Aberrant glycosylation of α-dystroglycan causes defective binding of laminin in the muscle of chicken muscular dystrophy

Fumiaki Saito[a,*], Martina Blank[b], Jörn Schröder[b], Hiroshi Manya[c], Teruo Shimizu[a], Kevin P. Campbell[d], Tamao Endo[c], Makoto Mizutani[e], Stephan Kröger[b], Kiichiro Matsumura[a]

[a] *Department of Neurology and Neuroscience, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*
[b] *Institute for Physiological Chemistry, University of Mainz, Duesbergweg 6, D-55099 Mainz, Germany*
[c] *Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-chyo, Itabashi-ku, Tokyo 173-0015, Japan*
[d] *Howard Hughes Medical Institute, Department of Physiology and Biophysics and Department of Neurology, The University of Iowa, Roy J. and Lucille A. Carver College of Medicine, 400 Eckstein medical research building, Iowa City, IA 52242-1101, USA*
[e] *Nippon Institute for Biological Science, 3331-114 kamisasao, kobuchisawa-chyo, Yamanashi prefecture 408-0041, Japan*

Received 17 February 2005; revised 9 March 2005; accepted 9 March 2005

Available online 25 March 2005

Edited by Sandro Sonnino

Abstract Dystroglycan is a central component of dystrophin–glycoprotein complex that links extracellular matrix and cytoskeleton in skeletal muscle. Although dystrophic chicken is well established as an animal model of human muscular dystrophy, the pathomechanism leading to muscular degeneration remains unknown. We show here that glycosylation and laminin-binding activity of α-dystroglycan (α-DG) are defective in dystrophic chicken. Extensive glycan structural analysis reveals that Galβ1-3GalNAc and GalNAc residues are increased while Siaα2-3Gal structure is reduced in α-DG of dystrophic chicken. These results implicate aberrant glycosylation of α-DG in the pathogenesis of muscular degeneration in this model animal of muscular dystrophy.

Keywords: Dystroglycan; Laminin; Muscular dystrophy; Glycosylation; Dystrophic chicken

1. Introduction

The dystroglycan complex is composed of two proteins, α- and β-dystroglycan (α- and β-DG), which are encoded by a single gene and cleaved by posttranslational processing [1]. α-DG is a highly glycosylated extracellular peripheral membrane protein and binds to several extracellular matrix (ECM) proteins including laminin, agrin, and perlecan [2–4]. In turn, the transmembrane protein β-DG anchors α-DG at the extracellular surface of the plasma membrane, while its cytoplasmic domain interacts with dystrophin, a large cytoplasmic protein that binds to F-actin [5]. Thus, the DG complex plays a crucial role to stabilize the plasma membrane by acting as an axis through which the ECM is tightly linked to the cytoskeleton.

Recently, primary mutations in the genes encoding putative glycosyltransferases have been identified in several types of congenital muscular dystrophies including Fukuyama-type congenital muscular dystrophy, muscle–eye–brain disease, Walker–Warburg syndrome, congenital muscular dystrophy 1C (MDC1C) and 1D (MDC1D) [6–10]. Because glycosylation and laminin-binding activity of α-DG are defective in these diseases [11], they are collectively called α-dystroglycanopathy [12]. However, the precise oligosaccharide structures defective in α-dystroglycanopathy have not been elucidated.

Muscular dystrophy in chicken was first described in 1956 [13]. Although dystrophic chicken has been established as an animal model of muscular dystrophy, the primary mutation has not yet been identified [14] and the pathomechanism leading to muscle cell degeneration remains unknown. We demonstrate here that glycosylation and laminin-binding activity of α-DG are defective in the skeletal muscle of dystrophic chicken. Extensive glycan structural analysis reveals that, compared to control chicken, the amount of Galβ1-3GalNAc and GalNAc residues are increased, whereas Siaα2-3Gal structure is reduced in α-DG of dystrophic chicken.

