Chapter 16

Oriented Immobilized Sialyloligo-macroligand Microarray

Satya Nandana Narla and Xue-Long Sun

Abstract

Silaic acid is the most common terminal glycan on cell surface glycoproteins and glycolipids, involving in many biological processes. Studying interactions between multivalent sialic acid scaffolds and proteins binding to it will give incredible information in understanding the biological process the sialic acid is involved in. Here we describe chemoenzymatic synthesis of chain-end functionalized sialyllactose-containing glycopolymers with different linkages and their oriented immobilization for glycoarray and SPR-based glyco-biosensor applications.

Key words Sialic acid, Glycan array, Glycopolymer, Lectin, Hemagglutinin, SPR

1 Introduction

Sialic acids are a family of 9-carbon containing acidic monosaccharides, often terminate oligosaccharide structures of cell surface glycoconjugates such as glycoproteins and glycolipids, and are involved in many biological recognition systems. For example, sialic acids expressed on respiratory epithelial cell surface are involved in influenza virus infection in both virus hemagglutinin-based attaching [1] and neuraminidase-based detaching processes [2]. Influenza virus contains about 350–400 HA trimmers [3] and 50 NA tetramers [4] on its surface, which facilitate strong binding affinity through multivalent interactions with the sialic acid receptors on the host cell surface. Previous efforts using sialic acid-bearing macromolecules provided a proof of the concept of multivalent hemagglutinin inhibition [5]. However, the monosaccharide sialic acid cannot account for the molecular determinant of virus receptor-binding specificity in the context of the whole sialyloligosaccharide receptor. Recently, it has been known that the host cell binding specificity and affinity of influenza viruses depends not only on sialic acid but also the penultimate sugar and the linkage of sialic acid to the penultimate sugar on the receptor.

Xue-Long Sun (ed.), *Macro-Glycoligands: Methods and Protocols*, Methods in Molecular Biology, vol. 1367,
DOI 10.1007/978-1-4939-3130-9_16,

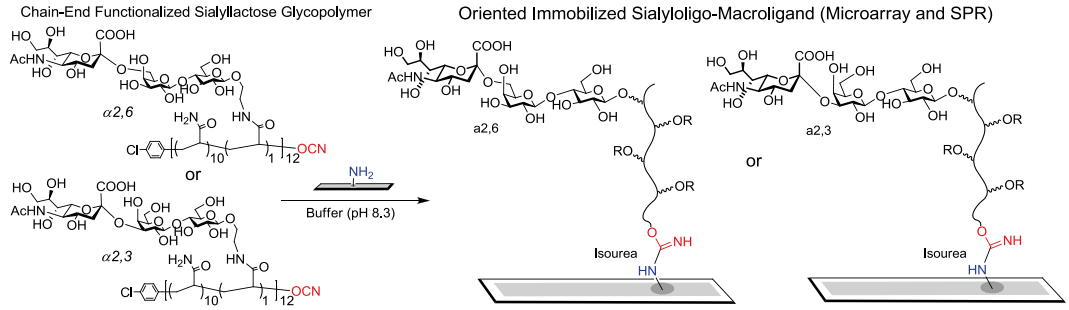

Fig. 1 Chemical structure of *O*-cyanate chain-end functionalized sialyllactose-containing glycopolymers and their orientally immobilized glyco-macroligand formation via isourea bond formation for microarray and SPR applications

Glycopolymers, namely polymers with carbohydrate pendent groups, have been extensively explored for different biological and biomedical applications [6, 7]. It is generally accepted that glycopolymers can mimic the functions of naturally occurring glycoconjugates [8, 9] and have been employed as cell-surface binding receptors [10]. Herein, we report a chemoenzymatic synthesis of *O*-cyanate chain-end functionalized sialyllactose-containing glycopolymers and their oriented sialyloligo-marcroligand formation for glycoarray and glyco-biosensor applications (Fig. 1). This oriented sialyloligo-macroligand platforms are expected to facilitate both affinity and specificity of protein binding and thus will provide a versatile tool for profiling glycan recognition for both basic biological research and practical applications.

2 Materials

2.1 Chemicals

1. 2-*N*-acryloyl-aminoethoxyl 4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranoside) is synthesized as previously described [11] (*see* **Note 1**).
2. 4-Chloroaniline.
3. Sodium nitrite ($NaNO_2$).
4. Tetrafluoroboric acid (HBF_4) (48 wt%).
5. Acrylamide.
6. Sodium cyanate (NaOCN).
7. Sulfuric Acid (H_2SO_4).
8. Cystamine.
9. Hydrogen peroxide (H_2O_2).
10. Hydrochloric Acid (HCl).
11. Sodium hydroxide (NaOH).
12. Phenol.

