GLYCOSYLATION OF LAMP2A: STRUCTURAL AND FUNCTIONAL INSIGHTS

Project Report (CHY597)

Submitted in partial fulfillment of the requirement for the degree of

Integrated Bsc – Msc (Research) Chemistry

By

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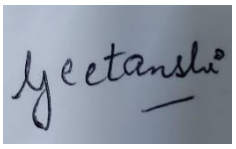
DECLARATION

This is to declare that the mid-term report entitled “Glycosylation of LAMP2A:Structural and Functional Insights” submitted by me in partial fulfillment for the award of the degree of Integrated Bsc -Msc (Research) in Chemistry is a record of work carried out by me at Shiv Nadar Institution of Eminence Deemed to be University, Delhi NCR. The work, in whole or in parts, has not been submitted to any other University/Institute for the award of any other degree. Any information/material used in the thesis from external sources has been appropriately acknowledged.



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CERTIFICATE

This is to certify that the mid-term report entitled "Glycosylation of LAMP2A:Structural and Functional Insights" submitted by Geetanshi (2110111229) to Shiv Nadar Institution of Eminence Deemed to be University, Delhi NCR, for the award of the degree of Integrated Bsc-Msc (Research) in Chemistry is a Bonafide record of research work carried out by her under my supervision. The contents of this report, in whole or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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ABSTRACT

Lysosome-associated membrane protein type 2A (LAMP2A) plays a vital role in chaperone-mediated autophagy (CMA), a cellular degradation pathway important for maintaining cellular homeostasis. Dysregulation of CMA results in various pathologies, including neurodegenerative diseases and cancer. The CMA functionality is intricately linked to LAMP2A protein's structure and dynamics. However, due to the membranous nature of the protein, its experimental characterization is challenging. Additionally, its heavy glycosylation imposes further constraints, making structural and functional studies more difficult. Glycosylation of LAMP2A is known to affect the structural integrity, stability, and functionality of the protein but the mechanism is uncertain. The current study is aimed to utilize computational resources to model the full-length glycosylated structure of LAMP2A in a biologically relevant lysosomal membrane environment and understand its conformational dynamics. Two independent MD simulations each of glycosylated and non-glycosylated protein, totalling to ~6μs of the data, suggests that the glycosylation induces both structural and dynamical changes in protein. It increases the distance between transmembrane domain of the protein and C-domain in the luminal region. Further, the flexibility of the linker region between N- and C- luminal domains were diminished in the glycosylated protein, likely to the presence of O-glycans in the region. Overall, the analysis can broaden our understanding of how glycosylation modulates LAMP-2A activity, thus contributing to a deeper understanding of CMA regulation.

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INTRODUCTION

Autophagy is a crucial cellular process. It preserves homeostasis by segregating cytoplasmic parts including ruined organelles and incorrectly folded proteins (called substrates) into lysosomes. The process is broadly divided into three types: Macro autophagy, Microautophagy and Chaperone-Mediated Autophagy (CMA). CMA is a highly selective pathway. It is essential in cellular stress response, protein quality control, and metabolic regulation. Unlike other autophagy pathways, CMA depends on recognizing a specific pentapeptide motif (KFERQ-like sequence) on the substrate protein by heat shock cognate protein 70 (HSC70) and coactivators. The target substrate is then delivered directly to the lysosomal membrane with the help of translocation receptor viz lysosome-associated membrane protein type 2A (LAMP2A)[1].

LAMP2A is a single transmembrane protein whose dynamic regulation is essential in CMA function. Its role extends from substrate recognition to the formation of translocation complexes that require protein oligomerization and interaction with other lysosomal components (Figure 1). Post-translational modifications, especially glycosylation, have been suggested to affect the stability and activity of LAMP2A, but the details of the mechanism remain unclear. Molecular dynamics simulations and computational analysis holds the potential to investigate the effect of glycosylation on LAMP2A structure and its role in regulating CMA activity. By exploring the structure-function relationship of LAMP2A, this study aims to improve our understanding of CMA regulation and its implications in health and disease.

Glycosylation plays a pivotal role in bolstering the stability of LAMP2A. It also plays a part in preventing potential protein damage and shielding the protein from harsh environmental of lysosome against proteolytic enzymes[2]. It extends the receptor's functional lifespan. It also enhances its efficiency. Specifically, it does this as a CMA receptor. Thus, glycosylation contributes to the stability of LAMP2A[3]. It is speculated to do so by keeping the integrity of extracellular loops. These loops are of considerable importance for substrate binding and recognition. Herein, we investigate the role of glycosylation in maintaining structural stability of LAMP2A.

