The Effect of T192M Mutation in Stability of Alpha Dystroglycan: Study with Molecular Dynamics Simulation

Simanti Bhattacharya, Amit Das, Rakhi Dasgupta and Angshuman Bagchi

Abstract Alpha-dystroglycan (α-DG), a cell surface receptor links extracellular matrix with cellular cytoskeleton. Its post translational modification is carried out with number of glycosyltransferases, depending on cell types to make the ligand specific mature α-DG receptor protein. However, T192M mutation in α-DG has been found to cause hypo-glycosylation of the protein disabling its Laminin binding form and thereby triggering the onset of a limb girdle muscular dystrophy affecting early childhood. Here for the first time we exploit the effect of this mutation in protein conformational stability. We have found that this mutation leads to significant changes in secondary structure of the protein as well as in the accessible surface area. All these changes also hamper the crucial disulfide bond that is required to maintain the globular fold at the N terminus of α-DG. This molecular insight will therefore be useful for developing new therapeutic approaches to overcome the disease state.

Keywords Alpha-dystroglycan · Mutation · Molecular dynamics simulation · Disulfide bond · Disease · Protein folding

Abbreviations

α-DG	Alpha dystroglycan
ECM	Extra Cellular Matrix
β-DG	Beta dystroglycan

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DAPC	Dystrophin Associated Protein Complex
LARGE	Like-acetylglucosaminyltransferase
MDDGC9	Muscular Dystrophy, Dystroglycanopathy, type C9
OPLS-AA	Optimized potential for liquid simulations all atom force field
RMSD	Root mean squared deviations
RMSF	Root mean squared fluctuations
SASA	Solvent accessible surface area

1 Introduction

Protein folding and post-translational modifications are essentials for the proper maturation of proteins. Cell surface glycoproteins, specifically, undergo extensive post translational modifications, like phosophorylations, glycosylations, by different enzymes depending on the cell types in higher vertebrates [1]. Alpha dystroglycan (α-DG) is one of such cell surface glycoprotein whose function broadly depends on its post translational modifications and active site folding. This protein serves as a receptor to the signals from extra cellular matrix (ECM) proteins. This signal is then transmitted to cellular actin cytoskeleton through its counterpart, beta dystroglycan (β-DG) which actually interacts with cytosolic Dystrophin. In this way, Dystrophin Associated Protein Complex (DAPC) converts the mechanical stimuli to electro-chemical signals and stands for muscle contraction in muscle cells [2]. The α-DG, being the founder-stone to this system, requires a functionally active maturation. It has been reported that proper glycosyation at 317/319 residues of the protein by LARGE (like-acetylglucosaminyltransferase) enables the protein to interact with ligand, Laminin [3]. Moreover, its N terminal part forms an independent globular fold and this architecture serves as the recognition site for LARGE as well as for Laminin [3, 4]. One natural mutation T192M in α-DG has been found to cause muscular dystrophy, dystroglycanopathy, type C9, MDDGC9 [OMIM:613818], an autosomal dystroglycanopathy that has an early onset affecting the childhood with limb development abnormality and is also associated with poor cognitive power, walking disability in affected children. This mutation causes hypoglycosylation of the protein and thereby reduces its interaction with Laminin [5, 6]. Our initial studies [6], interestingly, have found that T192M mutation changes the local hydrophobic nature of the protein as well as brings out structural changes in the vicinity of the aforementioned mutation site. However, the effects of the mutation on the overall integrity of the protein as well as on the stability of the active site architecture are broadly left untouched. In the current work, we have employed molecular dynamics simulations at physiological conditions to understand the conformational stability and the changes in the secondary structure and over all solvent accessibility of the protein due to the presence of the T192M mutation in a real time basis. And we have successfully addressed that the mutation alters the

structure of the protein and makes it more unstable. This study for the first time reveals the microscopic changes occurring in the system due to T192M mutation and also develops a deeper insight into the disease mechanism.

2 Materials and Methods

2.1 Homology Models for the Wild Type (WT) and T192M Mutated (MT) Protein

Homology models with the amino acid residues 60-304 of α-DG was generated as mentioned in the literature [6]. The mutated model of the protein was generated in the similar manner. These models were then taken for simulation analyses.

2.2 Molecular Dynamics Simulations

The structures for WT and MT protein were minimized with steepest decent minimization algorithm individually in GROMACS 5.0 package. Hydrogen atoms to each co-ordinate file were added using Optimized Potential for Liquid Simulations all atom force field (OPLS-AA) [7]. Each minimized structure was then solvated using sufficient water and a box was created where the structure was place in the centre and with 7Å equidistant from each surface. Proper counter ions were added to ensure the neural atmosphere. 1,000 ps production run was carried out after equilibrating the system with constant physiological temperature (310 K) and pressure sequentially for 100 ps each.

