Update on mucin-type O-glycan engineering in plants

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Glycosylation

**N-glycosylation:** Asn-linked glycosylation (Asn-X-Ser/Thr) – N-glycans

**O-glycosylation:** Ser/Thr-linked glycosylation – O-glycans
N-glycosylation

Function of N-glycans on proteins:

- Protein folding and stability
- Protein targeting (e.g. for lysosomal enzymes)
- Protein-protein interactions (e.g. effector functions of IgGs)
- “Masking function” (e.g. control of protein half-life)
- …
O-glycosylation

Function of mammalian-type O-glycans:

- Protein stability
- Protein-protein interactions
Glyco-engineering: WHY?

- Glycosylation affects the functional properties of therapeutic proteins.

- Current expression systems for recombinant proteins generate a mixture of different glycoforms.

- Aberrant glycosylation may generate unwanted immunogenic reactions.
Glyco-engineering of plants: AIM

Plant-based manufacturing platform for recombinant proteins with a customized glycosylation profile.

*N. benthamiana*
O-glycan engineering in *N. benthamiana*

**Generation of mucin-type O-glycans in plants:**

Erythropoietin (EPO)

3 N-glycosylation sites

1 O-glycosylation site: Ser-126

\[
\text{NeuAc}^\alpha_2 - 3\text{Gal}\beta_1 - 3\text{GalNAc}\alpha_1 - \text{Ser/Thr}
\]
Biosynthesis of mucin-type O-glycans

Plants lack mammalian GalNAc-type protein O-glycosylation machinery.
O-glycan engineering in *N. benthamiana*

O-glycosylation initiation: O-GalNAc formation:

Expression of human GalNAc-transferase 2

recombinant protein: EPO

**Diagram:**
- **UDP-GlcNAc-4-epimerase:**
  - UDP-GlcNAc → UDP-GalNAc
  - Cytoplasm

- **UDP-GlcNAc/UDP-GalNAc transporter:**
  - UDP-GalNAc

- **Golgi:**
  - UDP-GalNAc
  - Ser/Thr
  - GalNAc-transferase (GalNAc-T2)
  - GalNAcα1-Ser/Thr
O-glycan engineering in *N. benthamiana*

LC-ESI-MS analysis

recombinant protein: EPO

Castilho et al., 2012, JBC, 287, 36518-36526
O-glycan engineering in *N. benthamiana*

**O-glycosylation elongation:**

- Expression of human GalNAc-transferase 2
- Expression of *Drosophila* β1,3-galactosyltransferase

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**Diagram:**

- UDP-GlcNAc-4-epimerase
- Cytoplasm
- UDP-GalNAc
- Cytoplasm
- UDP-GlcNAc/UDP-GalNAc transporter
- Golgi
- UDP-GalNAc
- Ser/Thr
- GalNAc-transferase (GalNAc-T2)
- GalNAcα1-Ser/Thr
- UDP-Gal
- β1,3-galactosyltransferase (C1GALT)
- Galβ1-3GalNAcα1-Ser/Thr
- T O-glycan

**Recombinant protein:** EPO
O-glycan engineering in *N. benthamiana*

LC-ESI-MS analysis

recombinant protein: EPO
O-glycan engineering in *N. benthamiana*

**O-glycosylation elongation:**

- Expression of human GalNAc-transferase 2
- Expression of *Drosophila* β1,3-galactosyltransferase
- Expression of two sialyltransferases

**Enzyme Activities:**

- GNE: UDP-GlcNAc 2-epimerase/ManNAc kinase
- NANS: N-acetylneuraminic acid phosphate synthase
- CMAS: CMP-N-acetylneuraminic acid synthetase
- CST: CMP-N-acetylneuraminic acid transporter
O-glycan engineering in *N. benthamiana*

*In planta* synthesis of the disialyl core 1 structure!
N- and O-glycan engineering in *N. benthamiana*

![Diagram of glycan biosynthesis in N. benthamiana](image)
N- and O-glycan engineering in *N. benthamiana*

Expression of EPO in ΔXT/FT + sialic acid pathway + N- and O-glycan engineering enzymes

- sialic acid pathway (CMP-sialic acid biosynthesis)
- mammalian N-glycans
- mammalian O-glycans
- recombinant protein

GNE  NANS  CMAS  CST  GALT  ST  GnTIV  GnTV  GalNAc-T2  C1GALT1  2 x STs  EPO
N- and O-glycan engineering of EPO expressed in *N. benthamiana*

glycosylation site 3 (GQALLVNSSQPWEPLQHLVDK)
The O-linked tumor antigen STn can be produced on MUC1 in *N. benthamiana*.

chimeric MUC1-tandem repeat fused to the Fc-domain:

\begin{align*}
\text{DTRPAPG} & \text{STAPPAHGVT} \text{SAP}
\end{align*}
Summary

• Sialylated mucin-type O-glycans can be produced on different recombinant glycoproteins

• Glycoproteins with mucin-type core elongations can be generated in *N. benthamiana*

• O-glycan engineering does not interfere with N-glycan engineering
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