MUSCULAR DYSTROPHY

A LARGE dose of sugar

A sugar-transferring enzyme known as LARGE could help to restore muscle function in patients with muscular dystrophy, according to research from Kevin Campbell and colleagues published in *Nature Medicine* online on 6 June. And in a related *Cell* paper, published online on 3 June, Campbell and co-workers showed that LARGE might exert its effects by mediating the attachment of sugar molecules to α-dystroglycan, a key component of the dystrophin–glycoprotein complex that forms a link between the inside and outside of muscle cells.

Muscular dystrophies are a group of genetic disorders that are characterized by progressive weakening and wasting of muscles. In a subset of congenital muscular dystrophies (CMD), mutations in genes that encode glycosyltransferase enzymes, which add sugars to proteins, lead to muscle degeneration and defects in brain development. In these disorders, α-dystroglycan, which is normally heavily glycosylated, lacks its sugars and is unable to bind its ligands in the extracellular matrix. The disruption of this crucial link between the inside and outside of muscle cells is thought to make the cells more vulnerable to stress-induced damage.

In the *Cell* paper, experiments showed that an interaction between LARGE and α-dystroglycan is crucial for the production of functional, glycosylated α-dystroglycan and therefore the maintenance of healthy muscle. In their *Nature Medicine* paper, Campbell and colleagues used LARGE to investigate whether restoring glycosyltransferase activity in CMD could prevent the defects associated with this subset of muscular dystrophies.

They began their analysis using a mouse model of CMD, which has a mutation in the gene encoding LARGE. An adenovirus that was engineered to express the *LARGE* gene was injected into the muscle of mutated mice that were just a few days old. These muscle cells produced functional LARGE protein and α-dystroglycan that was enriched with sugar molecules. The ability of α-dystroglycan to bind to proteins in the extracellular matrix was restored, and when viewed under a microscope, the muscle looked like normal, healthy tissue. Furthermore, mice expressing the transferred gene were shown to suffer significantly less from exercise-induced muscle damage than untreated littermates. LARGE overexpression also increased the binding of

STRUCTURAL BIOLOGY

Not so crystal clear

Protein structures solved by X-ray crystallography might not be as accurate as originally thought, according to a recent article in *Structure*. The technique, which has been invaluable for characterizing protein interactions and for aiding structure-based drug design, relies on building models on the basis of an observed diffraction pattern that is created by the scattering of X-rays through a protein in a crystallized form. However, Mark A. DePristo and colleagues explain that if only a single model is used to fit the experimental data, the heterogeneous structure and anisotropic motion of proteins intrinsic to their function is unaccounted for, and the structure is therefore likely to be inaccurate.

In a crystalline form, proteins are ordered by packing within the crystal lattice, but the high solvent content in most crystals means that heterogeneity remains. Modelling of these features is currently only possible for proteins that diffract to high resolution, whereas most proteins diffract to worse than 1.6 Å resolution and are therefore solved as a single conformation with isotropic motion — a kind of 'one size fits all' approach. The accuracy of macromolecular models determined by X-ray crystallography is still a subject of debate, but now it seems that the limitations of the models used for theoretical calculations are partly to blame.

The authors generated an ensemble of alternative solutions for three previously solved protein structures using diffraction data obtained from the Protein Data Bank (PDB). These alternative solutions were refined to fit the experimental data at least as well as the original PDB structure, but remained different in detail from both the PDB structure and each other. This enabled the authors to study differences between the final models and estimate their accuracy. Because the same protocol was used to determine each structure, differences observed in the final models must result from intrinsic structural heterogeneity, because they cannot result from experimental variation or subjective human observations.

One example of heterogeneity was described for human interleukin-1β (h-IL1-β), which showed extensive main- and side-chain variability between the authors' models. Variation was most pronounced in the main chain, which differed by as much a 1 Å between models, and was consistent with previously reported regions of disparity. This demonstrates that it is not possible to accurately model the crystal structure of h-IL1-β using an isotropic protocol.

