Experiment title: Structural studies on mouse α-dystroglycan

Beamline: ID14-EH4 **Date of experiment**: 23rd -25th Nov 2000

Shifts: BAG 2 **Experiment number**: LS1824

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Report

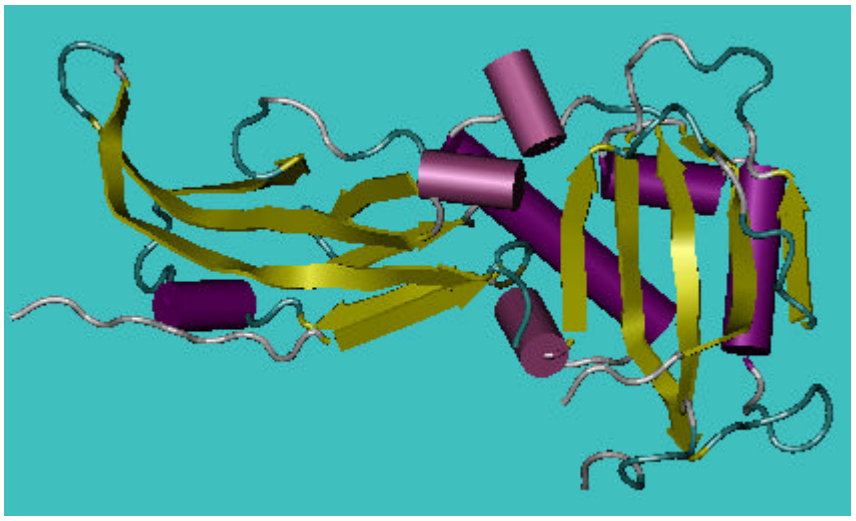
Dystroglycan (DG) - previously also identified as cranin - is a type-1 transmembrane protein expressed in muscles as well as in a wide variety of other tissues (for a recent review see Winder, 2001). Vertebrate DG is transcribed as a single mRNA corresponding to a 895 amino acid polypeptide The precursor molecule undergoes an early post-translational cleavage within the endoplasmic reticulum thus liberates α- and β-DG subunits which are both targeted separately to the plasma membrane. The two α- and β-DG subunits remain tightly associated *via* non-covalent interactions, and in skeletal muscle they form together with sarcoglycans, sarcospan, syntrophins and dystrobrevins the dystrophin-glycoprotein complex (DGC). This complex links the extracellular matrix with the actin cytoskeleton and provides stability to the muscle fibre sarcolemma against contractile forces.

α-DG is a highly glycosylated peripheral membrane protein whereas β-DG is the transmembrane subunit of the DG complex. The α-DG subunit was first characterized *via* electron microscopic techniques as a 20-30 nm long and dumbbell-shaped molecule. It contains a central mucine-like amino acid stretch, comprising approximately residues 315 to 485, flanked by two globular domains (Brancaccio *et al.*, 1995). The glycosylation pattern is suggested to play an important role in DG, especially for α-DG`s calcium dependent binding to laminin LG-like modules though the glycosylation is highly variable and heterogeneous even within the same tissue.

We have obtained reproducible crystals of the N-terminal region (52-315) of mouse α-dystroglycan. The crystals have unit cell dimensions of a=b=71.460Å c=144.248Å α=β=90.00° γ=120.00° and belong to the R3 space group. We have collected, at ESRF, a 2.3Å native data set with the following statistics: n° of measurements 63864, n° of observed reflections 32756, n° of unique reflections 12143, R_{sym} 6.7% (35.6% in the highest resolution shell), completeness 98.7% (98.1%), I/σ(I) 9.4 (3.7), multiplicity 2.7 (2.7).

The crystal structure of α-DG could be solved by SIRAS using a data set (2.9Å resolution) measured (in house source Cu K_α rotating anode) on an iodine derivative.

The α-DG N-terminal fragment shows an asymmetrically shaped two domain structure with an N-terminal domain having an immunoglobulin-like fold and a C-terminal domain that adopts a ribosomal RNA binding protein fold. The two domains are connected by a structurally not resolved flexible stretch.



The topology of α-dystroglycan

The new insight coming from this structural analysis has prompted us to investigate in more detail the interaction with laminin-1 (LN-1) as well as laminin-2/4 (LN-2/4) *via* solid-phase binding assays.

References

Winder, S.J. (2001). The complexities of dystroglycan. *Trend. Biochem. Sci.* **26**, 118-124.

Brancaccio, A., Schulthess, T., Gesemann, M., and Engel, J. (1995). Electron microscopic evidence for a mucin-like region in chick muscle α-dystroglycan. *FEBS Lett.* **368**, 139-142.