2.3 Molecular Analyses

The conformations generated by production run at physiological environment were next analyzed for (a) Root Mean Squared Deviations (RMSD), (b) Root Mean Squared Fluctuations (RMSF), (c) Changes in secondary structure and (d) changes in solvent accessible surface area (SASA). All these analyses were carried out using VMD 1.9.1 and GROMACS 5.0 package.

3 Results and Discussions

3.1 T192M Mutation Makes the Protein More Stable Conformations

Conformations were analyzed over time (Fig. 1). The RMSD variations for the conformations generated with production run show a stable pattern for the mutated (MT) structure. In comparison to this, the wild type (WT) structure has a relatively deviated RMSD plot (Fig. 1a). This indicates that the mutation itself stabilizes the protein structure. But the scenario indicates a worsen picture when we looked into the residue wise fluctuations of the two structures. The peak at residues 135-155 has fluctuated more than the WT residues (Fig. 1b). Similarly 205-225, 235 and 275-285 have shown deviations in residue level fluctuations higher than the wild type counter parts. This indicates in spite of being stable over the time, the MT structure has relatively unstable residues in the above mentioned regions.

3.2 Residue Level Fluctuations Weaken the Disulfide Bond Crucial to Structure

The T192M mutation has an impact in the secondary structure of protein in dynamics. The beta turn structures at residues 172 and 252 have been replaced by random coil structure. Similar scenario has been reflected in case of residues 200 and 224 where G-10 architecture has been replaced by turns (Fig. 2a). This emphasizes

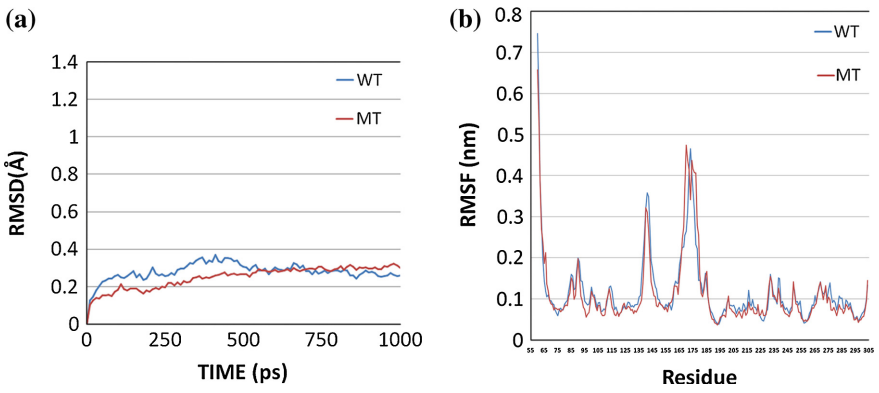
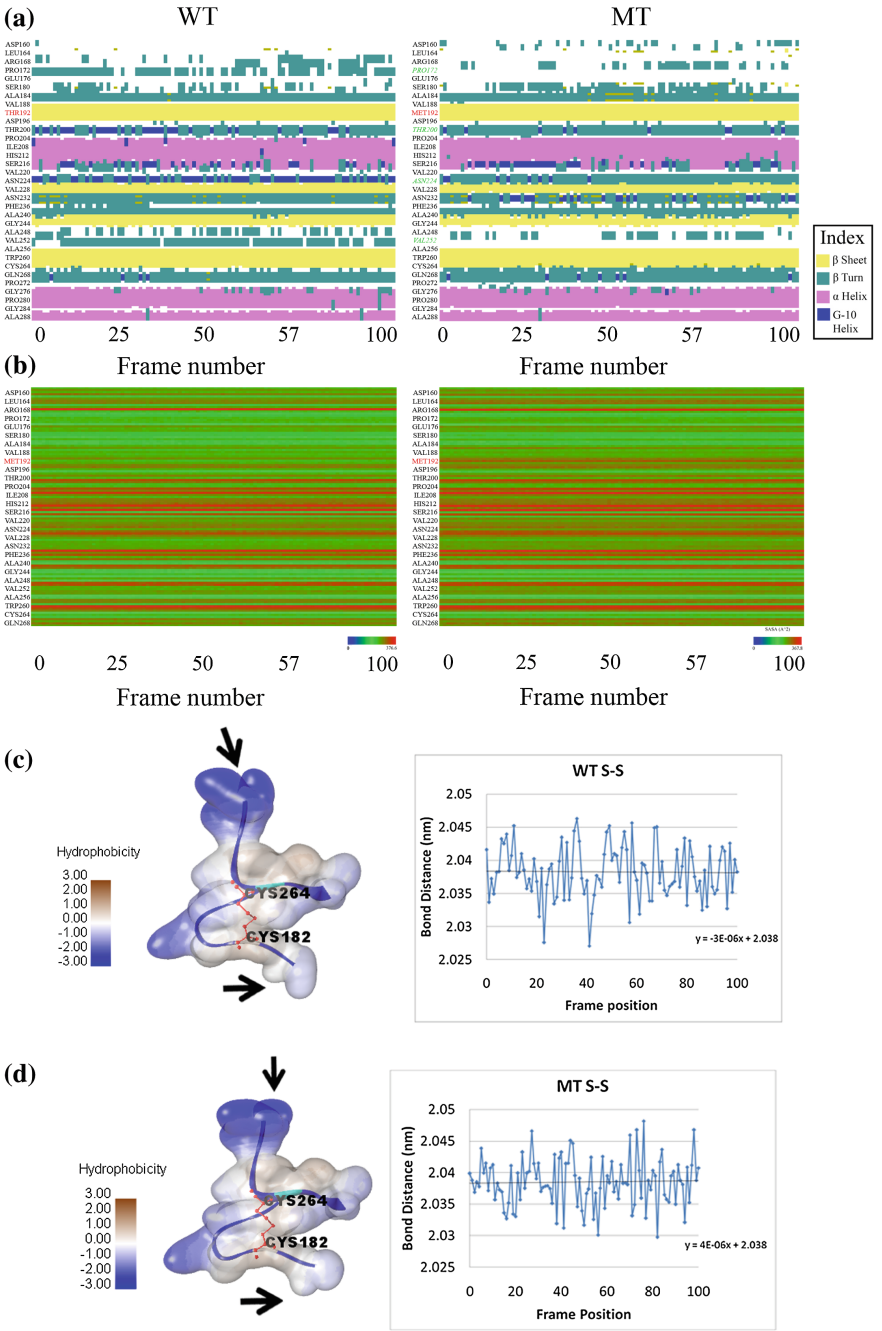