13. Sialic acid (Rose Scientific Ltd).
14. α2,3-Sialyllactose (V-Labs).
15. α2,6-Sialyllactose (V-Labs).

2.2 Other Commercial Reagents and Solvents

1. Methanol.
2. Ethanol (Fisher scientific).
3. Sephadex G-25 (Sigma Aldrich).
4. Tetrahydrofuran (THF).
5. Amine functionalized glass slides (Xenopore, Co).
6. MicroCaster microarray tool (Whatman, spot size 500 μm diameter).

2.3 Buffers

1. 0.10 M $NaHCO_3$ buffer (pH 10.3).
2. 0.10 M PBS buffer (pH 7.4) containing NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), KH_2PO_4 (1.8 mM).
3. 0.10 M PBST buffer (pH 7.4) containing 0.02 % Tween 20.
4. 0.10 M HEPES (pH 7.4) containing Triton X-100 (0.5 %), $MgCl_2$ (5 mM), $MnCl_2$ (2 mM), $ZnCl_2$ (5×10^{-5} M).

2.4 Proteins and Enzymes

1. MAA-FITC (*Maackia amurensis*, 1.4×10^{-3} mM, Bioworld).
2. SNA-FITC (*Sambucus nigra*, 1.3×10^{-3} mM, Bioworld).
3. PNA-FITC (*Arachis hypogaea*, 1.6×10^{-3} mM, Sigma).
4. CMP-Neu5Ac.
5. Calf alkaline phosphatase.
6. Bovine serum albumin.
7. α2,3-Sialyltransferase.
8. α2,6-Sialyltransferase.
9. H5N1 HA (A/Anhui/1/2005(H5N1)) (Sino Biological, China).
10. H3N2 HA (A/Brisbane/10/2007(H3N2)) (Sino Biological, China).

3 Methods

The synthesis of end-functionalized glycopolymers still poses significant challenges, including a requirement for serial protection/deprotection steps or further polymer derivatization after initial synthesis. The *O*-cyanate chain-end functionalized glycopolymers were synthesized *via* our previously developed cyanoxyl free radical mediated copolymerization scheme in one-pot fashion (Fig. 2) [12]. Briefly, 4-chloroaniline was used as initiator for the

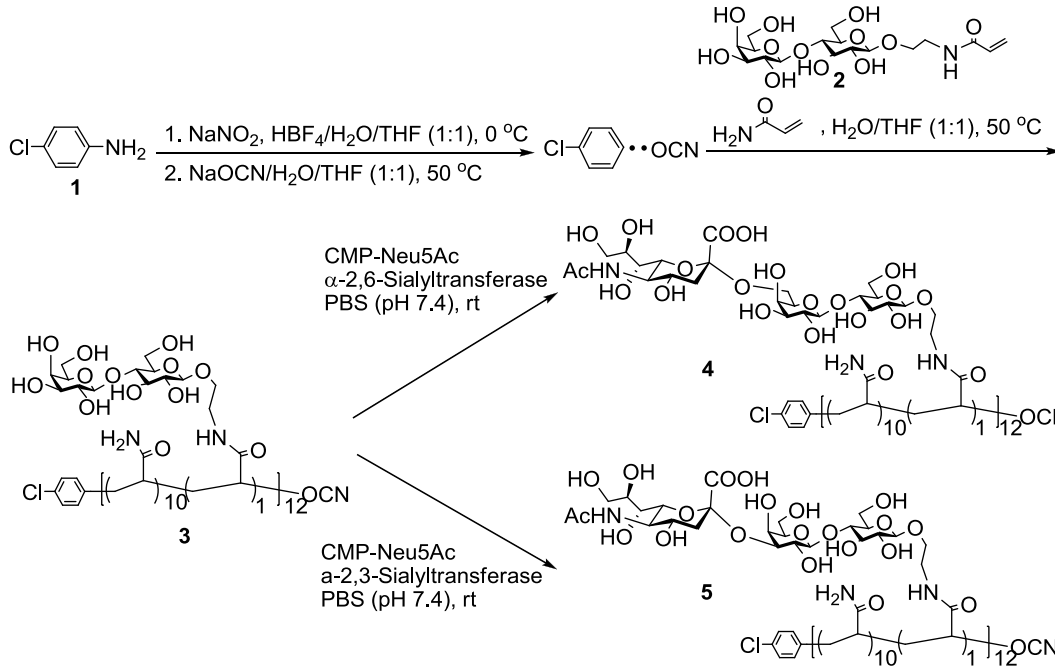

Fig. 2 Chemoenzymatic synthesis of *O*-cyanate chain-end functionalized sialyllactose-containing glycopolymers via cyanoxyl mediated free radical polymerization followed by enzymatic sialylation

