Tetrahedron 69 (2013) 3019–3026

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Glycosylated tris-bipyridine ferrous complexes to provide dynamic combinatorial libraries for probing carbohydrate–carbohydrate interactions

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ARTICLE INFO

Article history:
Received 14 November 2012
Received in revised form 27 January 2013
Accepted 31 January 2013
Available online 8 February 2013

Keywords:
Carbohydrate–carbohydrate interaction
Tris-bipyridine ferrous complex
Glycocluster
Dynamic combinatorial chemistry
Dynamic combinatorial library
Circular dichroism

ABSTRACT

2,2-Bipyridines having β-lactoside, β-D-glucoside, β-D-galactoside, and *N*-acetyl-β-D-glucosaminide were prepared and then, complexed with ferrous ion to afford trivalent glycoclusters having tris-bipyridine ferrous complex cores. Each glycocluster provides a dynamic combinatorial library composed of four diastereomeric stereoisomers (Δ*mer*, Δ*fac*, Λ*mer*, and Λ*fac*) whose ratios depend on their relative stabilities. CD spectral analyses of these glycoclusters showed that various cations (Na^+, Mg^{2+}, K^+ or Ca^{2+}) enriched Δ-forms of the glycocluster having β-lactosides and *N*-acetyl-β-D-glucosaminides possibly by cations-induced intramolecular carbohydrate–carbohydrate interactions.

1. Introduction

Carbohydrates ubiquitously exist as components of glycoproteins and glycolipids on cell surfaces and play substantial roles in various molecular recognition events including fertilizations, differentiations, and cell–cell adhesions.[1,2] Hitherto, these carbohydrates have been recognized as ligands for carbohydrate recognition proteins (lectins).[3] An increasing interest has been, however, placed on carbohydrate–carbohydrate interactions (CCIs) on cell surfaces, in which carbohydrates recognize carbohydrates in specific and, in most cases, Ca^{2+}-dependent manners.[4]

Glycosphingolipids (GSLs) on cell surfaces aggregate laterally to form micro domains to present densely packed carbohydrate clusters. Face-to-face interactions between such carbohydrate clusters from neighboring cells are the very first and an essential step in various cellular recognition events including compactions of embryos, cancer metastases and inflammations. It is of quite interest that these micro domains are associated with various signal transferring proteins, such as c-Src, FAK, Rho A, etc.[5] Since this fact strongly implies that these micro domains play important roles not only in cell–cell adhesions but also in cell–cell signal transductions, these micro domains are now widely called 'carbohydrate signaling domains'. Investigation on CCIs is of quite attractive, since it should supply useful information not only to understand mechanism of the cell-cell adhesions and the signal transductions but also to design new drugs to prevent various diseases triggered by unfavorable cell–cell adhesions (cancer metastases, inflammations, etc.). In spite of such importance, heterogeneity and fluidity of the cell membranes make it quite difficult to investigate CCIs in a detailed and quantitative manner. Simple and well-designed model systems are, therefore, highly required to obtain molecular-level information on CCIs.

Strength of CCIs also adds to the problem; that is, CCIs between two isolated carbohydrates are so weak that they are hardly detectable. Multivalent interactions between the clustered carbohydrates are, therefore, adopted in nature to overcome this problem. Most artificial systems also utilize densely packed carbohydrate clusters to probe CCIs.[6–9] For example, interactions between

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0040-4020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2013.01.095

Langmuir monolayers composed of G_M3 (NeuAc-α1,3-Gal-β1,4-Glcβ-ceramide) and dendrimers presenting multiple β-lactosides (βLac: Gal-β1,4-Glcβ-) were investigated by using π-A isotherm.[10] It has been also reported by Penadés et al. that aggregations of gold nanoparticles immobilized with Le^X-trisaccharides (Le^X_3: Gal-β1,4-(Fuc-α1,3-)GlcNAcβ-) were induced by Ca^{2+} in a specific manner.[11]

These excellent works successfully demonstrated specific CCIs between two carbohydrate clusters composed of the complex oligosaccharides (G_M3/βLac, Le^X_3/Le^X_3, etc.). Only limited information has been, however, obtained so far on contributions of each monosaccharide unit in these oligosaccharides. In the case of the Penadés's work, for example, gold nanoparticles immobilized with βLac- and β-maltoside (Glc-α1,4-Glcβ-) were used as negative references. Chemical structure of Le^X_3 is quite different from those of these disaccharides and therefore, little information can be obtained for structural criteria to induce the Le^X_3–Le^X_3 interactions.

It should be noted that these works are categorized into intermolecular approach. Since intermolecular bindings between two carbohydrate molecules accompany large entropic lose, so the multivalent interactions between the carbohydrate clusters are essential for these systems to probe CCIs. Almost no molecular-level information, such as spatial packings of the carbohydrates and stoichiometries between the carbohydrates and Ca^{2+}, has been, therefore, obtained to date suffering from the huge numbers of carbohydrates in these systems.

The other approach to investigate CCIs is intramolecular one. Since CCIs between two carbohydrates linked together with an appropriate spacer can minimize the entropic lose on their bindings, numbers of carbohydrates to induce their bindings can be also reduced in this approach.[12,13] For example, Schmidt et al. reported that two Le^X_3-units linked through an alkyl spacer took a cross-shaped packing on an addition of Ca^{2+} and this conformational change could be detected by using NOESY.[14] This work well exemplified one great advantage of the intramolecular approach; that is, accessibility toward the detailed molecular information on CCIs such as the spatial packings of carbohydrates on their bindings. We have been also pursuing the intramolecular approach to investigate CCIs by using various model systems.[15–18] One advantageous feature of our systems relies on their scaffolds; that is, scaffolds of our systems are designed to function as chromophores to monitor CCIs-induced conformational changes based on their UV–vis and/or CD spectra.

