

Structural dynamics of glycosylated isoforms of Glycodelin: a comparative study through molecular dynamics simulation

Muhammad Shoaib ¹, Mujeeb Alam Khan ¹, Muhammad Idrees ¹, Ehtesham ¹, Roshan Ali ¹, Muhammad Waqas Luqman ²

ABSTRACT

Objective: To study the structural dynamics of glycodelin (Gd) isoforms with distinct glycosylation patterns using molecular dynamics (MD) simulations to explore their potential roles in cancer.

Methods: We employed MD simulation to investigate the structural behavior of normal and aberrantly glycosylated glycodelin (PAEP). Protein sequence and glycosylation sites were retrieved from Universal Protein Resource (UniProt) and GLYCONNECT databases. Homology modeling and glycan attachment were performed using UCSF Chimera and Glycam, while molecular topology generated using CHARMM General Force Field. Simulations were performed using the Groningen package over a 50-nanosecond timescale. Post-simulation trajectory analyses included Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (Rg), hydrogen bonding analysis (HBA), and Principal Component Analysis (PCA) to evaluate the structural dynamics and stability of the glycodelin isoforms.

Results: RMSD and RMSF analyses demonstrated that glycosylated isoforms of both Gd1 and Gd2 exhibited greater structural stability, with reduced atomic fluctuations compared to their A-glycosylated counterparts. Remarkably, distinct residue fluctuations were observed at positions 30, 65, 110, and 142 in Gd1, and more broadly in Gd2. Rg analysis indicated increased compactness, particularly in glycosylated Gd2 isoform. PCA revealed higher structural randomness in A-glycosylated forms, while HBA further supported the enhanced stability of glycosylated variants. Overall, the glycosylated Gd2 isoform emerged as most stable, suggesting a potential role in cancer-associated conformational behavior.

Conclusion: Native glycosylation enhances Gd stability and compactness while reducing solvent exposure. Isoform-2-GP, in particular showed the most favorable dynamics, highlighting its potential as a cancer biomarker or therapeutic target.

Keywords: Glycodelin (MeSH); Molecular Dynamics Simulation; GROMACS (Non-MeSH); Root mean square fluctuation (Non-MeSH); Root mean square deviation (Non-MeSH).

THIS ARTICLE MAY BE CITED AS: Shoaib M, Khan MA, Idrees M, Ehtesham, Ali R, Luqman MW. Structural dynamics of glycosylated isoforms of Glycodelin: a comparative study through molecular dynamics simulation. *Khyber Med Univ J* 2025; 17(2):187-94. <https://doi.org/10.35845/kmu.2025.23776>

INTRODUCTION

Glycodelin (Gd), a member of the lipocalin superfamily,¹ is encoded by a gene located on chromosome 9q34,² a region that also harbors other lipocalin family genes, such as tear lipocalin I and 2, prostaglandin D synthase, and α 1-microglobulin.³ The Gd gene spans 5.05 kb and comprises seven exons, featuring a conserved retinol-binding motif characteristic of β -lactoglobulins.³ Its promoter region contains four putative glucocorticoid/progesterone

response elements (PREs) at positions -1799, -1071, -745, and -302, along with two additional putative PREs at +1912 and +1965.¹

Gd is composed of 180 amino acids, including an 18-residue signal peptide, and forms four intramolecular disulfide bonds at Cys66, Cys106, Cys119, and Cys160. It has two N-linked glycosylation sites at Asn41 and Asn81, which are glycosylated in its uterine (Gd-A) and seminal plasma (Gd-S) isoforms. The attached oligosaccharides include:

1: Department of Biochemistry, Institute of Basic Medical Sciences, Khyber Medical University (KMU), Peshawar, Khyber Pakhtunkhwa, Pakistan

2: Department of Biochemistry, KMU Institute of Medical Sciences, Kohat, Khyber Pakhtunkhwa, Pakistan

Email : dridrees104@gmail.com

Contact #: +92-333-9348678

Date Submitted: August 29, 2024

Date Revised: May 29, 2025

Date Accepted: May 31, 2025

- Gal β 1-4GlcNAc (LacNAc)
- GalNAc β 1-4GlcNAc (LacdiNAc)
- NeuAc α 2-6Gal β 1-4GlcNAc (Sialylated LacNAc)
- NeuAc α 2-6GalNAc β 1-4GlcNAc (Sialylated LacdiNAc)
- Gal β 1-4(Fuc α 1-3)GlcNAc (Lewis^x)
- GalNAc β 1-(Fuc β 1-3)GlcNAc (LacdiNAc analogue of Lewis^x) (Figure 1).³

Differentiated cells in the endometrium and other tissues exhibit the presence of glycodelin (Gd), although it is also occasionally found in malignant cells that are weakly differentiated. However, both normal and malignant cells in cancerous tissues may express Gd. Various histological samples have shown that Gd is expressed by epithelial components of tumors.⁴ Studies on Gd-negative cancer cell lines transfected with Gd complementary DNA (cDNA) revealed that the sense strand promoted epithelial development, while the antisense strand did not.⁵ Similar results were observed in the presence of basement membrane components when cancer cells were co-cultured with normal stromal cells. Both approaches led to Gd production in cancer cells, decreased cell proliferation, and a reversion of the malignant phenotype. These findings suggest that normal stromal cells, basement membrane components, and Gd each contribute to epithelial differentiation and glandular morphogenesis.⁶

