groups (Asp101 and Asp104) bridging between the calcium ions. In each monomer the calcium ions are just 3.75 Å apart and mediate the binding of fucose in a unique and sturdy carbohydrate-recognition mode (**Figure 11**). The fucose residue itself locks onto both calcium ions, with three of the fucose hydroxyl groups participating in both the coordination of the calcium ions and in hydrogen bonds with acidic groups forming the calcium binding site.

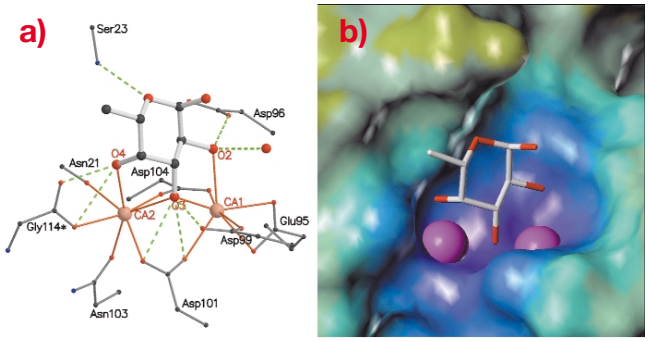

Fig. 11: Interactions of PA-IIL with calcium ions and fucose. (a) Stick representation of the amino acids involved in binding. Ca^{2+} coordination bonds are shown as solid orange lines; hydrogen bonds as dashed green lines. Colour coding: red, oxygen; blue, nitrogen; black, carbon; pink, Ca^{2+}. (b) Electrostatic surface representation of the PA-IIL binding site with Ca^{2+} as large pink spheres and fucose as a stick model.

In contrast with most lectins that display only weak affinity for monovalent sugar ligands, the PA-IIL-fucose interaction has an association constant in the micromolar range. The unusual ternary complex formed by the two Ca^{2+} ions, the side chains of amino acids that coordinate them, and the sugar provides the basis for such strong affinity. In order for sugars to bind to Ca^{2+} ions as found in the structure of PA-IIL, they must have a particular stereochemical arrangement of three carbohydrate hydroxyl groups. L-fucose, L-galactose, D-mannose and D-arabinose all have the necessary stereochemistry and all of them are recognised by PA-IIL, albeit with diverse affinities [2]. Subsequent modelling studies, based on the fucose complex structure, and binding studies have demonstrated that the preferred ligands of this bacterial lectin belong to the Le^a series. Such structure-based knowledge could be used for the design of efficient anti-bacterial compounds and, furthermore, the unusually high affinity interaction of this novel binding mode suggests that PA-IIL may be a useful target for oligosaccharide-based therapeutics.

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Principal Publication and Authors

E. Mitchell (a), C. Houles (b), D. Sudakevitz (c), M. Wimmerova (b), C. Gautier (b), S. Pérez (b), A.M. Wu (d), N. Gilboa-Garber (c) and A. Imberty (b), *Nature Structural Biology, Accepted for publication.*
(a) ESRF
(b) CERMAV – CNRS, Université Joseph Fourier, Grenoble (France)
(c) Bar-Ilan University, Faculty of Life Sciences, Ramat Gan (Israel)
(d) Institute of Molecular and Cellular Biology, Chang-Gung University (Taiwan)

Structure of the Bovine Lysosomal α-Mannosidase, the Enzyme Involved in the Lysosomal Storage Disease α-Mannosidosis

Lysosomal α-mannosidase (LAM) is a member of the glycosyl hydrolase family GH38. It hydrolyses all known α-mannosidic linkages in lysosomes. Lysosomes are cellular particles, which are responsible for the breakdown of cellular end products. They have an internal pH of 4.5 and are thus much more acidic than the rest of the cell. Errors in lysosomal processes lead to a number of inherited lysosomal storage diseases, many of which are very serious. These include α-mannosidosis, which is a rare disease in humans, cattle, cats and guinea pig. Lack of LAM activity causes swelling of the lysosomal vacuoles, and apparently leaking of the unhydrolysed sugars from these vacuoles causes the symptoms of the disease; mental retardation, skeletal changes, hearing loss and reduced immunity in humans [1, 2]. Two protein level α-mannosidosis mutations have been identified in cattle and six in humans.

We isolated the natural lysosomal α-mannosidase directly from bovine kidneys [2]. 10 kg of kidneys yields 5-20 mg of the enzyme. The LAM protein is a ~250 kDa homodimer which is both glycosylated and proteolytically cleaved during its maturation and transport to lysosomes. The protein crystallised in a fully-glycosylated form in a large hexagonal unit cell with cell dimensions a = b = 117.88 Å, c = 582.04 Å. Data were collected to 2.7 Å resolution at beamline **ID14-4**. We solved the structure in $P6_122$ space group by molecular replacement with a distant relative, the *Drosophila melanogaster* Golgi II α-mannosidase. The asymmetric unit of the crystal contains a single monomer and the crystallographic packing offered two possible LAM dimers. The correct solution dimer was identified by electron microscopy.