2. Materials and methods

2.1. Antibodies

Mouse monoclonal antibody against sugar chain moiety of α-DG (IIH6) and sheep polyclonal antibody against core protein of α-DG (sheep anti-α-DG) were described previously [2,15]. Mouse monoclonal antibody against sugar chain moiety of α-DG (IVA4-1) was obtained from Upstate Biotechnology. Mouse monoclonal antibody against β-DG (8D5), β-sarcoglycan (5B1) and γ-sarcoglycan (21B5) were kind gifts from Dr. L.V.B. Anderson (Newcastle General Hospital). Mouse monoclonal anti-dystrophin (MANDRA 1) and affinity isolated rabbit anti-laminin were obtained from Sigma. Mouse monoclonal anti-dystrobrevin was purchased from BD Biosciences.

2.2. Lectin chromatography

Dystrophic chicken used in this study is New Hampshire, line 413, the colony of which is maintained homozygously. Line GSN/1, was used as a control. Pectoralis muscle of dystrophic and control chicken of 3 months of age were used. Skeletal muscle was disrupted with a polytron followed by Daunce homogenization and incubation in 50 mM Tris–HCl, pH 7.4, 500 mM NaCl, 1% Triton X-100, 0.6 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 0.75 mM benzamidine, and 0.1 mM PMSF. The extract was incubated with lectin

*Corresponding author. Fax: +813 3964 6394.
E-mail address: f-saito@med.teikyo-u.ac.jp (F. Saito).

Abbreviations: DG, dystroglycan; DGC, dystrophin–glycoprotein complex

0014-5793/$30.00
doi:10.1016/j.febslet.2005.03.033

agarose, including wheat germ agglutinin (WGA), concanavalin A (Con A), peanut agglutinin (PNA), *Vicia villosa* agglutinin isolectin B_4 (VVA-B_4), *Maackia amurensis* lectin (MAM) and lentil lectin (LCA). Bound proteins were eluted by boiling the beads in sample buffer (65 mM Tris–HCl, pH 6.9, 3% SDS, 1% β-mercaptoethanol, 115 mM sucrose, and 0.0004% bromophenol blue) and the eluates were analyzed by Western blotting using sheep anti-α-DG.

2.3. Miscellaneous

Chemical deglycosylation was described previously [2]. Sialidase digestion was performed using sialidase from *Clostridium perfringens* (Roche) according to the procedure described elsewhere [16]. Immunofluorescent microscopic analysis, Western blotting and blot overlay assay were performed as described elsewhere [11]. The amount of glycosidically bound sialic acid was compared by periodate–resorcinol method [17] and statistical significance was evaluated by *t* test. Solid-phase assay was performed as previously mentioned [11] except that WGA eluates were coated on 96 wells EIA/RIA plates (Coaster) after measuring the band intensity of α-DG on Western blots so that each well contained the same amount of α-DG.

3. Results

3.1. Decreased immunoreactivity of α-DG in the skeletal muscle of dystrophic chicken

We first performed immunofluorescent microscopic analysis. The immunoreactivity of α-DG revealed by antibody against sugar chain moiety of α-DG was significantly decreased in dystrophic chicken, whereas the immunoreactivity of α-DG was indistinguishable between control and dystrophic chicken when detected by antibody against core protein of α-DG. The other components of dystrophin–glycoprotein complex (DGC) were normally expressed in dystrophic chicken (Fig. 1). Consistent with the immunofluorescent analysis, Western blotting with antibody against sugar chain moiety of α-DG demonstrated reduced immunoreactivity of α-DG in dystrophic chicken (Fig. 2). In addition, α-DG of dystrophic chicken migrated at 160 kD, faster than that of control which migrated at 200 kD (Fig. 2). The expression and molecular mass of the other components of the DGC were not altered (Fig. 2).