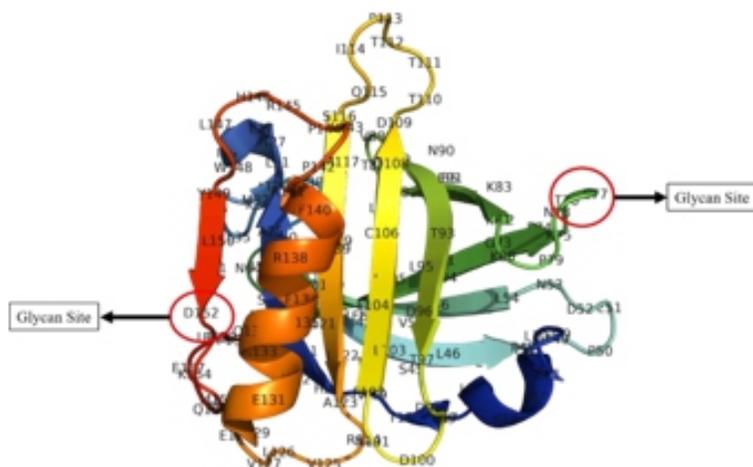


Figure 1: 3D Structure of Glycodelin (Gd) with the glycan marked positions

To date, four differential isoforms of Gd recognized by their glycan pattern, which are GdA⁷, GdS⁸, GdF⁹ and GdC.¹⁰

These four isoforms of Gd apply their particular biological function relying on their protein backbone as well as oligosaccharide post-translational extension.¹¹

Given the emerging evidence of Gd involvement in cancer, particularly through its glycosylation-dependent functions, we were motivated to investigate its protein structure, focusing specifically on variations in glycosylation patterns, using molecular dynamics (MD) simulations. Comparative analysis of the native (wild-type) and aberrantly glycosylated forms of Gd may provide critical insights into its functional roles in cancer biology. Aberrant glycosylation and truncation patterns commonly observed in malignancies emphasize the need for computational data to elucidate Gd's structural and functional dynamics. Since Gd isoforms play roles in key biological processes such as human reproduction, feto-maternal immune regulation, and tumor cell proliferation, this study aims to characterize their structural behavior and potential mechanistic link to cancer progression. These findings may support the future use of Gd isoforms as biomarkers or therapeutic targets in clinical oncology.

METHODS

Dataset retrieval: Fast-All (FASTA) sequence of progesterone-associated endometrial protein (PAEP) (Gd) was

retrieved from universal protein resource (UniProt) database (UniProt ID: 9606). Glycosylated regions were identified from the same sequence. The N-linked glycation with the Asparagine residues found at position Asn46 & Asn81 were observed using GLYCONNECT, a database that is designed to explore glycans and the proteins that carry or interact with them.

Homology modeling: 4ROB was downloaded in .pdb format from protein data bank and was homology constructed in Chimera software by removing ions and extra solvent molecules. Distinct oligosaccharides were added to the 3D PAEP structure through glycam.org (<https://dev.glycam.org/>). The added sugars, 3D PAEP structure diagrams are given below.

Carbohydrate topology: Carbohydrate topology was written through CHARMM (Chemistry at Harvard Macromolecular Mechanics) general force field (CGenFF) program V2.2.0.

Solvation neutralization: Water molecules were added around the protein in a rectangular configuration, sized to fit the protein with an additional distance of edge10 from its surface. System charges were neutralized using the Monte Carlo Ion Placing Method.

Energy minimization: Energy Minimization (EM) was performed to optimize the entire system i.e., carbohydrates attached and unattached

protein complex structures. This reduces the net force by moving atom of the system and distributes their local energy. In addition, the generated complex further helps in detailed structural information prior final run. EM is run through the “grompp” command by retrieving information from already prepared binary input file Step4.0_minimization.tpr, through Groningen Machine for Chemical Simulations (GROMACS) package 5.1.1. “Steep integrator”, “Verlet cutoff scheme” and “Linear Constraint Solver (LINCS) constraint-algorithm” were followed in minimization step.

Equilibration: The equilibration phase was carried out prior to the final molecular dynamics run to enable the solvent molecules to properly interact with the protein, whether carbohydrates are attached or not. This step also ensures that the engineered structure fits in simulation space and help reduce the noise in the protein dynamical analysis.

Engineered PAEP protein structure was equilibrated in Isothermal-isochoric ensemble (number of particles, volume, and temperature (NVT). The NVT Equilibration was performed by using “MD integrator” with 500000 N steps, “Van der Waals (Vdw) type cut-off”, “LINCS constraint- algorithm” and “Nose-Hover temperature coupling” with a reference temperature set to 300K. Temperature graphs are given below.

