analytical chemistry

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Functional Interaction Analysis of GM1-Related Carbohydrates and *Vibrio cholerae* Toxins Using Carbohydrate Microarray

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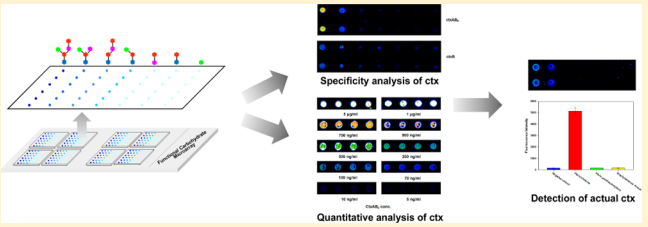
ABSTRACT: The development of analytical tools is important for understanding the infection mechanisms of pathogenic bacteria or viruses. In the present work, a functional carbohydrate microarray combined with a fluorescence immunoassay was developed to analyze the interactions of *Vibrio cholerae* toxin (ctx) proteins and GM1-related carbohydrates. Ctx proteins were loaded onto the surface-immobilized GM1 pentasaccharide and six related carbohydrates, and their binding affinities were detected immunologically. The analysis of the ctx-carbohydrate interactions revealed that the intrinsic selectivity of ctx was GM1 pentasaccharide ≫ GM2 tetrasaccharide > asialo GM1 tetrasaccharide ≥ GM3trisaccharide, indicating that a two-finger grip formation and the terminal monosaccharides play important roles in the ctx-GM1 interaction. In addition, whole cholera toxin ($ctxAB_5$) had a stricter substrate specificity and a stronger binding affinity than only the cholera toxin B subunit (ctxB). On the basis of the quantitative analysis, the carbohydrate microarray showed the sensitivity of detection of the $ctxAB_5$-GM1 interaction with a limit-of-detection (LOD) of 2 ng mL^{-1} (23 pM), which is comparable to other reported high sensitivity assay tools. In addition, the carbohydrate microarray successfully detected the actual toxin directly secreted from *V. cholerae*, without showing cross-reactivity to other bacteria. Collectively, these results demonstrate that the functional carbohydrate microarray is suitable for analyzing toxin protein-carbohydrate interactions and can be applied as a biosensor for toxin detection.



Most carbohydrates exist as complex forms of glycolipid, glycoprotein, and other glycoconjugates, and they are involved in diverse biological recognition events.[1−3] Molecular recognition processes between carbohydrates and proteins have significant roles in the infection mechanisms of pathogenic bacteria or viruses.[4] Many studies have been performed to understand the fundamental infection processes and to identify new pharmaceutical candidates.[5−7]

Cholera toxin (ctx) is a protein enterotoxin produced by the pathogen *Vibrio cholerae* and is the causative agent of cholera that leads to rapid dehydration, acidosis, and even death.[8] Whole cholera toxin ($ctxAB_5$) consists of a single A subunit (ctxA) and five identical B subunits (ctxBs). Studies show that ctxB is responsible for assisting the entrance of ctxA into a cell by binding to the GM1 ganglioside receptors on the cell membrane, and ctxA acts as an adenosine diphosphate-ribosyltransferase that causes osmotic imbalance through cyclic adenosine monophosphate accumulation in the cell.[9] The ctx-GM1 ganglioside interaction has been investigated using a variety of biophysical and biochemical technologies, including surface plasma resonance biosensing,[10,11] solid-phase and thin layer chromatography overlay assays,[9,12] atomic force microscopy,[13] flow cytometry,[14] X-ray crystallography,[15] and isothermal titration calorimetry.[16,17] Although these studies provided useful information on either the infection mechanism of ctx or the development of biomedical agents, they have shown several limitations in the functional analysis of ctx interactions. First, although ctx interacts only with the carbohydrate portion of the GM1 ganglioside receptor on the cell surface, most studies used a liposome- or lipid bilayer-based GM1 ganglioside or GM1-analogues rather than the carbohydrate portions.[10,11,13,14,16] However, the use of a liposome is associated with several limitations, including structural instability, particle size distribution, aggregation/fusion, sedimentation, and chemical instability.[18] In addition, it is difficult and time-consuming to prepare a GM1 ganglioside- or GM1-analogue-functionalized liposome with the uniform size and equal carbohydrate distribution necessary for proper interaction with ctx. The use of a lipid bilayer also exhibits poor stability and reproducibility.[19,20] Second, most studies have used ctxB as the target ctx to analyze the interactions with the GM1 ganglioside or its analogues; however, the complexation of the ctxA and ctxB subunits to form $ctxAB_5$ affects the ctx binding affinity to the GM1 ganglioside.[14] Thus, to obtain accurate and detailed information on the interactions between ctx and GM1-related carbohydrates, it is necessary to use both $ctxAB_5$ and ctxB as target ctx proteins.

