Carbohydrate Research 428 (2016) 31–40

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Glycan specificity of neuraminidases determined in microarray format

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ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 31 March 2016
Accepted 5 April 2016
Available online 8 April 2016

Keywords:
Sialic acids
Neuraminidase
Chemoenzymatic labeling
Microarray

ABSTRACT

Neuraminidases hydrolytically remove sialic acids from glycoconjugates. Neuraminidases are produced by both humans and their pathogens, and function in normal physiology and in pathological events. Identification of neuraminidase substrates is needed to reveal their mechanism of action, but high-throughput methods to determine glycan specificity of neuraminidases are limited. Here we use two glycan labeling reactions to monitor neuraminidase activity toward glycan substrates. While both periodate oxidation and aniline-catalyzed oxime ligation (PAL) and galactose oxidase and aniline-catalyzed oxime ligation (GAL) can be used to monitor neuraminidase activity toward glycans in microtiter plates, only GAL accurately measured neuraminidase activity toward glycans displayed on a commercial glass slide microarray. Using GAL, we confirm known linkage specificities of three pneumococcal neuraminidases and obtain new information about underlying glycan specificity.

1. Introduction

Sialic acids are a group of nine-carbon α-keto acids typically found at the non-reducing termini of glycoproteins and glycolipids.[1] N-acetylneuraminic acid (Neu5Ac) is the primary sialic acid found in human glycoconjugates, while N-glycolylneuraminic acid (Neu5Gc) and deaminoneuraminic acid (2-keto-3-deoxy-D-glycero-D-galactonononic acid; KDN) are produced by other species. Sialic acids can be removed from glycoconjugates through the action of neuraminidases, also known as sialidases. These enzymes hydrolyze the glycosidic bond between sialic acid and the underlying glycan. Desialylation of cell surface glycoconjugates can have dramatic effects on their activity, stability, and subcellular localization.[2] The human genome encodes at least four neuraminidases (NEU1-NEU4), which exhibit varying subcellular localizations, expression patterns, and glycan specificities.[3,4] Neuraminidases are also produced by both viral and bacterial pathogens, where they often function as virulence factors.[5,6] By desialylating host cell glycoconjugates, viral and bacterial neuraminidases can potently affect host cell signaling events.[7,8] The glycan specificity of neuraminidases determines which host glycoconjugates are desialylated, and thus dictates the effects of these enzymes on host cell biology.

A variety of approaches have been employed to examine the glycan specificity of neuraminidases.[9–14] In early efforts, neuraminidase activity toward individual glycans was examined in a one-by-one fashion, while more recent approaches have used microtiter plate assays to simultaneously examine a wider array of glycans. Recently, glycan microarrays have emerged as an important tool enabling high-throughput assessment of the specificity of glycan-binding proteins.[15–18] These successes suggest that glycan microarrays might also be used to examine the specificity of glycan-modifying enzymes, such as neuraminidases. Indeed, several groups have used glycan microarrays to evaluate specificity of parainfluenza and influenza neuraminidases. In these efforts, neuraminidase-induced changes in glycan structure were detected using fluorescently-labeled detection reagents, such as galactose-specific lectins or whole virus, to bind to desialylated glycans.[19–22] Notwithstanding these important studies, glycan microarrays have not yet been fully harnessed for analysis of neuraminidase specificity. One limitation is that commonly used detection reagents exhibit their own glycan specificities, restricting the set of glycans that can be analyzed.

We previously reported that a chemoselective glycan labeling reaction—periodate oxidation and aniline-catalyzed oxime ligation (PAL)[23]—could be used to interrogate neuraminidase specificity in microtiter plate format.[13] Here we examine the utility of PAL and a second chemoselective glycan labeling strategy—galactose oxidase and aniline-catalyzed oxime ligation (GAL)[24]—to evaluate neuraminidase specificity in microarray format. We find that GAL accurately reports on neuraminidase activity toward glycans

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http://dx.doi.org/10.1016/j.carres.2016.04.003
0008-6215/

displayed on microarrays. Thus, applying GAL to microarrays may offer a high-throughput approach to evaluate neuraminidase specificity through the use of emerging glycan microarray resources.

2. Results

We used our previously reported microtiter plate assay with PAL detection[13] to determine the substrate specificities of three pneumococcal neuraminidases—NanA, NanB, and NanC. The specificities of these neuraminidases for different sialic acid linkages are known: NanA is relatively non-selective, cleaving α2-3-, α2-6-, and α2-8-linked sialic acids, whereas NanB and NanC exhibit preferences for α2-3-linked sialic acids.[25,26] However, assessment of the effects of underlying glycan structures on the activities of these neuraminidases has been more limited. To determine the specificities of NanA, NanB, and NanC using the PAL microtiter plate assay, a set of biotinylated glycans (Fig. 1) was treated with neuraminidase or not, before incubation in individual wells of a streptavidin-coated 96-well plate. After binding glycans in each well, remaining sialic acids were labeled using PAL and fluorescence was measured.

