Carbohydrate – active enzymes (CAZymes) as drug targets and tools for medicinal lead compounds biosynthesis

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Context

Carbohydrates encode vital biological information
Seven most common hexose monosaccharides units:

- D-Glucose
- D-Galactose
- D-Mannose
- N-Acetyl-D-glucosamine
- N-Acetyl-D-galactosamine
- Sialic acid
- L-Fucose
CAZymes: What are they?

Enzymes that modify carbohydrates and glycoconjugates

Currently known to comprise: 113 Glycoside hydrolases, 91 glycosyltransferase, 19 polysaccharide lyase and 52 carbohydrate-binding module (CBM) families

www.cazy.org

>6000 CAZymes listed
CAZymes Sources:

Influenza A virus neuraminidase (Kalemera MSc thesis, 2011)


*Bacillus halodurans* alpha- amylase (Hashim S, PhD thesis 2006)

*B. strearothermophilus* alpha-galactosidase (Nisone A, Osanjo G, Glycoconjugate Journal)

*Thermotoga maritima* alpha-L-fucosidase (Osanjo et al 2007, Biochemistry)

*Glossina fuscipes* lectizyme (Abubakar et al, 2006 Insect Biochem Mol Biol)
Circulating influenza viruses in Kenya
Neuraminidase activity

A Host cell
- Budding virus
- Neuraminidase cleaves receptor
- Hemagglutinin
- Virion
- Neuraminidase
- Release of new virions
- Continued viral replication

B Neuraminidase inhibitor
- Receptor containing sialic acid
- Virion
- Neuraminidase inhibitors
- No virion release
- Halted viral replication
FIGURE 6. Proposed mechanism for the hydration of Neu5Ac2en to Neu5Ac.
Fig 11. Amplification of the recombinant plasmid (pGEMT-Na).
The plasmid was diluted 10 times and used in amplification of the
neuraminidase gene to confirm the presence of the neuraminidase
insert.

M = Molecular weight marker Promega 1Kb
Lane 1 = Recombinant Pgemt-Na
Lane 2-7 = Amplified neuraminidase using the recombinant plasmid
as template

Recombinant pGEMT with
the H3N2 neuraminidase
insert ( supercoiled and
circular forms

Amplified Neuraminidase

Aluvaala EK, unpublished
Possible Applications of influenza neuraminidase assays:

• Monitoring development of resistance to drugs

• Development of novel inhibitors

• Enzyme assay platforms during in vitro evolution
Applications in synthesis: Example of alpha- L-fucosidases
Transglycosylation using $\alpha$-L-fucosidase from *Thermotoga maritima* (Tm $\alpha$–fuc)

- Exoglycosidase, EC 3.2.1.51
- Family: GH29 (CAZy)
- Structure $(\beta/\alpha)_8$

Sulzenbacher et al. *J. Biol. Chem.*, 2004
\( \alpha \)-L-fucosidase

Autocondensation and transglycosylation

\( pNP \alpha \)-L-Fuc

\( pNPGal \)

\( \text{Fucose} 83\% \)

\( pNP \alpha \)-L-Fucp-(1→2)\( \beta \)-D-Galp

\( pNP \alpha \)-L-Fucp-(1→6)\( \beta \)-D-Galp

\( \sqrt{} \)
Mutagenesis (epPCR)

Library of mutated Tm α-fuc

Screen X-fuc (0.1 mM)

Selection of mutants with loss of hydrolysis

α-L-fucosidase Tm α-fuc
Molecular directed evolution

Analysis and selection of mutants with improved transglycosylation
**α-L-fucosidase**

Δ Directed evolution

Δ Separation of mutations

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**A**

Initial PCR amplification to generate 662 bp (A662) and 732 bp (B732) DNA fragments.

**B**

2nd PCR step: Overlapping extension

Transglycosylation (semi-quantitative)

<table>
<thead>
<tr>
<th>WT</th>
<th>R147G</th>
<th>P196L</th>
<th>Y237H</th>
<th>T264A</th>
<th>Y267L</th>
<th>L322P</th>
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Effect of distance of mutations to T264A

WT

mut T264A

Y64

R254

T264

A264

R254
Synthesis of antigen H analogue

\[
\text{pNP } \alpha -\text{L-Fucp-(1→2)- } \beta -\text{D-Galp-(1→3)- } \beta -\text{D-Glcp}
\]

Glycosynthase
TtβGly

98%  

\[
\text{pNP}\alpha -\text{L-Fucp-(1→2)- } \beta -\text{D-Galp-(1→3)- } \beta -\text{D-Glcp}
\]

64%  

\[
\text{pNP}\alpha -\text{L-Fucp-(1→2)- } \beta -\text{D-Galp-(1→3)- } \beta -\text{D-Glcp}
\]

Ph α -L-Fucp-(1→2)- β -D-Galp-(1→3)- β -D-Glcp
√ CAZymes have great potential in as drug targets

√ Directed evolution permits significant improvement of CAZymes as biocatalysts

√ Using these enzymes we have achieved synthesis of the glycotopes Fuc a(1→2)Gal et Fuc a(1→2)Gal b(1→3)Glc.
√ Francis Mulaa
√ Eva Aluvaala
√ Tirus Wanyoike
√ Wallace Bulimo
√ Francis Mulaa
√ Charles Tellier
√ Michel Dion
√ Vinh Tran
√ Julien Drone
√ Claude Rabiller
√ Claude Solleux
√ Coraline Rigouin