Because microarray-based technology enables the analysis of a large number of biomolecules quickly, quantitatively, and simultaneously, it has been utilized for high-throughput analysis of diverse biomolecular interactions A carbohydrate-based microarray system has been developed for analysis of carbohydrate-protein interactions.[21−25] However, to the best of our knowledge, the carbohydrate microarray has not been

Received: June 3, 2012
Accepted: July 4, 2012
Published: July 4, 2012

   dx.doi.org/10.1021/ac301511t | *Anal. Chem.* 2012, 84, 6884−6890

used for a functional interaction analysis between ctx proteins and GM1-related carbohydrates. In addition, only a few studies have reported the use of carbohydrate microarrays for the detection of ctx.[26,27] A carbohydrate microarray might have several advantages over other reported methods used for an interaction analysis of ctx and GM1 (or its analogues): (1) a large number of interactions between ctx and carbohydrates can be simultaneously analyzed by using a small amount of sample, (2) immobilized carbohydrates with the proper space and functional orientation are able to mimic carbohydrates on the cell surface and to facilitate multivalent interactions of ctx, and (3) surface immobilization of the carbohydrate portions of gangliosides by covalent binding after chemical modification can resolve the liposome- or lipid bilayer-induced limitations. The carbohydrate microarray can also provide other valuable information for disease diagnosis and for development of carbohydrate-based biomedical agents.

Previously, we developed a facile and efficient method for carbohydrate modification and immobilization onto gold or glass surfaces.[24,28] In the present work, we constructed a functional carbohydrate microarray containing the seven GM1-related carbohydrates based on our established immobilization method. Using the constructed carbohydrate microarray, we analyzed the interactions between ctx and GM1-related carbohydrates and investigated the effect of complexation on the affinity and selectivity of ctx. Finally, we applied the carbohydrate microarray to directly and specifically detect actual toxins from *V. cholerae*.

EXPERIMENTAL SECTION

Materials. Lactose, Neu5Ac, 4-(2-aminoethyl)aniline, ctxAB$_5$, and ctxB were purchased from Sigma-Aldrich (St. Louis, MO). Other carbohydrates used for modification consist of following: GM1 pentasaccharide and GM2 ganglioside from Alexis Biochemicals (San Diego, CA), asialo GM1 tetrasaccharide and Galβ(1 → 3)GalNAc from V-Labs (Los Angeles, CA), and GM3 trisaccharide from GeneChem Inc. (Daejeon, Korea). Rabbit polyclonal anti-ctx IgG, Alexa Fluor 546-conjugated goat anti-rabbit IgG, and ceramide glycanase were purchased from Abcam (Cambridge, MA), Invitrogen (Carlsbad, CA), and V-Labs, respectively.

Carbohydrate Modification. We followed a previously reported protocol to obtain GM2 tetrasaccharide from its ganglioside form.[17] Carbohydrate modification was performed under an identical protocol developed in our previous work.[24] Briefly, seven GM1-related carbohydrates (GM1 pentasaccharide, GM2 tetrasaccharide, asialo GM1 tetrasaccharide, GM3 trisaccharide, Galβ(1 → 3)GalNAc, lactose, and Neu5Ac) were dissolved in deionized water at a final concentration of 100 mM, and 4-(2-aminoethyl)aniline was dissolved in 100 mM acetic acid. Each carbohydrate solution was mixed with the 4-(2-aminoethyl)aniline solution in a 1:1 volume ratio and incubated in a sealed tube at 37 °C for 1 h. Next, freshly prepared reducing agent, 100 mM dimethylamine borane, was added to each reaction solution, and the tubes were incubated at room temperature for 1 h. Each product was then evaporated under a nitrogen gas stream by heating at 50 °C for 1 h. Without further purification steps, all modified carbohydrates were dissolved in printing buffer (150 mM phosphate, 5% (v/v) glycerol, 0.1 mg mL^{-1} BSA, and 0.04% (v/v) Tween 20; pH 8.5). Carbohydrate modifications were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis.

MALDI-TOF MS Analysis. To improve the ionization efficiency of MALDI-TOF MS, modified carbohydrate samples were desalted using Zip-tip C18 (Millipore, Billerica, MA) and eluted onto MALDI target plates using a matrix solution (10 mg of 2,5-dihydroxylbenzoic acid (DHB; Sigma-Aldrich) dissolved in 20% (v/v) acetonitrile and 0.1% (v/v) trifluoroacetic acid). A 1 μL sample of the desalted and eluted carbohydrate was mixed with the same volume of DHB matrix solution and loaded onto the MALDI target; sample spots were allowed to vacuum-dry at room temperature. All mass spectra were acquired in the reflection mode using a Voyager DE-STR (PerSeptive Biosystems, Framingham, MA) in the Korea Basic Science Institute (Daejeon, Korea). Spectra were obtained in the mass range 100−2 000 Da using ∼200 laser shots. Internal calibration was performed with the 4700 Cal Mix (Applied Biosystems, Foster City, CA).

