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C6-Modifications on chitosan to develop chitosan-based glycopolymers and their lectin-affinities with sigmoidal binding profiles

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ABSTRACT

Chitosan-based glycopolymers having multiple β-lactosides exclusively at their C6-positions were successfully synthesized from partially deacetylated chitin through perfect *N*-deacetylation/phthaloylation and C6-selective bromination/azidation to afford 6-azide-6-deoxy-*N*-phthaloyl-chitosan and the subsequent Cu^{+}-catalyzed Huisgen cycloadditions using alkyne-terminated β-lactoside and/or quaternary ammonium modules followed by dephthaloylations. Lectin-affinities of the resultant chitosan-based glycopolymers were assessed through fluorescence titration assays to show their unique sigmoidal binding profiles with amplified binding constants.

1. Introduction

Carbohydrates ubiquitously exist as components of glycolipids and glycoproteins on cell surfaces and play essential roles in various bioprocesses (Amon, Reuven, Ben-Arye, & Padler-Karavani, 2014; Dwek, 1996; Yu, Tsai, Ariga, & Yanagisawa, 2011). Multivalent or clustered structures of carbohydrates are adopted in nature to enhance their affinities (Dennis & Brewer, 2013) toward carbohydrate-binding proteins, such as lectins. Linear polymers carrying multiple copies of pendent carbohydrates are called glycopolymers (GPs) and they also show amplified lectin affinities through the multivalent carbohydrate–lectin interactions (Kohri et al., 2011; Miura, Koketsu, & Kobayashi, 2007; Nagatsuka, Uzawa, Ohsawa, Seto, & Nishida, 2010; Narumi & Kakuchi, 2008). They are, therefore, now widely recognized as one of the most fascinating materials, especially in bioorganic and medicinal chemistries (Nagatsuka et al., 2012; Sunasee & Narain, 2013). For example, polyacrylamides carrying P^{K} trisaccharides (Gal-α1,4-Gal-β1,4-Glc-β) (Gargano, Ngo, Kim, Acheson, & Lees, 2001; Lundquist, Debenham, & Toone, 2000) or sialyllactoses (Sia-α2,3-Gal-β1,4-Glc-β) (Tsuchida et al., 1998) have been developed to capture Shiga toxin and influenza virus, respectively. Some GPs having conductive and/or luminescent mainchains (polythiophene, polyphenylene, polyphenyleneethynylene, polycarbazole, etc.) have also received increasing research efforts because of their potential applications as sensory systems to detect the viruses and the toxins (Baek, Stevens, & Charych, 2000; Xue, Jog, Murthy, & Liu, 2006; Kim, Wilson, & Bunz, 2005; Disney, Zheng, Swager, & Seeberger, 2004; Chen, Cheng, Zhao, & Han, 2009). Other examples of GPs include polystyrenes having β-lactosides (Lac: Gal-β1,4-Glcβ-) and they are now commercially available as coating reagents for polystyrene culture dishes to cultivate hepatocytes. All these GPs can be categorized in a same group from a viewpoint of their mainchain structures; that is, their mainchains are composed of synthetic polymers those can be readily constructed through simple radical/ionic polymerizations and/or polycondensations of the corresponding monomers. Such readily accessible natures of these GPs have strongly accelerated their research progress and therefore, huge varieties of GPs in this group can be now found in the literature.

On the contrary, GPs having naturally occurring polymers (polynucleotides, polypeptides, and polysaccharides) as their

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polymeric scaffolds form the other group of GPs (Akasaka, Matsuura, & Kobayashi, 2001; Spinelli, Defrancq, & Morvan, 2013; Guo & Shao, 2005). These GPs are also attractive research targets in bioorganic/medicinal chemistry, green chemistry, and nano science, because of their biological functions, biodegradable natures, and unique superstructures. In spite of these potential applications, research progress on these GPs is relatively slow, possibly suffering from lack of simple synthetic strategy to access these GPs with desired structures. Recently, some research groups developed elaborate synthetic schemes to access polynucleotide-based GPs by applying solid phase synthetic techniques (Hunziker, 1999; Matsui & Ebara, 2012). In the case of polypeptide-based GPs, many research groups also reported successful syntheses of glycosylated Fmoc-amino acid monomers and their incorporations into multi glycosylated peptides through the solid phase peptide syntheses (Hasegawa & Sasaki, 2003; Maheshwari, Levenson, & Kiick, 2010; Ueki, Nakahara, Hojo, & Nakahara, 2007). On the contrary, no practical solid phase synthetic technique has been, however, established in carbohydrate chemistry. Direct modifications on native polysaccharides are sole alternative to access the polysaccharide-based GPs (Kurita, Shimada, Nishiyama, Shimojoh, & Nishimura, 1998). Such direct modifications are, however, still troublesome processes; that is, multiple hydroxy groups of the native polysaccharides have similar nucleophilicities and therefore, regioselective modifications are hardly achieved (Matsuzaki, Sato, Enomoto, & Yamamoto, 1986). Such random modifications cause troublesome problems to hinder developing the polysaccharide-based GPs. For example, when carbohydrate units are introduced onto the hydroxy groups of the polysaccharides those participate in essential hydrogen bonding networks to construct their superstructures, the resultant polysaccharide-based GPs would have disordered conformations and their potentials as chiral nano-materials would be strongly spoiled.

