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Bisecting GlcNAc restricts conformations of branches in model *N*-glycans with GlcNAc termini

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ABSTRACT

Bisected *N*-glycans play significant roles in tumor migration and Alzheimer's disease through modulating the action and localization of their carrier proteins. Such biological functions are often discussed in terms of the conformation of the attached *N*-glycans with or without bisecting GlcNAc. To obtain insights into the effects of bisecting GlcNAc on glycan conformation, a systematic NMR structural analysis was performed on two pairs of synthetic *N*-glycans, with and without bisecting GlcNAc. The analysis reveals that terminal GlcNAcs and bisecting GlcNAc cooperate to restrict the conformations of both the α1-3 and α1-6 branches of *N*-glycans. ^{1}H and ^{13}C chemical shift comparisons suggest that bisecting GlcNAc directly modulates local conformation. Unique NOE correlations between core-mannose and the α1-3 branch mannose as well as the $^{3}J_{C\text{-}H}$ constant of the glycoside linkage indicate that bisecting GlcNAc restricts the conformation of the 1–3 branch. The angles of the glycosidic bonds between core-mannose and α1-6 branch mannose derived from $^{3}J_{C\text{-}H}$ and $^{3}J_{H\text{-}H}$ coupling constants show that terminal GlcNAcs restrict the distribution of the ψ angle to 180° and the bisecting GlcNAc increases the distribution of the ω angle +60° in the presence of terminal GlcNAcs. It is feasible that restriction of branch conformations by bisecting GlcNAc has important consequences for protein-glycan interplay and following biological events.

1. Introduction

N-glycosylation takes place in a co- or post-protein translation manner, and therefore affects many vital aspects of glycoproteins, such as folding, degradation, transport, and localization [1,2]. During the maturation pathway of glycoproteins, particular *N*-glycans are modified with bisecting *N*-acetyl-D-glucosamine (GlcNAc) by the action of *N*-acetylglucosaminyltransferase (GnT)-III [3].

Addition of bisecting GlcNAc to specific proteins can influence the suppression or promotion of cancer and Alzheimer's disease. The presence of bisecting GlcNAc on proteins involved in tumor malignancy and migration, including epidermal growth factor receptor (EGFR), E-cadherin, and the integrins, plays a pivotal role in suppression of cancer progression and migration [4–8]; through inhibition of β1,6-GlcNAc transfer by GnT-V [5,9,10] and of core α1,6-fucosylation by α1,6-fucosyltransferase (Fut)8 [11,12]. A recent report indicates that overexpression of GnT-III suppressed α2,3-sialylation, which inhibits the migration of tumor cells [13]. The addition of bisecting GlcNAc to the *N*-glycan on β-site amyloid precursor protein cleaving enzyme-1 (BACE1) relates to formation of Aβ plaques in Alzheimer's disease patients by regulation of the cellular stability of BACE1 [14].

The biological function of bisecting GlcNAc can be extended by changes in *N*-glycan sequences and structures, or conformations [15,16]. Such features, including the number of branches, can be altered by the introduction of bisecting GlcNAc because of the substrate specificities of the glycosyltransferases acting on the *N*-glycan maturation process [4,17]. Furthermore, some biological aspects are likely influenced by shifts in the populations of back-folded and extended conformers at the 1,6-branch upon introduction of bisecting GlcNAc and/or core fucosylation [18,19]. Actually, the crystallographic data of a legume lectin Phytohemagglutinin from *Phaseolus vulgaris* (PHA-E) clearly reveals that PHA-E interacts with the bisecting GlcNAc, although most are with the Galβ(1–4)GlcNAcβ(1–2)Man unit on the 1–3 and 1–6 branches [20]; even though it is widely used for detecting bisected *N*-glycoproteins with a galactose terminus [21,22]. Such diverse glycan-lectin interactions can be rationalized, at least partly, as being due to

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conformational alterations induced by the bisecting GlcNAc [23]. A few X-ray crystal structures of glycoproteins with bisected *N*-glycans are available in the PDB, examples include, snake venom metalloproteinase and engineered immunoglobulin G Fc [24,25]. A comparison of their glycan structures shows remarkable differences - the ω angles (O6′-C6′-C5′-C4′) at the 1–6 branch are 56° (*gg*) and −168° (*gt*), respectively. Recently, three protein-bisected *N*-glycan complex structures became available, namely, C-type lectin dendritic cell inhibitory receptor 2 (DCIR-2), Calsepa lectin, as well as the above-mentioned PHA-E [20,23,26]. Currently, however, there are too few atomic structures of bisected glycans to understand their conformational range and complexity, particularly about conformational restrictions by bisecting GlcNAc.