Preparation of the Carbohydrate Microarray. NH_2-modified carbohydrates in printing buffer were spotted onto *N*-hydroxysuccinimide (NHS)-activated glass slides (GmbH, Jena, Germany) using a Microsys 5100 microarrayer (Cartesian Technologies, Ann Arbor, MI) with the Chip Marker 2 pin (Telecom International, Sunnyvale, CA) at 75% humidity in a class 10 000 clean room and incubated overnight under the same humidity conditions to achieve tight immobilization.

Analysis of Ctx-Carbohydrate Interactions on the Carbohydrate Microarray. Each prepared carbohydrate microarray was treated with a blocking solution (50 mM ethanolamine in 50 mM sodium borate buffer; pH 8.0) for 1 h to deactivate any remaining functional groups and to block nonspecific interactions. Next, each slide was removed from the blocking solution and rinsed with washing buffer I (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, and 0.5% (v/v) Tween 20; pH 7.5) and washing buffer II (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM KH_2PO_4; pH 7.5). For ctx-carbohydrate interaction analysis, 100 μL of 20 μg mL^{-1} ctxAB$_5$ or ctxB was applied onto a defined block of the carbohydrate microarray using a Gene Frame (Thermo Scientific, San Jose, CA), and after serial washing with washing buffers I and II, the microarray was sequentially immersed into a solution of rabbit anti-ctx IgG (50 μg mL^{-1}) and anti-rabbit IgG conjugated with Alexa Fluor 546 (50 μg mL^{-1}). To quantitatively analyze the ctx-carbohydrate interactions, various concentrations of CtxAB$_5$ (0−10 μg mL^{-1}) were used. A commercial confocal laser scanner (GSI Lumonics, Wilmington, MA) was used for image acquisition.

Detection of Ctx from *V. Cholerae* Culture. *V. cholerae* O1 (American Type Culture Collection (ATCC) 14035) was grown overnight in Luria−Bertani (LB) medium (0.5% (w/v) yeast extract, 1% (w/v) tryptone, and 1% (w/v) NaCl) at 37 °C and then subcultured in AKI medium (0.4% (w/v) yeast extract, 1.5% (w/v) peptone, and 0.5% (w/v) NaCl) in presence of freshly prepared 0.3% (w/v) sodium bicarbonate for 3 h.[29] *Vibrio parahemolyticus* (ATCC 17802) and *Staphylococcus aureus* (ATCC 6538) were cultured in LB medium at 37 °C for 12 h. Each cleared culture supernatant was obtained by centrifugation at 9 000*g* for 10 min and stored at 4 °C. Without further treatments including concentration, each cleared supernatant was directly applied onto the carbohydrate microarray.

RESULTS AND DISCUSSION

Analysis of the Interactions between Ctx Proteins and Carbohydrates. For ctx-carbohydrate interaction analysis,

dx.doi.org/10.1021/ac301511t | *Anal. Chem.* 2012, 84, 6884−6890

Table 1. Carbohydrates Used in This Work and Their Sequences

Carbohydrate	Saccharide	Sequence	Symbol[a]
GM1	pentasaccharide	Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc	
GM2	tetrasaccharide	GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc	
Asialo GM1	tetrasaccharide	Galβ1-3GalNAcβ1-4Galβ1-4Glc	
GM3	trisaccharide	Neu5Acα2-3Galβ1-4Glc	
Galβ1-3GalNAc	disaccharide	Galβ1-3GlcNAc	
Lactose	disaccharide	Galβ1-4Glc	
Sialic acid	monosaccharide	Neu5Ac	

[a] ■, glucose; ●, galactose; □, *N*-acetylgalactosamine; ★, *N*-acetylneuramic acid