Production MD: The final 50-nanosecond MD simulation was carried out for all four minimized and equilibrated structures, as described above. The complex system was maintained at a constant temperature by employing the velocity-rescaling temperature coupling (V-rescale) method and at constant pressure using the isotropic Parrinello–Rahman pressure-coupling scheme. “Vdwtype cut-off”, “LINCS constraint-algorithm” were used in linear order. The GROMACS package, “PyMol v1.7”, “Origin Pro Lab V2018” and “VMD” used for trajectories analysis and graphical representations.

Root mean square deviation: In bioinformatics molecular dynamic analysis, RMSD is one of the basic terms used in the measuring distances between the heavy atoms or their

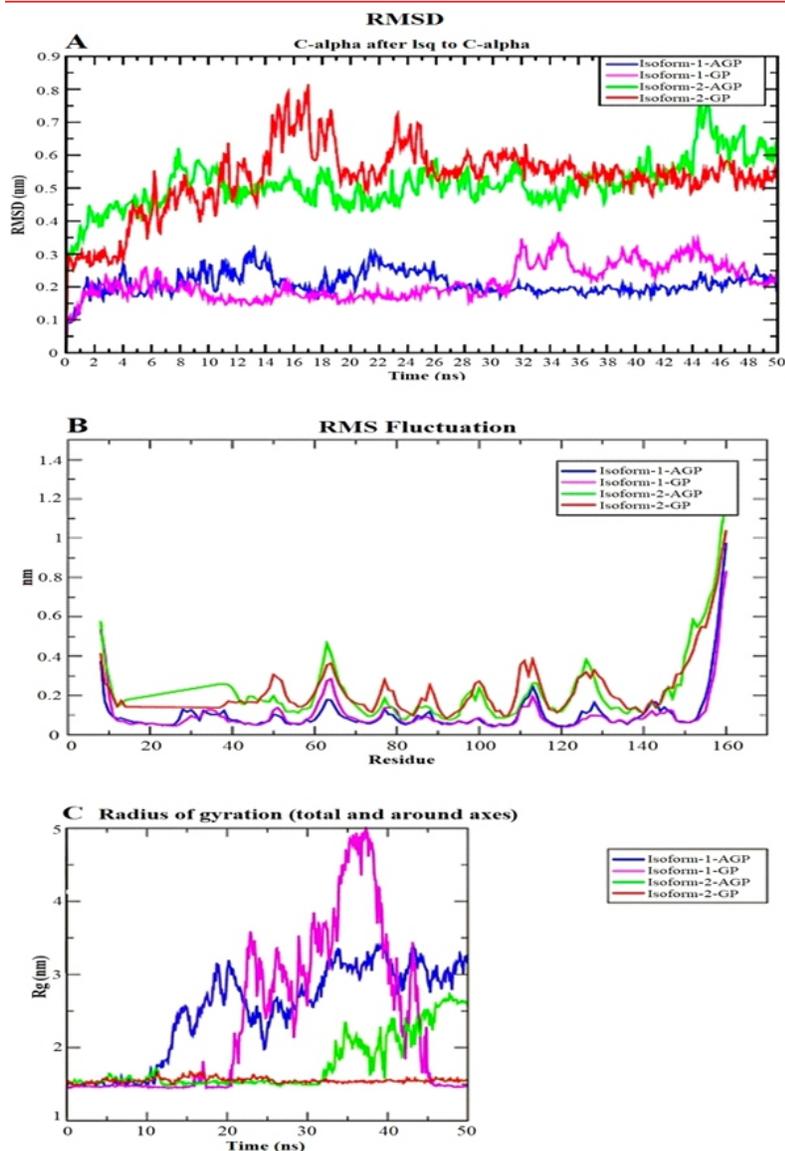


Figure 2: **(A)** RMSD scatter plot of the four proteins suggesting stability under different colour schemes (blue; Isoform 1 without glycan pattern, magenta; Isoform 1 with glycan pattern, green; isoform 2 without glycan pattern, red; Isoform 2 with glycan pattern, green). **(B)** RMSf scatter plot showing residue-level fluctuations for both the wild-type and cancer isoform of the protein. **(C)** Radius of gyration (Rgyr) scatter plot illustrating the compactness and stability of the wild-type versus cancer isoform.

deviation from their original structure over the timescale of molecular simulation run. It can be applied to non-protein part as well like glycans or ligands attached. Mostly, the RMSD calculations were done to carbon-alpha atomic coordinates, which are same in our analysis. In this study we find the RMSD of the normal wild type and their least fit carbon alpha cancer aberrant glycan attached isoform. Moreover, it is used to know how much a protein is stable in the system, having a plateau line

confirm this.

Root mean square fluctuations (RMSF):

Similar to RMSD, RMSF is another common analysis package used in MD analysis. Since, the RMSD deals with the stability of the protein, RMSF as the name suggests investigating the residue fluctuations in the molecular dynamic run. These fluctuations of the residues pointed out the important residues involved in the structural dynamic changes that can be bring by

any hypothesis questions raised during research designing. Hence, it is an important tool package, and we also consider it in our analysis.