We believe that C6-selective modifications of the polysaccharides should be the most appropriate strategy to avoid such conformational disorders, since hydroxymethyl groups (6OHs) of most polysaccharides do not participate in their essential hydrogen bonding networks. In this decade, we have been launching our research efforts to establish general synthetic procedures to access polysaccharide-based GPs carrying their pendent carbohydrates exclusively at their C6 positions. In a series of our works, we established two-steps chemical modifications on native polysaccharides to access various polysaccharide-based GPs; that is (1) C6-selective bromination/azidation on native polysaccharides to afford the corresponding 6-azido-6-deoxy derivatives and (2) the subsequent Cu^{+}-catalyzed Huisgen cycloadditions with alkyne-terminated carbohydrate modules. These two-steps chemical modifications were firstly established in curdlan (Cur, linear β-1,3-glucan) chemistry, in which we synthesized 6-azide-6-deoxycurdlan (Cur-N_3) through C6-selective bromination of native Cur by using triphenylphosphine (PPh_3) and carbon tetrabromide (CBr_4) followed by S_N2 reaction with sodium azide (Hasegawa et al., 2006, 2007). The most significant feature of Cur-N_3 is its perfect structural homogeneity that is clearly proven by its monosaccharide-like ^{13}C NMR spectrum composed of 6 predominant peaks. The subsequent Cu^{+}-catalyzed Huisgen couplings with the alkyne-terminated carbohydrate modules proceeded with perfect chemoselectivity to afford Cur-based GPs (Cur-GPs). We also applied this two-steps synthetic strategy in cellulose (Cel) chemistry to find that 6-azide-6-deoxycellulose (Cel-N_3) prepared through a similar bromination/azidation process also acts as an excellent key substrate in the following Huisgen cycloadditions (Yamashita, Okubo, Negishi, & Hasegawa, 2009; Negishi, Mashiko, Yamashita, Otsuka, & Hasegawa, 2011). Through this strategy, we successfully developed varieties of Cel-GPs whose carbohydrate modules are attached exclusively onto their C6-positions.

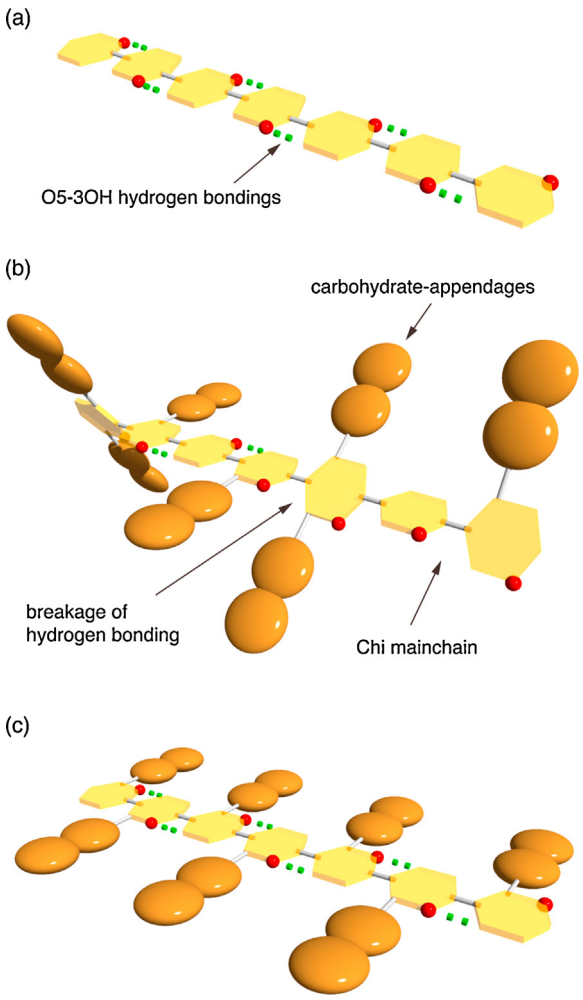

Fig. 1. 3D images of (a) native chitin/chitosan, and the Chi-GPs synthesized through (b) random and (c) C6-selective modifications.

These two successful results in hand, we then shifted our research effort to chitosan (Chi) chemistry to develop Chi-GPs. Not only biodegradability and biocompatibility but also unique immunopotentiative action of their Chi scaffolds would assure great potentials of the Chi-GPs in fields of biosciences and pharmaceutical chemistry. In additions, Chi derivatives would be also attractive research targets in nanoscience by taking advantages of 1D tape-like superstructures of their Chi mainchains kept by the intrastranded hydrogen bonding networks (Fig. 1). It is, therefore, of quite interest to developing the Chi-GPs whose carbohydrate-appendages are exclusively attached onto their C6 positions. We herein report preparation of such Chi-GPs and their properties including water-solubilities and lectin-affinities.

2. Results and discussion

2.1. Preparation of fully deacetylated chitosan

It is reasonably assumed that structural homogeneity of the starting material has critical impacts on those of the Chi-GPs. In this respect, fully deacetylated Chi whose mainchains are exclusively composed of β-1,4-linked glucosaminides (GlcN) would be the most appropriate starting material. Such Chi was, however, not available from commercial sources. Instead, we purchased partially deacetylated chitin (PDA–chitin, *Mw* = ca. 10 kDa) whose mainchains are composed of random mixtures of GlcN and