The Synthesis of Carbohydrate Microarrays by S-Alkylation of the Glass-supported 2-Bromoacetamides

Daisuke Takahashi,[1,†] Hiroshi Tanaka,[1] Emiko Nakane,[2] and Takashi Takahashi*[1]
[1]*Department of Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8552*
[2]*Pharmacology Laboratory, Research Division, Mitsubishi Tanabe Pharma Corporation, 3-16-89 Kashima, Yodogawa-ku, Osaka 532-8505*

(Received September 12, 2008; CL-080876; E-mail: ttak@apc.titech.ac.jp)

S-alkylation reaction of a thiol group on a carbohydrate probe with 2-bromoacetamide-coated glass slide as a loading reaction was developed under physiological conditions, and the synthesis of carbohydrate microarrays using the immobilization reaction was achieved.

Glycoconjugates have important roles in biological events on cell surfaces due to carbohydrate–protein interactions.[1] Analysis of these carbohydrate-mediated interactions is important for elucidating the biological importance of each oligosaccharide. In terms of structure–activity relationship studies, the use of chemically pure synthesized oligosaccharides as biochemical probes would be highly desirable owing to the possibility that such isolated glycoconjugates could be heterogeneous and/or contaminated with antigenic compounds. Recent development in the synthesis of oligosaccharides enables the preparation of various chemically pure oligosaccharides.[2] In addition, combinatorial chemistry enables the synthesis of carbohydrate libraries.[3,4] However, analysis of interactions between carbohydrate libraries and proteins are still difficult tasks due to these carbohydrate–protein interactions being relatively weak in comparison with DNA–DNA or protein–small molecule interactions.

Recently, microarray technology has been developed by utilizing nanotechnology, and allows the immobilization of a large number of a small amount of compounds in small area.[5–7] There have already been several reports indicating its applications in the analysis of carbohydrate–protein interactions.[8] In these publications, several immobilization techniques have been developed, for example non-covalent immobilization technology and covalent immobilization chemistry such as amidation reaction, the Diels–Alder reaction, 1,3-dipolar cycloaddition and the Michael addition of thiol groups on the solid surface with maleimide-functionalized carbohydrates or thiol groups on carbohydrates with maleimide-coated solid surfaces. The loading reactions of the carbohydrate probes should proceed chemoselectively with a proper functional group on the solid surface, even if the carbohydrate probes have other functional groups such as hydroxy and amino groups, and carboxylic acids. In addition, the surface of the solid supports involving the remaining functional groups for immobilization should not exhibit nonspecific interaction with proteins. Desirable features of the functional groups on slides are neutral and hydrophilic. Therefore, there is still a need for the development of an effective method for the synthesis of carbohydrate microarrays. Herein we report the synthesis of carbohydrate microarrays by S-alkylation reaction of glass-supported 2-bromoacetamides.

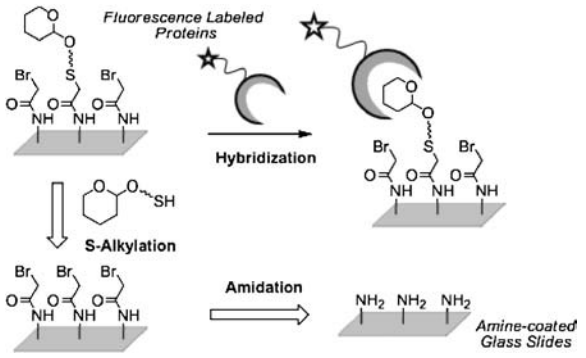

Figure 1. Synthetic strategy of carbohydrate microarray based on S-alkylation reaction on 2-bromo-coated glass slides.

Our designed microarray is composed of carbohydrate units linked through 2-alkylthioacetamide moiety on the glass slides (Figure 1). Preparation of the microarrays can be achieved by S-alkylation of solid-supported 2-bromoacetamides with carbohydrate probes containing a thiol group at the nonreducing end. The 2-bromoacetamides can be reacted with thiol groups under physiological conditions[9] in a chemoselecitve manner, even in the presence of various functional groups such as hydroxy and amino groups, and carboxylic acids. In addition, it is expected to exhibit relatively less nonspecific interaction due to the hydrophilicity of 2-alkylthioacetamide moieties. The solid-supported glass slides were prepared from commercially available amine-coated glass slides[10] by amidation with 2-bromoacetic acid or its equivalents.

The 2-bromoacetamide-coated glass slides were prepared as follows. Three kinds of commercially available amine-coated glass slides (A–C: 76 mm × 25 mm) were used for the synthesis. At first, the density of the amino functional groups on each glass slide was analyzed by a reported semiquantitative method[6,11] to be 1.9 (slide A), 1.1 (slide B), and 1.3 (slide C) unit/nm^2 respectively. Treatment of the three kinds of the amine-coated slides with 2-bromoacetyl succinimide in phosphate buffer (pH 7.4) for 1 h provided the 2-bromoacetamide-coated slides. Conversion of the amidation was estimated by analysis of remaining amino groups to be 61 (slide A), 95 (slide B), and 92% (slide C). Although two slides (slide B and C) were acceptable, further optimization is needed for amidation of slide A. The amidation of slide A was accomplished by treatment with 2-bromoacetic acid, HATU and DIEA in DMF for 14 h to be 72% convergent yield.[12]

