Discovering New Chlorovirus Glycoforms Using Polyclonal Antibodies

Tiffany Do[1], Irina Agarkova[2], James Van Etten[2]
[1]School of Biological Sciences, University of Utah
[2]Department of Plant Pathology and Nebraska Center for Virology, University of Nebraska-Lincoln

INTRODUCTION

Chloroviruses are large double-stranded DNA plaque forming viruses that infect chlorella-like green algae.

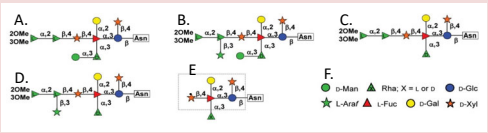
Chloroviruses have a linear DNA genome from 290 to 370 kilobases, with type species named PBCV-1 of 331 kilobases.

PBCV-1 genome encodes for approximately 410 proteins.

Figure 1: PBCV-1 cryo-EM reconstruction [virus diameter = 190 nm]. (Cherrier et al., 2009)

- Outer viral protein shell is sugar-coated with molecules called glycans.
- Many viruses have structural proteins that are glycosylated. All viruses studied to date use host-encoded glycosylation machinery to modify their proteins.
- Chloroviruses are the first viruses to encode most, if not all, of the machinery to glycosylate their proteins.

PBCV-1 virion packages 148 unique virus-encoded proteins. The most abundant protein is the major capsid proteins that makes up to ~40% of the total virion proteins and the protein is glycosylated.



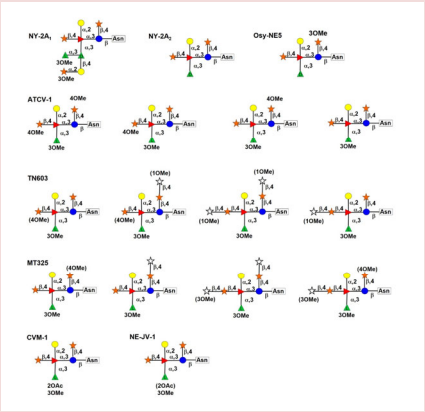


Figure 2 (Top panel): structure of PBCV-1 major capsid protein glycans. A-D.) different glycoforms of PBCV-1; E.) Common glycan core found in chloroviruses; F.) glycan sugar designation
(Bottom panel): Current known glycan structures of chloroviruses
(Van Etten et al., 2017)

PROTEIN GLYCOSYLATION

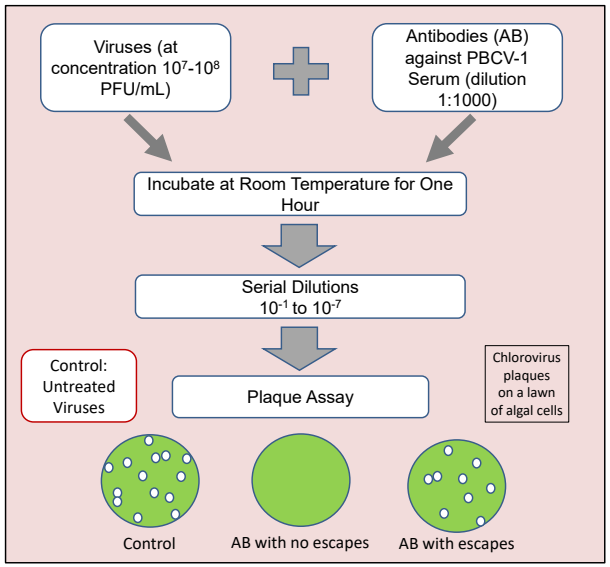
Glycosylation is a form of post-translational modification in which sugar moieties are covalently attached to specific amino acids on a target protein. It is also one of the most complex post-translational modifications because of the large number of enzymatic steps involved. Glycosylated proteins (glycoproteins) are found in all living organisms, including eukaryotes, eubacteria, and archaea. Protein glycosylation plays a critical role in cell functioning.

Glycosylation increases the diversity of the proteome to a level unmatched by any other post-translational modification because almost every aspect of glycosylation can be modified, including glycosidic linkage – the site of glycan (oligosaccharide) binding, glycan composition – the types of sugars that are linked to a particular protein, glycan structure – branched or unbranched chains, and glycan length – short or long-chain oligosaccharides. For a discovery of new chlorovirus glycogen forms, a study employing an antibody against type chlorovirus species PBCV-1 virus was designed.

We assumed that viruses possessing a unique glycan structure would not react with an antibody made against PBCV-1 virus, because as it was established previously that anti-chlorovirus antibodies react mainly with glycans of the major capsid protein (Wang et al., 1993). Thus, we can screen viruses for new glycan structure using antibodies made against chloroviruses with known glycan structures (Fig. 2). If a virus does not get neutralized by the anti-PBCV-1 virus antibody, it is an indication of a possible new and unique glycan structure.

Hypothesis: Viruses will react differentially with antibodies made against PBCV-1.

MATERIALS AND METHODS



RESULTS

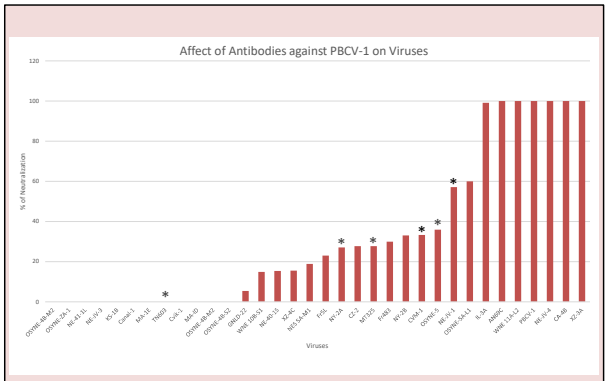


Figure 3: Neutralization of tested chloroviruses by antibodies made against PBCV-1 virus.

*represents known glycan structures other than PBCV-1

SUMMARY

Thirty-four chloroviruses were screened for new glycoforms using an antibody made against type chlorovirus species PBCV-1 with known glycan structure.

Viruses reacted differentially with antibodies made against PBCV-1. Therefore, the stated hypothesis was supported.

Chloroviruses with resolved glycan structure (NY-2A, MT325, CVM-1, OSYNE-5, NE-JV-1 and TN603), that have distinct glycoforms from PBCV-1 glycans, showed virus neutralization pattern varying from 0% to 57%.

Seven of the thirty-four assayed viruses were completely neutralized with the antibodies made against PBCV-1 virus. This indicates that these viruses have a glycan structure that resembles PBCV-1 glycans.

Applying 50% of neutralization as a cut off value, we identified twenty-four virus strains that are perspective candidates for following glycan composition and structure analysis using nuclear magnetic resonance (NMR) spectroscopy.

ACKNOWLEDGEMENTS

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