Fig. 1 Changes in the conformations. **a** Root Mean Squared Deviation (*RMSD*) is more stable in case of mutated structure. **b** Certain residues with higher Root Mean Square Fluctuations (*RMSF*) have been observed in mutated structure


(a)
WT
MT
Index
β Sheet
β Turn
α Helix
G-10 Helix
0
25
50
57
100
(b)
Frame number
(c)
Hydrophobicity
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
CYS264
CYS182
WT S-S
Bond Distance (nm)
2.05
2.045
2.04
2.035
2.03
2.025
y = -3E-06x + 2.038
0
20
40
60
80
100
Frame position
(d)
MT S-S
y = 4E-06x + 2.038
Frame Position

◀**Fig. 2** Relative instability conferred by T192M mutation. *WT* wildtype and *MT* mutated. **a** Changes in secondary structure. 192nd position has been marked with red. Altered sites have been marked with *green color* and *italic font*. **b** Solvent accessible surface area (SASA). The SASA has changed near mutation (*red*) site. **c–d** Changes in the surface architecture (*arrow*) near the Cys182-Cys264 disulfide bond. Graphical representation at *right* shows the nature of the change in bond distance

the fact that due to this single point mutation, secondary structure of the protein has been altered vastly. Moreover these structural changes have also affected the accessible surface area near the mutational site. Replacement of polar Threonine with non-polar Methionine makes the site more hydrophobic, but conformational changes have made the site more accessible (Fig. 2b) to solvent which is very unlikely and makes the structure unstable. It is worth mentioning here that disulfide bonds between Cysteine 182 and Cysteine 264 (Cys182-Cys264) is a crucial to maintain the architecture of the N terminal domain of alpha-dystroglycan [8, 9]. This N terminal region in turn is the recognition site for both LARGE and Laminin [3, 4]. This disulfide bond surpasses the mutation site too. So the above mentioned changes should have affected this important interaction. Time based simulation of the disulfide bond showed that in WT structure those two Cysteine residues stay close to each other and maintain the bond (Fig. 2c). But in MT structure the bond distance varies with the conformations (Fig. 2d). When changes in the bond distance have been plotted, the bond in WT conformations tends to converge (with a slope of −3) whereas the bond in the MT conformations tends to diverge more (slope = 4). The destabilization in the aforementioned disulfide bond hampers the N terminal architecture of the protein and makes it less recognizable to both its glycosylating enzyme, LARGE and ligand, Laminin.

4 Conclusions

Residue level fluctuations and change in secondary structure have been noticed in α-DG structure containing T192M mutation. Again, the hydrophobic region at Met192 becomes more accessible to water due to these changes making the protein unstable. More importantly, the conserved Cys182-Cys264 disulfide bond also gets destabilized in the presence of T192M mutation. These changes make the mutated structure, specifically its N-terminal domain, less accessible to LARGE. This also answers the reason behind the hypoglycosylation in presence of T192M mutation and thereby non-Laminin binding state in case of MDDGC9. Our study would therefore be helpful in designing new therapeutic approaches to overcome the effect of the disease.

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Conflict of Interest The authors declare no conflict of interests.

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