copolymerization of acrylaminoethyl lactoside and acrylamide. Specifically, cyanoxyl radicals were generated by an electron-transfer reaction between cyanate anions from a sodium cyanate aqueous solution and aryl-diazonium salts prepared in situ through a diazotization reaction of arylamine in water. In addition to cyanoxyl persistent radicals, aryl-type active radicals were simultaneously produced, and only the latter species was capable of initiating chain growth. A series of *O*-cyanate chain-end functionalized glycopolymers of varying sugar density were prepared by altering glycomonomer (GM) and acrylamide (AA) in good conversion yield (60–70 %) and low polydispersity ($1.1 < Mw/Mn < 1.6$) as described previously [13].

The *O*-cyanate chain-end functionalized lactose-containing glycopolymer (**3**) synthesized was then subjected to enzymatic sialylation of the terminal galactose (Gal) pending on the polymer for synthesizing *O*-cyanate chain-end functionalized sialyllactose-containing glycopolymer (Fig. 2). The enzymatic approach for transferring sialic acid from CMP-Neu5Ac to the non-reducing end of the carbohydrate acceptor was extensively reported [14]. The glycosidic linkage between the sialic acid and the acceptor carbohydrate is extremely controlled by the type of sialyltransferase selected. The transfer of sialic acid residue from CMP-Neu5Ac to 3- and 6-positions of terminal Gal of lactose glycopolymer (**3**), by enzymes α2,3-sialyltransferase and α2,6-sialyltransferase afforded

sialyllactose-containing glycopolymer α2,6SGP (**4**) and α2,3SGP (**5**), respectively (Fig. 2).

The resultant SGPs were characterized by ^{1}H NMR spectra. The successful sialylation of lactose glycopolymer was confirmed by the signals of protons from Neu5Ac (1.95 ppm, CH_3-Neu5NAc and 2.60 ppm, H_{3eq}-Neu5Ac), the degree of sialylation and the polymer length as well were calculated also using the ^{1}HMR spectra by comparing the integration value of proton signals from aromatic protons (7.21 and 7.06 ppm), anomeric protons (4.36 and 4.33 ppm) of Gal and Glc, and C3-equatorial proton (2.60 pm) of Neu5Ac as shown in Fig. 3. By comparing the integrated signal from the anomeric protons of Gal and Glc (4.36 and 4.43 ppm,) with that of the C3-equatorial proton of Neu5Ac (2.60 ppm, H3eq-Neu5Ac), the percentages of both sialylations were more than 90 % (Fig. 3).

SGP-based microarrays were fabricated by printing *O*-cyanate chain-end SGP onto amine functionalized glass slide. Next, the specific lectin binding ability of the SGP-based microarray was performed. The resultant α2,3 SGP and α2,6 SGP printed glass slides were incubated with respective FITC-labeled lectins, *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA) (Bioworld) in PBST buffer (pH 7.4) followed by extensive washing with PBST buffer (pH 7.4) and finally the glass slides were subjected to fluorescent imaging, respectively. To confirm the specific binding, competitive inhibition assays were performed by incubating the α2,3 SGP arrayed glass slides with lectin MAA that was pre-incubated with α2,6-sialyllactose, α2,3-sialyllactose and free sialic acid, respectively. Same pattern of inhibition assays were performed by incubating α2,6 SGP arrayed glass slides with SNA that was pre-incubated with α2,6-sialyllactose, α2,3-sialyllactose and free sialic acid, respectively. In addition, in order to determine the sialylation of lactose glycopolymer (**3**), both α2,3 SGP and α2,6 SGP printed glass slides were incubated with lectin PNA that has specificity to α-galactose on the starting glycopolymer (**3**). Overall, these observations (Fig. 4) indicate that arrayed SGPs are capable of distinguishing different glycan-binding protein based on their sialic acid linkage to the galactose in the SGPs.

