Production of Complex Viral Glycoproteins in Plants

Novel glyco-engineering technique for high-yield production of complex viral glycoproteins in plants

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Background

With costs, scalability and lengthy production times posing increasing limitations on conventional pharmaceutical production platforms, the use of plant expression systems has come to light as a cost-effective and scalable alternative.

Molecular farming of complex glycoproteins, however, poses a challenge due to differences between the host plant machinery and mammalian cells.

The production of complex viral glycoproteins in plants is currently hindered by major challenges such as low expression yields, non-native glycosylation and inefficient maturation (folding) of these proteins along the secretory pathway.

This has severely hampered the widespread implementation of molecular farming as a viable pharmaceutical production system, thus precluding the use of plants to consistently produce vaccines from complex heavily glycosylated viral glycoproteins.

Researchers at the University of Cape Town (UCT) have developed a novel approach to address these constraints by determining site-specific glycosylation of plant-produced viral glycoproteins followed by integrating various glyco-engineering strategies with chaperone co-expression.

This has enabled the production of a recombinant HIV Envelope gp140 trimer in plants which closely resembles the equivalent mammalian cell-production protein.

The invention is broadly applicable to multiple glycoproteins production and enables the production of heavily glycosylated complex glycoproteins in plants thereby facilitating the production of vaccines and therapeutics in plants that could not previously be produced.

Technology Overview

The present invention relates to methods for increasing the expression and glycosylation efficiency; reducing plant-specific modifications and aggregation; as well as promoting the correct folding of heterologous polypeptides of interest in a plant cell. Previous work in the group has demonstrated that the host chaperone machinery in plants does not support efficient production of complex glycoproteins. Accordingly, the researchers demonstrated that the co-expression of the lectin-binding chaperones (calnexin and calreticulin) improved production of several heavily glycosylated viral glycoproteins in plants. This work was patented to protect the underlying technology and to enable the pipeline to be further developed for commercialisation (WO 2018/220595). However, despite the improved yields of viral glycoproteins in plants, considerable aggregation of the recombinant proteins was observed suggesting further

constraints precluding their efficient production. HIV Envelope gp140 was transiently expressed in plants by co-expression of human calreticulin. The antigens were over-layed to compare their heterogeneity and efficiency of trimer formation. The data obtained demonstrates increased aggregation in plants following production of the recombinant glycoprotein. Following determination of the site-specific glycosylation of the plant-produced viral glycoproteins, an integrated expression approach was conceived to support improved production of a prototype HIV Envelope gp140 glycoprotein in plants. This approach was conceived to address host constraints precluding efficient production and glycosylation of the recombinant protein:

1. Human calreticulin was co-expressed to support protein folding and improve expression yields
2. *Leishmania major* LmSTT3D was co-expressed to improve glycan occupancyAn RNA interference construct was co-expressed to suppress Hexosaminidase 3 (HEXO3RNAi) which is responsible for the formation of truncated (paucimannosidic) glycans.

Protein production was conducted using *Nicotiana benthamiana* ΔXF plants which have been modified to mitigate activities of the enzymes responsible for imparting plant-specific complex glycans. These approaches were combined with the transient expression of HIV Env gp140. This integrated approach was compared to plants infiltrated with a) gp140 and calreticulin and b) gp140/CRT/LmSTT3D/HEXO3RNAi (glyco-optimized). In the absence of glyco-engineering, the protein produced in wildtype plants yielded a prominent aggregate peak which was not observed in the mammalian cell-produced sample or in the glyco-optimized sample. In contrast, both the glyco-optimized sample and the HEK293 sample yielded comparatively low levels of aggregates and the predominant peak was composed of trimers. Encouragingly, the elution profiles of the glyco-optimized protein overlaid perfectly with the HEK293 protein, suggesting that they were comparable. This data demonstrates that the aggregation was due to impaired glycosylation that occurred following expression in plants. The data also demonstrates that the integrated host engineering approaches improved the glycosylation and folding resulting in an antigen that was comparable to the mammalian cell-produced protein.The glycosylation of the glyco-optimized protein was similarly compared to the mammalian cell-produced antigen. The glycan occupancy of the 2 proteins was largely comparable, although subtle differences were observed at several sites. In some cases, the plant-produced protein had increased levels of occupancy.The glyco-engineered HIV Env gp140 vaccine is currently being tested in rabbits and compared to the equivalent mammalian cell-produced vaccine.

Benefits

- Not only do these approaches enable the production of well-folded and heavily glycosylated glycoproteins in plants, but addressing limitations in the glycosylation machinery resulted in improved folding (decreased aggregation).
- Decreased aggregation and improved protein folding.
- Enables the production of virus-like particles and synthetic nanoparticle vaccines and therapeutic glycoproteins.
- Solved the problem of under glycosylation and paucimannosidic glycan formation.

Applications

- Molecular farming of complex glycoprotein-based pharmaceuticals in plants.
- Can be used to produce glycoproteins with tailor-made glycosylation to improve potency of therapeutics and efficacy of vaccines.
- The technology is applicable to viral glycoproteins, such as antibodies which have value as therapeutics.

Opportunity

Available for licensing or collaboration

IP Status

- Patented

Seeking

- Development partner
- Commercial partner
- Licensing
- Seeking investment