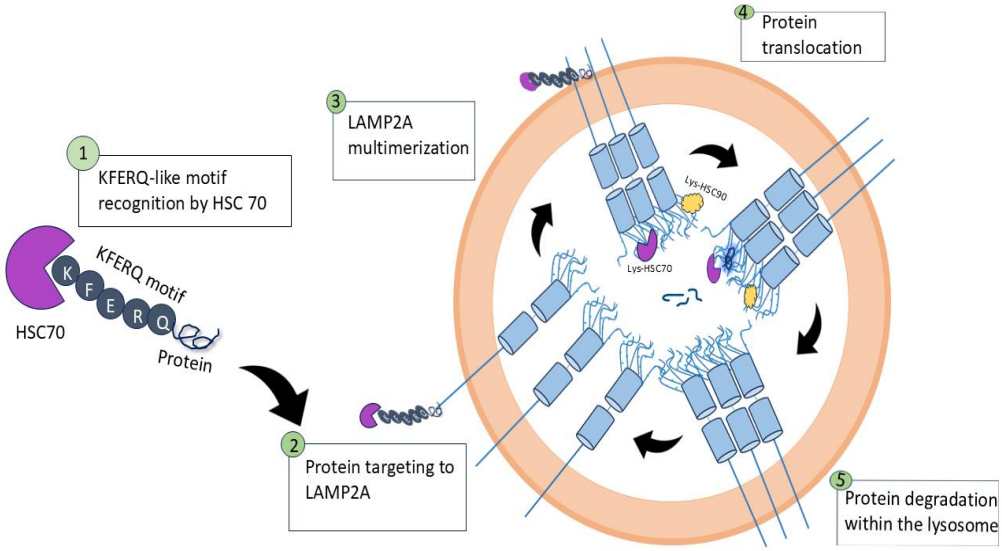

Figure 1: Role of LAMP2A in CMA mechanism.

OBJECTIVES

Objective 01: Structure Modeling of glycosylated LAMP2A in realisticlysosomal membrane

Precisely modeling the construction of LAMP2A is vital. It clarifies this protein's purpose in chaperone-mediated autophagy. The objective is to create a three-dimensional model. This model should be high-resolution. It should include LAMP2A in its glycosylated condition. It needs to represent the protein's natural state accurately.

Glycosylation, a post-translational modification, is crucial. It influences how proteins fold and interact. Thus, including this in the structural model is a must. It should be precise. This model would not be accurate without it.

Objective 02: Dynamics Analysis of the modelled structure

Elucidating the conformational dynamics of LAMP2A is crucial. It brings to light its functional mechanisms in CMA. This objective seeks to explore flexibility, movement, and structural rearrangements of LAMP2A under physiological conditions. All this is achieved through molecular dynamics (MD) simulations.

Objective 03: Effect of Glycosylation

Glycosylation is a post-translational modification. It is pivotal to proper protein folding and function. One of the most well-documented functions of glycosylation is its role in determining the stability and activity of proteins.

This project aims to investigate LAMP2A. It will scrutinize how glycosylation impacts its structural stability. It will observe how it impacts its interactions. It will also consider its overall role in CMA.

METHODOLOGY

The investigation used a series of computational tools to model complete structure of LAMP2A viz AlphaFold database, Modeller, CHARMM GUI, Gromacs and in-house scripts. The two States covered were with and without glycosylation. The amino acid sequence of LAMP2A was sourced from UniProt (ID: P13473). This is where glycosylation sites were noted. Initial structure was taken from AlphaFold database. The loops in the structure were refined using Modeller. Glycan chains were appended to the refined structure. CHARMM-GUI was used for this purpose[4].

System preparation began with the protein being embedded in a lipid bilayer. This bilayer was meant to be representative of the lysosomal membrane (Table 1). After this, the system was solvated in an explicit water box. Ions were also added to simulate physiological conditions.

Table 1: Lipid Composition Used for Simulating the Lysosomal Membrane

Lipid Types	No of lipids in Lower/Upper leaflet
PLPC	22
SAPC	36
SDPC	12
PLPE	14
SAPE	36
SAPI	12
SDPI	4
SAPS	4
SDPS	2
PSM	6
LSM	6
CHOL	36
BMP	14
Total	204

GROMACS was the software used for carrying out MD simulations[5]. The simulation duration range was from 1 to 1000 ns. The purpose was to capture conformation dynamics. The goal was also to evaluate system equilibrium.