Next, S-alkylation reaction on the glass slide C was examined utilizing α-mannoside probe **2** which possesses a di-

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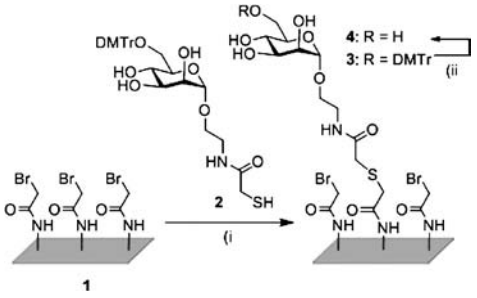

Scheme 1. Manual synthesis of carbohydrate microarrays based on S-alkylation reaction on 2-bromoacetamide-coated glass slides. Reagents and conditions: (i 2 (1.0 mM) in phosphate buffer (pH 8.0) containing 10% DMF, 25 °C, 3 h. (ii 30% aq. $HClO_4$, 5 min.

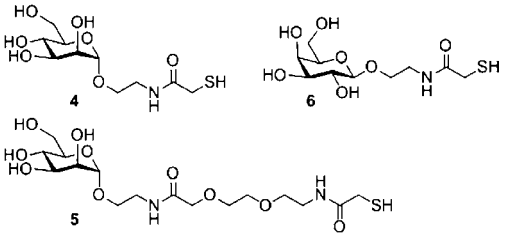

Figure 2. Structure of α-mannoside and β-galactoside probes.

methoxyltrityl (DMTr) group at C6 position (Scheme 1). Exposure of the glass slide to 1.0 mM ligand in DMF/sodium phosphate (pH 8.0) = 9/1 for 3 h provided the glass-supported saccharide **3a**. Conversion of this immobilization reaction was estimated by cleavage of the DMTr group to be 72%.

We next examined the synthesis of microarrays immobilized with two mannoside probes **4** and **5** containing different length of spacers on the three glass slides A–C (Figure 2). The reaction conditions for immobilization reaction were varied in pH (9.0, 8.6, 7.5, and 7.0), reaction time (1, 3, 6, and 18 h), and concentrations of the probes (10, 1.0, 0.10, and 0.010 mM). The printing of the mannoside probes on the glass slides was achieved by a GMS 417 arrayer. Figure 3a) shows a layout of a set of 40 spots with several reaction conditions. In this study, galactoside probe **6** was used as a negative control.

Validation of the microarrays was achieved by visualization of Alexa fluor 647-labeled concanavalin A (ConA)–α-mannoside interactions. The microarrays were incubated with ConA (200, 20, and 2 μg/mL) for 1 h at 37 °C. Fluorescent images of the selected microarrays are shown in Figure 3. Slide A was more effective for visualization of the interaction compared to slide B and C. Slide A only showed clear fluorescent spots at lower concentration of ConA (2.0 μg/mL). Only specific interaction of ConA–α-mannoside was observed. Moreover, the mannoside probe **5** possessing longer spacer exhibits stronger fluorescent than probe **4**. The pH conditions in the immobilization reaction did not show a remarkable change at this range. Longer immobilization time (18 h) enabled visualization of the interactions of lower concentrations of probes.

In conclusion, we describe an efficient synthesis of carbohydrate microarray using S-alkylation reaction of glass-supported 2-bromoacetamides. This technology enabled visualization of the specific interaction between ConA and α-mannoside with high sensitivity. We believe that this will lead to preparation of glycomicroarray possessing carbohydrate libraries obtained by combinatorial chemical synthesis.

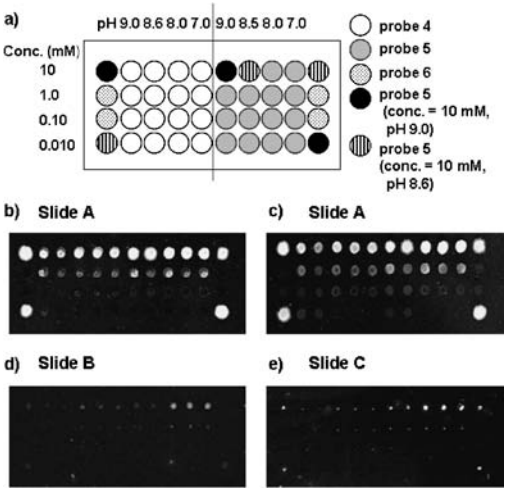

Figure 3. a) Layout for immobilization of carbohydrate probes on 2-bromoacetamide-coated glass slides. b) Imaging of glass slide A after printing for 1 h followed by hybridization for 1 h with ConA (2.0 μg/mL). c) Imaging of glass slide A after printing for 18 h followed by hybridization for 1 h with ConA (2.0 μg/mL). d) Imaging of glass slide B after printing for 18 h followed by hybridization for 1 h with ConA (20 μg/mL). e) Imaging of glass slide C after printing for 18 h followed by hybridization for 1 h with ConA (20 μg/mL).

References and Notes

† Present address: Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522.

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12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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