Gene 537 (2014) 108–114

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Hypoglycosylation of dystroglycan due to T192M mutation: A molecular insight behind the fact

Simanti Bhattacharya, Amit Das, Semanti Ghosh, Rakhi Dasgupta *, Angshuman Bagchi *

Department of Biochemistry and Biophysics, University of Kalyani, Kalyani, Nadia – 741235 WB, India

ARTICLE INFO

Article history:
Accepted 23 November 2013
Available online 18 December 2013

Keywords:
Laminin1
Alpha dystroglycan
Molecular modeling
Protein–protein interactions
Molecular docking simulation
Dystroglycanopathy

ABSTRACT

Abnormal glycosylation of dystroglycan (DG), a transmembrane glycoprotein, results in a group of diseases known as dystroglycanopathy. A severe dystroglycanopathy known as the limb girdle disease MDDGC9 [OMIM: 613818] occurs as a result of hypoglycosylation of alpha subunit of DG. Reasons behind this has been traced back to a point mutation (T192M) in DG that leads to weakening of interactions of DG protein with laminin and subsequent loss of signal flow through the DG protein. In this work we have tried to analyze the molecular details of the interactions between DG and laminin1 in order to propose a mechanism about the onset of the disease MDDGC9. We have observed noticeable changes between the modeled structures of wild type and mutant DG proteins. We also have employed molecular docking techniques to study and compare the binding interactions between laminin1 and both the wild type and mutant DG proteins. The docking simulations have revealed that the mutant DG has weaker interactions with laminin1 as compared to the wild type DG. Till date there are no previous reports that deal with the elucidation of the interactions of DG with laminin1 from the molecular level. Our study is therefore the first of its kind which analyzes the differences in binding patterns of laminin1 with both the wild type and mutant DG proteins. Our work would therefore facilitate analysis of the molecular mechanism of the disease MDDGC9. Future work based on our results may be useful for the development of suitable drugs against this disease.

1. Introduction

Muscular dystrophy (MD) stands for inherited genetic disease characterized by progressive degeneration and weakening of skeletal muscle affecting the control for muscular rhythm and functions (Shieh, 2013). Again muscular dystrophies generated owing to aberrant glycosylation of alpha-subunit of dystroglycan are collectively called dystroglycanopathies (Godfrey et al., 2011). Muscular dystrophy dystroglycanopathy of limb girdle, type C (MDDGC9, OMIM: 613818) is an autosomal recessive muscular disorder, characterized by severe mental retardation, delayed motor development and severe cognitive impairments in affected patients (Hara et al., 2011; Tabebordbar et al., 2013). Studies with diseased patients have revealed that a missense point mutation in human dystroglycan protein (DG) is the key abnormality behind this disease (Dinçer et al., 2003; Hara et al., 2011). DG is a trans-membrane protein found in muscle, brain, neuronal junctions and epithelial cells (Henry and Campbell, 1999; Ibraghimov-Beskrovnaya et al. 1992; Tisi et al., 2000; Yamada et al., 1994). The precursor DG molecule, translated from a single mRNA, is being cleaved posttranslationally into alpha subunit (α-DG) and beta subunit (β-DG) (Bozic et al., 2004; Henry and Campbell, 1999). α-DG, the extra-cellular peripheral glycoprotein part, remains non-covalently attached to β-DG (Dinçer et al., 2003; Hara et al., 2011). α-DG receives signals from a series of ligand molecules which include laminin, argin, perlecan and many more (Hohenester et al., 1999). α-DG contains two globular domains, viz., domain1 (amino acid residues 30–315) and domain2 (amino acid residues 486–654) which are separated by bristled mucin like domain (amino acid residues 316–485), the highly glycosylated part of the protein (Henry and Campbell, 1999; Moore and Winder, 2012). On the other hand, β-DG remains associated with the membrane (Fig. 1); thereby interacting with the cytosolic actin network through a number of downstream effecter partners, like dystrophin in muscle cells (Ilsley et al., 2001). They altogether form dystrophin dystroglycan complex (DGC) which is involved in a wide variety of cellular processes (Bozic et al., 2004) like membrane stability, cellular adhesion and intracellular signal propagations (Yurchenco, 2011). It also acts as an axis through which the extracellular matrix (ECM) is tightly coupled to the cellular actin cytoskeleton (Henry and Campbell, 1996). DG is the central part of the DGC system, which requires proper glycosylation at specified positions and proper ligand molecules to interact (Sciandra et al., 2003). In the case of the disease MDDGC9, there is hypoglycosylation of α-DG by LARGE