The interaction of lectin with orientally immobilized SGPs was also investigated with SPR (BI 2000, Biosensing Instrument). The major advantages of SPR assay are that it is a label free assay and monitors the binding in real time. Initially, the gold sensor chip was functionalized with amine by treating the sensor chip with cysteamine in ethanol overnight, the monolayer formation on the sensor chip was confirmed by increase in the resonance units upon modification of the chip. Next, α2,3 SGP was covalently immobilized onto the amine modified sensor chip. Then, a various concentrations of MAA in PBS buffer (pH 7.4) were injected over the α2,3 SGP modified sensor chip to establish the association and

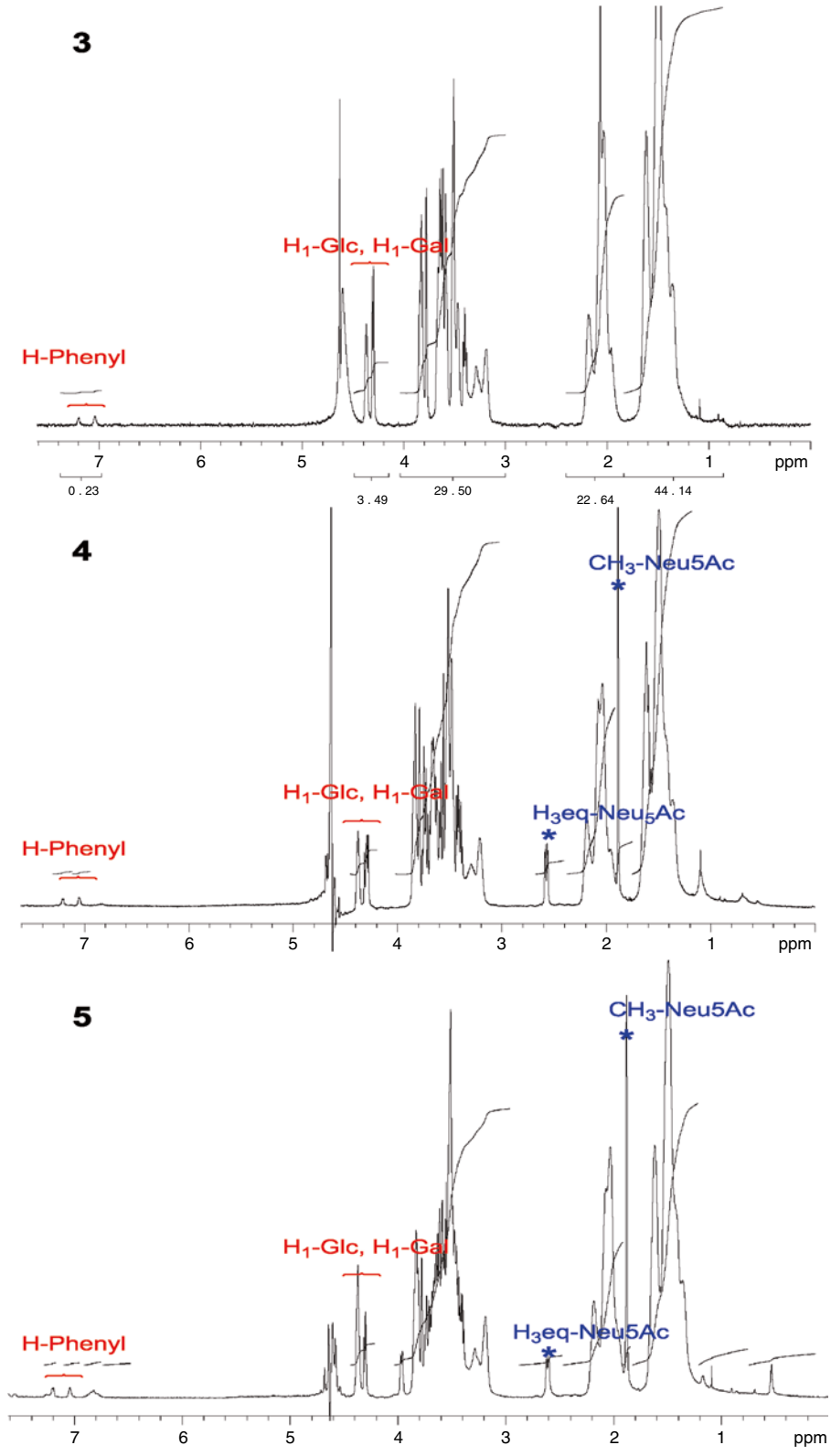

Fig. 3 ^{1}H NMR spectra of glycopolymers in D_2O: (**a**) lactose glycopolymer (**3**), (**b**) α2,6-sialyllactose glycopolymer (**4**), (**c**) α2,3-sialyllactose glycopolymer (**5**)

