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Synthesis of multivalent sialyllactosamine-carrying glyco-nanoparticles with high affinity to the human influenza virus hemagglutinin

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ABSTRACT

A series of multivalent sialoglyco-conjugated nanoparticles were efficiently synthesized by using highly-branched α-glucuronic acid-linked cyclic dextrins (GlcA-HBCD) as a backbone. The sialoglycoside-moieties, with varying degrees of substitution, could be incorporated onto the preformed nanoparticles. These synthesized particles, which are highly soluble in aqueous solution, were shown to have a spherical nanostructure with a diameter of approximately 15 nm. The interactions of the sialoglyco-nanoparticles (Neu5Acα2,6LacNAc-GlcA-HBCDs) with human influenza virus strain A/Beijing/262/95 (H1N1) were investigated using a hemagglutination inhibition assay. The sialoglyco-nanoparticle, in which the number of sialic acid substitution is 30, acted as a powerful inhibitor of virus binding activity. We show that both distance and multiplicity of effective ligand-virus formation play important roles in enhancing viral inhibition. Our results indicate that the GlcA-HBCD backbone can be used as a novel spherical nanocluster material for preparing a variety of glyco-nanoparticles to facilitate molecular recognition.

1. Introduction

Carbohydrate epitopes present in cell surface glycoconjugates play a significant role in viral and bacterial infection (Varki, 1993). Influenza viruses (IFVs) infect host cells through the binding of viral hemagglutinin (HA) to the specific sialylated glycoconjugates on the host cell surfaces (Suzuki et al., 1986). Hence, sialylated molecules having a high affinity for the influenza viral HA function are potent inhibitors of infection by IFV. During viral adhesion, the monovalent interaction between HA and sialic acid (SA) is relatively weak, with dissociation constants in the millimolar range (Pritchett & Paulson, 1989). Over recent decades, large numbers of repeating SA residues on artificial polymer scaffolds have been generated that display much enhanced binding potencies due to glycocluster effects (Mammen, Choi, & Whitesides, 1998; Sun, 2007; Wilson & Itzstein, 2003). Indeed, these synthetic multivalent inhibitors showed a powerful inhibitory effect against infection by IFV (Carlescu, Scutaru, Popa, & Uglea, 2009). Synthetic HA inhibitors have been reported to achieve high local concentrations of sialylated glycans with synthetic backbones such as linear polymers, spherical structures (dendrimers and liposomes) and nanostructures (Guo et al., 2002; Lemoine & Préat, 1998; Matsuoka, Oka, Koyama, Esumi, & Terunuma, 2001; Ogata et al., 2007; Suzuki et al., 2012; Tanaka et al., 2014; Umemura et al., 2008). Most commonly, these synthetic glycopolymers are a copolymer with acrylamide esters, acrylic acid esters and polystyrene that form a linear backbone with a rod-like structure (Choi, Mammen, & Whitesides, 1997; Gambaryan et al., 2005; Roy, Andersson, Harms, Kelm, &

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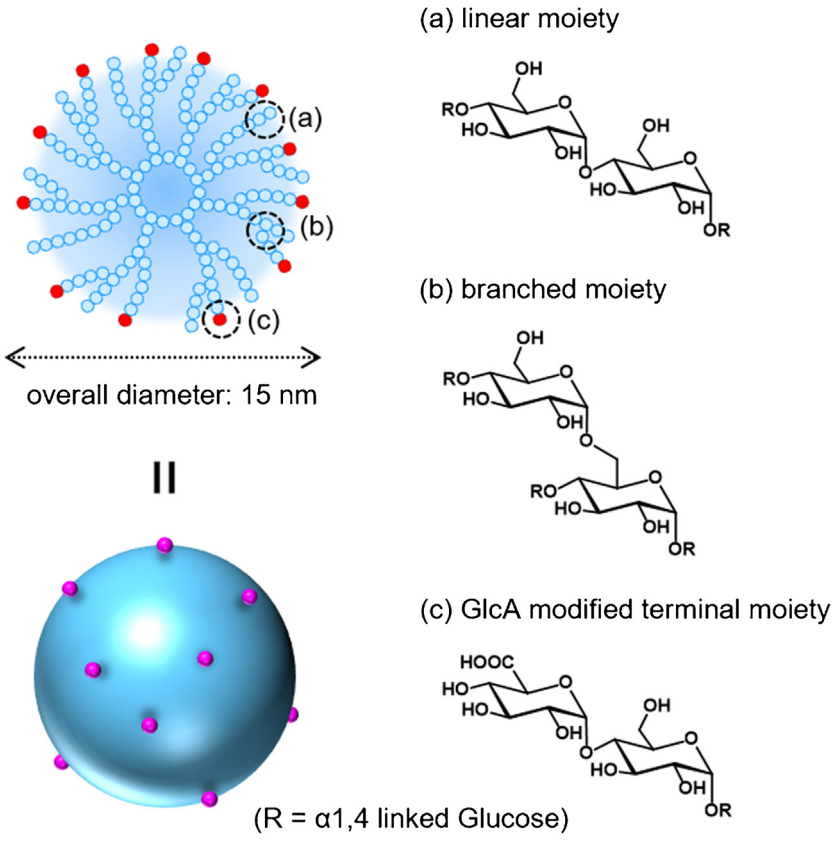

