CHEMO-ENZYMATIC SYNTHESIS OF $\beta$-(1,3)-GLUCANS: TAILORED LAMINARINASE AS "BLUE" BIOCATALYST.

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B-(1→3)-Glucanes: from traditional medicine to rational design
B-(1→3)-Glucanues: from traditional medicine to rational design
$\beta$-(1→3)-Glucanes: from traditional medicine to rational design

- Homopolymer of around 30 glucose units
- $\beta$-(1,3) linkage with $\beta$-(1,6) branch
- Reducing end either a manitol or a glucose (7/3)

Isolation

Structural analysis

Conformational analysis

Laminarine

Cereals

Lichens

Yeast

Mushrooms

Algae
B-(1→3)-Glucanes: from traditional medicine to rational design

Immunostimulation

Lentinane
✓ Colorectal cancer
✓ Gastro-intestinal cancer

Schizophyllane
✓ Gastric cancer
✓ Uterus cancer

Elicitors

Antibacterial effects

Biological properties
Receptors
Therapeutic applications
Clinical trials

Anticoagulants

Antiviral properties

⇒ Biological Response Modifiers
Structure-properties relationships

Still under investigation:

- Length of the polymers?
- Degree of substitution?
- Degree of purity?
- Tridimensional conformation?

Downfalls:

- Non reliable resources
- Heterogeneity of the structures
- Polydispersity

Development of synthetic tools to access analogs:

- shorter
- well defined
- bioactive

\[
\begin{align*}
X = O \text{ ou } S \\
6 < n < 2
\end{align*}
\]
Family of O-glucans

Activity from the tetrasaccharide

Activity increase with the chain length

Glycobiology 2005, 15, 393-407
Bioorg. Med. Chem. 2010, 18, 348-357
Development of a new family of glucans

With increased stability

With identical conformation in solution

With better affinity for the receptor

$\Rightarrow \beta-(1\rightarrow 3)$-Thioglucans
Family of S-glucanes

(Tri S1)

(Tetra S1)

(Penta S1)

(Tri S1,2)

(Tetra S1,2)
Family of S-glucans: \textit{in vivo} evaluation

![Graph showing percentage of positive cells for Macrophages, Monocytes, and Neutrophiles under different treatments.](image)

- Control
- Tri S1
- Tétra S1
- Tri S1,2
- Tétra S1,2

Family of S-glucans: *in vivo* evaluation

- **Activity from the trisaccharide**
- **Activity Tetra > Tri**
- **Activity S1,2 > S1**

*J. Med. Chem.* 2014, 57, 8280-8292
Optimization of oligoglucans synthesis

Aim: Bring the enzymatic catalyst to an organic chemistry laboratory

Chemistry / Biotechnology

Glycosidic Synthesis

Tailored biocatalyst extracted from marine ressources

http://www.idealg.ueb.eu/
β-(1→3)-glucans: Glycosidic synthesis thanks to new biocatalysts

Enzymatic Pool

Hydrolase

Synthase

Glucanase

Glycosynthase

Thioligase

Controlled the chain distribution
β-(1→3)-glucanases: *Zobellia* as model system

- Isolated first from the red algae *Delesseria sanguinea*
- Extreme behavior from symbiotic to pathogenic
- Highly active on the degradation of laminarine from brown algae

Seaweeds

*Zobellia galactanivorans* on *E. siliculosus*

Laminarinase

Source of carbone

Laminarine
5 identified laminarinases from the bacteria *Zobellia galactanivorans* (ZgLamA to E)

- GH16 and GH54 family
- Three are associated with Carbohydrate Binding Domain (CBM)
\( \beta-(1\rightarrow3)-\text{glucans} : \text{Glycosidic synthesis thanks to new biocatalysts} \)

**ZgLamC**

Crystallized with non hydrolyzable thioglucans

- Straight active cleft
- A non-processive endoglucanase
- Possibility to accommodate \( \beta-(1,6) \)-branch
- Excreted by *Zobellia*

Enzyme involved in early degradation of laminarine

\( \beta-(1\rightarrow3)\)-glucans: Glycosidic synthesis thanks to new biocatalysts

ZgLamA

EC#: 3.2.1.39

- Specific conformation of the active site
- Endoglucanase
- Additional loop
- 22 fold more active on laminarine than on lichenan
- Anchor to the membrane

Degradation of laminarine after debranching

*J. Biol. Chem.* **2014**, *289*, 2027-2042
Synthesis of $\beta$-(1→3)-glucans: ZgLamA as glycosynthase

ZgLamA

Endoglucanase with retention of configuration mechanism
Synthesis of $\beta$-(1\(\rightarrow\)3)-glucans: ZgLamA as glycosynthase

**ZgLamA**

Endoglucanase with retention of configuration mechanism

Catalytic site LamA
Synthesis of \( \beta-(1\rightarrow3) \)-glucans: ZgLamA as glycosynthase

ZgLamA

Endoglucanase with retention of configuration mechanism

Catalytic site LamA
β-(1→3)-glucans: Glycosidic synthesis thanks to new biocatalysts

Glycosynthase

Donor

\[ \text{F} \quad = \quad \text{OH} \quad \text{O} \quad \text{OH} \quad \text{OH} \quad \text{F} \]

Catalytic site LamA*

Mutation of nucleophilic glutamate

\[ \text{synthase} \]