The authors suggest that rather than concentrating on a single model, future X-ray crystallography models should actively incorporate heterogeneity, in a manner similar to the approach taken with nuclear magnetic resonance spectroscopy. Until then, features that depend on the exact positions of atoms, as well as small structural differences resulting from site-directed mutagenesis or ligand binding, should be treated with caution.

Joanna Owens

References and links

ORIGINAL RESEARCH PAPER DePristo, M. A., de Bakker, P. I. W. & Blundell, T. L. Heterogeneity and inaccuracy in protein structures solved by X-ray crystallography. *Structure* **12**, 831–838 (2004)

WEB SITE

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http://www.cryst.bioc.cam.ac.uk/tlb/research.html

α-dystroglycan to the extracellular matrix in the muscles of normal, healthy mice, without introducing any abnormalities.

The researchers then decided to test the effects of LARGE in the cells of patients with CMD. They treated the cells of patients with three different types of CMD — Fukuyama CMD, muscle–eye–brain disease and Walker–Warburg syndrome — with the adenovirus that carried the LARGE gene. And although these diseases are caused by mutations in different glycosyltransferase enzymes, expression of LARGE generated functional, glycosylated α-dystroglycan in all three cases.

These results, say the authors, suggest that stimulating the addition of sugar to α-dystroglycan by LARGE could provide a potential therapy for certain muscular dystrophies, regardless of the type of glycosyltransferase enzyme that has been mutated.

Clare Ellis

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HIGH-THROUGHPUT SCREENING

Free your assay

Many strategies for high-throughput screening (HTS) against enzyme targets require the analytes to be labelled, which has a number of potential disadvantages, such as interference of the label with the enzyme activity being assessed. Mass spectrometry (MS) methods do not have this problem, but the need for several time-consuming steps in analyte preparation has limited the use of such methods in HTS. Writing in *Nature Biotechnology*, Milan Mrksich and colleagues now describe a new MS-based assay strategy in which analyte preparation is sufficiently simple to allow rapid screening of chemical libraries, which they demonstrate by identifying a potent inhibitor of the anthrax lethal factor.

At the core of the authors' strategy are specially designed self-assembled monolayers (SAMs), which present enzyme substrates on a gold layer in a way that ensures that interactions with soluble enzymes only occur by way of the immobilized substrates. Then, after one washing step, these layers are suitable for analysis using matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) MS, allowing any change in the mass of the substrate owing to an enzymatic reaction to be readily detected.

To identify inhibitors of anthrax lethal factor, a protease with a key role in anthrax pathogenesis, Mrksich *et al.* produced 10 × 10 arrays of reaction wells lined with SAMs that displayed a peptide substrate for lethal factor. Analysis of the SAMs by MALDI-TOF MS (a procedure that the authors termed SAMDI) before and after treatment with lethal factor clearly showed a change in mass of the surface-linked peptide resulting from the loss of an N-terminal fragment by proteolytic cleavage.

Then, to screen a 10,000-compound library against lethal factor, the authors incubated cocktails containing lethal factor and 8 library compounds in each well on the 10 × 10 arrays. SAMDI analysis indicated that complete cleavage of the peptide occurred in most wells, but 11 of the wells (~1%) showed no or only partial proteolysis of the peptide, indicating the presence of an inhibitor in the cocktail. Repeating the assay with the 88 compounds present in these cocktails identified one compound, DS-998, that completely blocked lethal factor activity at 10 μm concentration. This low-molecular-mass compound was also shown to be active in cell-based assays, and so represents a potential lead for anti-anthrax drug discovery efforts.

So, how does the robustness of the SAMDI method, in terms of distinguishing positive signals from background, compare with others used in chemical screening? A widely accepted parameter for assay quality is the Z′ factor, which ranges from 1 for a 'perfect' assay to 0. The authors determined the Z′ factor of their SAMDI assay to be 0.83, which compares very favourably with established assays. It therefore seems that the SAMDI method has significant potential as a rapid and general label-free screening approach for enzyme targets, and it could be especially valuable for assays involving enzymes that are not easily monitored with labels, such as carbohydrate-modifying enzymes.

Peter Kirkpatrick

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