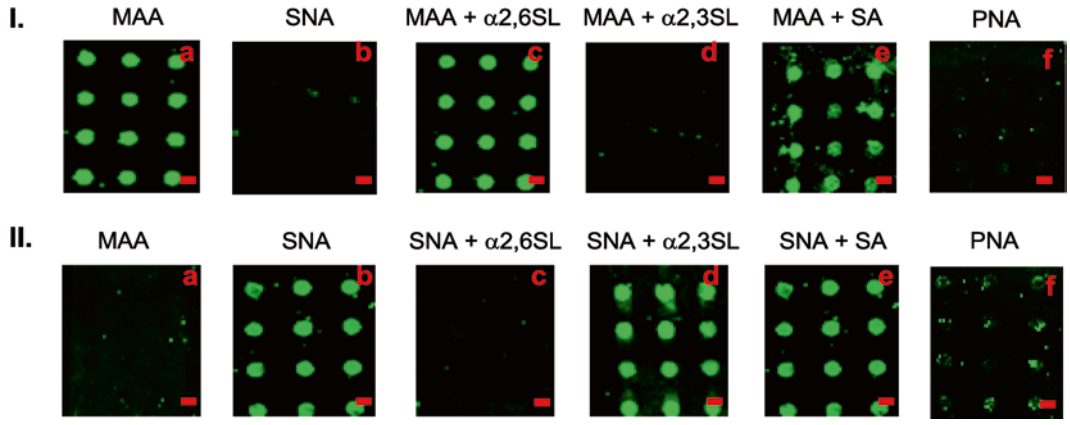

Fig. 4 Fluorescence images of α2,3 SGP and α2,6 SGP microarrays: (**I**) α2,3 SGP array incubated with FITC-labeled lectin, (**a**) MAA, (**b**) SNA, (**c**) MAA pre-incubated with α2,6-sialyllactose (α2,6SL), (**d**) MAA pre-incubated with α2,3-sialyllactose (α2,3SL), (**e**) MAA pre-incubated with free sialic acid (SA), and (**f**) PNA; (**II**) α2,6 SGP array incubated with FITC-labeled lectin, (**a**) MAA, (**b**) SNA, (**c**) SNA pre-incubated with α2,6SL, (**d**) SNA pre-incubated with α2,3SL, (**e**) SNA pre-incubated free SA, and (**f**) PNA. Bar size: 500 μm (*MAA Maackia amurensis*, *SNA Sambucus nigra*, *PNA Arachis hypogaea*)

Table 1

Binding kinetics of lectins to immobilized SGPs in SPR assays

Glycopolymer	Lectin	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
α2,3 SGP	MAA	4.39×10^{3}	9.08×10^{-4}	2.06×10^{-7}
α2,6 SGP	SNA	4.69×10^{3}	1.25×10^{-2}	2.60×10^{-6}

dissociation constants. Same experiment was conducted using α26 SGP with SNA lectin (Table 1).

With the binding specificity confirmed for immobilized SGPs, we next examined their specific binding to influenza viral hemagglutinins (HAs) of avian H_5N_1 (A/Anhui/1/2005(H5N1)) and human H_3N_2 (A/Brisbane/10/2007(H3N2)) with SPR technique, which bind to α2,3 SGP and α2,6 SGP, respectively. Human and avian HA in PBS buffer (pH 7.4) were injected over immobilized α2,3S GP and α2,6 SGP, respectively. As a result, avian HA showed stronger binding (response units) to α2,3 SGP than α2,6 SGP , and avian HA binds to α2,3 SGP much stronger so that there is no dissociation observed from α2,3 SGP.

3.1 Synthesis of Glycopolymer

1. Dissolve 4-chloroaniline (21.6 mg, 1.69×10^{-4} mol), sodium nitrite (14.1 mg, 2.04×10^{-6} mol) in a mixture of 2 mL water and THF (1:1, v/v) in a three-necked flask.
2. Then, degas the mixture under vacuum and refill with argon gas.