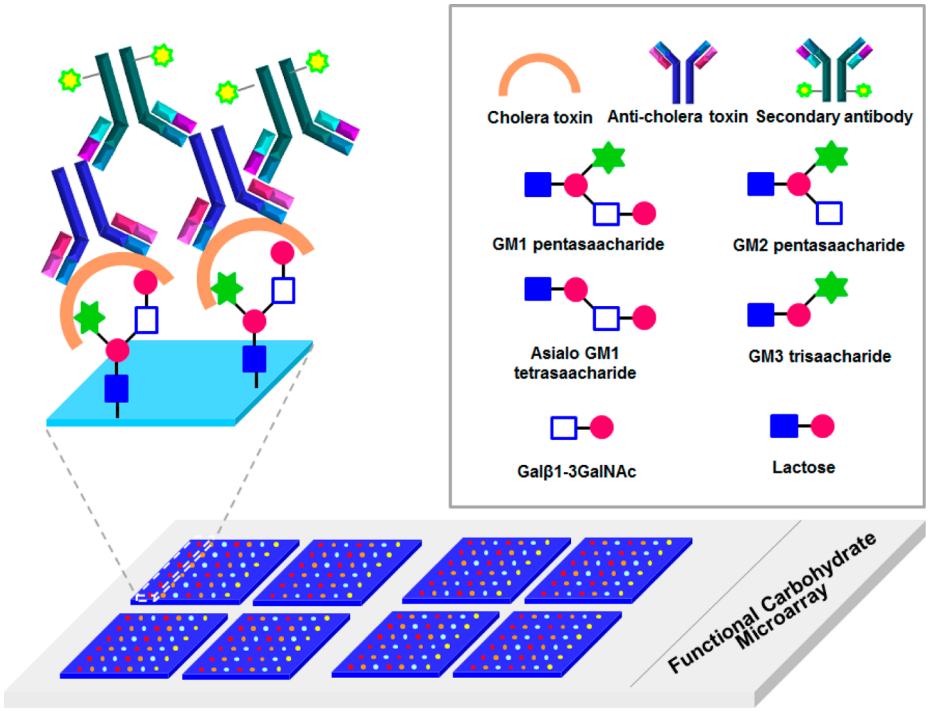


Figure 1. Schematic representation of the interaction analysis of ctx proteins and seven GM1-related carbohydrates and detection of ctx using a carbohydrate microarray combined with a fluorescence immunoassay.

seven types of GM1-related carbohydrates were modified (Table 1). A functional carbohydrate microarray was constructed by immobilizations of the modified carbohydrates (Figure 1) based on our previously established method.[24] Interactions between ctx and the carbohydrates were detected through fluorescence immunoassay. First, we performed an analysis of the intrinsic selectivity of ctxB by comparing the interactions of ctxB with the GM1-related carbohydrates on the carbohydrate microarray. We observed that the selectivity of ctxB was as follows: GM1 pentasaccharide ≫ GM2 tetrasaccharide > asialo GM1 tetrasaccharide ≥ GM3 trisaccharide (Figure 2). This result was consistent with previous reports in general, although the trend was somewhat different depending on the analytical tools used.[9,11−17] X-ray crystallography showed that the terminal galactose (Gal), *N*-acetylgalactosamine (GalNAc), and *N*-acetylneuraminic acid (Neu5Ac) residues contribute 39%, 17%, and 43% of the intermolecular contact in the ctxB-GM1 interaction.[15] In addition, the terminal Gal and Neu5Ac contribute 54% and 44% of the intrinsic binding energy of the ctxB-GM1

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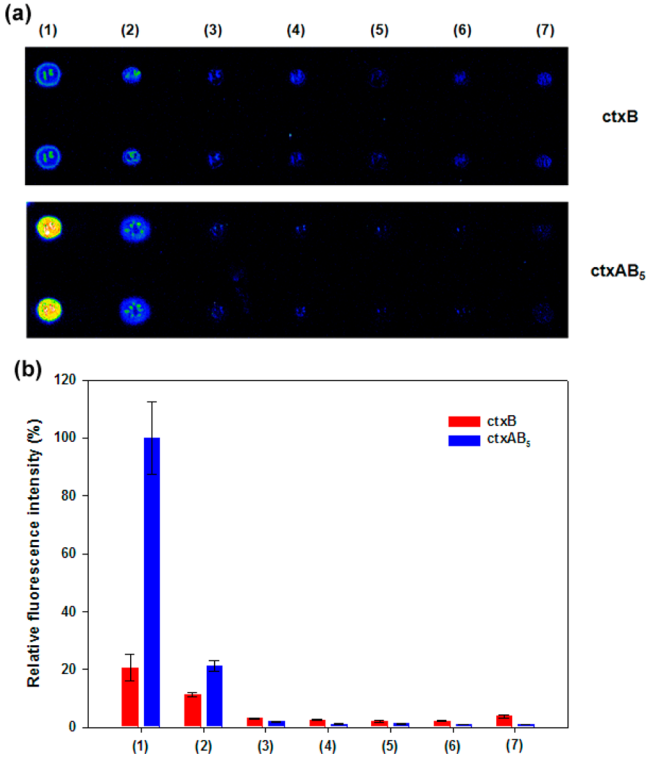

Figure 2. Interaction and affinity analyses of ctx proteins with seven GM1-related carbohydrates on the carbohydrate microarray. (a) Scanned raw images of the arrayed interaction and (b) the fluorescence intensity plot. (1) GM1 pentasaccharide, (2) GM2 tetrasaccharide, (3) asialo GM1 tetrasaccharide, (4) GM3 trisaccharide, (5) Galβ1-3GalNAc, (6) lactose, and (7) Neu5Ac. Each value is the mean of 24 independent spots, and the error bars represent the standard deviation.

