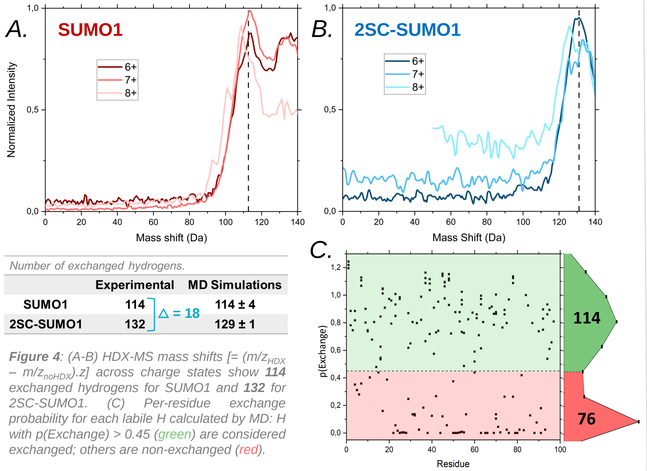


*Figure 3: ESI(+) MS of the SUMO1 (5 µM) / DEF in 50 mM AA after 24 hours at 37 °C. **Black labels** correspond to SUMO1 and **blue ones** to 2SC-SUMO1. This spectrum demonstrates the spontaneous in vitro succination.*

Solution-phase study: HDX-MS



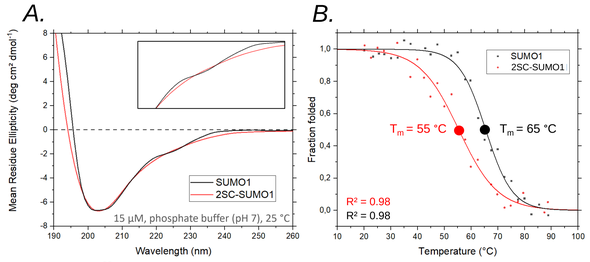
Number of exchanged hydrogens.

	Experimental	MD Simulations
SUMO1	114	114 ± 4
2SC-SUMO1	132	129 ± 1

△ = 18

*Figure 4: (A-B) HDX-MS mass shifts [= (m/z_{HDX} – m/z_{noHDX}).z] across charge states show **114** exchanged hydrogens for SUMO1 and **132** for 2SC-SUMO1. (C) Per-residue exchange probability for each labile H calculated by MD: H with p(Exchange) > 0.45 (green) are considered exchanged; others are non-exchanged (red).*

Solution-phase study: Circular dichroism



C.

Protein	T_m (°C)	Unfolding free energy (kcal.mol^{-1})
SUMO1	**65.4 ± 0.7**	**3.79**
2SC-SUMO1	**55.1 ± 1.2**	**1.66**

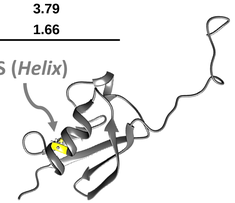


Figure 5: (A) UV CD spectra indicate overall tertiary structure conservation upon SUMO1 succination. Inset: zoom on the 225 nm shoulder (α-helix region). (B) Thermal melts of SUMO1 and 2SC-SUMO1, showing normalized ellipticity at 225 nm vs. temperature. (C) melting temperatures (T_m) and unfolding free energy (at 38 °C) for both variants.

*Molecular dynamics parameters calculated for SUMO1 and 2SC-SUMO1 **in solution** after 1 µs.*

	CYS distance from center-of-mass (Å)	Average Number of H-Bonds	Radius of gyration (Å)	Solution SASA (Å²)
SUMO1	**10.8 ± 1.3**	**34 ± 2**	**14.7 ± 0.5**	**7,517 ± 185**
2SC-SUMO1	**13.8 ± 0.1**	**29 ± 0**	**16.7 ± 0.3**	**7,750 ± 215**

Solution studies: SUMO1 succination leads to significant conformational and stability changes!

Gas-Phase study: IMS-MS

	$^{TW}CCS_{N2\rightarrow He}$ (Å²)	$^{TM}CCS_{He}$ (Å²)	Gas-Phase SASA (Å²)
SUMO1	**1,124 ± 5**	**1,170 ± 5**	**4,702 ± 16**
2SC-SUMO1	**1,143 ± 5**	**1,196 ± 5**	**4,755 ± 22**

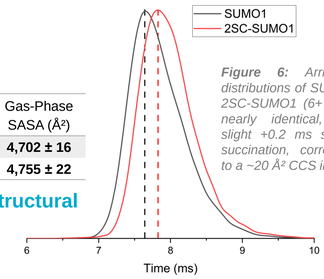


Figure 6: Arrival time distributions of SUMO1 and 2SC-SUMO1 (6+ ions) are nearly identical, with a slight +0.2 ms shift upon succination, corresponding to a ~20 Å² CCS increase.

Gas-phase study: No structural changes?

$CCS = \pi.R_{CCS}^2$

$SASA = S_{CCS} = 4.\pi.R_{CCS}^2$

	S_{CCS} (Å²)	V_{CCS} (Å³)	ρ_{CCS} (g.cm^{-3})	V_{SASA} (Å³)	ρ_{SASA} (g.cm^{-3})
Gas Phase	**4,496**	**28,347**	**0.64**	**19,326 ± 11**	**0.95**
Solution Phase	*I*	*I*	*I*	**22,964 ± 55**	**0.80**

Gas-phase compaction masks structural modifications!

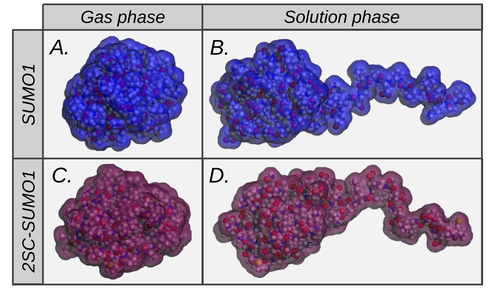


Figure 7: Final MD conformations in the gas phase (A, C) and in solution (B, D). Blue structures: wild-type SUMO1 (6+ in the gas phase, 4- in solution); purple structures: 2SC-SUMO1 (6+ in the gas phase, 4- in solution). Gas-phase ions adopt compact, globular shapes due to the charge solvation effect, resulting in reduced SASA.

Conclusions

Using both solution and gas-phase approaches supported by MD simulations, we found that succination induces SUMO1 unfolding, increasing its solvent accessibility, and altering cysteine orientation. These changes disrupt the tertiary structure without major loss of secondary structure. Although structural differences are visible in solution, they are masked in the gas phase due to the charge solvation effect. The altered conformation may impair SUMO1 role in cardiomyocytes, suggesting that fumarate-driven succination could contribute to cardiotoxicity.

References

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Acknowledgments

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