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Short Communication

Glyco-engineering Using Pneumococcal Exoglycosidases: A Pilot Study on Deglycosylation of Human Alpha-1-Acid Glycoprotein

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ABSTRACT

Glyco-engineering is an emerging field that focuses on modifying glycan structures to enhance or alter biological functions. Enzymatic glycan remodeling has numerous applications in biopharmaceuticals, vaccine development, and glycan-based diagnostics. Pneumococcal exoglycosidases, such as neuraminidase NanA and β-galactosidase BgaA, selectively hydrolyze terminal sugars from glycoconjugates, making them potential tools for glyco-engineering. However, their ability to modify human glycoproteins in vitro remains largely unexplored. In this study, we assessed the glyco-engineering potential of pneumococcal exoglycosidases by examining their role in deglycosylating human alpha-1-acid glycoprotein (AGP), a plasma glycoprotein involved in inflammation. Our results demonstrate that NanA removes terminal sialic acid residues from AGP, enabling subsequent galactose removal by BgaA, as confirmed by SDS-PAGE and lectin blot analysis. These findings provide experimental evidence supporting the utility of pneumococcal glycosidases for enzymatic glyco-engineering.

Keywords: Glyco-engineering, sialic acid, Streptococcus pneumoniae, glycosidases, NanA, BgaA, Lectin blotting, human alpha-1-acid glycoprotein (AGP1)

I. INTRODUCTION

Streptococcus pneumoniae exoglycosidases can modify host glycans

Glycans are key structural and functional components of glycoproteins and glycolipids, influencing diverse biological processes including cell signalling, immune recognition, and pathogen-host interactions (Varki, 2017). One of the key human pathogens which rely on human glycans for host-microbial interaction is Streptococcus pneumoniae (Hobbs et al., 2018). S. pneumoniae encodes several exoglycosidases which are known to modify host glycans and are crucial for both physiological and pathological processes (Mathew et al., 2023). S. pneumoniae exoglycosidases selectively hydrolyze terminal sugars from host glycoconjugates, altering glycan structures and functions (Hobbs et al., 2018; Mathew et al., 2023).

Among these, neuraminidase NanA removes terminal sialic acids, while β-galactosidase BgaA cleaves exposed β,1-4 linked galactose residues, thereby modifying host glycoproteins in a sequential manner (King, 2010). In addition to NanA and BgaA, other pneumococcal exoglycosidases also contribute to glycan modification. N-acetylglucosaminidase StrH cleaves terminal N-acetylglucosamine residues, which play a crucial role in host glycoprotein remodeling. Another enzyme, α-fucosidase, hydrolyzes terminal fucose residues, which are essential components of glycan structures involved in immune regulation (Hobbs et al., 2019). These enzymes act in a coordinated manner to degrade host glycan structures, facilitating colonization and immune evasion (Mathew et al., 2023).

Glyco-engineering is a broad term used to describe an advanced biotechnological approach aimed at modifying glycan structures to alter or enhance biological functions (Beals and Shanafelt, 2006; Mathew et al., 2023). Glyco-engineering is widely used in therapeutic glycoprotein optimization, vaccine development, and glycan-based diagnostics (Mathew et al., 2023; Zhang et al., 2016). Given their specificity in glycan cleavage, pneumococcal exoglycosidases have potential applications in glycoengineering. However, there is limited experimental validation of pneumococcal glycosidases as enzymatic tools for glycan remodeling in vitro.

In vitro deglycosylaytion of a human glycoprotein by S. pneumoniae NanA and BgaA

In this pilot study, we sought to provide direct biochemical evidence of the glyco-engineering potential of pneumococcal exoglycosidases, NanA and BgaA by investigating their role in the deglycosylation of human alpha-1-acid glycoprotein (AGP). AGP is a key plasma glycoprotein involved in inflammation and immune regulation, making it an ideal model substrate. Recombinant NanA and BgaA clones were generously provided by Dr. Anirudh Singh (SAM Global University, Raisen, MP, India) and purified using methods described earlier (Singh et al., 2014). Human AGP was obtained from Sigma-Aldrich. A total of 30 µg of AGP was incubated at 37°C for 4 hours in PBS (pH 7.4) with the following conditions: (1) NanA, (2) BgaA, (3) NanA + BgaA, and (4) PBS-only control. Following incubation, samples were mixed with 2X SDS-PAGE loading dye and subjected to reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% acrylamide gel. SDS-PAGE analysis revealed that AGP treated with NanA exhibited a lower molecular weight compared to the control, indicating the removal of terminal sialic acid. In contrast, AGP treated with BgaA alone showed no significant change in molecular weight, consistent with the enzyme's specificity for β,1-4 linked terminal galactose, which remains inaccessible due to sialic acid capping in N-linked glycans. When AGP was treated with both NanA and BgaA, an additional molecular weight reduction was observed, suggesting sequential removal of terminal sialic acid by NanA followed by exposure and removal of β,1-4 linked galactose by BgaA (Figure 1).

