May post-translational succination be involved in cardiac arrhythmia? A joint experimental and theoretical study combining CIU and molecular dynamics approaches

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During the Krebs cycle, the fumarate is converted into malate by the addition of a molecule of water 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 that present free thiol moiety (free cysteines), and this physiologically irreversible reaction is called succination. 2-succinocystein molecules are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed [2]. An immunoassay highlighting the reaction products of succination is available. However, no information about the spontaneity and the kinetics of these reactions can be deducted and their efficiency remains relatively controversial in the literature [3]. In this study, an MS-based approach was selected as a potential alternative to the current assay. Indeed, using this technique could increase the knowledge about succination by determining the reaction spontaneity and the maximal number of moieties that can be grafted into the target. During this study, the targeted protein is SUMO1 (Small Ubiquitin like MOdifier 1). SUMO1 is used in the stabilization of some target proteins, including SERCA2, a protein involved in Ca^{2+} regulation during cardiac contraction in healthy cardiac myocytes [4]. The objective of this work is to determine if succination may modify the structure of SUMO1 and therefore impact the cardiac contraction process. For this study, experimental and theoretical investigations are considered. For the experimental investigations, Ion Mobility Spectrometry Mass Spectrometry (IMS-MS) is used to determine the Collisional Cross Section (CCS) of protein ions. By measuring the CCS before and after succination, the impact of this side reaction on the 3D structure can be evaluated. However, IMS-derived CCS values alone often yield insufficient information to define the structures of proteins in detail. Therefore, to visualize the 3D structure modifications, molecular dynamics have been realized. Recently, to deepen our investigation, Collision Induced Unfolding (CIU) was implemented in our laboratory [5]. The principal aspect of this technique assesses stability changes before and after succination. This is characterized by the CIU_{50}, the inflection point of the fitted sigmoid. And in this case, if the succination affects stability, this point would undergo a shift in its position.

References

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