The N-terminal active site domain is a distorted 7-stranded α/β barrel and the active site is formed on the top of the barrel. Following the barrel domain, the structure consists of a 3-helix bundle, two further small β domains and a large 17 stranded β-domain (**Figure 12**). All known LAMs have a conserved glycosylation site following the 3-helix bundle. In our bovine LAM (bLAM) structure this site contains a high mannose type glycan, which rests against the 3-helix bundle. Two GlcNAc and eight mannose residues are visible in the electron density map. The bLAM structure also contains several salt bridge networks. As these networks are not conserved in related enzymes functioning at neutral pH, it seems likely that these networks are involved in stability and activation of the enzyme at the lysosomal pH (~4.5), which is close to the pK_a of aspartic and glutamic acids.

Mutations in the LAM amino acid sequence that cause α-mannosidosis in humans are scattered in different parts of the structure. Only H72L is in the active site itself, where it affects a metal-coordinating residue. Two other mutations (T355P and P356R) are located at the start of an α-helix in the active-site domain and presumably disturb the initiation of the helix and folding of the domain. Three further mutations (W714R, R750W and L809P) are located in the 17-stranded β-domain. Of these, R750W is possibly the most interesting, since it mediates an interaction between three domains.

Both mutations causing α-mannosidosis in cattle [2] are related to the active site of the enzyme. The R220H mutation in Galloway cattle affects R220, which is hydrogen-bonded both to an important residue in the catalysis, D196, as well as to the substrate mimic Tris. H220 would be able to form hydrogen bonds to D196 and to Y380 as R220 does, but the hydrogen-bonding to Tris and presumably to the substrate would be broken. This mutation will thus most likely affect the substrate binding and also the chemistry of D196. Another mutation, F320L, causes α-mannosidosis in Angus cattle and is also known to affect the stability of the enzyme. In the structure, the aromatic ring in A:F320 stacks against Y84 in a loop which is involved in dimer formation. Its mutation to Leucine would presumably weaken monomer-monomer interactions in the physiological dimer. However, this mutation might also affect catalysis since it follows another active-site residue, D319, in the amino acid sequence.

Our structure determination thus helps to explain how particular mutations on the LAM amino acid sequence can result in α-mannosidosis and is the first step in understanding the biological mechanism behind the disease. It also provides a first structure of a mammalian enzyme in this class of glycoside hydrolases and provides an interesting new way of low pH activation.

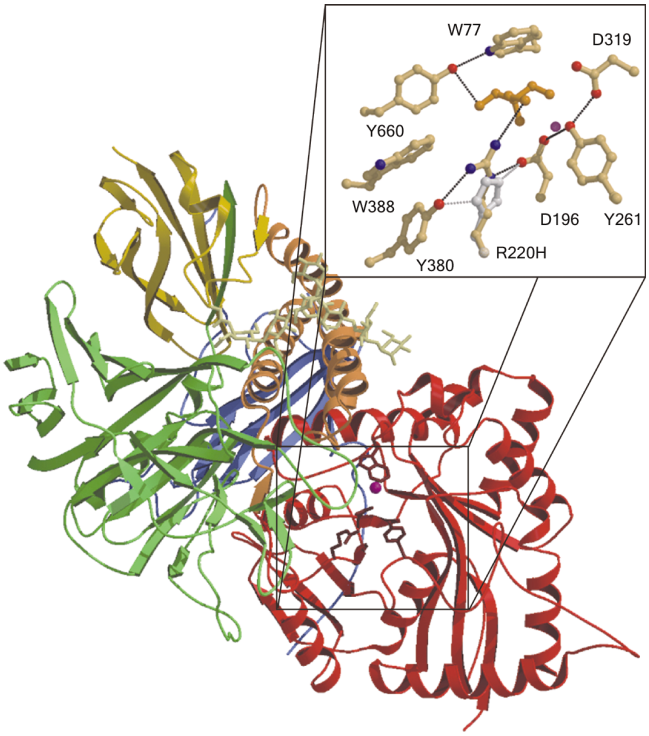

Fig. 12: The three dimensional structure of bovine α-mannosidase. The N-terminal active-site domain is shown in red, the three-helix bundle in brown and the two small ß domains in blue and khaki. The large 17-stranded ß-domain is shown in lime green. The high-mannose sugar found at the glycosylation site at the end of the three-helix bundle is shown in stick representation. The insert shows, in ball-and-stick representation, the residues involved in substrate binding and catalysis (the substrate mimic, Tris, is shown in brown) and demonstrates how the R220H mutation would lead to a loss of a hydrogen bond to the substrate.

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Authors

P. Heikinheimo (a), R. Helland (a), H-K. Schrøder Leiros (a), I. Leiros (a), S. Karlsen (a), G. Evjen (a), R. Ravelli (b), G. Schoehn (b,c), R. Ruigrok (b), O-K. Tollersrud (a), S. McSweeney (b,d) and E. Hough (a).

(a) Universitetet i Tromsø, Tromsø (Norway)

(b) EMBL, Grenoble (France)

(c) IBS, Grenoble (France)

(d) ESRF