Radius of Gyration (Rgyr)

(gyradius): Radius of gyration (Rgyr) is an investigative tool of the simulation run that predicts the compactness of the protein throughout the molecular run. This is used to know the overall protein confirmation in the box designed for the specialized protein. In addition, it also correlated with the RMSD.

Secondary structure: Since, the protein has four types of structures. While the functional structures are tertiary or quaternary but in bioinformatics analysis researcher mainly analyzed secondary structure of the protein and their behavior in total production run. Therefore, for this purpose Define Secondary Structure of Proteins (DSSP) determination package was used to find the possible outcome.

Principal component analysis:

Principal Component Analysis (PCA) is a statistical technique used to reduce the number of variables in large datasets. It achieves this by transforming the original variables into a smaller set of new variables, called principal components, which retain most of the original information.

In this study PCA was employed to investigate the major conformational motions of the glycosylated and non-glycosylated PAEP (Glycodelin) protein structures during molecular dynamics simulations. PCA helped us find the main movements by looking at changes in the protein's backbone atoms. This made it easier to compare different forms of the protein and see how sugar chains (glycosylation) affect its behavior.

RESULTS

Structural Dynamics of the differential Gd structure: RMSD compares two structures, one native isoform with the distinct glycan pattern and it's another counter isoform without glycan attached pattern, by computing each C-alpha structure trajectory (-f) and comparing it. In the Figure 2(A), overall isoform 1 differs in stability from the isoform 2 structured proteins. In between isoform 1 A-glycated and glycated isoforms both shows initial

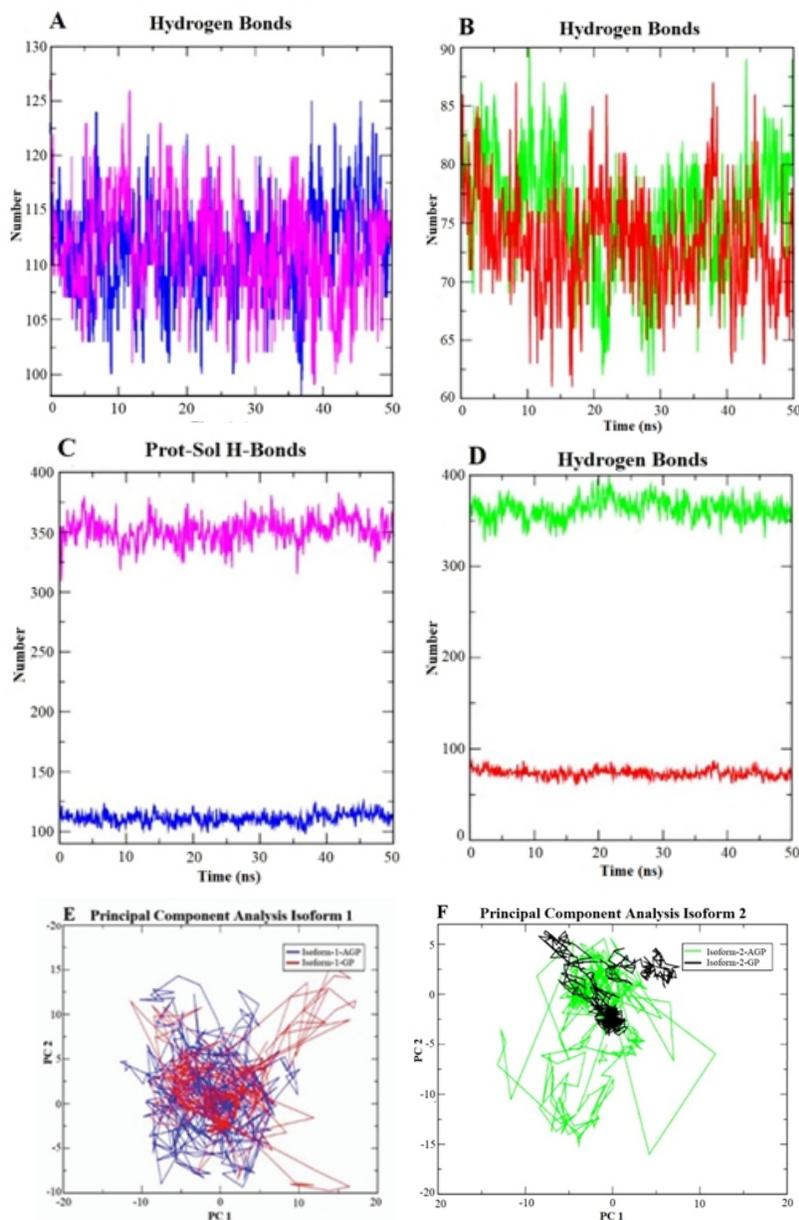


Figure 3: (A) H-bonding after 50 ns MD simulation of the Isoform 1, (B) H-bonding after 50 ns MD simulation of the Isoform 2. (C) solvent-protein H-bonding after 50 ns MD simulation of the Isoform 1, (D) solvent-protein H-bonding after 50 ns MD simulation of the Isoform 2. (E) PCA Scatter Plot of Isoform 1 protein residues, (F) PCA Scatter Plot of Isoform 2 protein residues.

