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ORIGINAL ARTICLE

Effects of Normal and Aberrant Glycosylation on the Stability of α_1-Anti Trypsin Through Molecular Dynamic Simulation

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ABSTRACT

The Alpha-1 antitrypsin belonging to serpin family is a protease inhibitor, the level of which rises by a factor of ten during inflammation

Purpose: To investigate the stability of normal and aberrantly glycosylated α_1-antitrypsin through molecular dynamics simulation

Study Design: Experimental study

Methodology: Current project was conducted in the department of Biochemistry at Institute of Basic Medical Sciences Khyber Medical University, Peshawar. A1AT FASTA sequence was retrieved from UniProt database (UniProt ID: **P01009**). Post-Translational Modifications (PTM) regions were identified from the same database. GLYCONNECT database was used to understand N-linked glycation with the asparagine residues found at position 70, 107, and 271 amino acid residue regions

Statistical analysis: Different bioinformatics analyses such that Root Mean Square Deviation, Radius of gyration, Root Mean Square fluctuation, Hydrogen-bonding, Secondary Structure Determination, and Principal Component Analysis were executed for 100 ns molecular dynamics simulation run

Results: RMSd, RMSf, and Rgyr significantly differ between the native type and cancer isoform. More H-bonding and strong protein stability and folding were seen in the native type. PCA analysis further confirms native type compact motion in the parallel direction during MD simulation

Conclusion: It was concluded that glycated protein appears to have high structural stability than its aberrant glycated protein. However, it will be utilized for the prompt production of the anti-cancer drugs to effectively treating cancer disease

Key Words: Alpha-1 Antitrypsin, Aberrant Glycosylation and Molecular Dynamic Simulation.

INTRODUCTION

Alpha-1antitrypsin (α_1AT) protein is a protease inhibitor, also known as α_1-proteinase inhibitor (Pi), is the major circulating serine anti-protease related to the serpin superfamily[1]. Serpins are a group of proteins that have been discovered for their ability to block certain proteases[2]. Since the primary serpins are to be differentiated on chymotrypsin-like serine proteases, the abbreviation serpin was first coined[3]. They're famous for their unusual mode of action, in which they inflict irreversible damage to their targeted protease by undergoing a broad conformational shift that disrupts its dynamic location. In contrast to the more widely employed competitive method, which involves protease inhibitors binding to and blocking access to the active site of the protease[4]. Serpin-mediated protease inhibition regulates a variety of biological processes, including coagulation and inflammation, and as a result, these proteins are a focus of medical study[5]. Their unique conformational shift is also of interest to structural biology and protein folding researchers. There are several advantages to a conformational change, it does, however, have certain drawbacks: Serpinopathies, such as protein misfolding and the production of inert long-chain polymers, are caused by mutations in serpins[6]. Serpin polymerization lowers the number of active inhibitors while simultaneously causing polymer aggregation, which leads to cell death and organ failure. Serpin-structured proteins perform several roles in addition to regulating proteolytic reaction, including storage, transport, and molecular chaperoning. Even though they are non-inhibitory activities, these members are frequently referred to as serpins[7]. Serpins have been found in over 1000 species, including 36 human proteins, as well as molecules from all five kingdoms of life, including animals, plants, fungus, bacteria, and archaea, as well as many viruses[8]. A systematic nomenclature for classifying members of the serpin superfamily according to their evolutionary connections was created in the early 2000s. As a result, serpins are the most abundant and varied protease inhibitors[9]. Extracellular activities are performed by around two-thirds of human serpins, which block proteases in the circulation to control their activity[10]. Extracellular serpins, for example, play a role in blood coagulation, inflammatory and immunological responses, and tissue remodeling via regulating proteolytic reaction[11]. In Pakistan, the reference range of serum α_1AT in healthy human blood is 2.47 g/L[12].

Alpha-1 antitrypsin deficiency is a common genetic cause of liver disease, affecting 180,000 people worldwide. This dangerous mutation occurs in 1 in every 3500 live births[13]. In newborns, its deficiency is the most prevalent hereditary cause of liver illness and transplantation[13]. Glycosylation is the most complex post-translational modification of peptides, involving the attachment of carbohydrate residues to the protein backbone chain[14]. Protein synthesis is genetically regulated. However, because glycosylation is a posttranslational process, peptide glycoforms have a lot of diversity and variability[15]. About 13 distinct monosaccharides, 8 different amino acids, and 16 types of enzymes are used to produce. About 41 different types of glycosidic linkages[16]. Specific sugars are complexed in the glycosylation pathway within the endoplasmic reticulum (ER) serve a variety of important

roles, including protecting certain residues from protease cleavage[17] and serving as recognition structures in certain peptide folding pathways.

OBJECTIVES

To investigate the stability of normal and aberrantly glycosylated A1AT through molecular dynamics simulation.

