Synthesis of a novel class of glycocluster with a cyclic α-(1→6)-octaglucoside as a scaffold and their binding abilities to concanavalin A

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ABSTRACT

The synthesis of small glycoclusters with high affinity toward lectins is one of the important subjects in glycotechnology. Although cyclic α-(1→6)-D-octaglucoside (CI8) is an attractive scaffold on which to put glycosyl pendants, the compound has only secondary hydroxyl groups, which are relatively unreactive for substitution reactions. The oxidation of the vicinal diols of CI8 and reductive amination of the resultant dialdehydes with 2-aminoethyl mannoside gave mannose-CI8 conjugates with a variety of average mannose incorporation numbers (2–7). The average numbers were deduced from MALDI-TOF mass and ^{1}H NMR spectroscopy. The binding ability of mannose-CI8 conjugates to concanavalin A increased with the increasing numbers of average mannose incorporation, reaching a plateau at tetravalence, as estimated from a latex bead-based agglutination lectin assay. Toxicity tests demonstrated the biocompatibility of mannose-CI8 conjugates.

1. Introduction

The interactions of cell-surface carbohydrates with lectins on other cells are central to some biotargeting processes such as pathogen invasion to specific cells and migration of leukocytes to injured tissues.[1] Carbohydrate ligands that strongly and specifically bind to the lectins therefore can be antibacterial or anti-adhesive agents.[2] Although the interaction of a lectin with a carbohydrate in a monovalent form is generally weak, multivalent carbohydrates attached on scaffolds often show relatively strong affinities to lectins because of the cluster effect.[3] To harness the cluster effect, peptides,[4] dendrimers,[5] cyclodextrins (CD*n*),[6,7] synthetic polymers,[8] proteins[9] etc. have been used as scaffolds to lend multivalency to the covalently linked saccharides. Among them, glycopolymers and glycoproteins often show outstanding affinities to lectins, affording more than three orders of magnitude higher potency than the monomers in inhibiting agglutination by lectins. The high molecular weights of these molecules, however, may be problematic when they are required to penetrate through the blood–organ barriers as anti-adhesives. Bioincompatibility of some synthetic glycopolymers may be another problem. In this sense, peptides- and CD*n*-based multivalent carbohydrate conjugates are relatively small in size and biocompatible, leaving a room for further studies to improve the affinities to lectins.

In the course of searching new scaffolds for multivalent glyco-conjugates, we were interested in cylcodextrans, cyclic isomalto-oligosaccharides with 7–12-mers of α-(1→6)-linked glucopyranose residues (CI*n*) shown in Figure 1, which are produced from dextran by the action of cyclodextran glucanotransferase (CITase) from *Bacillus circulans*.[10] CI*n* and their derivatives may find applications in pharmaceutical and food industries based on their beneficial properties such as a cariostatic activity, anti-HIV activity, and the ability to form inclusion complexes with hydrophobic substances.[10,11] The cariostatic activity is due to their inhibitory effect against dextransucrase and this activity has not been observed with cyclodextrins (CD*n*). The specific inhibitory activity of CI*n* as opposed to CD*n* can be explained by the conformational flexibility of CI*n* owing to the freely rotatable methylene groups involved in the loop, which might help induced-fit insertion into the enzyme pocket. The flexibility of CI scaffolds might also be beneficial for induced fitting of the pendant multivalent sugars to lectins if these sugars can be attached to CI*n* scaffolds. We thus started to develop the methods of incorporating multivalent sugar ligands into CI*n*.