In general, chemical structures of mammalian *N*-glycan chains consist of concatenated pyranose rings linked through flexible glycosidic bonds. Therefore, glycan conformations are defined by the dihedral angles of the glycosidic linkages, namely, φ (O5-C1-O(*n*)'-C(*n*)'), ψ (C1-O(*n*)'-C(*n*)'-C(*n*-1)'), and ω [27]. NMR is a powerful technique for analyzing the structure and conformation of glycans in aqueous media [28–38]. The φ angle often defines *gauche* conformations through the so-called exo-anomeric effect [39]; whereas ψ and ω angles mostly depend on steric effects and surrounding environments.

Herein, we report an NMR structural analysis of four synthetic glycans, bisectGN-Man$_3$GN$_2$ **1**, Man$_3$GN$_2$ **2**, bisectGN-Man$_3$ **3**, and Man$_3$ **4**, respectively (Fig. 1). Conformational properties of the *N*-glycans, especially the effect of bisecting GlcNAc, are systematically analyzed by NMR-based parameters including chemical shifts, NOE distance, and scalar coupling constants.

2. Results and discussion

2.1. Chemical shift comparison of bisected vs. non-bisected N-glycans

Chemical shift is a sensitive NMR parameter to analyze differences in local magnetic environments of biomacromolecules as well as small organic molecules including glycans [40]. Previous studies have evaluated the effect of bisecting GlcNAc on glycan conformation by ^{1}H chemical shift perturbations [30,35]. The correlation between ^{13}C chemical shifts of the anomeric carbon and ψ angle of the glycosidic linkage has been studied previously [41]. In this study, ^{1}H- and ^{13}C-chemical shifts of bisected *N*-glycans (**1** and **3**) and the non-bisected counterparts (**2** and **4**) in ^{1}H-^{13}C HSQC spectra were compared after standardization using weighted chemical shift differences (Fig. 2). Shift differences between Man$_3$ **3** and bisectGN-Man$_3$ **4** are summarized in Fig. 2A. C3,4-(Man1), C1-(Man2) and C1-(Man3) exhibit a large chemical shift difference ($\Delta\delta_{avg} > 0.15$), which seems directly induced by the bisecting GlcNAc (GN1). Moderate chemical shift differences

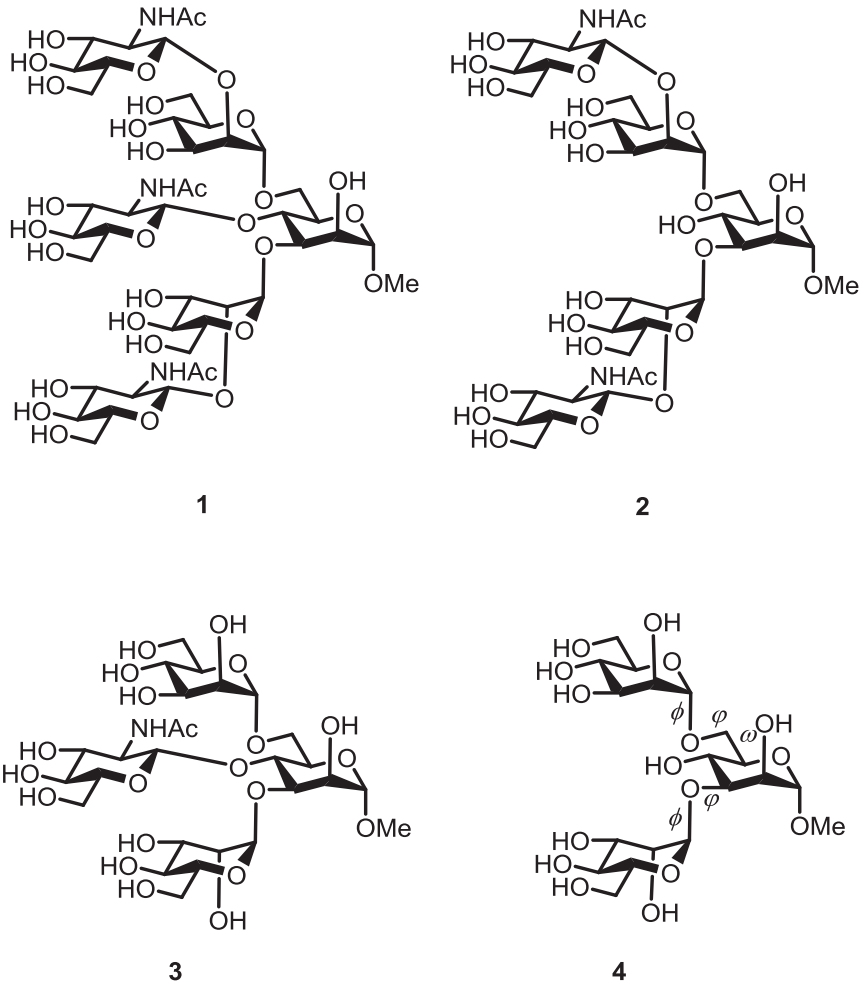

Fig. 1. Structures of bisected *N*-glycans (bisectGN-Man$_3$GN$_2$) **1** and (bisectGN-Man$_3$) **3**, and corresponding Man$_3$GN$_2$ **2** and Man$_3$ **4**.