MD simulations were analyzed for their trajectories. This was done to evaluate the stability and flexibility of LAMP2A. Both the glycosylated and non-glycosylated forms were studied. Different metrics were used for this analysis. Some of them were the Root Mean Square Deviation (RMSD). Root Mean Square fluctuation (RMSF) and the radius of gyration were also used.

Assessment of structural stability and flexibility was another goal. Visualization tools were used for this. Tools used included Vmd and ChimeraX. They were used to explore specific conformational changes and interaction patterns.

RESULTS AND DISSCUSION

Structural Modelling of LAMP2A in lysosomal membrane

The complete structure of LAMP2A is not resolved experimentally till date. Figure 2(I) depicts the domain architecture of LAMP2A along with the sections of the protein experimentally resolved in different species. We have used AlphaFold-predicted structure from AlphaFold database as a starting structure. The loop refinement was further carried out using Modeller. Figure 2(II) depicts the structure after refinement. Thereafter the structure was embedded in a realistic composition of mammalian lysosomal membrane containing 13 different lipid types and 204 total lipids. The model has been further analysed to understand key structural features, including the impact of glycosylation and conformational flexibility, which are critical for its functionality. This structure is a foundation for further studies on the allosteric regulation and interaction of LAMP2A within the CMA pathway.

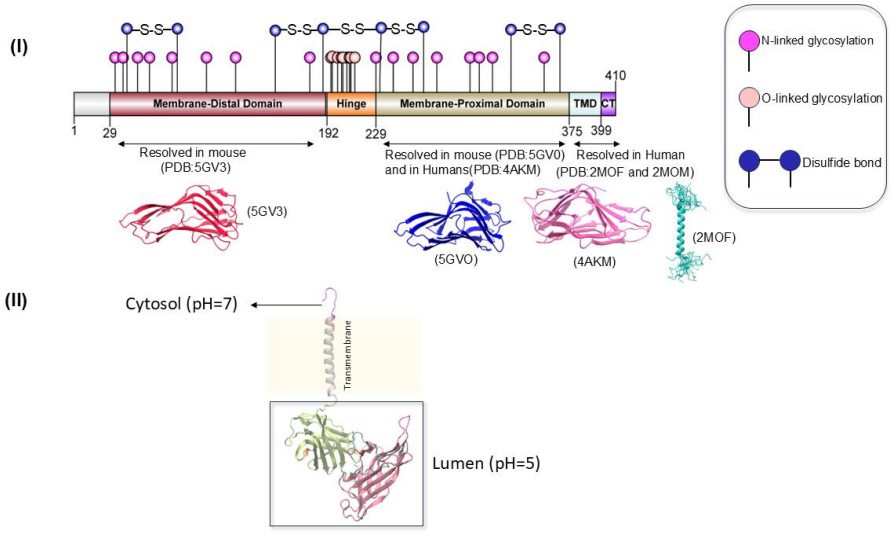

Figure 2. Structure of LAMP2A (I) Domain architecture of LAMP2A showing C-terminal in purple, a single transmembrane region in light blue colour while the Membrane distal and Proximal domain is represented in brown and golden colour the N and O glycosylated sites are represented by dark and light pink colour (II) Represents the modelled structure of LAMP2A

Stability of Simulated system: RMSD Analysis

Root mean square deviation (RMSD) is used to measure the structural stability of the simulated system in molecular dynamic simulation. The time variation of the RMSD of two replicas for both the glycosylated and non-glycosylated systems, as shown in Figure 3, indicates that both systems appear to have reached equilibrium during the last 500 ns of the trajectory. Additionally, it is interesting to observe that RMSD of glycosylated systems is lesser compared to the non-glycosylated ones, suggesting enhanced structural stability attained by the glycosylated protein.