Fig. 1. Schematic representation of GlcA-HBCD.

Schauer, 1992; Tsuchida et al., 1998). Unfortunately, many artificial polymeric HA inhibitors display low solubility, cytotoxicity and immunogenicity (Iurovskii, Bovin, Safonova, Vasilov, & Khorlin, 1986; Kuperman, 1958; Reuter et al., 1999). In order to address these problems, Papp et al. (2011) recently reported the synthesis of biocompatible SA-conjugated polyglycerol-based nanoparticles and demonstrated effective cluster effects against IFV-HA. This development made it possible to perform a conformation activity correlation between the sialylated nanoparticles and IFV. Although the first example by Papp et al. (2011) was published, application of particulate biocompatible backbone to IFV infection inhibitors has been much less popular than the use of amorphous linear polymers.

Recently, Takemoto et al. (2013) reported the enzymatic synthesis of highly branched α-glucuronic acid-linked cyclic dextrins (GlcA-HBCD; Fig. 1). Moreover, GlcA-HBCD can be produced in good yield and the molecular weight, diameter and GlcA functionalization are easily controlled. The resulting cyclic-polymers offer some advantages as a spherical backbone. Specifically, these cyclic polymers; (i) display a higher level of *endo*-group functionality than linear glycopolymers (Voit & Lederer, 2009), (ii) are highly soluble with low polydispersity indices, and (iii) are suitable as biocompatible materials for use in human because all their components are derived from sugar units. In this study, we synthesized sialoglyconanoparticles based on the GlcA-HBCD backbone as HA inhibitors against human IFV. By using a backbone with a highly controlled GlcA functionalization (Fig. 1 and Table 1) we achieved potent inhibition of hemagglutination of IFV, which depended on the number of sialylation. This is the first study to report the synthesis of multivalent HA inhibitors using a spherical nanocluster backbone made up exclusively of sugar units.

2. Results and discussion

2.1. Chemoenzymatic synthesis of glyco-nanoparticles carrying Neu5Acα2,6LacNAc-glycosides (Neu5Acα2,6LacNAc-GlcA-HBCD) with GlcA-HBCD backbones

Our strategy of molecular design was to construct spherical and multivalent glyco-nanoparticles by coupling a sialoside unit to a hydrophilic branched glycopolymer GlcA-HBCD, which is a highly-branched α-glucuronic acid-linked cyclic dextrin in the form of a nanoparticle (Fig. 2). The efficient incorporation of LacNAc-glycoside into the GlcA-HBCD backbone was the key step in the present study.

As a first step, *N*-(γ-trifluoroacetamidobutyryl)-β-LacNAc **1** was easily prepared using our previously reported method (Ogata, Hidari, Kozaki et al., 2009). The aglycon trifluoroacetamide group of compound **1** was deacylated to *N*-(γ-aminobutyryl)-β-LacNAc **2** by hydrolysis in an alkaline solution. The resulting amino function was coupled with the carboxy groups of GlcA-HBCD in the presence of the condensation reagents benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) and 1-hydroxybenzotriazole hydrate (HOBt). In this study, three kinds of GlcA-HBCDs ($GlcA_{8\%}$-HBCD, $GlcA_{22\%}$-HBCD and $GlcA_{53\%}$-HBCD) with different molecular weights (141,000, 142,000 and 149,000, respectively) and number of terminal GlcA residues (NTGs; 4.8, 13 and 32, respectively) were prepared and used as the backbone of LacNAc-carrying glyco-nanoparticles (Table 1). Each reaction solution was applied to a column of Sephadex G-25M PD-10 to separate the target LacNAc-GlcA-HBCD (**3a–c**) from the low molecular weight reactants. The structures of the synthesized asialoglyconanoparticles were confirmed by ^{1}H and ^{13}C NMR analyses. The assigned ^{1}H NMR spectrum of **3c** is shown in Fig. 3a as a rep-