stability up-to 10 nano seconds by 0.2 nm and then isoform 1 A-glycated moves to 0.15 nm by 30 ns, the follow higher path till final run (50 ns). In contrast, isoform 1 glycosylated moves at higher RMSD 0.3 nm until 28 ns, and moves to stability (0.15 nm) by until 50 ns. In conclusion, isoform 1 glycosylated was more stable in the end, suggesting that the structure is more stable than the A-

glycosylated structure. Similarly, isoform 2 A-glycated and glycosylated were compared in another portion of the graph. Isoform 2 A-glycated structure begins with an unstable RMSD of 0.6 nm until 10 ns, then drops to 0.45 nm before jumping back to 0.75 nm. In comparison, the glycosylated structure of Isoform 2 starts off steady at 0.3 nm until 5 ns, then rapidly rises to 0.8 nm at 20

ns, before gradually dropping and reaching a stable level (0.5 nm) till the finish of desired run. Same it suggesting that glycosylated structure is more stable than the A-glycosylated structure. The scatter plot Figure 2(B), representing different color schemes for the specified trajectories, such that blue; Isoform 1 without glycan pattern, magenta; Isoform 1 with glycan pattern, green; isoform 2 without glycan pattern, red; Isoform 2 with glycan pattern, green. X-axis denotes 160 amino acid residues of Gd, and Y-axis suggest "nm" fluctuations. Isoform 1 A-glycated and glycosylated residue observe fluctuations at 30, 65, 110 & 142 positions. Isoform 2 A-glycated and glycosylated residue observe more fluctuations ranging between 10-60, 80-90 & 110 positions. This suggested that glycan can bring stability changes towards protein structures. The Rgyr was used to calculate the gyration radius of a collection of atoms (weighed in mass) as a function of time. The different color scheme in the above figure represented distinct isoform explained above. Rgyr is associated with protein folding stability by expressing their plotted line at a reasonably constant value. Figure 2(C) clearly indicates that the red line (isoform-2-GP) maintains their linear trajectory, implying higher compactness and optimal protein folding, whereas isoform-2-GP was stable over 35ns time, implying protein unfolding afterwards. In addition, Isoform 1 AGP & GP were not compact, showing fluctuations. In conclusion, this finding demonstrated that the isoform 2 is more compact than the isoform 1.

Clustered motion trajectories:

Hydrogen bonds play an important role in stabilizing protein folding, maintaining structural integrity, and facilitating molecular recognition. The protein backbone commonly consists of secondary elements such as α -helices and β -sheets. These sheets are stabilized by hydrogen bonds formed between the carbonyl oxygen and the amide nitrogen of the main chain. These interactions provide structural stability and specificity in molecular interactions. Hence the "gmx hbond" tool in GROMACS was employed to analyze hydrogen bonds between atom groups or specific donor-hydrogen-acceptor (D-H-A) triplets, based on defined distance and angle criteria. Output data

showed an increased number of hydrogen bonds forming over time in A-glycated isoform groups compared to other glycated variants. These result assumptions are further clarified at the next H-bonding analysis (Figure 3 A-D). Furthermore, solvent-protein H-bonding analysis represent the dissociation of the protein structure by the interacting exposed residue. The more H-bonding denotes suggests more dissociation. As in the above figure isoform 1 AGP has lowered H-bonding compared to its glycated counterpart. While on the other plot, isoform 2 glycated has low H-bonding as compared with the A-glycated isoform. In Figure 3(E) Isoform-1 AGP PC1+PC2 cluster around -10 & +10, while, Isoform-1 GP PC1+PC2 cluster around -5 & +15. On the other hand cancer isoform-2 AGP PC1+PC2 residue cluster moves around -12 & +10 and isoform-2 GP PC1+PC2 -5 and +8. This suggested that randomness in the protein structure are found more in the isoform-1 AGP, Isoform-1 GP, isoform-2 AGP compared to the isoform-2 GP.

DISCUSSION

This study utilized MD simulations to explore the structural dynamics and stability of Gd isoforms with distinct glycosylation patterns, aiming to understand their potential roles in cancer-related processes. Our results demonstrated that glycosylation significantly influences protein stability, compactness, and intramolecular interactions. Particularly, the glycated variants of both isoforms exhibited improved structural stability over their A-glycated counterparts, as evidenced by lower RMSD fluctuations, consistent radius of gyration, and hydrogen bonding profiles.

