Tetrahedron xxx (2015) 1–14

Contents lists available at ScienceDirect

Tetrahedron

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

Glycoclusters as lectin inhibitors: comparative analysis on two plant agglutinins with different folding as a step towards rules for selectivity

Sabine André [a,*], Shane O'Sullivan [b], Hans-Joachim Gabius [a], Paul V. Murphy [b,*]

[a] Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Veterinärstr. 13, 80539 Munich, Germany
[b] School of Chemistry, National University of Ireland Galway, University Road, Galway, Ireland

ARTICLE INFO

Article history:
Received 25 April 2015
Received in revised form 19 June 2015
Accepted 6 July 2015
Available online xxx

Keywords:
Glycoprotein
Lectin
Modelling
N-Acetylglucosamine
Valency

ABSTRACT

The emerging physiological significance of carbohydrate (glycan)–protein (lectin) recognition engenders the interest to design synthetic inhibitors with a high level of selectivity among natural sugar receptors. Plant agglutinins are common models to determine structure–activity relationships. Focussing on the contribution of valency towards selectivity, copper-catalysed azide (sugar derivative)–alkyne (scaffold) cycloaddition yielded a panel of 10 bi- to tetravalent glycoclusters with *N*-acetylglucosamine as the bioactive headgroup. They were introduced into assays using (neo)glycoproteins and cell surfaces as platforms to study carbohydrate-dependent lectin binding. The ability of the bivalent compounds, which exhibit a distance profile of the sugar headgroups of about 16–21 Å, for intramolecular bridging of two contact sites from the eight hevein domains of wheat germ agglutinin led to comparatively high enhancements of inhibitory potency relative to a tetrameric leguminous lectin (distance profile of 50–70 Å between sugar-specific sites), especially for a β-S-glycoside. The extent of inhibition at fixed concentrations of the sugar depended on the type of matrix used for the assay. Increases to tri- and tetravalency played a less important role than the anomeric position to keep cross-reactivity low, these tested topologies enabling cross-linking for both lectins. The potential for cis-interactions (intramolecular interactions), with glycoclusters serving as molecular rulers, is suggested to help designing selective blocking reagents.

1. Introduction

Synthetic chemistry substantially contributes to the progress in unravelling structure–activity relationships of carbohydrate (glycan)–protein (lectin) interactions, also referred to as cracking the sugar code.[1] A major question to be answered concerns the origin of the apparent specificity and selectivity of carbohydrate-binding proteins (lectins) for distinct cellular glycans presented by specific glycoconjugates (counterreceptors), in structural and topological terms. Growing insights into the natural occurrence of diverse sugar receptors is facilitating to classify them into groups, either formed by homologous family members or by non-related proteins sharing at least monosaccharide specificity, for example, β-galactoside-specific receptors defined as C-type lectins or galectins.[1f,2] Due to their functional cooperation in vivo, versatile tools are needed to comparatively dissect recognition properties of each constituent of such a network. Towards this end, glycoclusters (glycosylated scaffolds with at least bivalency) with galactosides as headgroups have proven their applicability in the test case of galectins, revealing routes towards selectivity among the three types of structural design of these lectins and natural/engineered variants (for examples, please see Ref. 3). Such data strengthen the assumption that these synthetic products can be potent sensors of the topological and/or spatial aspects of lectin binding and that their design can achieve differential reactivity to various types of receptors for compounds presenting the same carbohydrate epitope. Herein, we test the concept on a further letter of the sugar alphabet and two folding patterns of lectins.

N-Acetylglucosamine (GlcNAc), like galactose, can be spatially accessible when positioned at branch termini of N- and mucin-type O-glycans as well as on glycolipids. Physiologically, N-glycans with terminal β-GlcNAc are abundant in basophils and eosinophils, and an increase in such GlcNAc-terminated structures in several types of carcinoma intimates a value as tumour marker, with potential functional significance.[4] In fact, a panel of

* Corresponding authors. E-mail addresses: sabine.andre@lmu.de (S. André), paul.v.murphy@nuigalway.ie (P.V. Murphy).

http://dx.doi.org/10.1016/j.tet.2015.07.020
0040-4020/

endogenous lectins is known with specificity for GlcNAc, among them soluble host defence effectors (collectins, ficolins), transmembrane pattern recognition receptors such as the tandem-repeat-type macrophage mannose receptor, the trimeric langerin, chitinase 3-like-1/2 (YKL-40/39) and also the trefoil factor 2.[5] Truncated N-glycan structures are also presented by viral glycoproteins, turning them into docking sites for ensuing virus entry. With this route defined, the *l*iver/lymph node *s*inusoidal *e*ndothelial *c*ell C-type lec*tin* (LSECtin, CLEC4G) with its micromolar affinity to the GlcNAcβ1,2Man disaccharide becomes a prominent potential target for therapeutic lectin blocking, as the chitin-binding protein GbpA of *Vibrio cholerae* is, which acts as adherence factor to intestinal mucins.[6] These examples underscore the emerging interest in developing bioactive and selective GlcNAc-presenting glycoclusters. In this respect, previous research has proven the particular usefulness of the plant lectin wheat germ agglutinin (WGA) as a model.[7] This sugar receptor with a total of eight hevein domains (about 14 Å apart) binds terminal GlcNAc irrespective of its anomeric linkage and also interacts with the innermost core GlcNAc moiety of complex-type N-glycans.[8] Intriguingly, bivalent (dumb-bell-like) compounds can be accommodated by two sites of the same protein, if linker length (somehow) matches distance between contact sites, or cross-link two proteins, in both cases saturating GlcNAc-binding sites.[7]