As expected, NanA was able to cleave α2-3-linked N-acetylneuraminic acid (Neu5Ac). NanA displayed similar activities toward Neu5Ac α2-3-linked to lactose (Lac) and to N-acetyl-D-lactosamine (LacNAc) (Fig. 2a). NanA was also active against α2-3-linked N-glycolylneuraminic acid (Neu5Gc), although cleavage of Neu5Gc appeared to be slightly less efficient than cleavage of Neu5Ac at similar enzyme concentrations. Interestingly, while NanA was highly active against sialyl Lewis X (sLe^X, Neu5Ac-α2-3-Gal-β1-4-(Fuc α1–3)-GlcNAc-), it displayed slightly less activity toward sialyl Lewis a (sLe^a, Neu5Ac-α2-3-Gal-β1-3-(Fuc α1–4)-GlcNAc-), a glycan in which the positions of sialylated galactose and fucose attachment to GlcNAc are swapped relative to sLe^X (Fig. 1). While the promiscuous NanA was also able to cleave α2-6-linked Neu5Ac from both Lac and LacNAc, as expected, the enzyme showed slightly higher activity toward the LacNAc-containing glycan. Reports indicate that NanA collaborates with other glycosidases to cleave Neu5Ac-α2-6-LacNAc from host cells surfaces during infection,[27] suggesting that pneumococcus may have evolved to achieve efficient recognition of this important glycan. Surprisingly, NanA did not appear to be active against Neu5Gc α2-6-linked to LacNAc, even at the highest enzyme concentration. Taken together with results from α2-3-linked Neu5Gc, pneumococcal NanA appears to prefer Neu5Ac over Neu5Gc. Finally, NanA displayed no activity against the GM1 oligosaccharide headgroup or a glycan containing KDN, a sialic acid species that occurs mainly in bacteria and lower vertebrates. Overall, these results emphasize the importance of the underlying glycan, as well as the sialic acid species, in determining sialidase specificity.

Activity of NanB against Neu5Ac α2-3-linked to Lac or LacNAc was high, as expected (Fig. 2b). While NanB was also able to cleave

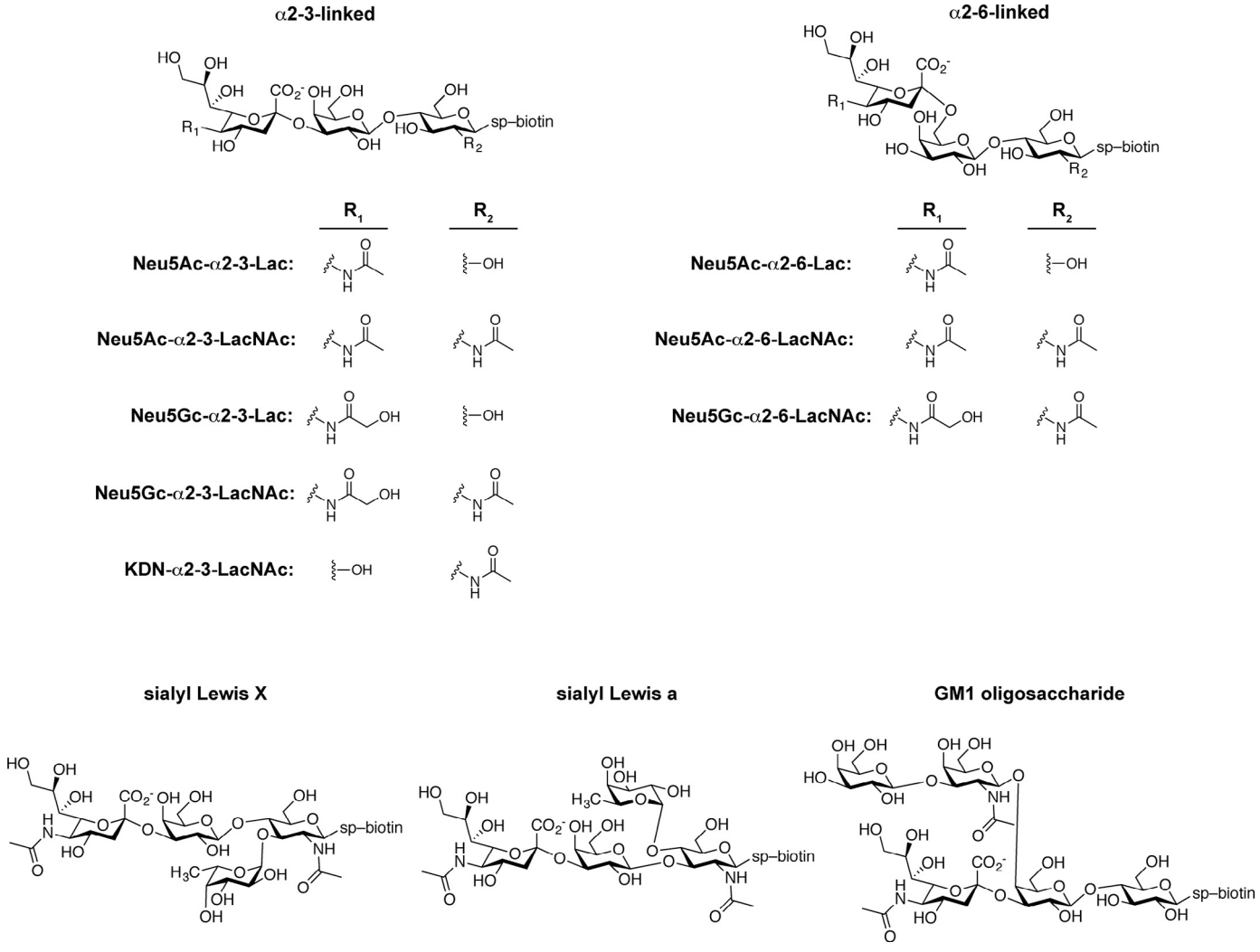

Fig. 1. Structures of glycans used in microtiter plate assay.