Sequence Curation and Glycoform Analysis of SARS-CoV-2 RBD Domains Produced in Mammalian Cell Lines

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Introduction

The SARS-CoV-2 spike (S) protein's receptor binding domain (RBD) mediates the interaction with the ACE2 receptor on host cells and is target to immune response and diagnostic tools.

We characterized recombinant RBDs on the levels of released glycans, glycopeptides, intact mass analysis with glycan-enzymatic dissection and Top-Down Sequencing for comprehensive annotation of RBD proteoforms (**1**).

This work not only offers insights into RBD structural and functional features but also provides a workflow for characterization of new RBDs and batch-to-batch comparison.

References

1. C Gstöttner, T Zhang, A Resemann, S Ruben, S Pengelley, D Suckau, T Welsink, M Wuhrer, E Domínguez-Vega. Anal. Chem. 2021, 93, 17, 6839–6847

Methods

His_6-tagged RBDs were expressed in CHO and in HEK293 cells (InVivo). N-linked glycans were removed with PNGase F (Promega) and O-glycans with OglyZOR and SialEXO (Genovis).

MALDI Top-Down Sequencing (TDS) spectra were obtained from approx. 40 pmol RBD in sDHB matrix using rapifleX and analyzed using BioPharma Compass 2021b and BioTools 3.2 SR7 (all Bruker).

Sequence, O-Glycosylation

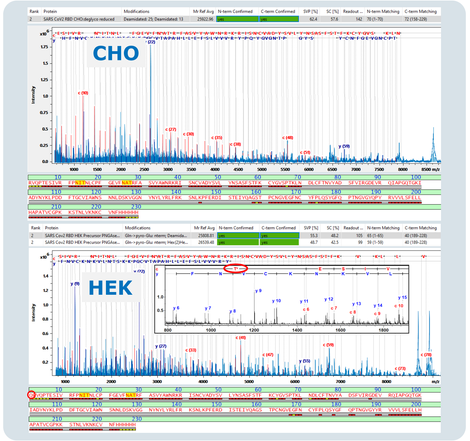

Fig. 1 MALDI-ISD spectra of deglycosylated CHO-RBD confirms the expected sequence; of HEK-RBD establishes N-terminal pyroGlu as leftover from unexpected pro-peptide processing and Thr-6 with core-2 O-glycosylation remaining from OglyZOR/SialExo digestion (T*, see insert). Ser-8 is not glycosylated.

O-glycan alditols released from RBD via reductive β-elimination were analyzed by amazon ETD speed ion trap (Bruker) MS/MS in negative ion mode after PGC nano-LC separation (3 µm Hypercarb 100 µmx150 mm, GraceDiscovery Sciences).

Released O-glycan analysis

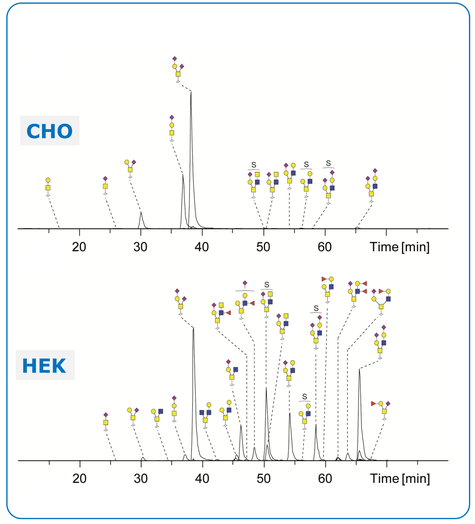

Fig. 2 PGC-MS analysis of released O-glycan alditols from CHO-RBD showing nearly exclusively core-1 O-glycosylation and from HEK293-RBD comprising a mixture of core-1 and core-2 O-glycosylation.

ESI data of intact RBDs were obtained after CE separation (CESI 8000, Sciex) and ESI-QTOF analysis (Impact II, Bruker)).

Glycoforms were assigned based on average mass shifts before and after glycosidase treatment.

Intact RBD analysis

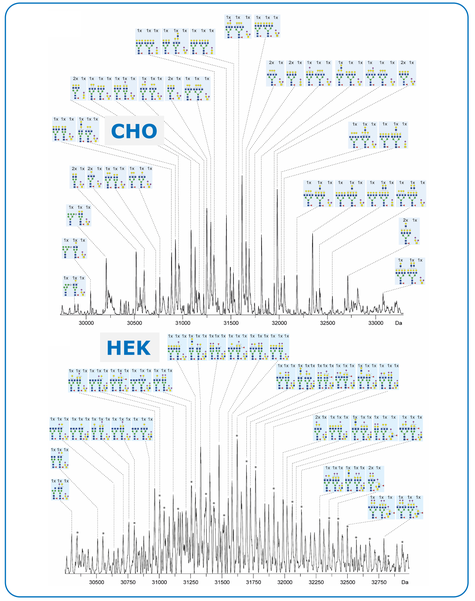

Fig. 3 Deconvoluted mass spectrum of CHO-RBD and HEK293-RBD following CE-qTOF MS separation.

Summary

- Complementary MS workflows combined with separation techniques and enzymes were successfully used to assign RBD proteoforms
- MALDI-ISD-TOF established protein sequences including unexpected N-terminal pGlu of the HEK-RBD and pinpointed the O-linked glycosylation site (Thr 323)
- PGC-MS analysis showed key differences in O-linked glycosylation between HEK293- and CHO-expressed RBD
- CE-qTOF analysis clearly shows significant differences in overall glycosylation between HEK and CHO allowing batch-to-batch comparison

Conclusions

- Recombinantly produced CoV-2-RBDs in CHO and HEK293 cells exert distinct and complex glycosylation patterns, which include 2 N- and 1 O-glycosylation sites
- The CHO-RBD exclusively expressed core-1 O-glycans and HEK293-RBD core-2 structures in addition
- A single O-glycosylation site at Thr-6 was identified, Ser-8 was not O-glycosylated
- The CHO-RBD sequence was confirmed by Top-Down Sequencing while an unexpected pyroGlu was N-terminally added in the HEK293-RBD

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