Dynamic characteristics of totally glycosylated human glucocerebrosidase carrying N370S substitution

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Abstract. Full-atom models of a totally glycosylated wild type human glucocerebrosidase (GBA) and its mutant form N370S were constructed. Molecular dynamics simulations in explicit water box have revealed high flexibility and mobility of glycans bonded to the enzyme; glycans was able to spread a rather large volume determined by their length. Amino acid substitution N370S decreased the flexibility of GBA polypeptide chain region forming Loop 1 (residues 311-319), while the mobility of catalytic residues remained at the similar level both in wild type and N370S GBA mutant form.

1. Introduction

Gaucher disease (GD) is a hereditary lysosomal storage disease associated with glucocerebrosidase enzyme deficiency, most often caused by mutations in the GBA1 gene, leading to amino acid substitutions L444P and N370S [1]. As a result, the enzyme’s undegraded substrate, glucocerebroside, accumulates in lysosomes. Macrophages are the most affected cells in GD patients. Swelled macrophages can infiltrate the spleen, liver, and bone marrow, and cause inflammation and organomegaly [1]. Along with enzyme-replacement and substrate-reducing therapies, the use of small chemical compounds capable of restoring the activity of the mutant form of the enzyme is one of the promising methods for reducing the symptoms of GD. Virtual screening of such compounds requires construction of full-atom model most closely reconstituting the native structure of the enzyme [2]. Mature GBA enzyme is a glycoprotein containing 497 amino acid residues and having molecular weight varying from 59 to 69 kDa, considering to differential glycosidic remodeling [3]. There are four glycosylation sites on the GBA surface (amino acid residues Asn19, Asn59, Asn146 and Asn270). Glycosylation seems to be critically needed for proper spatial organization of amino acid residues in the GBA active center, and consequently for the enzyme activity [3]. The previous study on effect of wild-type enzyme (WT) glycosylation level on its polypeptide chain flexibility showed the importance of glycosylation at the first site (Asn19) [3].

2. Methods

Enzyme models were constructed based on spatial structure of human glucocerebrosidase deposited in Protein Data Bank (entry 2NT1). Asparagine at position 370 was substituted with serine to produce the mutant form of the enzyme. Glycan chains with the following sequence DNeup5Aca2-3DGalpb1-4DGlcNAcb1-6[DNeup5Aca2-3DGalpb1-4DGlcNAcb1-2]DManpa1-6[DNeup5Aca2-3DGalpb1-4DGlcNAcb1-2DManpa1-3]DManpb1-4DGlcNAcb1-4DGlcNAcb1-OH that was previously described by a combined Smith degradation and chromatography study [4], were added using Glycoprotein builder at Glycam web server (www.glycam.org). The influence of amino acid substitution N370S on mobility of
the enzyme polypeptide chain and catalytic amino acid residues was studied by the method of molecular dynamics (MD) simulations in explicit solvent with periodic boundary conditions (AMBER16 software package, ff14sb force fields for amino acids and GLYCAM for carbohydrate part, TIP3P water molecule model, 300K, 1 atm, PME periodic boundary conditions, 10 Å cutoff distance for nonbonded interactions, LINCS, 2 fs integration step). Two independent 100 ns simulations were run for each of the models.

3. Results
In this study, we investigated the effect of glycosylation on the dynamic characteristics of the polypeptide chain of GBA mutant form N370S. We have constructed full-atom models of WT GBA and its mutant form N370S bounded with triantennary, trisialylated, complex-type oligosaccharide at all four sites mentioned above.

Analysis of MD trajectories has shown that, due to their high flexibility and mobility, glycans do not occupy fixed positions on the surface of the enzyme, but spans a certain volume restricted by the length of the carbohydrate chain. The glycan chains occupy different conformations and interact with distinct parts of the GBA surface. The glycan attached to the residue N146 is located in proximity to both the active site and the predicted membrane associated region of the GBA. None of the glycans were found to block the entrance to neither the active site nor the membrane binding site.

On the modelled time frame (100 ns), glycosylation does not cause any sufficient conformational changes of the GBA tertiary structure. However, it should be noted that the N370S amino acid substitution provokes positional stabilization of amino acid residues 316 and 317 associated with the Loop 1 (composed of residues 311-319 according to conventional classification [2]), and Loop 2 (composed of residues 345-349) located next to the active site (Figure 1). This result is in good agreement with the X-ray crystallography data [5] and validates constructed model. Apart from this stabilization, the rest of enzyme’s secondary structures do not show noticeable changes.

![Figure 1. RMSF curves of WT (blue) and N370S mutant (red) forms of GBA. Grey horizontal line displays RMSF average value.](image)

Both WT GBA and N370S mutant form have distinguishable root-mean-square fluctuations (RMSF) of a number of backbone regions that are higher than the sum of average RMSF value and its deviation 0.6±0.22 Å (Figure 1). For the WT enzyme, these are amino acid residues: 1, 29-34, 60-64, 171, 240-245, 345, 347-349, 439-443, 497. For the mutant form N370S, these are residues: 1, 29-34, 58-64, 189-194, 239-246, 407-408, 441-443, 452-454, 497. Regions with high rate of fluctuations specific to WT or N370 mutant form are marked by italic. Indicated residues compose not only unstructured loops connecting an elements of secondary structures, but termini of alpha-helices of the domain II and beta-sheets of the domains I and III.

Introduction of substitution N370S can lead to significant changes in mobility of several backbone regions (Figure 2) displayed by molecular dynamics simulations. Main chain atoms of residues 58-63, 88-94, 127-128, 137, 187-196, 239, 274, 383, 386-387, 393, 406-409 and 451-497 increased, while that of residues 1, 2, 32, 45-47, 64-65, 171, 201-202, 242-243, 254, 300, 313-319, 343-349, 398, 429 and 439-442 decreased their mobility in comparison with WT.
Residues 313-319 and 343-349 compose Loop 1 and Loop 2 respectively forming the entrance to the active site of the enzyme.

Figure 2. The median structure of a totally glycosylated N370S mutant form of GBA obtained by cluster analysis of the MD trajectory. Depending on changes of RMSF value backbone regions were considered more (red) or less (blue) flexible in comparison with WT enzyme. Glycan chains and essential amino acid residues are shown in balls and sticks representation. Catalytic residues (E235, E340) are colored in green, residues bonded with glycans (N19, N59, N146, N270) are in yellow and serine at position 370 is in magenta.

The distances between carbon atoms of carboxylic groups of the catalytic residues (E235 and E340) are 7.93±0.96 Å and 7.54±1.42 Å for WT and mutant form of GBA, respectively. RMSFs of E235 are 28.0 Å and 32.9 Å and RMSFs of E340 are 16.2 Å and 10.3 Å for WT and mutant form of GBA, respectively.

4. Conclusion
Since the catalytic amino acid residues have the similar mobility both in the WT human glucocerebrosidase and its mutant form N370S, it can be assumed that decay of enzyme activity resulting from amino acid substitution is primarily caused by decrease of Loop 1 flexibility, located at the entrance to the active site of the enzyme.

Acknowledgements
This work was supported by the Russian Science Foundation under grant 17-75-20159. The authors acknowledge the use of resources of the Peter the Great Saint-Petersburg Polytechnic University Supercomputing Center (www.scc.spbstu.ru).

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