Stoichiometry of Laminin, α-Dystroglycan and Dystrophin in Skeletal Muscle

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Introduction

- **Muscular dystrophy** is a category of hereditary diseases that are characterized by progressive muscle weakness, muscle degeneration and muscle fibrosis.
- **Dystrophin-Glycoprotein Complex (DGC)** is a multi-protein complex that links the extracellular matrix (ECM) to the cortical actin cytoskeleton, stabilizing sarcolemma when muscle contracts.
- **Laminin (Lam)**, **dystroglycan (DG)** and **dystrophin (Dys)** make up the axis of the DGC.
- In most types of muscular dystrophy, dystrophin and other components of the DGC are reduced or even absent on sarcolemma.
- It is thought that these three proteins always associate with each other into the DGC, but recent studies showed the possible existence of "free DG" without incorporated into the DGC. [1]

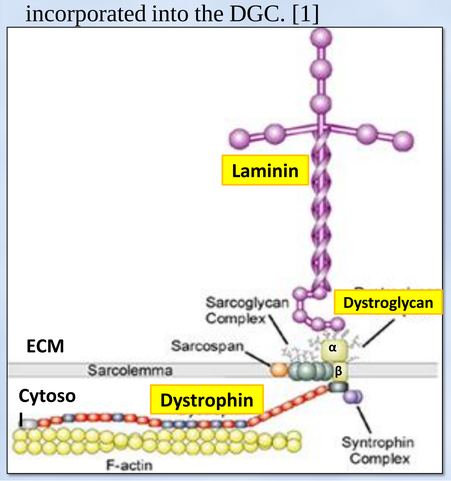
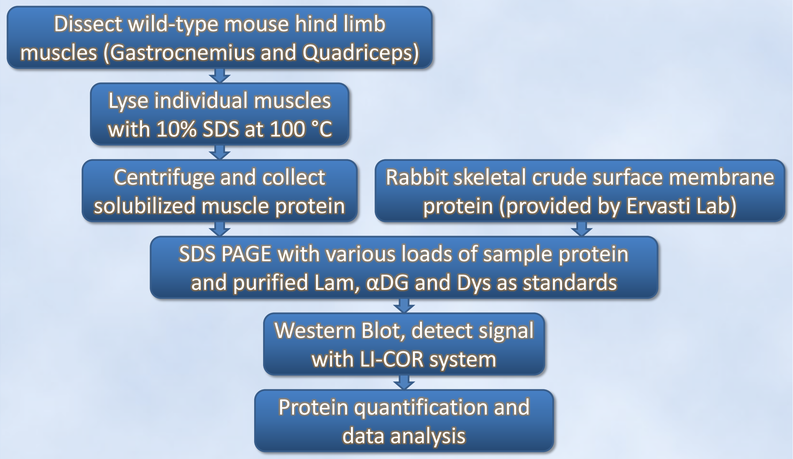


Figure 1. Model of the Dystrophin-Glycoprotein Complex (DGC) [2]. Components of the DGC and their positions are illustrated. Laminin, dystroglycan and dystrophin form an axis that link extracellular matrix across sarcolemma to F-actins in cytosol. Sarcoglycan and sarcospan are thought to stablize the DGC. Syntrophin is an adapter protein that allows other proteins to dock on the DGC.

Acronyms

αDG	→ α subunit of dystroglycan	ECM	→ extracellular matrix
DG	→ dystroglycan	Lam	→ laminin
DGC	→ dystrophin-glycoprotein complex	Ms SKM	→ mouse skeletal muscle
Dys	→ dystrophin	Rb CSM	→ rabbit crude surface membrane

Methods



Results

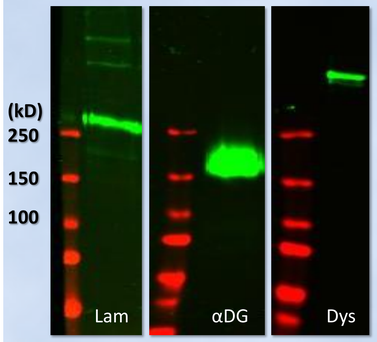
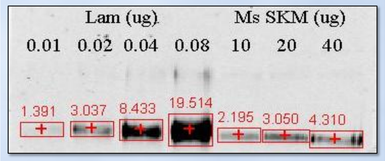
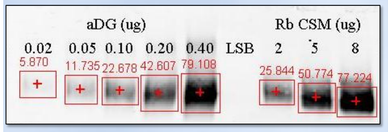
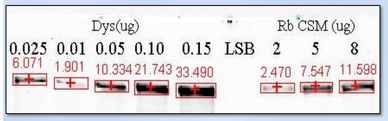
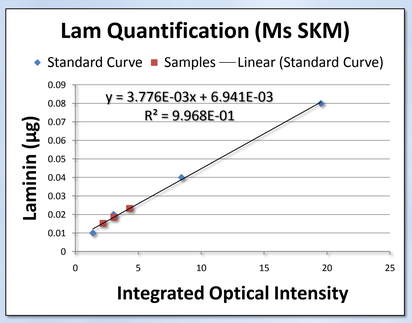


