The Structure of the T190M Mutant of Murine α-Dystroglycan at High Resolution: Insight into the Molecular Basis of a Primary Dystroglycanopathy

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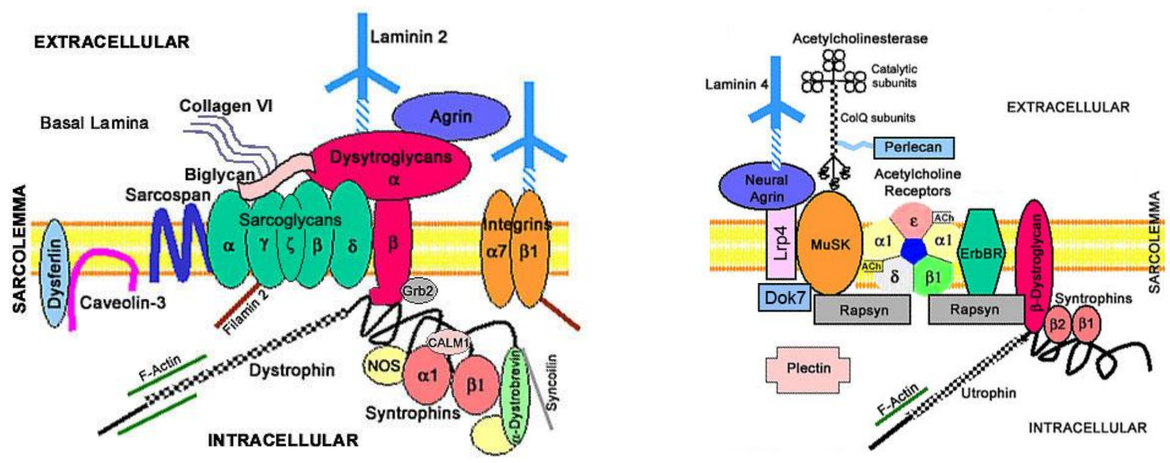
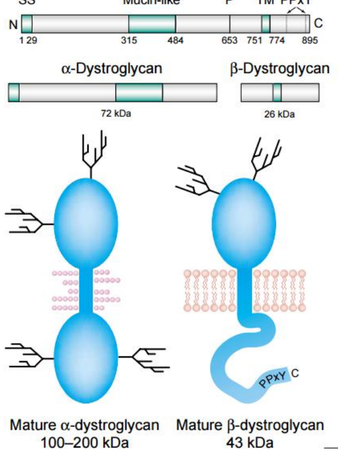
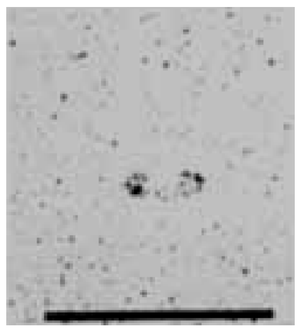
Dystroglycan (DG) is a cell surface receptor consisting of two subunits: α-dystroglycan, extracellular and highly glycosylated, and β-dystroglycan, spanning the cell membrane. It is a pivotal member of the dystrophin-glycoprotein complex and is involved in a wide variety of important cellular processes such as the stabilization of the muscle fiber sarcolemma or the clustering of acetylcholine receptors [1,2].

Extrajunctional muscle membrane | **Neuromuscular Junction**



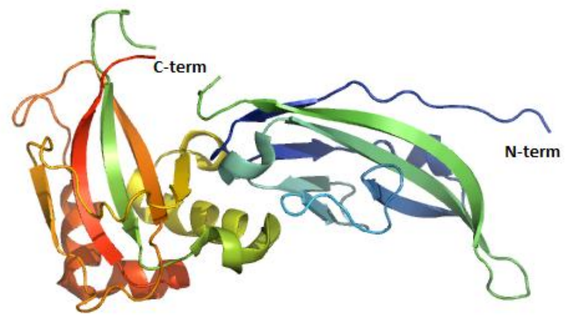
Electron microscopy investigation on the shape of α-dystroglycan suggests that the core protein consists of two roughly globular domains connected by a segment which most likely corresponds to a mucin-like central region also predicted by sequence analysis on mammalian isoforms [3]. This segment may act as a spacer in the dystrophin-associated glycoproteins complex exposing the N-terminal domain of α-dystroglycan to laminin in the extracellular space.



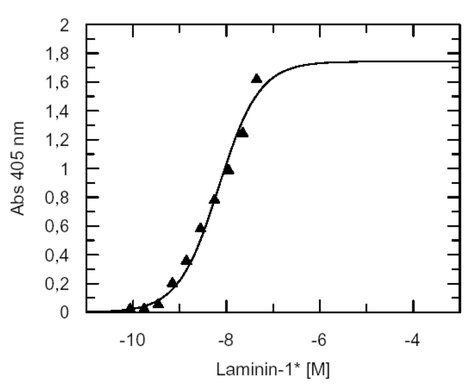


The bar represents 100 nm

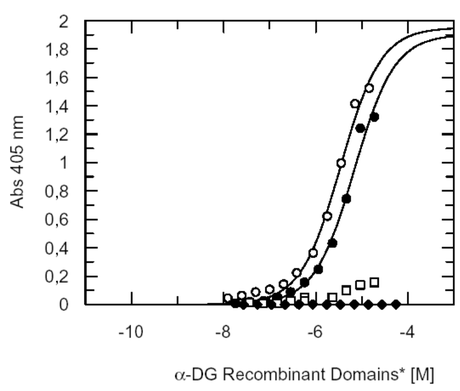
We previously reported the 2.3Å resolution crystal structure of the murine skeletal muscle N-terminal α-DG region (52-315), which unveiled the presence of two autonomous domains architecture; the first identified as an Ig-like (I-set) and the second resembling ribosomal RNA-binding proteins (Ribosomal protein S6 fold) [4].