Selection of ZgLamA\textsubscript{E269S} as synthase

β-(1,3)-glucoside
Self-condensation reactions catalyzed by $\text{ZgLamA}_{E269S}$

$$\text{Lam2F} + \text{LamA}^* \xrightarrow{100 \text{ mM phosphate buffer (pH 7.2)}} \text{Lam4-8OH}$$

$m = 3-7$

Self-condensation
Synthesis of the required donors

Glucose $\rightarrow$ Laminaribiose (90%) $\rightarrow$ Laminaritetraose (91%)

Conditions: $\text{Ac}_2\text{O}$, DMAP, Pyridine, RT, 12h
Synthesis of the required donors

Conditions: HF.Pyridine
Synthesis of the required donors

Conditions: NaOMe, MeOH
Self-condensation reactions catalyzed by ZgLamA_{E269S}

Glc-F $\xrightarrow{\text{LamA}^*}$ No oligoglucoside

Endoglucanase: minimum of a disaccharide in positive and negative site

Hydrolysis site

-4 -3 -2 -1 1 2 3 4
Self-condensation reactions catalyzed by ZgLamA_E269S

\[ \text{Glc}_2\text{-F} \xrightarrow{\text{LamA}^*} \text{Glc}_4\text{-F} \]
\[ \text{Glc}_4\text{-OH} \]
\[ \text{Glc}_2\text{-F} \]
\[ \text{Glc}_2\text{-OH} \]
Self-condensation reactions catalyzed by ZgLamA<sub>E269S</sub>

(2h, 37 °C)

Stability of Glc-2F at 37 °C without enzyme
Transglycosylation reactions catalyzed by ZgLamA^{E269S}

Transglycosylation

\[ \text{Lam}2F + \text{Lam}2pNP \xrightarrow{\text{Lam}A^*} \text{Lam}4-8pNP \]

\[ m = 3-7 \]

Self-condensation

\[ \text{100 mM phosphate buffer (pH 7.2)} \]
\[ 25 ^\circ C \]

\[ \text{Lam}4-8\text{OH} \]
\[ \text{Lam}4-8\text{F} \]

\[ m = 3-7 \]

\[ \text{Transglycosylation} \]

\[ \text{Self-condensation} \]
Transglycosylation reactions catalyzed by ZgLamA<sub>E269S</sub>

\[
\text{Glc2-F + Glc2-OpNP} \rightarrow \text{Glc-F + Glc-OH + Glc-OpNP}
\]

1 : 1

Auto Trans

\[\Rightarrow \text{Reaction over at +10h}\]
\[\Rightarrow \text{Lam2F quickly consumed or hydrolysed}\]
\[\Rightarrow \text{Transglycosylation > self-condensation}\]
Transglycosylation reactions catalyzed by ZgLamA_{E269S}

\[ \text{Glc2-F + Glc2-OPNP} \rightarrow \text{Glcn-F + Glcn-OH + Glcn-OPNP} \]

Influence of the temperature

10h, 25 °C

10h, 10 °C

\[ \Rightarrow \text{Decreasing the temperature slowed the kinetics of the reaction} \]
\[ \Rightarrow \text{More hydrolyses at 10 °C} \]
Transglycosylation reactions catalyzed by ZgLamA_{E269S}

Glc2-F + Glc2-OpNP → Glcn-F + Glcn-OH + Glcn-OpNP

Influence of D:A ratio

1:1 D:A, 25 °C

1:1.5 D:A, 25 °C

⇒ Less LamnOH & and more Lam4pNP with [1:1.5]
  • Lam2F is more consumed than hydrolysed
  • Higher rate of transglycosylation
  • Low polydispersity
Transglycosylation reactions catalyzed by ZgLamA_{E269S}

Glc2-F + Glc2-OpNP → Glcn-F + Glcn-OH + Glcn-OpNP

Influence of D:A ratio

1:1.5 D:A, 25 °C

1:2 D:A, 25 °C, dropwise addition

⇒ Successive Lam2F additions more efficient for transglycosylation
⇒ Increased transglycosylation, reduced hydrolysis and self-condensation
Transglycosylation reactions catalyzed by ZgLamA<sub>E269S</sub>

- Transglycosylation > self-condensation, favored with buffer
  - Need of mild buffered media for enzyme activity
- Best results obtained with slow addition of the acceptor: 47% of total conversion
Transglycosylation reactions: Scale up

Lam2F + Lam2pNP → LamA* → Lam4pNP

100 mM phosphate buffer (pH 7.2)

11% overall yield

Possible inhibition of the laminarinase
Transglycosylation reactions: Scale up

One regio-isomer

No contamination with β-(1,4)-linkage
Conclusion

**ZgLamA**: Endoglucanase from marine origin

- Specific conformation of the active site
- A non-processive enzyme
- Degradation of linear laminarine

**ZgLamA_{E296S}** is able to produce small oligo-β-(1→3)-glucans starting from Glc2-F

- Self-condensation: Glc4, Glc6
- Transglycosylation: Glc4, Glc6, Glc8

**Controlled oligomerization**

When the acceptor is in excess, LamXpNP (X=4-8) products are preferably synthesized rather than LamXOH

- Transglycosylation is favored (vs. autocondensation)
- Low polydispersity – total regioselectivity
- Laminaritetraose could be isolated with up to 10% yield.
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