3. Add HBF_4 (66 mg, 7.51×10^{-4} mol) and allow it to react for 30 min at 0 °C under Ar atmosphere to generate diazonium salt with red to yellow color observed (*see* **Note 2**).
4. Dissolve 2-*N*-acryloyl-aminoethoxyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (186 mg, 2.65×10^{-5} mol), acrylamide (210 mg, 2.65×10^{-4} mol), and NaOCN (55.2 mg, 8.49×10^{-4} mol) in 1 mL of water in another round bottom flask. Then, degas the mixture by freeze–pump–thaw procedure three times and refill with argon gas.
5. Transfer the solution of the above degassed mixture into the flask containing diazonium salt.
6. Stir the reaction mixture at 65 °C for 16 h under argon atmosphere.
7. Transfer the reaction mixture to a round bottom flask and connect to rotary evaporator to remove the THF.
8. Filter the reaction mixture through syringe filter to remove any precipitates.
9. Dialyze the resultant mixture (3500 da cutoff) against deionized water for 2 days (change the deionized water in a timely manner) at room temperature to remove any inorganic salts and impurities.
10. Lyophilize the solution to afford glycopolymer (248 mg, 60 % converting yield) (*see* **Note 3**).

3.2 Synthesis of Sialyl Glycopolymer

1. Dissolve O-cyanate chain-end functionalized lactose-containing glycopolymer (40 mg, 1 μmol, 10 μmol of lactose) in 2 mL of 0.10 M HEPES buffer (pH 7.4) containing (Triton X-100 (0.5 %), $MgCl_2$ (5 mM), $MnCl_2$ (2 mM), and $ZnCl_2$ (5×10^{-5} M).
2. To the dissolved polymer solution, add CMP-Neu5Ac (6.5 mg, 100 μmol), calf alkaline phosphatase (10 U), bovine serum albumin (0.8 mg), and α2,3-sialyltransferase (0.5 U).
3. Stir the mixture gently at room temperature for 2 days (48 h).
4. Purify the resultant α2,3-sialyllactose glycopolymer (α2,3 SGP) by gel-filtration with Sephadex G-25 column using DI water as an eluent.
5. Confirm the fractions containing SPG with phenol sulfuric acid assay (*see* **Notes 4** and **5**).
6. Follow the same protocol above to synthesize α2,6-sialyllactose glycopolymer (α2,6 SGP) by using α2,6-sialyltransferase.

3.3 Microarray Fabrication of Sialyllactose Glycopolymer on Amine Functionalized Glass Slide

1. Dissolve α2,3 SGP in 0.10 $NaHCO_3$ buffer (pH 10.3) for a concentration of 2.1×10^{-2} mM.
2. Ink MicroCaster microarray tool with α2,3S GP in pH 10.3 $NaHCO_3$ buffer (2.1×10^{-2} mM).
3. Press onto MicroCaster microarray tool amine functionalized glass slides at room temperature for 10 min.
4. Incubate the glass slides in a humidifying chamber at 37 °C for 4 h.
5. Wash the glass slides with 0.10 $NaHCO_3$ buffer (pH 10.3) (30 min, 3 times).
6. Then wash with 0.10 $NaHCO_3$ buffer containing 0.2 % PBST (pH 7.4).
5. Incubate the array glass slides with lectin MAA-FITC (*Maackia amurensis*, 1.4×10^{-3} mM, Bioworld) in 0.10 M PBST buffer (pH 7.4) containing 0.02 % Tween 20 at room temperature for 3 h.
6. Wash the array glass slides with 0.10 M PBST buffer (pH 7.4) containing 0.02 % Tween 20 for 30 min.
7. Finally, subject the glass slides to fluorescent imaging using Typhoon 9410 Variable Model Imager (Amersham Biosciences, USA).
8. As control experiments, print α2,3 SGP on glass slides and incubate with lectin SNA-FITC (*Sambucus nigra*,1.3×10^{-3} mM, Bioworld), PNA-FITC (*Arachis hypogaea*, 1.6×10^{-3} mM, Sigma), MAA-FITC (0.2 mg, 1.4×10^{-3} mM) that is pre-incubated with α2,3-sialyllactose (1.5×10^{-2} mM, V-Labs), α2,6-sialyllactose (1.5×10^{-2} mM, V-Labs), and free sialic acid (3.2×10^{-2} mM, Rose Scientific Ltd) in 0.2 % PBST buffer (pH 7.4) at room temperature for 2 h, respectively, and followed by extensive washing with 0.2 % PBST buffer (pH 7.4) for 30 min and finally subject to fluorescent imaging..
9. The same protocol should be used for α2,6 SGP microarray fabrication and followed by incubation with lectin SNA-FITC (1.3×10^{-3} mM).
10. As control experiments, print α2,6 SGP on glass slides and incubate with lectin MAA-FITC (1.3×10^{-3} mM), lectin SNA-FITC--> (1.4×10^{-3} mM) that is pre-incubated with α2,6-sialyllactose (1.5×10^{-2} mM), α2,3-sialyllactose (1.5×10^{-2} mM), and free sialic acid (3.2×10^{-2} mM), PNA-FITC (*Arachis hypogaea*, 1.6×10^{-3} mM, Sigma) in 0.2 % PBST buffer (pH 7.4) at room temperature for 2 h, respectively, and followed by extensive washing with 0.2 % PBST buffer (pH 7.4) for 30 min and finally subject to fluorescent imaging.