Solid-phase laminin binding assays showed the occurrence of protein-protein type of interactions involving the Ig-like domain of α-DG [4].



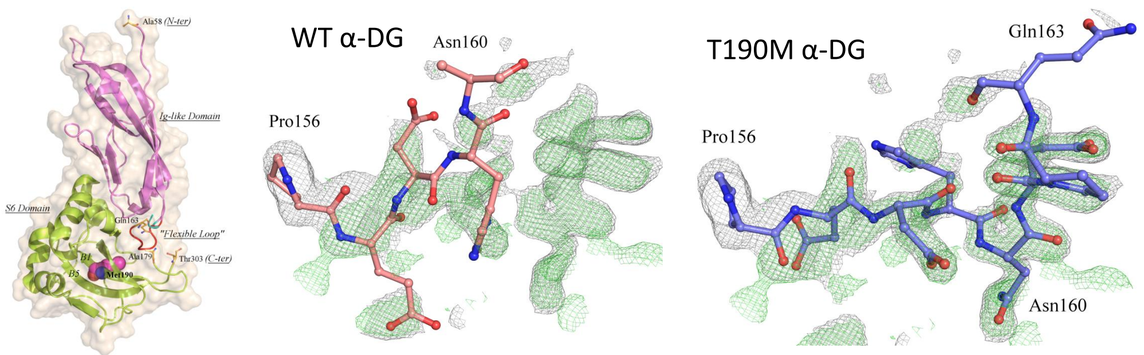
Biotinylated Laminin-1 and native chick skeletal muscle α-DG (▲).
The apparent K_d is 6nM



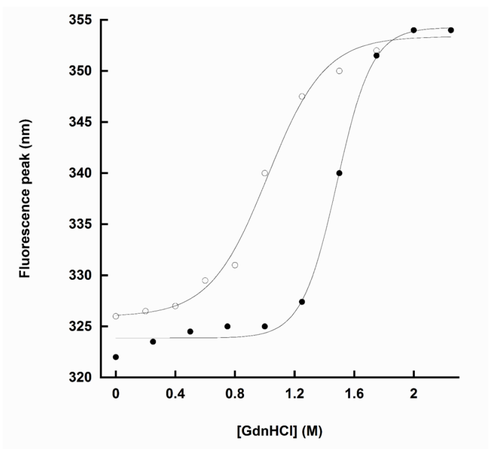
Biotinylated recombinant α-DG (28-313) (○); (28-313) (●); (28-313) (□); BSA (♦)
The apparent K_d is 3.5μM (○) and 7μM (●)

The severe dystroglycanopathy known as a form of Limb-Girdle Muscular Dystrophy (LGMD2P) is an autosomal recessive disease caused by the point mutation T190M in α-dystroglycan. Functional expression analysis in vitro and in vivo indicated that the mutation was responsible for a decrease in posttranslational glycosylation of dystroglycan, eventually interfering with its extracellular-matrix receptor function and laminin binding in skeletal muscle and brain [5].

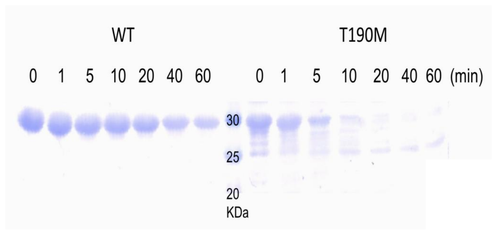
The X-ray crystal structure of the missense variant T190M of the murine N-terminal domain of α-DG (50-313) has been determined at 1.6Å resolution [6], and showed an overall topology very similar to that of the wild-type structure [4]. Despite showing very similar crystal structures, WT α-DG and T190M α-DG differ in the conformation of the flexible loop linking the two domains (residues 159–179).



Chemical denaturation and limited proteolysis experiments point to a decreased stability of the T190M variant with respect to its wild-type counterpart [6].



Equilibrium unfolding curves of WT α-DG and T190M mutant. The red shifts in the fluorescence peaks upon addition of GdnHCl indicate a progressive exposure of the tryptophan residues to the solvent: the data fit to a single cooperative transition for both WT α-DG (○), and T190M mutant (●), with a difference in midpoints (Gdn50%) of 0.45M, that reveals the T190M mutant to be less stable than the wild-type α-DG .



T190M α-DG was rapidly cleaved by trypsin and completely degraded within 10 minutes of digestion. WT α-DG instead was less susceptible to tryptic cleavage and showed a clear degradation only after 40 minutes of digestion. The T190M mutant is clearly more susceptible to proteolysis than WT α-DG .

The exact molecular mechanism leading to hypoglycosylation of α-DG carrying the T190M mutation and causing a severe dystrophic phenotype, accompanied by some degree of intellectual impairment, is currently unknown. The effect of the T190M mutation, which is next to the flexible hinge connecting the two sub-domains, may "propagate" to the Ig-like domain by changing its relative orientation with respect to the S6-like domain also influencing its binding properties. This mutation may render the entire L-shaped protein architecture less flexible. The overall reduced flexibility and stability may affect the functional properties of α-DG via negatively influencing its binding behavior to factors needed for DG maturation, and may lay the molecular basis of the T190M-driven primary dystroglycanopathy.

References

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