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Structure and dynamic studies of lunatic, manic and radical fringe

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ABSTRACT

O-Fucosylpeptide-3-beta-N-acetylglucosaminyltransferase (fringe) belongs to the family of glycosyltransferases. The modulator fringe participates in Notch signaling pathway. Various studies have shown that this modulator is involved in many developmental processes. The mutation or overexpression of fringe can lead to various diseases including cancer. Lunatic fringe (Lfng), manic fringe (Mfng) and radical fringe (Rfng), the paralogues of fringe, have not yet been studied at any level of computational biological research. The work accomplished in this study has been a step forward towards cutting edge research in the field of computational biology. Homology modeling and molecular dynamics simulation have been employed in order to study different structural, theoretical and dynamical properties of the fringe protein family. The 3D structures of fringe protein family which have not been reported previously in any of the structural database have been predicted by utilizing MODELLER tool. Structures were analyzed and validated through different structural and physicochemical characterization tools. Molecular dynamics simulation (MD simulation) has been employed on all three paralogues—Lfng, Mfng and Rfng—to investigate the protein dynamics of the fringe family at the atomic level reaching till the total production run of 15 ns. Time dependent behavior of protein on different time scales has been noticed through root mean square deviation (RMSD) and root mean square fluctuation (RMSF). The average RMSD for Lfng, Mfng and Rfng calculated was 3.07, 2.82 and 3.35 Å, respectively, for the production run of 5 ns each, depicting the stable structural dynamics of protein models. RMSD, RMSF, β factor and radius of gyration graphs showed the similar pattern of protein dynamics throughout the production run of 5 ns each for Lfng, Mfng and Rfng. The DxD (aspartate any residue aspartate) motif, a hallmark active site of glycosyltransferases, remained well stabilized throughout the simulation run of fringe glycosyltransferase. This study is significantly important to interpret theoretical and dynamical behavior of the fringe protein family that also infers the conservation of similar dynamic pattern among same family of proteins which further demonstrates the sequence to dynamic similarity of proteins.

1. Introduction

Glycosyltransferases (Gts) belong to the transferases class of enzymes. They transfer glycosyl group to form glycoconjugates. Gts are type II transmembrane proteins working in combination to form different glycoconjugates. Gts consist of a short amino terminal cytoplasmic domain, transmembrane domain and a stem region connecting to catalytic domain of Gts, which faces the luminal side of the Golgi body [1]. Most of the eukaryotic glycosyltransferases are Golgi apparatus and endoplasmic reticulum resident proteins, They play vital role in glycan synthesis and signaling events. Cytoplasmic domain, transmembrane domain and stem region (CTS) of Gts are helpful in their Golgi retention, targeting them to different functional areas, and enzyme's susceptibility to intracellular proteolysis. Glycosylation is one of the most important and complex forms of post-translational modification to occur in proteins. It is involved in most important biological events such as embryonic development, cell to cell communication and blood group type variations. Hence the process of glycosylation is a key to various signaling transduction pathways which regulates the normal functioning of the body [2]. Knockout studies in various model organisms have revealed that aberrations in genetic loci related to the process of glycosylation severely impair the process of development and categorize the congenital disorders of glycosylation [3]. Stem region of Gts contains several O-glycosylation and N-glycosylation sites rich in cysteine residues. N-glycosylation can serve as a signal to transport the protein to the cell surface; if unglycosylated, they stay as inactive. Glycosyltransferases transfer the sugar moiety from an activated nucleoside – sugar to an acceptor which may be a growing oligosaccharide, a lipid or a protein [4]. Notch signaling pathway involves various modulators which help in different developmental events occurring in the body. One of these modulators is fringe O-fucosylpeptide-3-beta-N-acetylglucosaminyltransferase (EC:2.4.1.222) that was discovered in Drosophila. Its three homologues have been discovered in mammals namely lunatic fringe (Lfng), manic fringe (Mfng) and radical fringe (Rfng) [5–7]. Fringe has O-fucose specific