interaction.[17] Taking into consideration previously reported contribution levels of each monosaccharide residue for the ctxB-GM1 interaction, it can be speculated that the GM2 and asialo GM1 tetrasaccharides, which lack the terminal Gal and the Neu5Ac from the GM1 pentasaccharide structure, respectively, will yield similar fluorescence intensities. However, we found that the GM2 tetrasaccharide (~21% fluorescence intensity of the GM1 pentasaccharide) showed higher fluorescence intensity than the asialo GM1 tetrasaccharide (~2% fluorescence intensity of the GM1 pentasaccharide) (Figures 2(2),(3)). These results indicate that the ctxB-carbohydrate interaction could not be explained simply by the contribution levels of the GalNAc, Gal, and Neu5Ac monosaccharides and that the carbohydrate conformation may also affect the interaction. A study describing the substrate specificity of ctxB toward the GD1a and GD1b hexasaccharides (an addition of Neu5Ac to the terminal Gal and the Neu5Ac of the GM1 pentasaccharide structure, respectively) also supports this explanation.[10] Because the Gal binding site provides conformational flexibility in ctxB-substrate binding, a terminal GalNAc residue of GM2 may interact with the Gal binding site, and a Neu5Ac residue maybe repositioned within ctxB to form an alternative binding mode.[9,12,17,30] This hypothesis is reinforced by the report that the FucGM1 hexasaccharide (an addition of fucose (Fuc) to the terminal Gal of the GM1 pentasaccharide structure) and GM1 have a similar binding affinities to ctxB.[16] The asialo GM1 tetrasaccharide and the GM3 trisaccharide showed low fluorescence intensities, but Galβ1-3GalNAc, lactose, and Neu5Ac showed very weak intensities (Figure 2). Although lactose itself interacts with ctx weakly, the internal lactose unit of GM1 pentasaccharide may be attributed to form the structure of GM1 pentasaccharide suitable for ctx binding.[31] Overall, we surmise that both the "two-finger grip (bivalent)" formation[15] and the constituent terminal monosaccharides (GalNAc, Gal, and Neu5Ac)[15,17] play significant roles in ctx-carbohydrate interactions.

Next, we compared the binding affinity and substrate specificity of two toxin proteins, $ctxAB_5$ and ctxB, with the GM1-related carbohydrates. Only a few studies have examined this comparison.[14,32] In this study, the molar concentration of ctxB used was approximately 5 times higher than that of $ctxAB_5$. Importantly, we found that $ctxAB_5$ had a stronger (~5-fold) binding affinity toward the GM1 pentasaccharide than did ctxB (Figure 2(1)). The tight binding of $ctxAB_5$ to the GM1 pentasaccharide results from a positive cooperation between the subunits and from the multivalent interaction of $ctxAB_5$ with the GM1 pentasaccharide.[33−37] This result was strongly supported by previous reports that a ctxB pentamer had a higher binding affinity than a ctxB monomer due to interactions among neighboring ctxB molecules,[14] and the GM1-$ctxAB_5$ interaction showed a higher binding force than GM1-ctxB.[32] We found that $ctxAB_5$ had the same intrinsic selectivity with ctxB toward GM1-related carbohydrates (GM1 ≫ GM2 > asialo GM1 ≥ GM3) (Figure 2). However, ctxB showed relatively higher fluorescence intensities than $ctxAB_5$ on several GM1 analogues, not including the GM2 tetrasaccharide, although these differences in fluorescent intensities were not significant. From these results, we hypothesize that $ctxAB_5$ has strict substrate specificity on only bivalent-forming carbohydrates, while ctxB has flexible substrate specificity on monovalent- as well as bivalent-forming carbohydrates. This result may be due to complexation of the cholera toxin protein. With these results, we confirmed that our developed carbohydrate microarray has powerful potential for the functional analysis of protein-carbohydrate interactions and can provide important information on substrate specificity and ctx complexation effects.

Quantitative Analysis of $CtxAB_5$-GM1 Pentasaccharide Interaction. For quantitative analysis of ctx-GM1 pentasaccharide interactions, $ctxAB_5$ was used as the target ctx because it is the actual complete toxin produced and secreted by *V. cholerae*. First, we investigated the sensitivity of $ctxAB_5$-GM1 interactions on the carbohydrate microarray using various concentrations (0−10 $\mu g\ mL^{-1}$) of applied $ctxAB_5$. The limit-of-detection (LOD) was defined as the lowest concentration of the sample that produced a fluorescence intensity 3-fold higher than the standard deviation of the intensity without a sample, as determined from a dose−response curve. We determined that the LOD for the $ctxAB_5$-GM1 interaction was approximately 2 ng mL^{-1} (≈ 23 pM), while the visual limit was approximately 5 ng mL^{-1} (≈ 58 pM) (Figure 3). This LOD value is much more sensitive than the reported values from monosaccharide-based microarrays[38] and disaccharide-based colorimetric assays[39] and is comparable to other high sensitivity liposome-based assay systems.[40−46] The spot intensities increased according to the applied $ctxAB_5$ concentrations, showing a dynamic analytical range between 5 ng mL^{-1} and 1 $\mu g\ mL^{-1}$ (Figure 3). Importantly, a strong linear correlation between the fluorescence intensity and the $ctxAB_5$ concentration was shown in the range of 10−200 ng mL^{-1} (inset of Figure 3b). When a concentration greater than 1 $\mu g\ mL^{-1}$ was