As proof-of-principle for a sensor activity of topological aspects, we added a structurally different (tetrameric) leguminous lectin to our test panel, i.e., *Griffonia* (*Bandeiraea*) *simplicifolia* agglutinin-II (GSA-II). It prefers the α-anomeric linkage of GlcNAc to its β-isomeric form.[8b,9] The common distance profile between sugar-binding sites in leguminous lectins of 50–70 Å makes intramolecular bridging impossible for the glycoclusters presented herein. Integrating a specificity control with scaffold-presented *N*-acetylgalactosamine (GalNAc) to exclude carbohydrate-independent interactions to the linker and building on previous experience with WGA and two bivalent compounds,[7g] we prepared a panel of 10 bi- to tetravalent glycoclusters bearing GlcNAc (Fig. 1). To ensure comparability, the nature of the linker was strictly kept constant and only one parameter was altered. Structural variations were introduced by changing anomeric configuration and valency, and using either an O- or S-glycosidic linkage. The products were assayed under identical conditions (e.g., aliquots of the same lectin solution or cell suspensions per experimental series) to determine their capacity to block binding of these two structurally dissimilar model lectins to surface-immobilised (neo)glycoproteins and to cells. The obtained data in the inhibition studies reveal glycocluster- and lectin-type-dependent results, underscoring the suitability to use glycoclusters as molecular rulers and as source of selectivity.

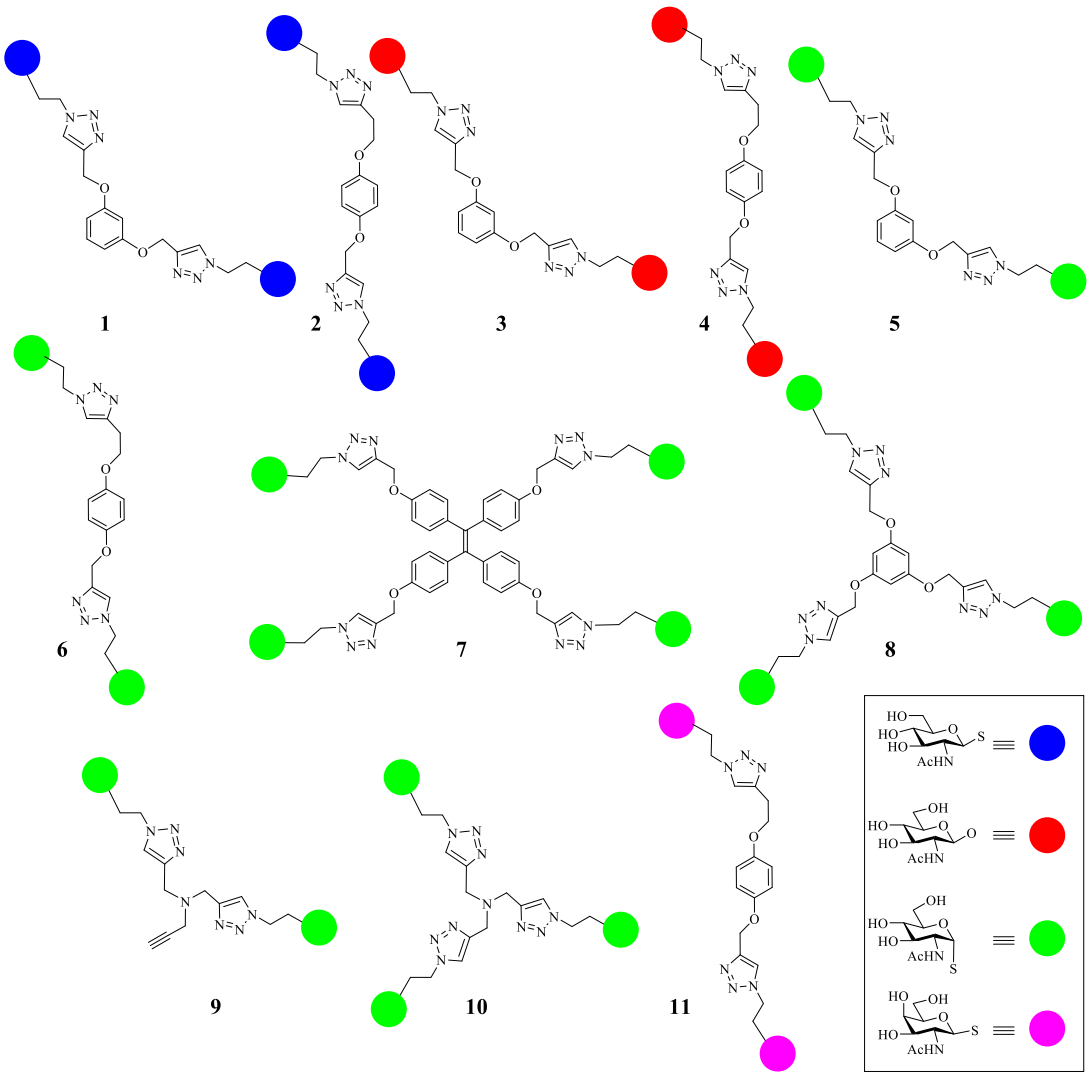

Fig. 1. Structure of glycoclusters **1**–**11**.

Please cite this article in press as: André, S.; et al., Tetrahedron (2015), http://dx.doi.org/10.1016/j.tet.2015.07.020