Relationship between Muscular Dystrophy and Sugar Chains Containing Ribitol Phosphate Newly Found in Mammals

Ribitol, a sugar alcohol, is a major cell wall component of gram-positive bacteria, but has not been found in mammals. We recently found that the muscular dystrophy-related O-mannosyl glycan of α-dystroglycan contains a tandem ribitol phosphate (RboP) structure that is formed by fukutin and fukutin-related protein (FKRP), mutations of which cause muscular dystrophy. To elucidate the molecular mechanism underlying tandem RboP synthesis and its relationship with disease, the X-ray crystal structure of FKRP was determined. Structural and mutational analyses revealed that tetramer formation is required for FKRP function, and two FKRP protomers recognize an acceptor substrate by different manner.

The dystrophin-glycoprotein complex (DGC) in the plasma membrane connects the actin cytoskeleton with the extracellular matrix, such as laminin. In the muscle cells, it protects the muscle plasma membrane from physical stress associated with contraction and relaxation of muscles. The deficiency of DGC components causes muscular dystrophy [1]. A protein called α-Dystroglycan (α-DG) in DGC, is modified with various sugar chains. Among them, the core M3 type sugar chain (**Fig. 1**), which is a characteristic *O*-Man type sugar chain, plays a role in binding the extracellular matrix molecules. The deficiency of the sugar chain synthesis is responsible for causing muscular dystrophy. The core M3 type sugar chain on α-DG is synthesized in cells as follows (**Fig. 1**): First, the Thr residue on α-DG is Man-modified by POMT1/POMT2, whereas POMGNT2 and B3GALNT2 transfer GlcNAc and GalNAc, respectively. In addition, POMK adds a phosphate at the 6-position of Man. Subsequently, fukutin (FKTN) and fukutin-related protein (FKRP) add two ribitol phosphate (RboP) units. After that, RXYLT1 (TMEM5) and B4GAT1 add Xyl and GlcA, respectively. Finally, LARGE forms a repeat structure of GlcA and Xyl (**Fig. 1**).

It was remarkable to find that the core M3 type sugar chain contains tandem RboP, because it was the first report of RboP being found in mammals [2]. FKTN and FKRP, which encode RboP transferases, are responsible for muscular dystrophy. To elucidate the molecular mechanism of synthesizing a very unique tandem RboP structure and its relationship with the disease, it is necessary to clarify the structure and function of its synthesizing enzymes, FKTN and FKRP. In a joint research, members of the Structural Biology Research Center in IMSS, KEK performed an X-ray crystal structure analysis of FKRP, the second RboP transferase, and clarified its three-dimensional structure and substrate recognition mechanism [3].

FKRP is a type II transmembrane protein localized in the Golgi apparatus. We determined the X-ray crystal structure of the luminal domain (45-451 amino acids) containing the catalytic active center. The overall structure of FKRP is composed of an N-terminal stem domain and a C-terminal catalytic domain (**Fig. 2**). Interestingly, FKRP forms a tetramer in the crystal. Size exclusion chromatography in combination with small angle X-ray scattering (SEC-SAXS) confirmed that FKRP

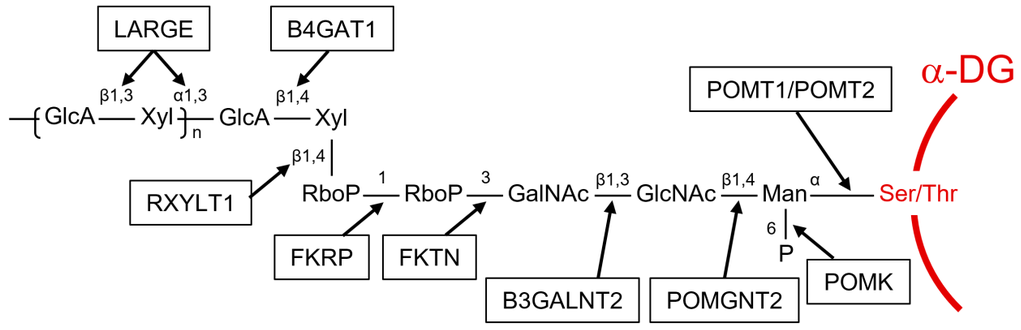

Figure 1: Structure and synthesis of the core M3 type sugar chain. α-Dystroglycan (α-DG) is indicated by the red brown line. Enzymes that perform the biosynthesis of the sugar chain are indicated in boxes. Abbreviations are as follows: Man - mannose; GlcNAc - *N*-acetylglucosamine; GalNAc - *N*-acetylgalactosamine; RboP - ribitol phosphate; Xyl - xylose, and GlcA - glucuronic acid.