Gd is a lipocalin protein found mostly in epithelial cells. It has previously been demonstrated to promote cell differentiation in different types of the cancer cells. This was linked to a reduction in xenograft development in cancer patients. Moreover, in a *denocarcinoma*, immunohistochemical labelling revealed higher Gd staining when compared to squamous cell carcinoma; however, there was no apparent difference in staining when compared to normal tissues. Both normal and melanoma samples showed weak Gd

staining. Additionally, it contains two possible N-linked glycosylation sites: Asn 41 and Asn 81. Therefore, its structures on glycoproteins are changed in cancer, according to new research. On the plasma protein -I-proteinase inhibitor, fucosylation groups and sialylations have been related to malignancy. These glycosylation modifications in a tumor-secreted protein might be owing to changes in glycosylation enzymes' basic function, either through changing enzyme levels or enzymatic activity. Changes in glycan structure may also be exploited as cancer biomarkers for early detection.

RMSD is the initial analysis step of the molecular dynamic simulation, which suggest an overall look of the structure dynamics through the whole procedure done. Here in this study, we investigated RMSD of the four isoforms, out of which two were glycated and two were un-glycated. It seems from the results that glycated isoforms were more stable compared to their A-glycated counterparts. The reason could be glycation achieve stability to the protein structure and this was shown by many studies including.^{20,21} Now, if we look at the glycated in-between comparison the isoform-1 is more stable than the isoform-2 glycated isoform. This is because the isoform-2 has truncated portion which makes it a little un-stable folding mechanistic compared to the isoform-1. The same results were explained by.²² Therefore, in conclusion isoform-1 glycated can be considered for the future drug interaction studies. Taking the glycated isoforms in the RMSD as a reference plot, we see fewer fluctuations in the isoform-1 compared to the isoform-2.²³ Which further denoted that glycation has a greater impact on the isoform-2. The said result is supported by the gyration plot (Figure 2) denoted a very straight linear red line isoform-2, where the abovementioned plots were taken as a reference value. The study result is also explained by Lobnov M, et al.,¹⁷ showing a great compactness of the structure due to post-translational modifications.

The hydrogen bonds inside the protein between A-glycated and other glycated isoforms, especially near the chiral centers of the dipeptide group, may be important for telling apart different chiral forms. In Figure 3, we showed

where the hydrogen bonds start and end, and how many hydrogen bonds were found during the MD simulations. The H-bond utility package in GROMACS was used to identify hydrogen bonding. In the hydrogen bond analysis, the distance between heavy atoms forming hydrogen bonds was set to 3.5, and the angle between donor and acceptor atoms was set to 30 degrees. During the MD simulation, when the monomers were placed into ASN pockets, the glycated isoforms showed fewer hydrogen bonds on average. Because hydrogen bonding interactions are reduced, any pocket is unlikely to be the favored chiral recognition site. When A-glycated isoforms were bound, a substantial increase in hydrogen bonding was seen in MD simulations. The highest number of hydrogen bonds between molecules was seen in the Asn pocket of the A-glycated isoforms. This result suggests that this pocket is the main place where ligands attach and where chiral recognition happens. The findings of intermolecular hydrogen bond analysis for the four isoforms simulations are also shown in Figure 3. First and foremost, there were less intermolecular hydrogen bonds between isoform-1-AGP and isoform-2GP. Both isoform-1-GP and isoform-2-AGP have the greatest intermolecular hydrogen bonding interactions with the solvent, as shown by this finding. When the two isoforms were examined, it was discovered that isoform-2-AGP formed H-bonds with a considerably higher % occupancy than isoform-1-GP. As previously stated, this result is consistent with the A-glycated isoform's higher stickiness to the exposed solvent, which eventually leads to more dissociation.²⁴

The 3D structure of human Glycodelin (Gd) showed a tightly packed dimer form. This agrees with earlier research that observed dimeric stoichiometry in all isolated glycoforms of Gd.^{8,25} The arrangement of charged and hydrophobic amino acids on the surface of the glycated isoforms shows two areas with opposite charges and one side that is more hydrophobic. This pattern suggests that without glycosylation, the protein may have a higher chance of aggregation, especially when the ionic strength is low. These observations are strongly supported by the analysis results.^{18,26} Since the critical

Glutamic acid (Glu) residue at position 89 is replaced by Alanine (Ala) in Gd, the conformational change typically influenced by pH may not occur in the same way. Furthermore, the tiny pocket size combined with the sterically restricted nature of Gd implies a limited affinity for physiologically relevant ligands found in other human lipocalins, such as fatty acids, retinoic acid, retinol, etc. This is consistent with the fact that retinol has a 100-fold higher KD value than β -lactoglobulin. As a result, it appears that Gd binding of a small molecule ligand to Gd might not be crucial for its biological role.²⁸ As a result, it appears that both the kind and distribution location of connected oligosaccharides are critical. Both monomers are positioned such that all the sugar chains face the same side and point in the same direction of the structure. Depending on the type of glycosylation, it may bind to glycan receptors (lectins) located on the sperm head's cell surface. This interaction likely leads to a strong avidity effect, which could explain why the thick sugar matrix Gd-A glycosylated can outcompete in the zona pellucida's glycocalyx. Due to the flexible nature of large oligosaccharides, their terminal sugar units can interact with glycan receptors located up to 100 nm away. Besides humans, chimpanzees, and gorillas, another group of glycodelin-related sequences-lacking the two N-glycosylation sites-can be found throughout the ape evolutionary lineage. These sequences correspond to primate orthologues of β -lactoglobulin, a well-known member of the lipocalin protein family in mammals.^{29,30} In humans, the β -lactoglobulin gene is inactive and exists only as a pseudogene.³⁰ The consistent presence of the two N-glycosylation sites in all Gd sequences highlights their importance and suggests they appeared within a relatively short period of time.³⁰ Our structural and bioinformatic analyses indicate further study of Gd glycan structures could provide new insights into evolution and diseases such as cancer.