3.4 SPR Analysis of Specific MAA and SNA Binding to Immobilized α2,3 SPG and α2,6 SGP

1. The specific binding of lectin to immobilized SGP is analyzed with SPR with a BI 2000 biosensor system (BI Biosensing Instruments).
2. To prepare the sensing surface, clean the commercial SPR gold chip (BI Biosensing Instruments) by rinsing with piranha solution of sulfuric acid and hydrogen peroxide (1:1, v/v), followed by DI water and ethanol (3 times) and dry under nitrogen.
3. Incubate the dried gold chip with 1 mg/mL of cystamine solution in ethanol at 4 °C overnight and followed by washing with ethanol and DI water to remove weakly adsorbed cystamine to generate amine functionalized gold chip surface.
4. Assemble the amine functionalized gold chip onto the chip holder and dock into the SPR instrument.
5. To covalently immobilize α2,3SGP onto the amine functionalized gold chip surface, inject the polymer solution (2 mg/mL, $NaHCO_3$ buffer (pH 10.3)) at a flow rate of 10 μL/min for 600 s and repeat at least three times until the surface is saturated with the polymer, using $NaHCO_3$ buffer (pH 10.3) as a running buffer.
6. Inject a serial of solution of lectin MAA in concentration ranging from 0.5 to 10 μM in 0.01 mM PBS running buffer (pH 7.4) over the immobilized α2,3SGP at a flow rate of 20 μL/min for 2 min (120 s, association phase).
7. For regeneration of the surface, inject 0.1 M HCl (pH 1.5) at a flow rate of 20 μL/min for about 30 s and repeat twice to remove bound lectin completely if necessary.
8. Determine the association and dissociation constants of the lectin binding to immobilized SGP by standard BI 2000 (scrubber) evaluation software.
9. For the experiments with α2,6 SGP, the same surface modification procedure should be followed as above. For lectin SNA binding, the same procedure should be used as above except different concentrations ranging from 1 to 10 μM must be used.

3.5 Hemagglutinin (HA) Binding to Immobilized SGP

1. Both α2,3 SGP and α2,6 SGP immobilized on the gold sensor chip following the same procedure as Subheading 3.4.
2. Prepare solutions of H5N1 HA (A/Anhui/1/2005(H5N1)) and H3N2 HA (A/Brisbane/10/2007(H3N2)) (Sino Biological, China) in 0.01 mM PBS buffer (pH 7.4, 420 nM),
3. Inject the HA solutions over the two immobilized α2,3S GP and α2,6 SGP at a flow rate of 20 μL/min for 180 s, respectively.

4. As control, both H5N1 HA and H3N2 HA are pre-incubated with α 2,3-sialyllactose (PBS buffer (pH 7.4), 5×10^{-6} mM) and α 2,6-sialyllactose (PBS buffer (pH 7.4), 5×10^{-6} mM) for 30 min, and then inject over immobilized α2,3 SGP and α2,6 SGP, respectively.
5. Inject 100 mM NaOH for regeneration of surface between each HA injections.
6. Determine the association and dissociation constants of the protein to immobilized SGP by standard BI 2000 (scrubber) evaluation software.