Abbreviations: MD, muscular dystrophy; DG, dystroglycan; MDDGC9, muscular dystrophy dystroglycanopathy of limb girdle, type C; OMIM, online mendelian inheritance in man; α-DG, alpha dystroglycan; β-DG, beta dystroglycan; DGC, dystrophin dystroglycan complex; ECM, extracellular matrix; LARGE, like-acetylglucosaminyltransferase; LG 4–5 domain, laminin G like domain 4 and 5; PDB, protein data bank; BLAST, basic local alignment search tool; WT, wild type; MT, mutant.

* Corresponding authors. Tel.: +90 51948843; fax: +91 33 2582 8282.
E-mail addresses: rdgadg@gmail.com (R. Dasgupta), angshuman_bagchi@yahoo.com (A. Bagchi).

0378-1119/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.11.071

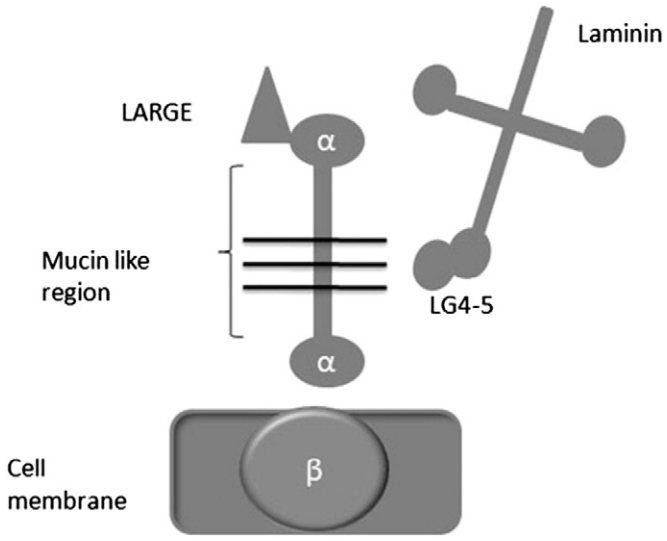

Fig. 1. The interaction scheme of laminin–DG-LARGE: LARGE recognizes and binds to N terminal part of α-DG and thereby phosphorylates the latter. This in turn helps in laminin binding and proper signal propagation for cellular development. Mutation at T192M in α-DG portion leads to hypoglycosylation and causes MDDGC9.

(like-acetylglucosaminyltransferase) enzyme due to the presence of the point mutation T192M in α-DG, which ultimately leads to weak interaction with laminin and therefore weakening of the flow of the extracellular signals (Dinçer et al., 2003; Hara et al., 2011). Human LARGE interacts with the N terminal part of the α-DG (Fig. 1) where it recognizes and glycosylates the mucin rich region of the α-DG protein and this glycosylation is crucial to establish a functional laminin–α-DG interaction for the flow of extracellular signals (Kanagawa et al., 2004; Patnaik and Stanley, 2005; Peyrard et al., 1999). Laminin belongs to the family of heterotrimeric proteins comprising of α, β and γ subunits and plays important role in membrane assembly and signal transduction. Three subunits of laminin form a cruciform structure and the carboxy terminal part of alpha subunit of laminin protein possesses globular LG domains (LG1 to LG5). In this context, laminin1 has been named according to its alpha subunit (i.e. alpha1). It has been experimentally observed that α-DG preferably interacts with LG4-5 domain of laminin alpha 1 subunit (Barresi and Campbell, 2006; Colognato et al., 1999; Durbeej et al., 2001; Tisi et al. 2000). Interestingly, not only the mucin like domain but several residues distributed across the entire N terminal domain of α-DG (30–315 amino acid residues) have been identified to be involved in its interactions with laminin1 to form laminin1–α-DG complex (Bozic et al., 2004). To be precise, amino acid stretches 30–168 make the essential contacts with laminin1 while the other region, comprising of 169–315 residues do not interact with laminin1 (Bozic et al., 2004). So far there are no previous reports that elucidate the molecular details of the interactions of α-DG, both its wild type and mutant proteins, with laminin1. In this context, we have employed in silico molecular modeling to build the yet to be identified three-dimensional structures of wild type (WT α-DG) and mutant α-DG (MT α-DG) proteins from human. Thereafter the structural aberrations in the α-DG protein occurring due to the mutation T192M (in the α-DG protein) have been analyzed. Structural comparisons of the models of WT and MT α-DG have shown remarkable differences in their secondary structural arrangements both upstream and downstream to the mutation site as well as the mutation has effect on the hydrophobic character of the protein. We then have employed molecular docking approaches to study the binding interactions of laminin1 with both WT and MT α-DG separately. The WT α-DG has been found to be interacting strongly with laminin1, compared to the MT α-DG protein. In this regard, our study may be considered to be the first of its kind which gives a residue level insight into the interactions of α-DG protein with laminin1. Our study also gives a rationale behind the onset of the disease MDDGC9 for which there were no such reports available. This study would therefore pave the pathway to future genetic and biochemical studies with α-DG and laminin1 to bring forth a suitable therapeutic approach targeting this disease.