CONCLUSION

Our MD simulations revealed that native glycosylation significantly improves the structural integrity of Gd

isoforms by enhancing their stability, compactness, and resistance to solvent exposure. Isoform-2-GP, in particular, exhibited the lowest residue fluctuations, consistent hydrogen bonding patterns, and tightly clustered PCA trajectories, indicating minimal conformational randomness and optimal folding behavior. These structural advantages suggest a potential functional relevance in cancer biology, positioning isoform-2-GP as a promising candidate for therapeutic targeting and biomarker development in glycan-related diseases.

REFERENCES

1. Seppälä M, Taylor RN, Koistinen H, Koistinen R, Milgrom E. Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. *Endocr Rev* 2002; 23(4): 401-30. <https://doi.org/10.1210/er.2001-0026>
2. Yeung WSB, Lee K-F, Koistinen R, Koistinen H, Seppala M, Ho PC, et al. Roles of glycodelin in modulating sperm function. *Mol Cell Endocrinol* 2006;250(1):149-56. <https://doi.org/10.1016/j.mce.2005.12.038>
3. Seppälä M, Koistinen H, Koistinen R, Chiu PCN, Yeung WSB. Glycodelin: a lipocalin with diverse glycoform-dependent actions. In: *Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Available from URL: https://www.ncbi.nlm.nih.gov/books/NBK6332/*
4. Kämäräinen M, Halttunen M, Koistinen R, von Boguslawsky K, von Smitten K, Andersson LC, et al. Expression of glycodelin in human breast and breast cancer. *Int J Cancer* 1999;83(6):738-42. [https://doi.org/10.1002/\(sici\)1097-0215\(19991210\)83:6%3C738::aid-ijc7%3E3.0.co;2-f](https://doi.org/10.1002/(sici)1097-0215(19991210)83:6%3C738::aid-ijc7%3E3.0.co;2-f)
5. Koistinen H, Seppälä M, Nagy B, Tapper J, Knuutila S, Koistinen R. Glycodelin reduces carcinoma-associated gene expression in endometrial adenocarcinoma cells. *Am J Obstet Gynecol* 2005; 193(6): 1955-60. <https://doi.org/10.1016/j.ajog.2005.05.073>
6. Arnold JT, Lessey BA, Seppälä M, Kaufman DG. Effect of normal endometrial stroma on growth and differentiation in Ishikawa endometrial adenocarcinoma cells. *Cancer Res* 2002;62(1):79-88.
7. Julkunen M, Rutanen EM, Koskimies A, Ranta T, Bohn H, Seppälä M. Distribution of placental protein 14 in tissues and body fluids during pregnancy. *Br J Obstet Gynaecol* 1985; 92(11): 1145-51. <https://doi.org/10.1111/j.1471-0528.1985.tb03027.x>
8. Koistinen H, Koistinen R, Dell A, Morris HR, Easton RL, Patankar MS, et al. Glycodelin from seminal plasma is a differentially glycosylated form of contraceptive glycodelin-A. *Mol Hum Reprod* 1996; 2(10): 759-65. <https://doi.org/10.1093/molehr/2.10.759>
9. Tse JY, Chiu PC, Lee KF, Seppala M, Koistinen H, Koistinen R, et al. The synthesis and fate of glycodelin in human ovary during folliculogenesis. *Mol Hum Reprod* 2002; 8(2): 142-8. <https://doi.org/10.1093/molehr/8.2.142>
10. Chiu PC, Chung MK, Koistinen R, Koistinen H, Seppala M, Ho PC, et al. Cumulus oophorus-associated glycodelin-C displaces sperm-bound glycodelin-A and -F and stimulates spermatozoa-zona pellucida binding. *J Biol Chem* 2007; 282(8): 5378-88. <https://doi.org/10.1074/jbc.M607482200>
11. Seppälä M, Koistinen H, Koistinen R, Chiu PC, Yeung WS. Glycosylation related actions of glycodelin: gamete, cumulus cell, immune cell and clinical associations. *Hum Reprod Update* 2007; 13(3): 275-87. <https://doi.org/10.1093/humupd/dmm004>
12. Vanommeslaeghe K, Hatcher E, A-

