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Inulin-based glycopolymer: Its preparation, lectin-affinity and gellation property

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ABSTRACT

The glycopolymer composed of an inulin scaffold and pendent β-lactosides was developed from commercially available inulin through sequential chemical modification processes composed of tosylation, azidation, and the subsequent Huisgen cyclocoupling with an alkyne-terminated β-lactoside. The resultant inulin-based glycopolymer has unique dual affinity towards β-galactoside and α-glucoside specific lectins which is attributable to its pendent β-lactosides and terminal α-glucoside. Its gellation property was also accessed to find that the inulin-based glycopolymer forms hydrogels whose critical gellation concentration (CGC) was lower than that required for hydrogels made from native inulin. Drug release properties of the inulin-based glycopolymer were also discussed in this paper.

1. Introduction

Oligosaccharides widely exist as components of glycoproteins and glycolipids on cell surfaces to play essential roles in various bioprocesses such as cell–cell adhesions.[1] In spite of these critical biological functions, affinities and specificities between single oligosaccharide and a carbohydrate-binding protein (lectin) are usually quite low especially in aqueous media, suffering from competitive hydrogen bondings with water molecules. Multivalent, or clustered, oligosaccharides are highly required to induce strong and specific lectin-affinities. It is well exemplified that branched oligosaccharides of glycoproteins or glycolipids show strong lectin affinities arising from their multiple non-reducing terminals. Synthetic and naturally-occurring polymers carrying multiple copies of pendent carbohydrates (glycopolymers) also show amplified lectin affinities and various glycopolymers based on polyacrylamides,[2,3] polystyrenes,[4–7] polynucleotides,[8–11] polypeptides,[12–15] and polysaccharides,[16–18] etc. have been so far reported in the literature.[19–22] In the very beginning of glycopolymer science, researchers in this scientific field focused their research efforts onto developing glycopolymers with enhanced lectin affinities. The research efforts, then, shifted to developing advanced glycopolymers by combining the amplified lectin-affinities arising from their multiple copies of carbohydrate appendages and distinctive properties intrinsic to their polymeric mainchains. For examples, the polystyrenes having β-lactoside (βLac: Gal-β1,4-Glcβ-) as their pendent groups are now commercially available as coating reagents for polystyrene culture dishes. Hydrophobic interactions between their polystyrene scaffolds and the polystyrene dish surfaces are driving forces for the coatings and the resultant dish surfaces immobilized with densely packed βLacs show excellent properties to cultivate hepatocytes expressing asiallo-glycoprotein receptors (βLac-specific lectins). Recently, quite interesting paper has been published in which the authors reported that the Lac-appended polystyrene is quite effective to differentiate mouse embryonic stem cells toward hepatocytes.[7] Polyphenylene-,[23], polyphenyleneethynylene-[24,25] and polythiophene-based glycopolymers[26] have been also developed as fluorometric/colorimetric probes to detect lectins, toxins, viruses, and microorganisms by taking advantages of their intrinsic nature of the polymeric scaffolds whose fluorometric/colorimetric properties are quite sensitive to their conformations and/or aggregations. We also developed some glycopolymers having unique properties originating from their polymeric scaffolds. Helical polyisocyanides-based glycopolymers[27–29] can be, for example, applied as coating reagents for silica gels that can be used as chiral stationary phases for HPLC. The glycopolymers having β-1,3-glucan mainchains (schizophillan[30,31] and curdlan[17,32,33]) interacts with certain polynucleotides to form stable triple-stranded macromolecular

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complexes and therefore, have great potentials as carriers of various polynucleotide-based drugs.

In spite of the great progress in this research field, no report has been found in the literature for the glycopolymers having inulin (Inu: β-2,1-fructan) scaffolds, except for our previous communication.[34] Inu is an energy reserving material in various plants including dahlia, chicory, and Jerusalem artichoke and 2OH terminal of their β-2,1-fructan mainchains is sometimes end-capped by an non-reducing α-D-glucopyranoside.[35] Inu has relatively short chain length with its degree of polymerization (DP) values ranging from 22 to 60, depending on sources of Inu, times of harvest, and processes of production. Since higher animals including human have no inulinase in their digestive systems, Inu can pass through human metabolic system inert. Together with these properties, gellation abilities of Inu to form hydrogels whose texture, mouth-feel, and appearance suitably imitate fat make itself an excellent alternative to replace fat, sugar, and flour. Inu-based drug carrier has been also developed to cure colon cancer by taking advantages of these properties.

It is reasonably expected that the Inu-based glycopolymers should have great potentials as cell-targeted drug carriers, since both excellent lectin affinities and gellation properties can be expected for them.[36] The aforementioned low DP values of Inu are, however, formidable obstacles to develop the Inu-based glycopolymers and mild reaction conditions are highly required to avoid decomposition of Inu-scaffolds, since Inu has oligomeric rather than polymeric nature and further decrements in their DP values should critically spoil their intrinsic gellation properties. We, herein, report detailed synthetic protocols to overcome these problems and to introduce multiple copies of carbohydrate units into Inu scaffolds. Lectin affinity and gellation properties of the resultant Inu-based glycopolymers are also discussed.