Recently, a fascinating strategy, that is, so-called dynamic combinatorial chemistry (DCC), was emerged especially in a field of pharmaceutics.[19] One can define DCC as combinatorial chemistry under thermodynamic control and all members of dynamic combinatorial libraries (DCLs) are in equilibrium in which ratios of these DCLs members depend on their relative stabilities. Reasonably quick interconversions among DCLs members are required to attain such DCC and therefore, varieties of reversible exchange reactions, such as ester,[20] hydrazone,[21] disulfide,[22] and metal–ligand exchange ones[23] are widely used to construct DCLs.

The success of DCC in pharmaceutics encouraged us to develop new model systems to investigate CCIs by combining the intramolecular approach and DCC. In this respect, we designed monoglycosylated 2,2′-bipyridines (glycobpys), which spontaneously assemble onto Fe^{2+} to construct the corresponding tris-bipyridine ferrous complexes carrying trivalent glycoclusters (glycometalloclusters). Taking advantages of the reversible metal–ligand exchange reactions, these glycometalloclusters can produce suitable DCLs as follows.[24] (1) The glycometalloclusters exist as mixtures of four diastereomeric stereoisomers (Δ*mer*, Δ*fac*, Λ*mer*, and Λ*fac*) having individual spatial distributions of carbohydrate appendages (Fig. 1). Although these abbreviations (Λ*mer*, Λ*fac*, Δ*mer*, and Δ*fac*) are widely used in metallo-complex chemistry, it should be herein clearly defined that Λ and Δ mean left- and right-handed spatial arrangements of their bpy units around the Fe^{2+} core,

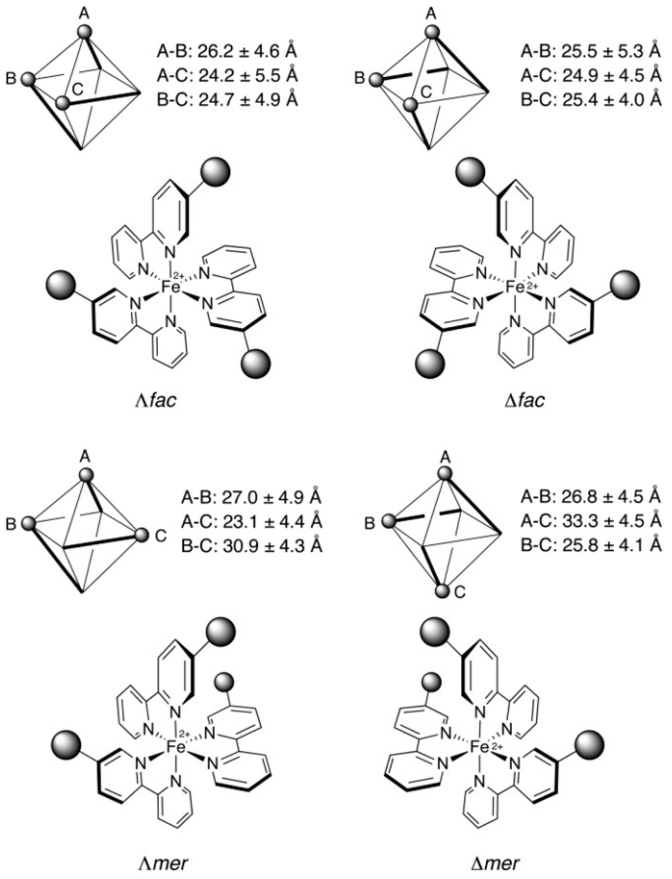

Fig. 1. Four diastereomeric stereoisomers of the tris-bipyridine ferrous complexes presenting trivalent βLacs and averaged distances between three non-reducing βGal terminals (C4) in the complexes during molecular dynamics calculations (See Figs. S1–S3).

respectively. In additions, *mer* and *fac* mean meridional and facial isomers in which each set of three pyridine units on which carbohydrate-unit attached occupies a plane passing through Fe^{2+} and one face of the octahedron surrounding Fe^{2+}, respectively. (2) Time-scales for their interconversions are reasonably short (~1 h) and new equilibrium can be quickly achieved on external stimuli. (3) Since these glycometalloclusters are CD active, their CD spectra offer useful information on their Δ–Λ ratios. (4) The DCLs members of the glycometalloclusters are diastereomeric and therefore, they can be quantified through HPLC analyses without using any chiral stationary phases.

If a certain DCLs member has a spatial carbohydrate arrangement, that is, suitable for intramolecular CCIs, such CCIs should act as adhesive forces to stabilize this DCLs member and to increase its ratio (Fig. 2). In fact, Sasaki et al. reported in their pioneering works that similar glycometalloclusters are effective to probe spatial structures of carbohydrate binding sites of lectins.[25,26]

Herein, we report the first successful example of such DCC-based intramolecular approach to investigate CCIs. In this work, we synthesized various glycometalloclusters having βLacs, β-glucosides (βGlcs), β-galactosides (βGals), and *N*-acetyl-β-D-glucosaminides (βGlcNAcs) to assess CCIs among these carbohydrates. Note that, in these carbohydrates, βLac exists as a carbohydrate unit of lactosylceramide, that is, the most abundant glycosphingolipid on the cell surfaces and βLac–βLac interactions has been reported by many research groups using the intermolecular/intramolecular approaches.[27] Meanwhile, βGlcNAc is an essential structural component of Le^X, that is, widely recognized as the glycosphingolipids to induce strong CCIs.