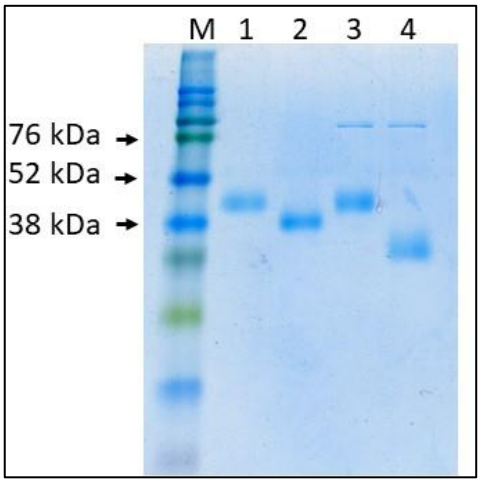

Figure 1. Deglycosylation of human AGP with S. pneumoniae NanA and BgaA. SDS-PAGE analysis of AGP treated with S. pneumoniae NanA and/or BgaA. A total of 10 µg of each treated sample was separated on a 10% SDS-polyacrylamide to analyse AGP deglycosylaytion. Lane M: Molecular weight marker, 1: No treatment, 2: NanA, 3: BgaA, and 4: NanA + BgaA. Expected size of glycosylated human AGP is 41-43 kDa which is evident in the untreated Lane 1. Note

the decrease in size of AGP in Lane 2 and a further decrease in size in Lane 4.

To further confirm sialic acid removal, lectin blot analysis was performed using Sambucus nigra agglutinin (SNA), which specifically binds to sialylated glycans. AGP samples on replica polyacrylamide gel from Figure 2a were transferred on to a polyvinylidene fluoride (PVDF) membrane and probed with SNA using the DIG Glycan Detection Kit (Roche) following the manufacturer's protocol. While the control and BgaA-only lanes showed positive SNA binding, NanA and NanA+BgaA treated AGP exhibited no detectable signal, confirming the enzymatic removal of terminal sialic acid (Figure 2).

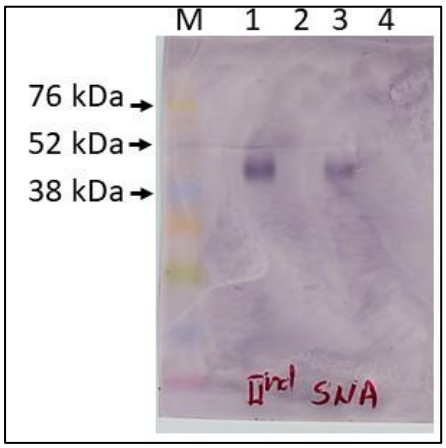

Figure 2. Deglycosylation of human AGP with S. pneumoniae NanA and BgaA. Lectin blot to detect terminal sialic acid on untreated and NanA and/or BgaA treated AGP. A total of 10 µg of each AGP sample was separated on 10% SDS-polyacrylamide gel and transferred on PVDF membrane. The membrane was then probed with SNA which recognizes terminal α,2-6 sialic acid linked to galactose. Lane M: Molecular weight marker, 1: No treatment, 2: NanA, 3: BgaA, and 4: NanA + BgaA. The positive signals in Lane 1 and 3 indicate presence of terminal sialic acid. The absence of positive signals in Lane 2 and 4 are due to removal of terminal sialic acid by NanA.

II. CONCLUSION

Our findings provide direct biochemical evidence of human glycoprotein deglycosylation by pneumococcal exoglycosidases underscoring the potential of these enzymes for glyco-engineering applications. The ability of NanA to remove terminal sialic acid, thereby exposing underlying galactose residues for BgaA activity, demonstrates a sequential mechanism of glycan processing. Pneumococcal exoglycosidases may serve as valuable tools for glycan remodeling in biopharmaceuticals, vaccine development, and glycan-based diagnostics. Further studies with additional glycoprotein substrates and in-depth glycomic analyses will be crucial to fully explore their biotechnological potential.

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