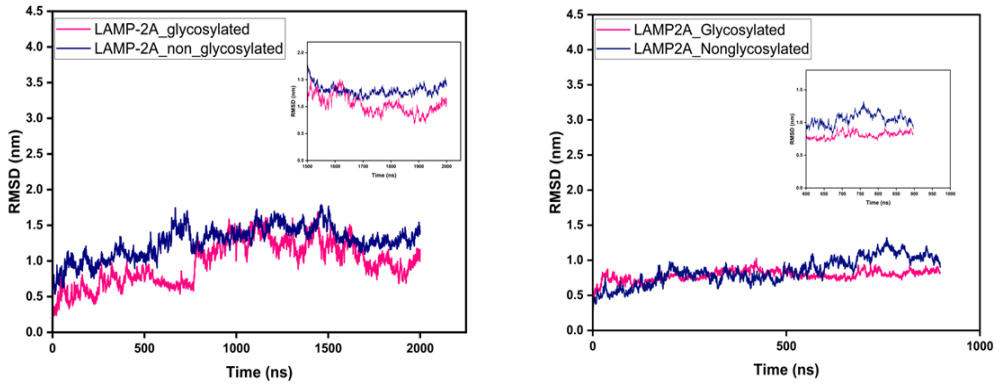

Figure 3: Root mean square deviation (RMSD) for glycosylated LAMP2A and non-glycosylated LAMP2A for two replicas

Residue Level Dynamics of Simulated System

To analyze the dynamics of the simulated systems at residue level, we calculated root mean square fluctuation (RMSF) for each system. The left and right panel of Figure 4 represented the calculated RMSF for both the replica containing glycosylated and non-glycosylated systems. Higher RMSF values represent more fluctuations, while lower RMSF values represent low fluctuations. Both replicas show that fluctuations in glycosylated LAMP2A are majorly reduced in the hinge region compared to non-glycosylated LAMP2A. This indicates that glycosylation stabilizes the protein. The reason for reduced fluctuations could be the presence of O-glycans in the hinge region.

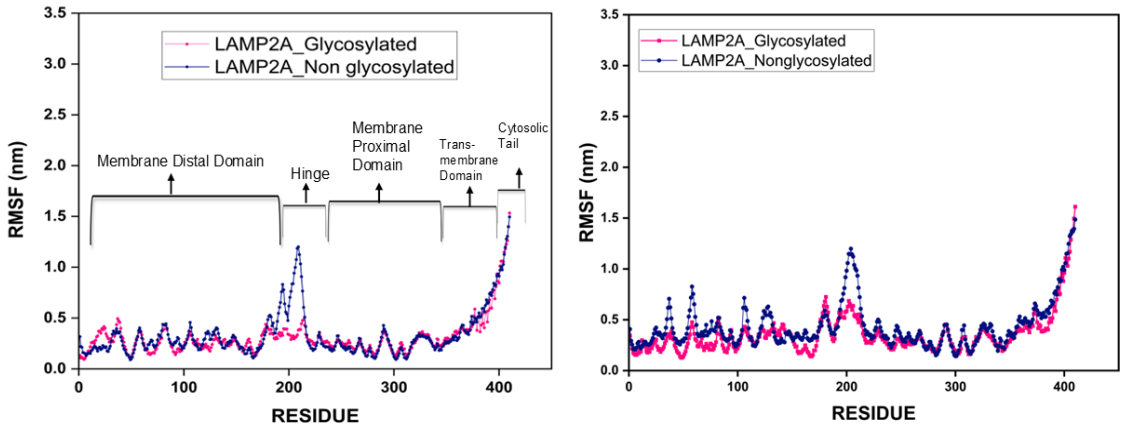

Figure 4:Root mean square fluctuation (rmsf) of glycosylated LAMP2A and non-glycosylated LAMP2A for two replicas

Radius of Gyration

It measures how spread out the molecule's atoms are around its center of mass. The protein's radius of gyration (Rg) was observed to be higher in glycosylated system compared to the non-glycosylated one (Figure 5). This variation can be attributed to changes in the relative orientation (Figure 6) of the protein's domains and conformational adjustments. Rg was calculated using:

$$R_g = \sqrt{\frac{1}{N}\sum_{i=1}^{N} m_i \cdot (r_i - r_{\mathrm{cm}})^2}$$

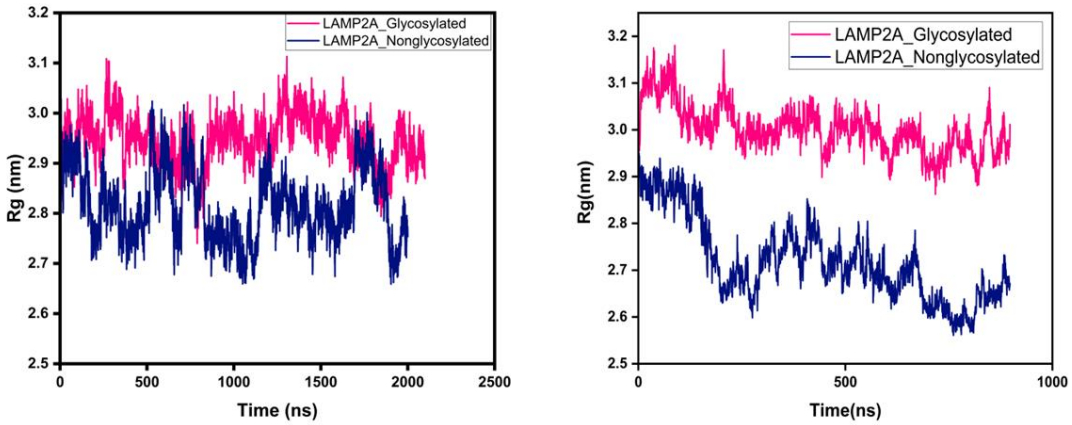

Figure5: Rg plot of glycosylated LAMP2Aand non-glycosylated Lamp2A

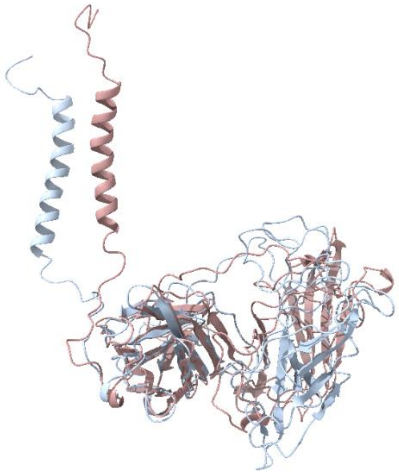

Figure 6: Overlapped structure of glycosylated (blue) and non-glycosylated LAMP2A

Membrane Density

Lipid bilayer density refers to the mass of lipids and associated molecules per unit area or volume of the membrane. It quantitatively measures the compactness and molecular arrangement within the bilayer, reflecting the influence of lipid composition, packing, and membrane environment. Peaks indicate the presence of headgroups and cholesterol near the bilayer surface. Valleys indicate the hydrophobic core of the membrane. Both glycosylated and non-glycosylated systems exhibited similar curves indicating similar lipid bilayer density.

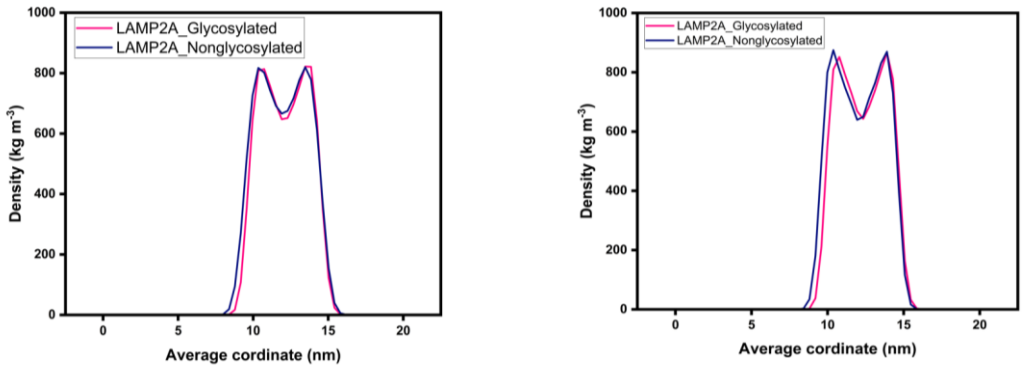

Figure 9: Graph representing the density profile of glycosylated and non-glycosylated LAMP2A

CONCLUSION

We have Modelled the complete of glycosylated LAMP2A in a biologically relevant lysosomal membrane environment. Few structural and dynamical changes including enhanced radius of gyration and reduced conformational dynamics were observed in the glycosylated protein. The study can provide a deeper comprehension of role of glycosylation in LAMP-2A and can be tuned for therapeutic implications. These post-translational modifications regulate LAMP-2A's activity.

FUTURE PLAN

The current study has provided valuable insights into the structural and dynamic properties of LAMP-2A and its role in the chaperone-mediated autophagy (CMA) mechanism. Building upon this foundation, the next research phase will involve an extended molecular dynamics simulation and extensive analysis to explore the effect of glycosylation on LAMP2A. This step is critical, as glycosylation is known to modulate protein folding, stability, and interactions, potentially influencing the CMA pathway.

Specifically, the extended simulations aim to:

1. Analyze how glycosylation affects the structural stability and conformational dynamics of LAMP2A.
2. Explore the allosteric effects in protein as a result of glycosylation (if any).

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Thank You

Geetanshi

Integrated Bsc-Msc Chemistry (Research)

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