METHODOLOGY

A1AT FASTA sequence was retrieved from UniProt database (UniProt ID: **P01009**). Post-Translational Modifications (PTM) regions were identified from the same database. GLYCONNECT database was used to understand N-linked glycation with the asparagine residues found at position 70, 107, and 271 amino acid residue regions. The 3D structure of A1AT (PDB Id: **1ATU)** was retrieved from the protein data bank. It was further input for the energy minimization through Molecular Operating Environment (MOE) software package ® 2015. After that, the homology structure was subjected to charge correction and ionization, followed by the insertion of hydrogen bonds using Molecular Operating Enviroment software package® 2015. This process was done to adjust pH, minimize atomistic clashes, and H-Bonding to the generated structure using the same software. Water particles were added to the structure in a rectangular fit manner to that of protein size at the distance of edge 10. Charges were neutralized using the Monte Carlo ion placing method. Energy minimization (EM) was done to optimize the whole system i.e., the carbohydrate attached and the un-attached carbohydrate-protein complexed structures. The equilibration phase is achieved before the final molecular run of the system, a reason to adjust the solvent molecules with protein in either carb-attached or unattached configurations. Moreover, this allows the engineered structure to fit in simulation space(it's the area where constructed protein is placed for the 100ns run), and stop the noisy trajectories in the protein dynamical analysis. The final 100 nanosecond MD simulation was run for all minimized and equilibrated structures as discussed in the above paragraph. The complex system was designed under constant temperature by using V-Rescale t-coupling group and under constant pressure by adjusting to Parrinello-Rahman.

Statistical Analysis: Different bioinformatics analyses such that Root Mean Square Deviation, Radius of gyration, Root Mean Square fluctuation, Hydrogen-bonding, Secondary Structure Determination, and Principal Component Analysis were executed for 100 ns molecular dynamics simulation run.

RESULTS

Root Mean Square Deviation (RMSD): For least-square fitting and computing RMSD, we choose an index group that contains all atomic positions to increase the acceptness of the result and remove any bias. The cancer isoform structure was first stable up to 40 nanoseconds, then moved to 0.25 Å by 45 nanoseconds, and subsequently decrease to 0.15 Å and continued the same lower course over the 100 ns simulation run.

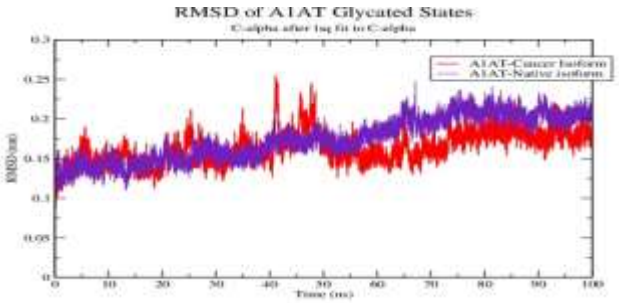

Figure-1: RMSD of alpha1-Antitrypsin Glycosylated States Root mean square fluctuation (RMSF)

The function RMSf computes the atomic locations after (optionally) fitting to a reference frame. A group is chosen for the RMSf computation after accepting the command below; in this case, the C-alpha atoms were used (Group 3). The scatter plot depicts several colour schemes for the given trajectories, such as red (A1AT-cancer isoform) and magenta (A1AT-Native isoform).

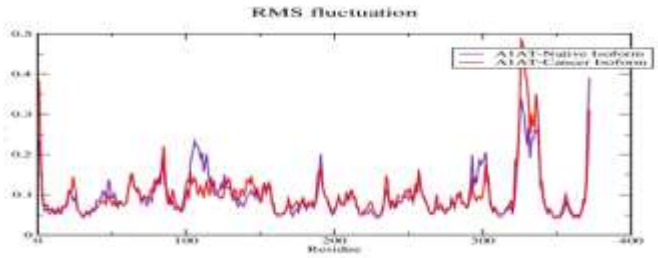

Figure-2: RMSf scatter plot of the protein stability A1AT-Native Type and Cancer isoform

X-axis represented that 354-amino acid A1AT protein, whereas the Y-axis represented variations during simulation trajectory. Native A1AT was taken as a representative reference, and the cancer isoform was compared to it. The cancer isoform's behaviour pattern of fluctuations is noted by 100-120, 296-300, and 325-340. This suggested that the cancer isoform deviates during the 100 ns trajectory run.

Principal Component Analysis: The PCA was performed and displayed to find the most notable structural alterations in each structure caused by the binding of glycan complexes (discussed earlier). The goal of PCA analysis was to collect information on the conformational states of native and cancer isoforms using a 0-100 ns Molecular Dynamic simulation trajectory. The native type moved in three clusters, whereas the cancer isoform moved in one single compacts form. Native type spans a region of -2, +3, and -1, +4 in PC1 and PC2. The cancer isoform PCA plots, on the other hand, revealed a compact kind of motion along PC1 (-10, +15) and PC2 (-20, +25). It is possible that cancer complexes, as a result of their abnormal glycosylation, exhibit irregular mobility rather than cluster motion, as indicated by PCA plots.