2. Materials and methods

Amino acid sequence of DG precursor protein from human was obtained from UniprotKB (id: Q14118). The sequence of alpha subunit of DG (α-DG) from human was extracted from the total sequence of dystroglycan precursor protein of human (amino acid residues 60 to 304). This was done to study the interactions between alpha dystroglycan and laminin1. Previously found studies reported that the aforementioned portion of the alpha dystroglycan was mainly responsible for recognition and binding to laminin (Bozic et al., 2004; Henry and Campbell, 1999; Moore and Winder, 2012). We used this sequence of α-DG as input to the program BLAST (Altschul et al., 1990) in order to find its suitable homologue(s) from protein data bank (PDB) (Berman,

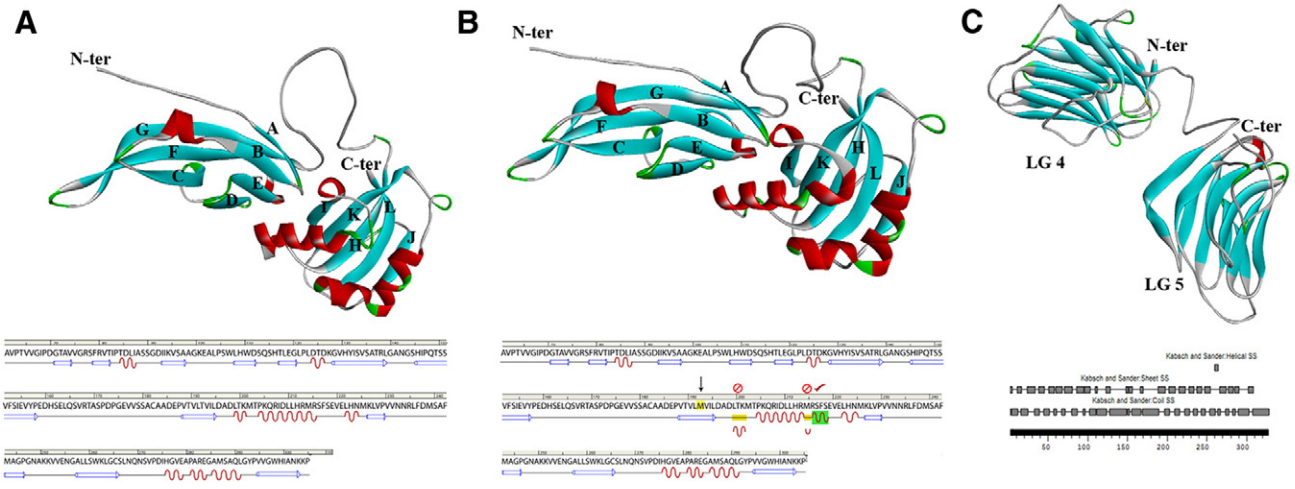

Fig. 2. Simulated structures of α-DG and laminin1: (A) WT α-DG, (B) MT α-DG and (C) laminin1 LG4-5. These models have been constructed by molecular modeling. The lower panel for each figure shows the predicted secondary structures. The WT α-DG and MT α-DG differ at their secondary structural level. Figures have been prepared in DS2.5. A high resolution figure for the amino acid sequences showing differences between WT α-DG and MT α-DG has been given as Supplementary Fig. 2A (WT α-DG) and 2B (MT α-DG).