3.2. Altered glycosylation of α-DG in the skeletal muscle of dystrophic chicken

The results described above raise the possibility that the glycosylation, rather than expression, of α-DG in dystrophic chicken may be altered. In order to test this possibility, α-DG was enriched by WGA chromatography and chemically deglycosylated with trifluoromethanesulfonic acid. Similar to the antibody against sugar chain moiety of α-DG, antibody against core protein of α-DG recognized α-DG species migrating around 200 and 160 kD in control and dystrophic chicken, respectively (Fig. 3, deglycosylation −). In addition, however, the anti-core protein antibody also detected α-DG species with a lower molecular mass of 110 kD in control and 70–120 kD in dystrophic chicken (Fig. 3, deglycosylation −). In this report, we tentatively call the larger and smaller α-DG species as L-α-dystroglycan (L-α-DG) and S-α-dystroglycan (S-α-DG), respectively. Upon chemical deglycosylation, the molecular mass of α-DG was reduced to 55 kD both in control and dystrophic chicken equally, eliminating the difference in molecular mass (Fig. 3, deglycosylation +). These data indicate that α-DG is aberrantly glycosylated in the skeletal muscle of dystrophic chicken. We also examined various tissues of dystrophic chicken to see if defective glycosylation of α-DG was present. Western blot analysis using antibody against core protein of α-DG demonstrated a

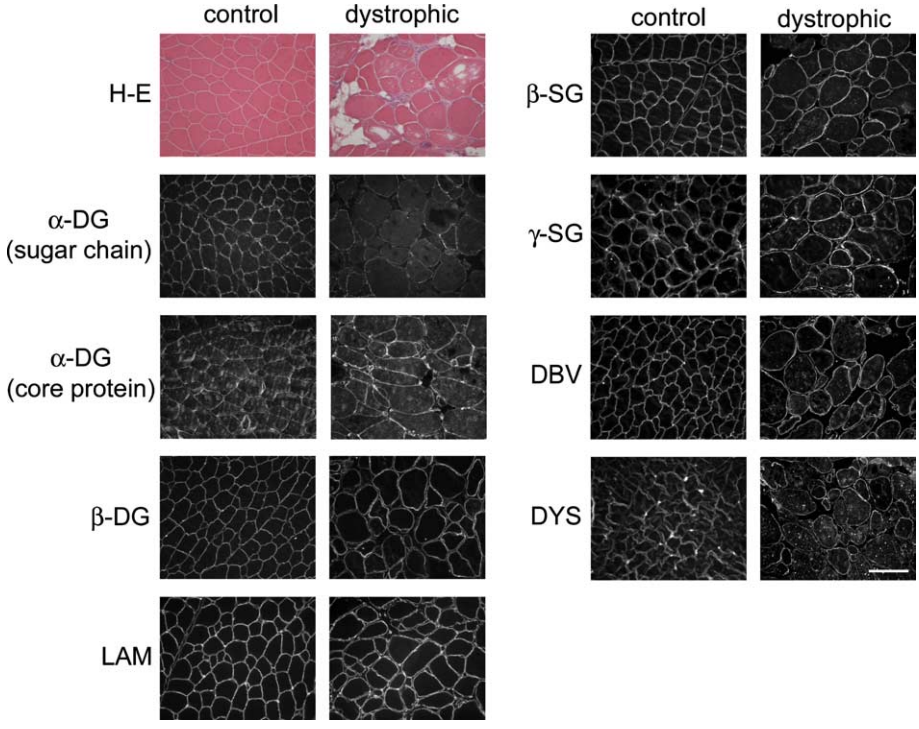

Fig. 1. Immunoreactivity of α-dystroglycan is reduced in the skeletal muscle of dystrophic chicken when probed by antibody against sugar chain moiety. Expression and localization of each component of the DGC were analyzed by immunofluorescent microscopy. The immunoreactivity of α-DG, as revealed by antibody against sugar chain moiety of α-DG (IIH6), is reduced in dystrophic chicken. However, the expression of α-DG core protein is not altered. DG, dystroglycan; LAM, laminin; SG, sarcoglycan; DBV, dystrobrevin; DYS, dystrophin. Bar indicates 100 μm.