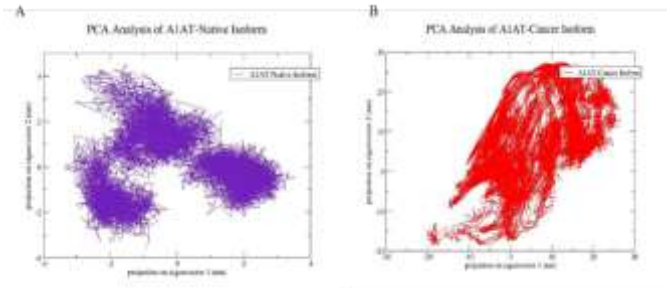

Figure-3: A) PCA of the A1AT-Native Type; B) PCA of the Cancer isoform pr

DISCUSSION

α1AT is the most prevalent circulating serpin in the body, and in certain persons, its absence causes emphysema to develop early. It also causes inflammation, immunological changes, apoptosis, and perhaps alters cellular processes as well. These events interact to maintain health and their failure adds to the development of emphysema, cirrhosis, etc as previously noted[17, 18]. The most frequent therapy for A1ATD is a parenteral weekly infusion of plasma-derived AAT (60 mg/ml). Despite producing an increase in stability, this results in a reduction in the protein's inhibitory activity[19].

The remarkable level of conservation demonstrates the significance of these amino acids in the function and structure of serpins. According to earlier research, the majority of activity-affecting mutations are found at the RCL or the loop insertion site on the A sheet; in other words, mutations in the -helix A have little effect on protein inhibitory activity and stability[20]. As a result, A -helix was removed from the protein structure. The AAT structure has a glycosylation site and an attachment loop between helix A and S6B (the sixth strand from the β-sheet) (amino acid 46). Previous research has revealed that glycosylation influences protein half-life, and when this site is removed, the glycosylation pattern changes, and the recombinant protein generated by P. pastoris may be less stable than the native AAT[21].

It is thought that due to glycosylation variations and mutation, domain residues engage with neighboring vicinity residues, causing the structure in-stability in a protein structure. As a consequence, our RMSD and RMSf investigation revealed molecular changes in the mutant protein over some time. These results are similar to the previous study suggesting the deviation of RMSD and RMSf due to mutation[22]. The results were also further confirmed by the Radius of gyration analysis, showing higher compactness in the wild type compared to the native structure. Now to further dig the stability reason because of the cancer variation H bonding analysis, a strong theoretical approach, was applied. When compared to the original structure, the altered structure had less H-bonding. These findings are similar to an earlier study by Doss CG et al[23].

Furthermore, the PCA results indicated a more streamlined motion of the native A1AT isoform, confirming important predictions. Higher Glycosylation induces higher stability in a protein structure, supported by the studies[24,25]. Most of the naturally occurring proteins are glycosylated, but only 4% of X-ray crystallographic structures in the Protein Data Bank (as of March 2021) include covalently bonded N- or O-glycans. This minor number of glycoproteins in the PDB is mostly because most target proteins are partly or completely truncated before crystallization to eliminate the glycosylation. glycans that inhibit or decrease the formation of advantageous crystal contacts and other possible explanations include glycosyl micro-heterogeneity, glycan flexibility, and utilization of non-native protein production methods[26].

Finally, after all this discussion we can say based upon our results and previous research data that aberrant A1AT is truncated has lost negative charge on it, blunts immune response, and promotes tumor growth and metastasis by weakening cell to cell adhesions and increase production of TGF. Keeping in view all these points if we can somehow stop this aberrancy in A1AT then in this way we can clear the target for drugs/immune system to easily find and attack the cancer cells and neutralize them.

Limitations: No liquid chromatography-isotope and mass spectrometry techniques were used.

CONCLUSION

It was concluded that glycated protein appears to have high structural stability than its aberrant glycated protein. Moreover, a link between fewer structural fluctuations identified by MD simulations and enhanced protein stability after glycosylation as measured by experimental measures. As a result, simulation techniques may be used to explore the impact of glycans on target protein structural stability computationally. Hence, the methodology proposed in this research will enable researchers to examine numerous key disease-related processes that occur in a short period using just conventional, medium-sized computer clusters found in most computing facilities.

Author's Contribution: MAK&E: Conceptualized the study, analyzed the data, and formulated the initial draft.

MS&RA: Contributed to the histomorphological evaluation.

MI: Contributed to the analysis of data and proofread the draft.

