Display of Azido Glycoside on a Sensor Chip

Toshinori Sato,* Sinya Fujita, Maria Carmelita Z. Kasuya,† Kenichi Hatanaka,† and Tatsuya Yamagata††
Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522
†*Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Meguro-ku 153-8505*
††*Shenyang Pharmaceutical University, P. O. Box 29, 103 WenHua Road, Shenyang 110016, P. R. China*

(Received February 16, 2004; CL-040172)

Using 12-azidododecyl β-mannoside, we successfully immobilized azido glycoside onto a sensor chip by either the Staudinger reaction or reduction of azido group followed by condensation reaction. Specific binding of Concanavalin A to the sensor chip proved immobilization of the glycoside by either method.

It is known that oligosaccharides are important molecules that are correlated with various cell functions and diseases. To accelerate the research on the function of carbohydrate, the development of saccharide microarray to comprehensively detect carbohydrate recognition has long been sought for. For the development of a saccharide microarray, a saccharide library consisting of a variety of oligosaccharides is required to immobilize individual oligosaccharides on the microplate. For the construction of a saccharide library, we have developed biocombinatorial synthesis of oligosaccharides by animal cells in culture.[1] From a single building block chemically synthesized, a large number of oligosaccharides can be constructed by various kinds of cells. This is possible since animal cells have a potential to produce particular glycoconjugates depending on their origin. Dodecyl β-lactoside is one of the building blocks which can be glycosylated after incorporation into cells,[1] and such a building block is also called as saccharide primer. In order to utilize the oligosaccharide on a sensor chip, oligosaccharides in saccharide library must possess a functional group amenable to covalent reaction. It was recently found that 12-azidododecyl β-lactoside, a saccharide primer having azido group, was as well glycosylated by cells as dodecyl β-lactoside.[2]

Several approaches on saccharide arrays have been reported. Fukui et al. presented the neoglycolipids on nitrocellulose membrane.[3] Houseman and Mrksich prepared carbohydrate array by the Diels–Alder mediated immobilization of carbohydrate–cyclopentadiene conjugates.[4] Park and Shin reported the attachment of maleimide-linked carbohydrates to a thiol group-coated glass slide.[5] Furthermore, Faizio et al. described the attachment of oligosaccharides by 1,3-dipolar cycloadditions between azides and alkynes to microtiter plate.[6]

We synthesized 12-azidododecyl β-mannoside (Man-C12-N_3), and immobilized it to sensor chip by two methods, the Staudinger reaction (Method 1) and conversion of azides to amines followed by condensation reaction (Method 2).

Man-C12-N_3 was synthesized as follows. 1-Bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose was prepared from mannose through acetylation and subsequent treatment with HBr–HOAc. 12-Azidododecanol was synthesized from 1,12-dodecandiol.[7] The *O*-acetyl mannosyl bromide was reacted with 12-azidododecanol in CH_2Cl_2 in the presence of Ag_2CO_3 and molecular sieves 4A for 21 h. The product was purified by silica gel chromatography (ethyl acetate:*n*-hexane = 1:1) and reprecipitation (chloroform/n-hexane).[8] Deacetylation was carried out in dry methanol in the presence of NaOMe. The total yield from mannose was 29%. The molecular mass determined by MALDI–TOF–MS (Autoflex, Bulker) was 413.06 (exact mass as $[M + Na^+] = 412.47$).

Immobilization of the Man-C12-N_3 was carried out on the sensor chip of a surface plasmon resonance-based IAsys[9] (Thermo Labsystems) as shown in Figure 1.

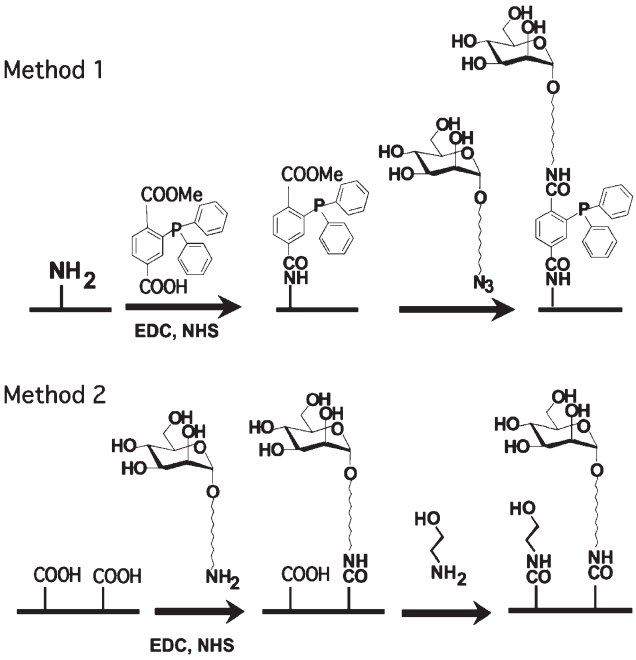

Figure 1. Immobilization of Man-C12-N_3 (method 1) and Man-C12-NH_2 (Method 2) on sensor chip.