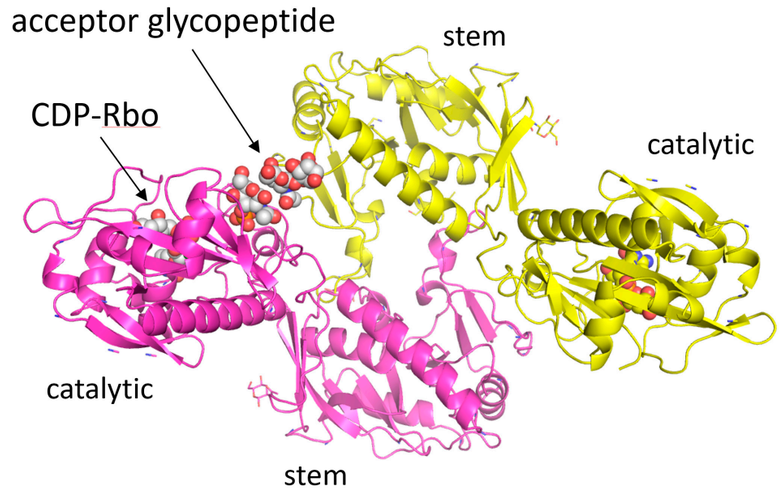

Figure 2: Crystal structure of FKRP with CDP-Rbo and an acceptor glycopeptide. Two protomers (purple and yellow) are shown. Each protomer consists of an N-terminal stem domain and a C-terminal catalytic domain.

exists as a tetramer in the solution. Disease mutations of FKRP, such as Tyr88Phe, Ser221Arg, and Leu276Ile, are located at the interface between the FKRP protomers and between domains. Leu276Ile is the most common FKRP disease mutation in the US and Europe. Each mutant protein shows a reduced oligomer formation and enzymatic activity, suggesting that the tetramer formation of FKRP is important for the enzyme activity.

In the catalytic domain, there are two Mg^{2+} coordination sites (site I: Asp360, Asp362, Asp364; site II: Asp362, Asp364, Asp416) that are considered to be enzyme active sites. When these Asp are replaced with Ala, each mutant protein completely loses its enzymatic activity. These results suggest that the binding of the divalent metal ion is essential for the enzyme activity.

Next, the complex structure of FKRP with CDP-Rbo, which is the donor substrate, was determined. As compared to the non-bound structure, the structures surrounding CDP-Rbo were changed by its binding. In particular, the loop region from Leu433 to Gln437 interacts with the Rbo portion. Furthermore, the crystal structure of the complex with CDP-Rbo and a glycopeptide containing an acceptor sugar chain structure (RboP-3GalNAcβ1-3GlcNAcβ1-4(phospho-6)Manα1-) was determined (**Fig. 2**). Two FKRP protomers grab the sugar chain. His252 and Lys256 in the stem domain of one FKRP protomer interact with the phosphate group at position 6 of Man, which is the root of the sugar chain. Arg295 and Val300 in the catalytic domain of another FKRP protomer interact with the phosphate group at the tip of the sugar chain (phosphate group of RboP). To confirm the importance of these interactions, the enzymatic activity of each mutant was examined. His252Ala and Lys256Ala mutant proteins showed remarkably reduced activity, whereas Arg295Ala completely lost its activity.

From these results of the FKRP structure-function analysis, the unique tandem RboP-containing sugar chain synthesis mechanism has become clear. It was revealed that FKRP has a unique sugar chain recognition mechanism in which FKRP binds the acceptor sugar chain by forming a tetramer. It is expected that these findings can be applied to elucidate pathological conditions and develop therapeutic methods.

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BEAMLINES

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