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REFERENCES

1. Hashemi, M., et al., Alpha-1 Antitrypsin: Its Role in Health and Disease. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents), 2010. 9(4): p. 279-288.
2. Kellici, T.F., E.S. Pilka, and M.J. Bodkin, Small-molecule modulators of serine protease inhibitor proteins (serpins). Drug Discovery Today, 2020.
3. Bao, J., et al., Serpin functions in host-pathogen interactions. PeerJ, 2018. 6: p. 4557.
4. Schmidt, C.Q., J.D. Lambris, and D. Ricklin, Protection of host cells by complement regulators. Immunological reviews, 2016. 274(1): p. 152-171.
5. Chen, H., et al., The serpin solution; targeting thrombotic and thrombolytic serine proteases in inflammation. Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders), 2013. 13(2): p. 99-110.

6. Chang, Y.-P., et al., Targeting serpins in high-throughput and structure-based drug design. Methods in enzymology, 2011. 501: p. 139-175.
7. Gatto, M., et al., Serpins, immunity and autoimmunity: old molecules, new functions. Clinical reviews in allergy & immunology, 2013. 45(2): p. 267-280.
8. Rehman, S., et al., Genome-Wide Identification and Comparative Analysis of the Serpin Gene Family in Brachypodium and Barley. Plants, 2020. 9(11): p. 1439.
9. Heit, C., et al., Update of the human and mouse SERPIN gene superfamily. Human genomics, 2013. 7(1): p. 1-14.
10. Craik, C.S., M.J. Page, and E.L. Madison, Proteases as therapeutics. Biochemical Journal, 2011. 435(1): p. 1-16.
11. Gaci, N., et al., Patented biotechnological applications of serpin: an update. Recent Patents on DNA & Gene Sequences (Discontinued), 2013. 7(2): p. 137-143.
12. Dunlea, D., The role of glycosylated alpha-1 antitrypsin in resolution of inflammation. 2018, Royal College of Surgeons in Ireland.
13. Strnad, P., N.G. McElvaney, and D.A. Lomas, Alpha1-antitrypsin deficiency. New England Journal of Medicine, 2020. 382(15): p. 1443-1455.
14. Matamala, N., et al., Characterization of novel missense variants of SERPINA1 gene causing alpha-1 antitrypsin deficiency. American journal of respiratory cell and molecular biology, 2018. 58(6): p. 706-716.
15. Daura, X., et al., Peptide folding: when simulation meets experiment. Angewandte Chemie International Edition, 1999. 38(102): p. 236-240.
16. Sangachini, E.D., et al., Construction of an engineered alpha 1-antitrypsin with inhibitory activity based on theoretical studies. Electronic Journal of Biotechnology, 2012. 15(2): p. 8-8.
17. Silverman, G.A. and D.A. Lomas, Molecular and cellular aspects of the serpinopathies and disorders in serpin activity. 2007: World Scientific.
18. Sarkar, A. and P.L. Wintrode, Effects of glycosylation on the stability and flexibility of a metastable protein: The human serpin α1-antitrypsin. International journal of mass spectrometry, 2011. 302(1-3): p. 69-75.
19. Karnaukhova, E., Y. Ophir, and B. Golding, Recombinant human alpha-1 proteinase inhibitor: towards therapeutic use. Amino Acids, 2006. 30(4): p. 317-332.
20. Kumar, A., et al., Molecular dynamic simulation reveals damaging impact of RAC1 F28L mutation in the switch I region. PloS one, 2013. 8(10): p. e77453.
21. Doss, C.G.P., et al., Screening of mutations affecting protein stability and dynamics of FGFR1—A simulation analysis. Applied & translational genomics, 2012. 1: p. 37-43.
22. Solá, R.J. and K. Griebenow, Effects of glycosylation on the stability of protein pharmaceuticals. Journal of pharmaceutical sciences, 2009. 98(4): p. 1223-1245.
23. Shental-Bechor, D. and Y. Levy, Effect of glycosylation on protein folding: a close look at thermodynamic stabilization. Proceedings of the National Academy of Sciences, 2008. 105(24): p. 8256-8261.
24. Watanabe, I., et al., Glycosylation affects the protein stability and cell surface expression of Kv1. 4 but not Kv1. 1 potassium channels: a pore region determinant dictates the effect of glycosylation on trafficking. Journal of Biological Chemistry, 2004. 279(10): p. 8879-8885.
25. Tan, N.Y., et al., Sequence-based protein stabilization in the absence of glycosylation. Nature communications, 2014. 5(1): p. 1-7.
26. Lee, H.S., Y. Qi, and W. Im, Effects of N-glycosylation on protein conformation and dynamics: Protein Data Bank analysis and molecular dynamics simulation study. Scientific reports, 2015. 5(1): p. 1-7.