Method 1: 2-Diphenylphosphonylterephthalic acid 1-methyl ester was employed as linker to immobilize azido glycoside.[10] 1-Methyl hydrogen 2-iodoterephthalate was synthesized from 1-methyl hydrogen 2-aminoterephthalate through the treatment with $NaNO_2$ followed by KI.[11] The linker was synthesized by coupling 1-methyl hydrogen 2-iodoterephthalate and diphenylphosphine in the presence of palladium acetate and triethylamine in acetonitrile under a nitrogen atmosphere for 5 h at 85 °C.[12] The linker was first conjugated with amino silane cuvette (Si–C_6H_{12}–NH_2, sensor chip) in 200 mM acetate buffer (pH 4) containing 400 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 100 mM *N*-hydroxysuccinimide (NHS). Thereafter, the linker was coupled with 20 mM Man-C12-N_3 dissolved in methanol. The amount of immobilized linker was estimated to be 0.1 ng/mm^2 from response due to mass increase on the surface of sensor chip, while the amount of immobilized Man-

C12-N_3 was below the detection limit (0.14 pg/mm^2).

Method 2: Azido group of Man-C12-N_3 was reduced to amino group in the presence of triphenylphosphine in DMF/$H_2$0 for 6 h at 50 °C. The production of 12-aminododecyl α-D-mannopyranoside (Man-C12-NH_2) was confirmed by molecular mass ([M + H^+] = 364.20) and ninhydrin test. Man-C12-NH_2 was immobilized on carboxylate cuvette in 5 mM maleic acid buffer (pH 6) containing 400-mM EDC and 100-mM NHS. Unreacted carboxyl groups were blocked with ethanolamine. When the concentrations of Man-C12-NH_2 in the reaction solution were 5, 7.5, and 10 mM, the immobilized amounts were 0.14, 1.09, and 2.35 pmol/mm^2, respectively.

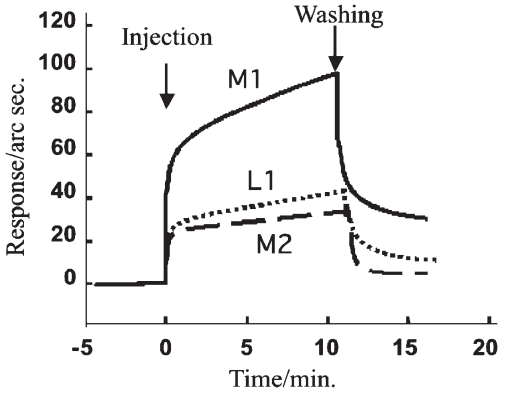

Figure 2. Binding of ConA (M1) and BSA (M2) to mannose immobilized by method 1. L1 represents the binding of ConA to linker without mannose. [ConA] = [BSA] = 10 μM.

Whether or not mannose was successfully fixed and displayed on the sensor chip by Methods 1 and 2 was confirmed by analysis with an IAsys. Figure 2 showed the typical sensorgrams for the binding of mannose–specific lectin, Concanavalin A (ConA), to mannose displayed by Method 1. Though the amount of mannose immobilized on a sensor chip was below detection limit of instrument, a specific binding of ConA (curve M1 in Figure 2) was observed. The binding of ConA was inhibited by the addition of 100 mM mannose, and no significant binding of ConA to the linker surface without mannose was observed. The bindings of ConA were measured at the ConA concentrations of 1, 3, 5, and 10 μM, and the kinetic parameters (binding rate k_1, dissociation rate k_{-1}, dissociation constant K_d, and response) were determined by FASTfit attached to IAsys. Those results were summarized in Table 1. The K_d value for ConA-binding was 8.3 μM, and 10 times lower than that for BSA-binding. The mannose-immobilized sensor chip prepared by Method 2 also showed specific binding of ConA. The specificity of ConA-binding depends on the density of immobilized mannose. For the mannose density of 0.14 pmol/mm^2, no significant differences in the K_d values between ConA and BSA were observed, though response due to the binding amount of ConA was higher than that of BSA. For the mannose density of 1.09 pmol/mm^2, significant differences in K_d values between ConA and BSA were observed. These values well agreed with those obtained by Method 1. For the mannose density of 2.35 pmol/mm^2, the specific binding of ConA was observed, though the differences in K_d value between ConA and BSA was not so large. From these results, it is considered that the optimum density of mannose is about 1 pmol/mm^2, which corresponds to 13% displacement of carboxyl group on the sensor chip with mannose.