dx.doi.org/10.1021/ac301511t | *Anal. Chem.* 2012, 84, 6884−6890

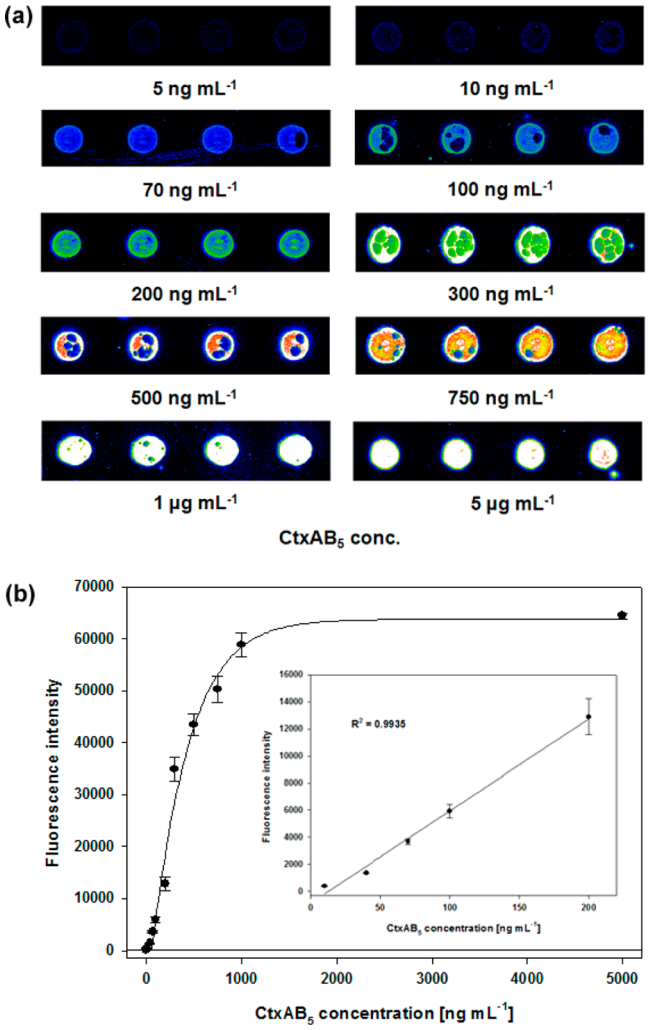

Figure 3. Quantitative analysis of the ctxAB$_5$-GM1 pentasaccharide interaction on the carbohydrate microarray. (a) Scanned raw images of the ctxAB$_5$-GM1 interactions on the carbohydrate microarray immobilized with 10 mM GM1 pentasaccharide according to various ctxAB$_5$ concentrations (0−5 μg mL^{-1}). (b) Dose−response curve describing the fluorescence intensity in relation to ctxAB$_5$ concentration (inset, plot for lower (10−200 ng mL^{-1}) ctxAB$_5$ concentrations). Each value is the mean of 24 independent spots, and the error bars represent the standard deviation.