2. Results and discussion

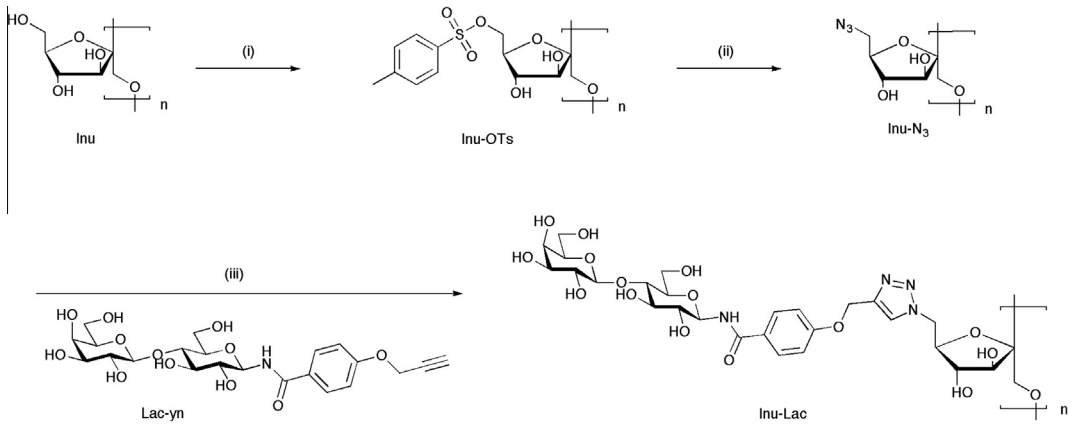
2.1. Structural analysis of inulin used in our synthetic route

Introductions of multiple copies of carbohydrate appendages onto Inu strands were achieved through a synthetic route shown in Scheme 1. In this scheme, Inu is firstly tosylated to afford Inu tosylates (Inu–OTs) and then azidated to give Inu azides (Inu–N_3) that is finally converted to the Inu-based glycopolymers. We obtained Inu (from dahlia tuber) from a commercial source and used it in our synthetic route without any purifications. Before we started the chemical modifications, chemical structures and purities of the Inu were assessed in detail by using various analytical techniques, including ^{1}H (Fig. 1a) and ^{13}C (Fig. 2a) NMR spectral analyses. In the ^{1}H NMR spectrum, several peaks attributable to its β-2,1-fructan strand can be observed although highly broadened nature of these peaks strongly hindered their detailed assignments. Small peaks at 5.17 and 103.78 ppm, in ^{1}H and ^{13}C NMR, respectively, are assignable to anomeric proton and carbon of the terminal α-Glc unit, respectively. A comparison between peak area of this ^{1}H peak and those of β-2,1-fructan strand offers useful information about DP value of the Inu sample, that is, the Inu has relatively low DP value (DP = 32.7) which is slightly higher than that (DP = 27.5) obtained GPC analysis, implying that a small amount of Inu without the α-Glc terminal might be contained in this Inu sample although its percentage is quite low (Table 1). High purity of this Inu sample was also supported by the ^{1}H NMR spectrum having no sharp peak arising from low molecular weight organic impurities.

2.2. Synthesis of inulin tosylates

The Inu was treated with tosyl chloride (TsCl) in *N*,*N*-dimethylacetoamide (DMAc) under conditions summarized in Table 2. In the first stage of our investigation, conventional re-precipitation procedures using methanol were applied to retrieve pure Inu–OTs samples from the reaction mixtures. We, however, found that no or little precipitate could be obtained through these procedures, possibly suffering from the relatively low DP values of Inu–OTs. We then established a two-step purification procedure composed of (1) dialysis (MWCO1000, water) of the reaction mixtures followed by lyophilization to afford crude Inu–OTs powders and (2) the subsequent washings of these powders with chloroform. Since large amounts of precipitates including water-insoluble tosylate were formed during the first step, the second washing step is essential to obtain pure Inu–OTs. The solvent of choice for the second step also has great impacts on yields of the final products, that is, washing with methanol instead of chloroform extremely lowered the yields of Inu–OTs, mainly due to partial dissolution of Inu–OTs into the waste methanol.

The introductions of tosyl groups (–OTs) onto the Inu strands were clearly proven by appearances of two doublet peaks assignable to *ortho*- and *metha*-protons of the –OTs in ^{1}H NMR spectra of Inu–OTs (Fig. 1b). Peak areas of these protons and those of the Inu strands were quite useful to assess their degree of substitution (DS: averaged number of introduced functional units on one fructose repeating unit) values to indicate that Inu–OTs with DS values ranging from 0.008 to 0.413 were obtained through our protocol (Table 2). Although we carried out the tosylation reactions with



Scheme 1. Sequential chemical modifications of Inu to afford the Inu-based glycopolymers having multiple copies of βLacs (Inu–Lac): (i) TsCl, Et_3N, DMAc, rt, 24 h; (ii) NaN_3, DMAc, 60 °C, 24 h; (iii) Lac-yn, $CuBr_2$, ascorbic acid, propylamine, DMSO, rt, 24 h.