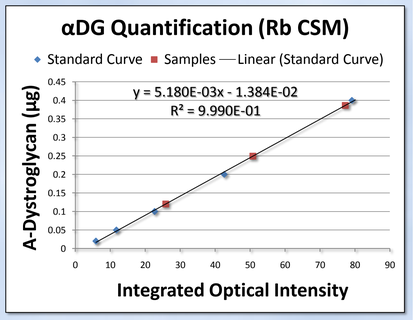
Figure 2. Position of Lam, αDG and Dys on Western Blot. Protein samples were subjected to SDS PAGE, transferred to nitrocellulose membrane and blotted with anti-laminin antibody, IIH6 (anti-αDG), or DYS1 (anti-Dys). The fluorescence intensities were detected by the LI-COR system. Lam ran at apparent MW of 255 (combined signal of β and γ subunits). αDG ran at apparent MW of 156kD. Dys ran at apparent MW of 250kD. These signals were later used to quantify each protein.
Red: molecular weight marker. Green: Lam, αDG or Dys as indicated.











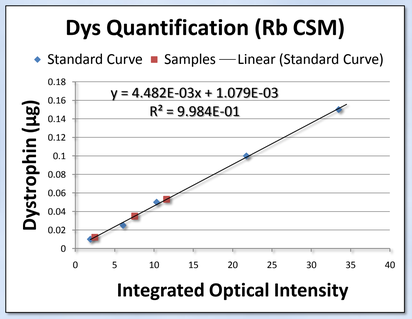


Figure 2. Quantification of Lam, αDG and Dys. Various loads of protein samples were subjected to Western blotting along with purified protein standard. The integrated optical intensity was measured by the LI-COR system. The amount of purified protein loaded was plotted against integrated optical intensity. A linear regression was then applied to the data and generate a standard curve. The integrated optical intensity of the protein samples were then compared to the standard curve to calculate the amount of target protein in the protein samples. Representative blots used to quantify Lam (A), αDG (B) and Dys (C) and the corresponding standard curve plots (D, E and F) are shown.

	Ms SKM		Rb CSM	
	Concentration (nmol/μg total protein)	n	Concentration (nmol/μg total protein)	n
Lam	2.198 ± 0.223	14	N/A	N/A
aDG	89.72 ± 15.04	9	692.67 ± 57.39	15
Dys	2.197 ± 0.069	2	16.11 ± 0.69	11
	Lam:aDG:Dys = 1:41:1		**aDG:Dys = 43:1**	

Table 1. Summary of Lam, αDG and Dys concentrations in Ms SKM and Rb CSM. The concentrations are shown as mean ± standard error with the unit of nmol per μg of total Ms SKM protein or Rb CSM protein.

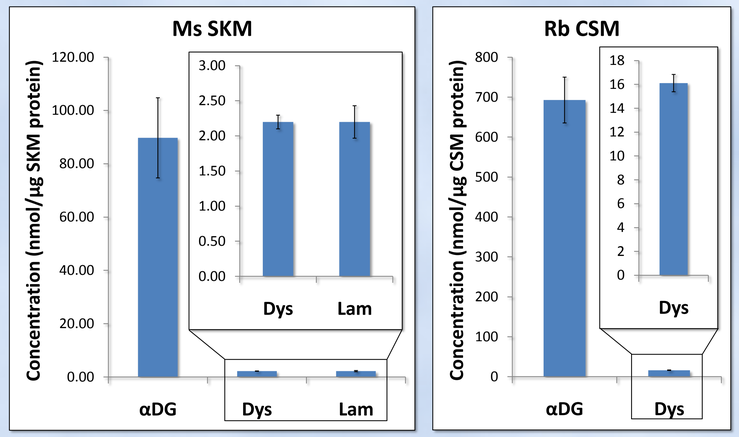


Figure 4. Graphic Representation of Mean Concentrations of αDG, Dys and Lam in Ms SKM and Rb CSM. These plots were constructed with data from Table 1. Insets shows the amplification of the boxed region (note that insets have different scales).

Discussion

- Our data suggest that DG is highly excessive on sarcolemma, indicating that "free DG", which is not incorporated into DGC, indeed exists.
- Future studies will focus on what role "free DG" plays on the sarcolemma.
- We have some concerns on the purity of our αDG standard used, which might falsely increase the concentration of αDG we calculated.
- To further confirm the presence of excessive αDG in skeletal muscle, we are currently working on coimmunoprecipitation of DGC using Dys antibody as bait to determine if the entire DG population coimmunoprecipitates with Dys in muscle extract.

References

1. Ervasti, J., & Sonnemann, K. J. (2008). Biology of The Striated Muscle Dystrophin-Glycoprotein Complex. *International Review of Cytology , 265*, 191-255.
2. http://www.cbs.umn.edu/BMBB/faculty/Ervasti.J.M.shtml

Acknowledgement

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