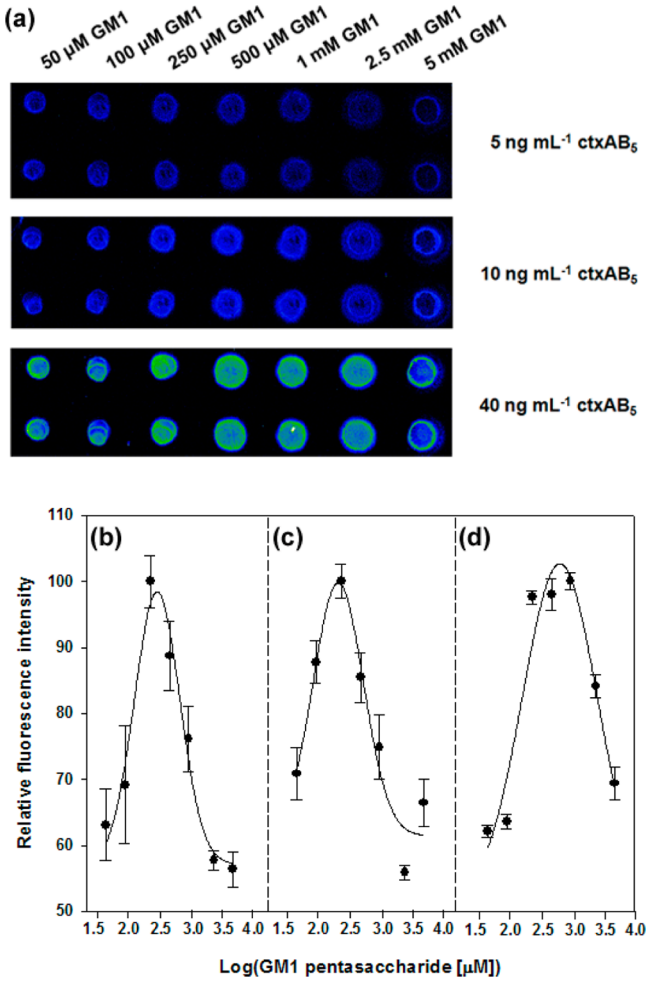

Figure 4. The effect of arrayed GM1 pentasaccharide concentration on the sensitivity of the ctxAB$_5$-GM1 interaction. (a) Scanned raw images of the ctxAB$_5$-GM1 interactions on the carbohydrate microarray immobilized with various concentrations (50 μM−5 mM) of the GM1 pentasaccharide according to several ctxAB$_5$ concentrations (5, 10, 40 ng mL^{-1}). Fluorescence intensity plots of (b) 5 ng mL^{-1}, (c) 10 ng mL^{-1}, and (d) 40 ng mL^{-1} ctxAB$_5$. Each value is the mean of 24 independent spots, and the error bars represent the standard deviation.

applied on the carbohydrate microarray, the fluorescence intensity reached saturation. In addition, we also performed quantitative analyses of the GM1 analogues-ctxAB$_5$ interactions. The GM2 tetrasaccharide-ctxAB$_5$ interaction was visually detectable up to 750 ng mL^{-1} of ctxAB$_5$, while the other GM1 analogues interacted with ctxAB$_5$ only at high concentrations (>5 μg mL^{-1}) (data not shown).

The amount of immobilized GM1 pentasaccharide may also affect the sensitivity of the ctxAB$_5$-GM1 interaction on the carbohydrate microarray. Various concentrations of the GM1 pentasaccharide (50 μM−5 mM) were immobilized on the microarray to interact with various concentrations of ctxAB$_5$ (5−40 ng mL^{-1}). Interestingly, there was an optimal GM1 pentasaccharide concentration range (~250−500 μM) for efficient interactions with all tested ctxAB$_5$ concentrations (Figure 4). We speculate that the density of the GM1 pentasaccharide affects its interaction with the toxin protein on the carbohydrate microarray, which is a key factor in determining the sensitivity of the ctxAB$_5$-GM1 interaction. In multivalent binding systems, the ligand density is an important factor because it influences the ligand distribution and the distance between ligands.[47−49] The study of the GM1 clustering effect on ctx binding revealed that the binding signal decreased with increased GM1 density.[50] In addition, ctx binding to an intestinal microvillus membrane with varying GM1 densities showed stronger binding signals at lower GM1 concentrations.[51] These previous studies support our speculation that the GM1 pentasaccharide density plays an important role in the effective interaction of ctxAB$_5$-GM1. Therefore, the sensitivity of the ctxAB$_5$-GM1 interaction can be enhanced by using the optimal amount of immobilized GM1 pentasaccharide. This enhanced interaction of ctxAB$_5$ and GM1 can increase the feasibility of using a carbohydrate microarray for the efficient detection of actual ctx from contaminated water or foods.

Functional Detection of Cholera Toxin in *V. Cholerae* Culture. Because many people around the world still suffer from *V. cholerae* contaminated water or foods and because even

dx.doi.org/10.1021/ac301511t | *Anal. Chem.* 2012, 84, 6884−6890

very small amounts of ctx can be fatal,[8,52] sensitive and specific detection methods are required.[38−46] We investigated the applicability of our developed carbohydrate microarray system for the specific detection of actual ctx proteins produced by *V. cholerae*. Without further steps, cleared supernatant from the *V. cholerae*-culture medium was applied directly onto the carbohydrate microarray, which was spotted with seven GM1-related carbohydrates as capture probes. We found that the actual toxins from the cultured medium were efficiently detected on the GM1- and GM2-immobilized spots of the carbohydrate microarray (Figure 5a). The high sensitivity of the carbohydrate microarray enabled the detection of ctx within a relatively short incubation time (~3 h). Next, the specificity of our carbohydrate microarray was evaluated by checking for cross-reactivity with the culture media of two other pathogens: *V. parahemolyticus* and *S. aureus*. Importantly, no fluorescence intensities were shown for the other pathogen samples (Figure 5b,c), indicating that we can use the developed carbohydrate microarray system for highly specific detection of the actual cholera toxin (Figure 5d). We checked feasibility of the developed carbohydrate microarray as a practical biosensor for ctx detection. Detailed studies on specificity and sensitivity of the carbohydrate microarray system for ctx detection in food or water sample will be carried out in the future.