- charya C, Kundu S, Zhong S, Shim J, et al. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J Comput Chem* 2010;31(4):671-90. <https://doi.org/10.1002/jcc.21367>
13. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ. GROMACS: fast, flexible, and free. *J Comput Chem* 2005;26(16):1701-18. <https://doi.org/10.1002/jcc.20291>
14. Roe DR. RMSD Analysis in CPPTRAJ. Formerly known as "Tutorial A1: Setting up an advanced system. July 2014. [Accessed on: March 30, 2021]. Available form URL: https://ambermd.org/tutorials/analysis/tutorial1/index.php?utm_source=chatgpt.com
15. Wooding SJ, Howe LM, Gao F, Calder AF, Bacon DJ. A molecular dynamics study of high-energy displacement cascades in α -zirconium. *J Nucl Mat* 1998;254(2):191-204. [https://doi.org/10.1016/S0022-3115\(97\)00365-6](https://doi.org/10.1016/S0022-3115(97)00365-6)
16. BioChemCoRe. RMSD/RMSF Analysis. [Accessed on: March 30, 2021]. Available form URL: <https://ctlee.github.io/BioChemCoRe-2018/rmsd-rmsf/>.
17. Lobanov M, Bogatyreva NS, Galzitskaia OV. Radius of gyration is indicator of compactness of protein structure. *Mol Biol (Mosk)*. 2008;42(4):701-6.
18. CD Computa Bio. Radius of gyration (Rg) analysis services. [Accessed on: March 30, 2021]. Available form URL: <https://www.computabio.com/radius-of-gyration-rg-analysis-services.html>
19. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. *Philos Trans A Math Phys Eng Sci* 2016;374(2065):20150202. <https://doi.org/10.1098/rsta.2015.0202>
20. Alizadeh-Rahrovi J, Shayesteh A, Ebrahim-Habibi A. Structural stability of myoglobin and glycomyoglobin: a comparative molecular dynamics simulation study. *J Biol Phys* 2015;41(4):349-66. <https://doi.org/10.1007/s10867-015-9383-2>
21. Lee HS, Qi Y, Im W. Effects of N-glycosylation on protein conformation and dynamics: protein data bank analysis and molecular dynamics simulation study. *Sci Rep* 2015;5(1):8926. <https://doi.org/10.1038/srep08926>
22. Wang W, Xi W, Hansmann UHE. Stability of the N-terminal helix and its role in amyloid formation of serum amyloid A. *ACS Omega* 2018;3(11):16184-90. <https://doi.org/10.1021/acsomega.8b02377>
23. Sirangelo I, Iannuzzi C. Understanding the role of prote-in glycation in the amyloid aggregation process. *Int J Mol Sci* 2021;22(12). <https://doi.org/10.3390/ijms22126609>
24. Morris KF, Billiot EJ, Billiot FH, Gladis AA, Lipkowitz KB, Southerland WM, et al. A molecular dynamics simulation study of the association of 1,1'-Binaphthyl-2,2'-diyl hydrogenphosphate enantiomers with a chiral molecular micelle. *Chem Phys* 2014;439:36-43. <https://doi.org/10.1016/j.chemphys.2014.05.004>
25. Vigne JL, Hornung D, Mueller MD, Taylor RN. Purification and characterization of an immunomodulatory endometrial protein, glycodelin. *J Biol Chem* 2001;276(20):17101-5. <https://doi.org/10.1074/jbc.m010451200>
26. Qin BY, Bewley MC, Creamer LK, B-aker HM, Baker EN, Jameson GB. Structural basis of the Tanford transition of bovine beta-lactoglobulin. *Biochemistry* 1998;37(40):14014-23. <https://doi.org/10.1021/bi981016t>
27. Breustedt DA, Schönfeld DL, Skerra A. Comparative ligand-binding analysis of ten human lipocalins. *Biochim Biophys Acta* 2006;1764(2):161-73. <https://doi.org/10.1016/j.bbapap.2005.12.006>
28. Fugate RD, Song PS. Spectroscopic characterization of beta-lactoglobulin-retinol complex. *Biochim Biophys Acta* 1980;625(1):28-42. [https://doi.org/10.1016/0005-2795\(80\)90105-1](https://doi.org/10.1016/0005-2795(80)90105-1)
29. Sawyer L, Kontopidis G. The core lipocalin, bovine beta-lactoglobulin. *Biochim Biophys Acta*. 2000;1482(1-2):136-48. [https://doi.org/10.1016/s0167-4838\(00\)00160-6](https://doi.org/10.1016/s0167-4838(00)00160-6)
30. Schiefner A, Rodewald F, Neumaier I, Skerra A. The dimeric crystal structure of the human fertility lipocalin glycodelin reveals a protein scaffold for the presentation of complex glycans. *Biochem J*. 2015;466(1):95-104. <https://doi.org/10.1042/bj20141003>

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

MS, MAK & MI: Conception, acquisition, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

Eh & RA: Concept and study design, critical review, approval of the final version to be published

MWL: Analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request



This is an Open Access article distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

KMUJ web address: www.kmuj.kmu.edu.pk

Email address: kmuj@kmu.edu.pk