Glyco-engineered CHO cell lines producing alpha-1-antitrypsin and C1 esterase inhibitor with fully humanized N-glycosylation profiles

Recombinant Chinese hamster ovary (CHO) cells are able to provide biopharmaceuticals that are essentially free of human viruses and have N-glycosylation profiles similar, but not identical, to humans. Due to differences in N-glycan moieties, two members of the serpin superfamily, alpha-1-antitrypsin (A1AT) and plasma protease C1 inhibitor (C1INH), are currently derived from human plasma for treating A1AT and C1INH deficiency. Deriving therapeutic proteins from human plasma is generally a cost-intensive process and also harbors a risk of transmitting infectious particles. Recombinantly produced A1AT and C1INH (rhA1AT, rhC1INH) decorated with humanized N-glycans are therefore of clinical and commercial interest.

Here, we present engineered CHO cell lines producing rhA1AT or rhC1INH with fully humanized N-glycosylation profiles. This was achieved by combining CRISPR/Cas9-mediated disruption of 10 gene targets with overexpression of human ST6GAL1. We were able to show that the N-linked glyco-structures of rhA1AT and rhC1INH are homogeneous and similar to the structures obtained from plasma-derived A1AT and C1INH, marketed as Prolastin®-C and Cinryze®, respectively. rhA1AT and rhC1INH produced in our glyco-engineered cell line showed no detectable differences to their plasma-purified counterparts on SDS-PAGE and had similar enzymatic in vitro activity. The work presented here shows the potential of expanding the glyco-engineering toolbox for CHO cells to produce a wider variety of glycoproteins with fully humanized N-glycan profiles. We envision replacing plasma-derived A1AT and C1INH with recombinant versions and thereby decreasing our dependence on human donor blood, a limited and possibly unsafe protein source for patients.

General information
Publication status: Published
Organisations: CHO Cell Line Engineering and Design, Novo Nordisk Foundation Center for Biosustainability, CHO in Silico Engineering of Glycosylation and Protein Quality (CiSe), CHO Core, Pre-Pilot Plant, iLoop, Section for Synthetic Biology, Network Engineering of Eukaryotic Cell factories, Department of Biotechnology and Biomedicine
Corresponding author: Hansen, A. H.
Number of pages: 10
Pages: 143-152
Publication date: 2019
Peer-reviewed: Yes

Publication information
Journal: Metabolic Engineering
Volume: 52
ISSN (Print): 1096-7176
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
Original language: English
Keywords: Chinese hamster ovary (CHO) cells, CRISPR/Cas9, Glyco-engineering, Biotechnology, Multiplexing, Plasma proteins
DOIs: 10.1016/j.ymgen.2018.11.014
Source: FindIt
Source-ID: 2442102452
Research output: Contribution to journal ‒ Journal article ‒ Annual report year: 2019 ‒ Research ‒ peer-review