4 Notes

1. 2-*N*-acryloyl-aminoethoxyl 4-*O*-(*β*-D-galactopyranosyl)-*β*-D-glucopyranoside) should be synthesized from lactose peracetate as described previously [11]. Briefly, glycosylation of lactose peracetate (Sigma) with 2-azidoethanol in the presence of boron trifluoride etherate gives the *β*-azidoethyl-lactose peracetate, which is then converted to *β*-aminoethyl-lactose via catalytic hydrogenation and deprotection with aqueous sodium hydrogen carbonate. Finally, *N*-acryloylation of an aminoethyl aglycon with acryloyl chloride gives the product.
2. The red to yellow color is the indicator of diazonium salt formation when HBF_4 is added to the solution of 4-chloroaniline, sodium nitrite in a mixture of 2 mL water and THF (1:1, v/v).
3. The resultant glycopolymer must be characterized by ^{1}H NMR spectroscopy. It is noteworthy that the phenyl group at the end of the glycopolymer provides a convenient mechanism for calculating carbohydrate content and the molar mass through comparison of integrated phenyl proton peaks with those of carbohydrate and backbone protons, respectively (Fig. 3).
4. To 0.05 mL of each fraction add 0.45 mL of DI water, 0.5 mL of 5 % aq. phenol solution, and 2.5 mL of H_2SO_4 (96 %). Change in color from colorless to yellow or brown indicates SGP in the fraction.
5. The resultant SGPs can be characterized by ^{1}H NMR spectra. The successful sialylation of lactose glycopolymer is confirmed by the signals of protons from Neu5Ac (1.95 ppm, CH3-Neu5NAc and 2.60 ppm, H_{3eq}-Neu5Ac), the degree of sialylation and the polymer length as well are calculated also using the ^{1}HMR spectra by comparing the integration value of proton signals from aromatic protons (7.21 and 7.06 ppm), anomeric protons (4.36 and 4.33 ppm) of Gal and Glc, and C3-equatorial proton (2.60 pm) of Neu5Ac as shown in Fig. 3. By comparing the integrated signal from the anomeric protons

of Gal and Glc (4.36 and 4.43 ppm) with that of the C3-equatorial proton of Neu5Ac (2.60 ppm, H_{3eq}-Neu5Ac), the percentages of both sialylations are more than 90 %.

Acknowledgements

This work was supported from AHA Grant-in Aid grant, Ohio Research Scholars Program grant, NSF MRI grant, and Doctoral Dissertation Research Expense Award Program (Cleveland State University). Thanks to Dr. Dale Ray at Case NMR Center for NMR studies.

References

1. Skehel JJ, Wiley DC (2000) Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem 69:531–569
2. Palese P, Tobita K, Ueda M, Compans RW (1974) Characterization of a temperature-sensitive influenza B virus mutant defective in neuraminidase. Virology 61:397–410
3. Wiley DC, Skehel JJ (1987) The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Annu Rev Biochem 56:365–394
4. Murti KG, Webster RG (1986) Distribution of hemagglutinin and neuraminidase on influenza virions as revealed by immunoelectron microscopy. Virology 149:36–43
5. Matrosovich M, Klenk H-D (2003) Natural and synthetic sialic acid-containing inhibitors of influenza virus receptor binding. Rev Med Virol 13:85–97
6. Ting SSR, Chen G, Stenzel MH (2010) Synthesis of glycopolymers and their multivalent recognitions with lectins. Polym Chem 1:1392–1412
7. Voit B, Appelhans D (2010) Glycopolymers of various architectures-more than mimicking nature. Macromol Chem Phys 211:727–735
8. Jiang X, Housni A, Gody G, Boullanger P, Charreyre MT, Delair T, Narain R (2010) Synthesis of biotinylated α-D-mannoside or *N*-acetyl β-D-glucosaminoside decorated gold nanoparticles: study of their biomolecular recognition with Con A and WGA lectins. Bioconjugate Chem 21:521–530
9. Narain R, Armes SNP (2003) Synthesis and aqueous solution properties of novel sugar methacrylate-based homopolymers and block copolymers. Biomacromolecules 4:1746–1758
10. Gupta SS, Raja KS, Kaltgrad E, Strable E, Finn MG (2005) Virus-glycopolymer conjugates by copper(I) catalysis of atom transfer radical polymerization and azide-alkyne cycloaddition. Chem Commun 34:4315–4317
11. Sun X-L, Grande D, Baskaran S, Chaikof EL (2002) Glycosaminoglycan mimetic biomaterials. 4. Synthesis of sulfated lactose-based glycopolymers that exhibit anticoagulant activity. Biomacromolecules 3:1065–1070
12. Brown JM, Rogers CJ, Matson JB, Krishnamurthy C, Rawat M, Hsiesh-wilson LC, Lee SG (2010) End-functionalized glycopolymers as mimetics of chondroitin sulfate proteoglycans. Chem Sci 1:322–325
13. Hou S, Sun X-L, Dong C-M, Chaikof EL (2004) Facile synthesis of chain-end functionalized glycopolymers for site-specific bioconjugation. Bioconjugate Chem 15:954–959
14. Muthana S, Yu H, Huang S (2007) Chemoenzymatic synthesis of size-defined polysaccharides by sialyltransferase-catalyzed block transfer of oligosaccharides. J Am Chem Soc 129:11918–11919