The low reactivity of hydroxyl groups, especially of secondary ones, relative to amino and carboxyl groups makes it difficult to
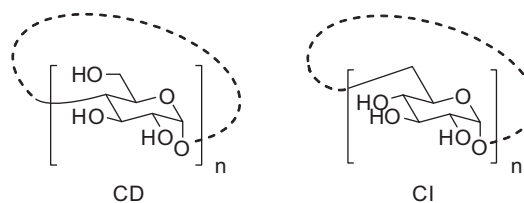
Figure 1. Structures of cyclodextrins (CD*n*; *n* = 6−8) and cyclic α-1,6-oligogluco-sides (CI*n*; *n* = 7−12).

attach pendant components to neutral oligo- and polysaccharides. Cyclic α-(1→6)-oligoglucosides CIn bear only secondary hydroxyl groups and thus incorporation of sugar ligands by direct substitution reactions appears very difficult. Among the limited numbers of methods to functionalize hydroxyl groups, periodate oxidation of vicinal diols and triols and subsequent imination or reductive amination of the resulting dialdehydes seemed to be the best way for modification.[12] These serial reactions have been employed for the synthesis of morpholine derivatives from the carbohydrates having vicinal triols, in which the dialdehyde group produced from the triols through periodate oxidation, reacts with an amine to form a morpholine ring through double reductive amination (Fig. 2).[13] The morpholine ring formation is also expected for the same serial reactions toward each glucopyranoside unit of CIn and could be used for the synthesis of multivalent sugar–CIn conjugates.

The purpose of this study is to synthesize a novel class of glycoclusters using a CIn as a scaffold through NaIO$_4$ oxidation and the reductive amination with an amino-functionalized sugar derivative. We describe herein the optimization of the reaction conditions for morpholine formation with regard to CIn and 2-aminoethyl mannoside. The lectin-binding ability of these multivalent glycoconjugates is also discussed on the basis of latex bead-based agglutination lectin assays (LALA)[14] for concanavalin A (ConA).

2. Results and discussion

We selected the octamer CI8 for the scaffold of glycoclusters, because CI8 is most easily isolatable from the CIn mixture in a fermentation broth. When this compound is subjected to NaIO$_4$ oxidation and reductive amination with an amino sugar so that the glucose unit is converted into a morpholine derivative, up to eight amino sugar units can be incorporated into CI8. However, this incorporation may be inefficient, resulting in the production of a number of incomplete adducts, because the flexible loop of CI possibly buries the reactive groups inside a folded structure. Even if the incorporation is perfect, the resulting sugar cluster may cause signal broadenings in NMR spectra owing to the conformational flexibility of the CI loop. We thus started by testing the amino sugar incorporation process using methyl α-D-glucopyranoside (1) as the simplest scaffold model, to optimize the reaction conditions and to obtain a standard NMR spectrum. Compound 1 was treated with excess NaIO$_4$ to produce the dialdehyde intermediate 2, which was then treated with 2-aminoethyl mannoside 3 and NaCNBH$_3$ in water to afford the morpholine derivative 4 in 35% yield (Scheme 1). The result was the best among those obtained with slightly different conditions and several different workup methods.

A dialdehyde with the structure like 2 is known to exist as the mixture of hydrate isomers, complicating the structure elucidation by NMR. To estimate the yield of the oxidation step, we reduced the aldehyde groups into alcohols with NaBH$_4$ to give triol 5 in 73% from 1. The good yield suggests that the low yield of the morpholine 4 is attributable to the reductive amination step. When methanol or DMSO was used as a solvent in the reductive amination, the yield of 4 was less than 5%. The better yield in water than that in organic solvents was unexpected for the dehydration reaction, which usually avoids water as illustrated by a number of similar reductive amination reactions that have been reported.

The above result predicts that morpholine formation by the coupling of an amino sugar and an α-(1→6)-glucose derivative

would generally be inefficient. Figure 3 shows the ^{1}H NMR spectrum of compound 4, highlighting the characteristic CH$_2$N protons, in which the ring protons (H$_{2a}$, H$_{2b}$, H$_{4a}$, and H$_{4b}$) are clearly apparent at 3.40, 2.86, 3.33, and 2.67 ppm, respectively, while the chain protons (H$_{Na}$ and H$_{Nb}$) are converged at 3.14 ppm. This spectrum is used as a standard to analyze the more complicated ^{1}H NMR spectra of 3-CI8 conjugates.

