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Interconversion of the Specificities of Human Lysosomal Enzymes

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Abstract
Fabry disease (FD) is an X-linked recessive lysosomal storage disorder (LSD) known to affect approximately 1 in every 40,000 males, and a smaller number of females. FD results from a deficiency of functional α-galactosidase (α-GAL), which leads to the accumulation of terminally α-galactosylated substrates in the lysosome. The predominant treatment is Enzyme Replacement Therapy (ERT), requiring the regular infusion of recombinant human α-GAL. More than half of individuals receiving ERT experience a range of adverse infusion reactions, and it has been reported that as many as 88% of patients receiving ERT develop neutralizing IgG antibodies against the drug.

In aim of designing a non-immunogenic treatment candidate for Fabry disease ERT, we have engineered the active sites of α-GAL and another homologous family 27 exoglycosylase named α-N-acetylgalactosaminidase (α-NAGAL) to have interconverted substrate specificities. 11 of 13 active

site residues are conserved between these two enzymes, and we have shown that their substrate specificities can be interconverted by mutating the two non-conserved active-site residues. We report the kinetic properties of these two mutants along with wild type controls, and use western blotting to show that both mutant enzymes retain their respective wild type enzyme antigenicity. Structural data obtained by X-ray crystallography on the α-GAL mutant (called α-GALSA) reveals the mechanism by which substrate specificity is dictated between these two proteins, and provides explanations for the mutant's reduced catalytic efficiency.

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