Table 1. Kinetic parameters (k_1, k_{-1}, K_d, and response) for immobilized mannose

		k_1 /M^{-1} s^{-1}	k_{-1} /s^{-1}	K_d /μM	Response[a] /arc sec
Method 1					
	ConA	2516	0.021	8.3	28
	BSA	459	0.037	80.6	4
Method 2					
Man = 0.14[b]	ConA	885	0.043	48.6	38
	BSA	514	0.020	38.9	19
Man = 1.09[b]	ConA	2825	0.025	8.8	17
	BSA	531	0.034	64.0	1
Man = 2.35[b]	ConA	3564	0.023	6.5	10
	BSA	1567	0.024	15.3	2

[a] Response after washing with buffer. [ConA] = 10 μM.
[b] The density of the immobilized mannose (pmol/mm^2).

In conclusion, we developed two technologies to immobilize azido glycoside on sensor chip. These technologies will lead to the preparation of saccharide microarray using a strategy that involves immobilization of the saccharide library obtained by the saccharide primer method. Method 1 was very favorable for us to immobilize azide-bearing saccharide library. We are preparing mictotiter plates displaying azide-bearing oligosaccharide produced by animal cells.

This work was partly supported by Special Coordination of Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government (T. S.)

References and Notes

1 a) Y. Miura, T. Arai, and T. Yamagata, *Carbohydr. Res.*, **289**, 193 (1996). b) H. Nakajima, Y. Miura, and T. Yamagata, *J. Biochem.*, **124**, 148 (1998).
2 M. C. Z. Kasuya, L. X. Wang, Y. C. Lee, M. Mitsuki, H. Nakajima, Y. Miura, T. Sato, K. Hatanaka, S. Yamagata, and T. Yamagata, *Carbohydr. Res.*, **329**, 55 (2000).
3 S. Fukui, T. Feizi, C. Galustian, A. M. Lawson, and W. Chai, *Nat. Biotechnol.*, **20**, 1011 (2002).
4 B. T. Houseman and M. Mrksich, *Chem. Biol.*, **9**, 443 (2002).
5 S. Park and I. Shin, *Angew. Chem., Int. Ed.*, **41**, 3180 (2002).
6 F. Faizo, M. C. Bryan, O. Blixt, J. C. Paulson, and C.-H. Wong, *J. Am. Chem. Soc.*, **124**, 14397 (2002).
7 ^{1}H NMR (500 MHz, $CDCl_3$); δ 3.75 (2H, t, HOCH_2–), 3.23 (2H, m, N_3CH_2–), 1.58 (5H, m, $HOCH_2CH_2$–, $N_3CH_2CH_2$–, –OH), 1.38–1.28 (16H, m, –CH_2–). ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.73 (s, 1C, –CH_2OH), 51.31 (s, 1C, –CH_2N_3).
8 MS (MALDI-TOF): m/z = 581.30 [M + Na^+]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (16H, s, –CH_2–), 1.57 (4H, m, –CH_2–), 1.75 (3H, s, –CH_3), 2.05 (3H, s, –CH_3), 2.08 (3H, s, –CH_3), 2.12 (3H, s, –CH_3), 3.26 (2H, t, –CH_2N_3), 3.47 (2H, m, O–CH_2–), 3.68 (1H, m, H5), 4.14 (1H, dd, H6), 4.24 (1H, dd, H6′), 4.59 (1H, dd, H2), 5.15 (1H, dd, H3), 5.30 (1H, t, H4), 5.47 (1H, d, J = 2.44 Hz, H1).
9 N. Athanassopoulou, R. J. Davies, P. R. Edwards, D. Yeung, and C. H. Maule, *Biochem. Soc. Trans.*, **27**, 340 (1998).
10 a) E. Saxon, J. I. Armstrong, and C. R. Bertozzi, *Org. Lett.*, **2**, 2141 (2000). b) E. Saxon, S. J. Luchansky, H. C. Hang, C. Yu, S. C. Lee, and C. R. Bertozzi, *J. Am. Chem. Soc.*, **124**, 14893 (2002).
11 MS(FAB): m/z = 306 [M + H^+]. ^{1}H NMR (300 MHz, $CDCl_3$); δ 3.67 (1H, s, –CH_3), 7.83 (1H, d, J = 8.05 Hz, Ar–H), 8.11 (1H, d, J = 8.63 Hz, Ar–H), 8.68 (1H, s, Ar–H).
12 MS (MALDI-TOF): m/z = 365.31 [M + H^+]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.41 (3H, s, –CH_3), 7.20 (4H, m, Ar–H), 7.40 (6H, m, Ar–H), 7.52 (1H, d, J = 3.78 Hz, Ar–H), 8.02 (2H, m, Ar–H). ^{31}P NMR (300 MHz, DMSO-d_6) δ −5.72 (P:), 28.13 (P=O).

Published on the web (Advance View) April 19, 2004; DOI 10.1246/cl.2004.580