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ß 1-3 N-acetylglucosaminyltransferase activity that adds N-acetylglucoseamine to O-linked fucose on epidermal growth factor repeats of Notch [8]. Fringe is a Golgi resident glycosyltransferase that requires a specific DxD active site motif to function. It specifically binds to uridinediphosphate (UDP). Binding to UDP and presence of DxD motif are two significant characteristics of glycosyltransferases [9,10]. Conserved stretch of hydrophobic amino acid residues near the amino terminus is a feature of Drosophila and vertebrate fringe that predicts that it is a secreted protein. Some of the fringe proteins are localized at the periphery of the expressing cells and some of them are secreted out of the cell. The molecular dynamic simulations being carried out in this study is based on the fact that fringe is a secreted protein [8,9]. Class of evolutionary conserved transmembrane receptors encoded by the gene of Notch receptor family transmits signals affecting development in organisms ranging from sea urchins to humans and is involved in the most important biological events such as apoptosis, cellular proliferation, and organization of tissue boundaries. Mutations in Notch receptors and their ligands may result in various diseases ranging from different forms of cancer to various other life threatening diseases such as multiple sclerosis and spondylocostaldysostosis [11–14]. Multiple homologues of both Notch receptor (Notch 1, 2, 3, and 4) and ligands (Delta like and serrate/jagged like) have been identified in mammals [15]. Both Notch receptors and ligands are transmembrane proteins. The extracellular portion of Notch receptors has 36 EGF like repeats and three Lin12 repeats. O-fucose is present between second and third conserved cysteine residue at C2-X-X-G-G-S/T-C3 of EGF like repeat; it exists as mono-, di- or trisaccharide. Fringe increases the incorporation of GLcNAc to fragments containing these EGF like repeats [5,7,8,16]. EGF repeats 27–36 and LNR domain of Notch are found to be specific for binding fringe [7]. Characterization of fringe in humans as per chromosomal localization is as follows: Mfng maps to human chromosome 22q13.1, Lfng is located at 7p22 arm of human chromosome and Rfng is localized at 17q25 arm of human chromosome [17]. In a recent study on a carrier of Asperger's syndrome it has been proposed that duplication of 380 kb at 7p22.3 arm of human chromosome which contains Lfng with other eight genes may have remarkable effect on the occurrence of Asperger's syndrome [18]. The purpose of this entire study revolves around the need to predict 3D structures of the fringe protein family and to investigate the protein dynamics at atomic level. Molecular modeling or computational chemistry encloses all the theoretical approaches and computational techniques to enact or simulate the molecular or chemical system at atomic level [19], whereas homology modeling is based on predicting the protein's three-dimensional structures on the basis of already known structure of a protein called template [20]. Molecular dynamics simulation is the technique through which interactions between molecules and atoms over a period of time are studied through computer simulations [21]. It is the principle method in theoretical studies of biological molecules that calculates the time behavior of the molecular system while exhibiting the detailed information on the fluctuations and conformational changes of proteins and nucleic acids. Structural validation, dynamics and thermodynamics of the system can be interpreted by the MD simulations [22]. The study presented in this work focuses on computational methods, namely, homology modeling and molecular dynamics simulations which respectively build structures and simulate the natural motion of biological macromolecules. Goals are to assess capabilities and limitations of current protein modeling servers, highlight promising areas, assess the performance of comparative modeling, and thereby enhance progress in the field. The major goals to be achieved are comparative structural modelling of fringe family (Lfng, Mfng, Rfng) and comparison of structures build through MODELLER and web based servers, molecular dynamics simulation of fringe family (Lfng, Mfng, Rfng) and to determine their dynamic patterns. Structural variations in these proteins in an aqueous environment with respect to time are also the major attributes of this study.

Table 1
Blast results of fringe protein with template selected to build structures.

	Template ID	Query coverage	Sequence identity
Lfng	2J0A	72%	64%
Mfng	2J0A	86%	86%
Rfng	2J0A	83%	57%

2. Material and methodology

2.1. Homology modeling

MODELLER 9.9 [23,24] is utilized to predict three-dimensional structure of the fringe protein. MODELLER is based on knowledge based protein structure prediction method. It requires the amino acid sequence of the target protein. Fringe-O-fucosylpeptide-3-beta-N-acetylglucosaminyltransferase has three paralogues: manic fringe (Mfng), radical fringe (Rfng) and lunatic fringe (Lfng). The sequences of human fringe-O-fucosylpeptide-3-beta-N-acetylglucosaminyltransferase for all of three paralogues were retrieved from National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). The sequences were subjected to Basic Local Alignment Search Tool (BLAST) [25] individually against a Protein Databank (PDB) (www.rcsb.org/) [26]. The PDB ID 2J0A was selected as template having 72% of query coverage and 64% of sequence identity for Lfng, 86% of query coverage and 86% of sequence identity for Mfng, and 83% of query coverage and 57% of sequence identity for Rfng (Table 1). One by one the sequence for each paralogue in PIR format was loaded with manually edited alignment files, required for MODELLER 9.9 to generate models. Five models for each of the paralogues (Lfng, Mfng and Rfng) were generated through automated function of the MODELLER. The web servers used to generate three-dimensional structures were 3D JIGSAW [27], ESyPred3D, I-TASSER [28], ModWeb [29] and SWISS MODEL [30].

For structure validation different web based tools were utilized and manually the models were checked for steric contacts. Ramachandran

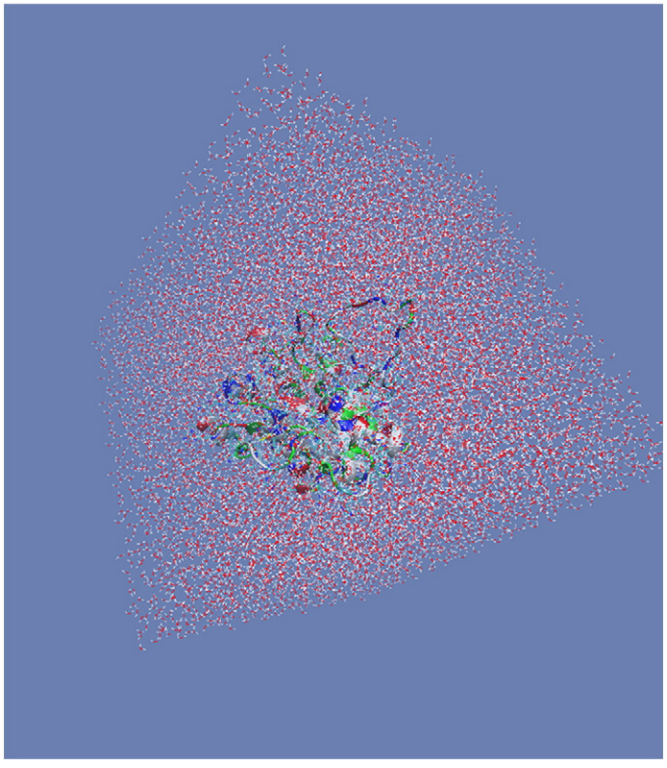

Fig. 1. 3D structure of fringe glycosyltransferase inside TIP3P water box.