We tested the above method of morpholine formation for a cyclic tetraglucoside 6 with the repeating unit of α-(1→6)-Glc-α-(1→3)-Glc (Scheme 2), in which only the (1→6)-Glc unit with a vicinal triol can react with NaIO$_4$. Though NaIO$_4$ oxidation was satisfactory to give the tetraaldehyde 7 in 94% yield, the reductive amination again gave a low yield (26%) of the dimannose adduct 8. From the decreased yield relative to that for methyl glucoside 1, we can extrapolate that larger α-(1→6)-glucosides such as CI8 would scarcely have a chance of complete conversion into permorpholine derivatives. The ^{1}H NMR spectrum of 8 shows the ring protons (H$_{2a}$, H$_{2b}$, H$_{4a}$, and H$_{4b}$) at 3.52, 2.89, 3.33, and 2.58 ppm and the chain CH$_2$N protons (H$_{Na}$, H$_{Nb}$) at 3.17 ppm (Fig. 4); the integrals of these signals indicate that two mannose units are incorporated and two morpholine structures are formed.

We treated CI8 with the same conditions of NaIO$_4$ oxidation for methyl mannoside 1 to give polyaldehyde 9 (Scheme 3). As inactivation or removal of NaIO$_4$ is essential to the next reduction reactions, several methods were examined. This included the addition of reducing agents (e.g., Na$_2$S$_2$O$_3$ or ethylene glycol), filtration through silica gel or ion exchange resins, dialysis, and gel filtration. Among them the gel filtration with BioGel P2 gave the most satisfactory results leaving no signs of oxidation activity on starch–iodine test. To assess the efficiency of the NaIO$_4$ oxidation, we treated 9 with NaBH$_4$ to give polyalcohol 11 in 56% yield from CI8. The structure of 11 was elucidated by ^{1}H NMR spectroscopy and ESI-MS. The triplet at 5.33 ppm in the ^{1}H NMR spectrum clearly indicates that the anomeric proton has adjacent methylene protons. The yield was satisfactory for this large polyfunctional compound.

Table 1 shows the degrees of amino sugar incorporation for the reductive amination of the polyaldehyde 9, with the varied reaction conditions: reaction time, temperature, pH, and sonication. Low efficiency in the reductive amination was again observed for the polyaldehyde 9 as with the model case for dialdehyde 2, giving incomplete incorporation of amino sugar 3. Thus, the products were mixtures of CI8-amino sugar conjugates 10$_{m,n,o}$, where the subscripts m, n, and o (m + n + o = 8) stand for the numbers of the unit structures produced by full, half, and no reductive aminations, respectively (Fig. 5). The ^{1}H NMR spectra of 10$_{m,n,o}$ indicate that the peaks for the aldehyde are negligibly small and the unreacted aldehyde groups mostly exist as hemiaminal (n) or hydrate (o). For simplicity, Figure 5 shows only a representative structure with regard to the hemiaminal (n) and hydrate (o) components.

The hemiaminal (n) can be the regioisomer and the hydrate (o) an acyclic dihydrate as reported for similar dialdehyde derivatives.[15] The hemiaminal (n) and hydrate (o) could exist as seven-membered rings owing to a single NaIO$_4$ oxidation. The load, a degree of mannose incorporation, was calculated by dividing the weight of a product mixture by the theoretical weight of the complete CI8-(amino sugar)$_8$ conjugate 10$_{8,0,0}$ assumed to be obtained from a given amount of CI8 via the complete reaction. The low reactivity of the mannose incorporation can be explained by the large and flexible
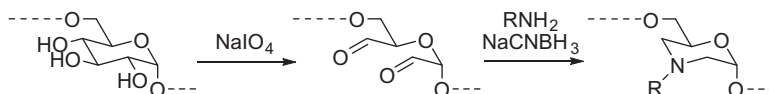

Figure 2. Synthesis of morpholine derivatives from α-(1→6)-glucosides.