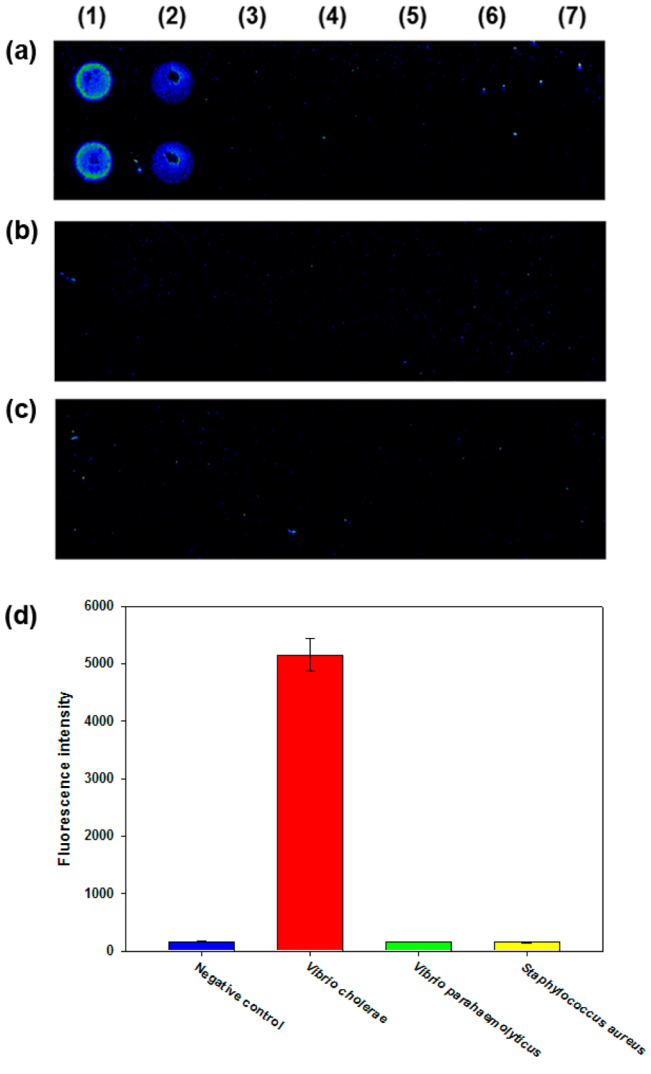

Figure 5. Specific detection of the actual cholera toxin from *V. cholerae* culture using the carbohydrate microarray. Scanned raw images for (a) *V. cholerae*-, (b) *V. parahemolyticus*-, and (c) *S. aureus*-cultured samples, and (d) fluorescence intensity plot for GM1 pentasaccharide spots. Each cleared culture supernatant was applied to the carbohydrate microarray without further treatments. (1) GM1 pentasaccharide, (2) GM2 tetrasaccharide, (3) asialo GM1 tetrasaccharide, (4) GM3 trisaccharide, (5) Galβ1-3GalNAc, (6) lactose, and (7) Neu5Ac. Each value is the mean of 24 independent spots, and the error bars represent the standard deviation.

CONCLUSIONS

In the present work, we demonstrated the potential of a carbohydrate microarray as a functional analytical tool for quantitatively describing interactions between ctx proteins and GM1-related carbohydrates. The carbohydrate microarray enabled us to analyze the interactions of $ctxAB_5$ or ctxB with several carbohydrates simultaneously using a small amount of sample and provided important information on the interactions. First, both the conformation of the carbohydrate and its constituent terminal monosaccharides affected the interaction significantly. Second, the $ctxAB_5$ complex had ~5-fold stronger binding affinity and a stricter substrate specificity than ctxB alone. Finally, the immobilized carbohydrate density was an important factor for the interaction, and there was an optimal range for the immobilized GM1 density (~250−500 μM). The carbohydrate microarray showed high sensitivity in detecting ctx-GM1 interactions, with an LOD of about 23 pM. This high sensitivity increased the feasibility of applying the carbohydrate microarray as a biosensor for actual ctx detection from *V. cholerae* culture without cross-reactivity. Therefore, our proposed carbohydrate microarray system can be successfully used as a versatile tool for functional analysis of diverse protein-carbohydrate interactions and as a biosensor for carbohydrate-protein interaction-based infection.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Marine Biomaterials Research Center grant from the Marine Biotechnology program funded by the Ministry of Land, Transport and Maritime Affairs, Korea.

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