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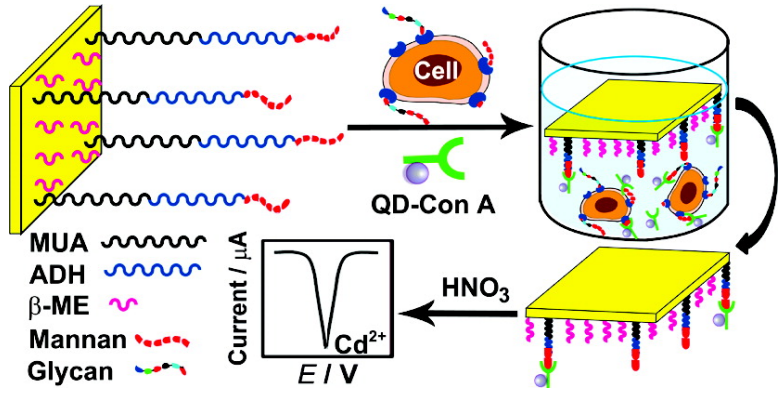
J. Am. Chem. Soc., **2008**, 130 (23), 7224-7225 • DOI: 10.1021/ja801468b • Publication Date (Web): 20 May 2008



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Published on Web 05/20/2008

Carbohydrate Monolayer Strategy for Electrochemical Assay of Cell Surface Carbohydrate

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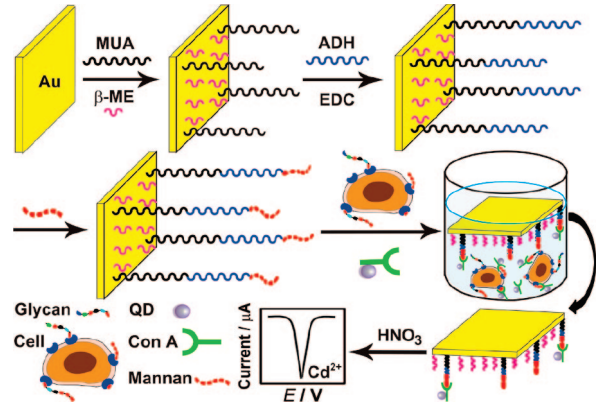
The main objective of chemical biology is to unravel the complex chemical mechanisms underlying biological processes. Carbohydrates that exist on most cells play important roles in cell growth and development,[1] immune recognition/response,[2] signal transduction,[3] cell−cell communication,[2] and tumor growth and metastasis.[2] The principal obstacle for elucidation of carbohydrate functions is the lack of toolboxes for reliable identification of carbohydrate expression patterns on cell surfaces due to the complexity of the glycocode itself.[2]

Some carbohydrate microarrays have been presented for the high-throughput evaluation of carbohydrate/protein interactions,[4] and several analytical strategies based on mass spectrometry,[5] fluorescence microscopy,[6a] flow cytometry,[6b] and lectin array-based platform[7] have also been proposed for analysis of cell surface carbohydrates. Mass spectrometry can categorize the glycomes, but it faces intrinsic difficulties owing to isomeric variations of oligosaccharides and is not amenable to living cell interrogation due to its destructivity.[5] Lectins are a class of proteins that exhibit highly specific binding affinity for carbohydrates, thus they provide valuable tools for glycan analysis.[6b,7,8] The lectin array-based platform simplifies the detection of glycans on cells.[7] However, the detection process involves change of living cell behaviors by the used fluorescent labels. More importantly, the issues of active site accessibility and lectin denaturation in the surface immobilization format with high density impair the sensitivity and stability of this method.[4a,9]

Herein we report a strategy for in situ analysis of cell surface carbohydrate by integrating biomimetic carbohydrate monolayer, competitive recognition in a one molecule−two surfaces format, and elemental analysis of QDs by electrochemistry and using a mannose moiety as a model analyte (Scheme 1). Self-assembled monolayer (SAM) has the ability to present a wide range of organic functionality with nearly atomic-scale precision[10] and offers exquisite control over molecular density, pattern, and orientation.[4c] The accurate reflection of carbohydrate composition of the cell surface can be achieved on the artificial carbohydrate monolayer through the selective binding of concanavalin A (Con A), a lectin, to mannan. The monolayer shows good stability and high sensitivity because carbohydrates do not suffer from above issues associated with protein arrays, due to the participation of only small ligand structural motifs in biochemical interactions.[11] The surface-confined mannan competes effectively with cell surface mannose moieties to recognize quantum dot (QD)−Con A conjugates, which is then detected by anodic stripping signal of QDs[12,13] for the quantitative assay of cell surface carbohydrate.

The QD−lectin conjugates were prepared by coupling CdTe QDs with Con A (see Supporting Information). The conjugates could

Scheme 1. Schematic Representation of the Monolayer Fabrication and the Competitive Assay



selectively bind to mannose moieties on cell surface, which was verified by a blocking experiment (Figure S2 in Supporting Information), using a type of adherent tumor cell (BGC-823) for convenient manipulation. After reaction with the conjugates for 1 h, the cells displayed distinct fluorescence; in contrast, initial reaction of Con A with cells led to occupation of the mannose sites, which prevented subsequent binding of the conjugates.

The fabrication of a robust multivalent carbohydrate scaffold is critical for emulating the natural settings of cell surface carbohydrates[14] to perform the competitive assay. A SAM of 11-mercaptoundecanoic acid (MUA) with proper density was first formed by using β-mercaptoethanol (β-ME) as the diluent molecule. Mannan was then coupled to MUA by employing adipic dihydrazide (ADH) as a linker. The step-by-step construction of the carbohydrate monolayer could be demonstrated with an electrochemical probe (Figure S3 in Supporting Information) and atomic force microscopic (AFM) images (Figure 1A−C). After reacting with QD−Con A, the mannan-derivatized surface morphology showed a larger particle size (Figure 1D), indicating effective recognition of QD−Con A to mannan, while QD−nonmannose-binding lectin or protein conjugates could not bind to the mannan monolayer (Figure S4 in Supporting Information).

For validation of the methodology, K562 cells, whose surface has abundant mannose moieties, were chosen for competition with the mannan-derivatized gold substrate to bind the QD−Con A conjugates. After the competition, QDs captured on the gold substrate were dissolved with HNO_3 to produce a solution containing Cd^{2+} for anodic stripping voltammetric detection of the cell surface mannose moieties. In comparison of the responses of the QD−Con A conjugates to mannan, mannose, and K562 cells in solutions, only mannan and K562 cells could show efficient binding to the conjugates (Figure S5 in Supporting Information), thus this

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10.1021/ja801468b CCC: $40.75

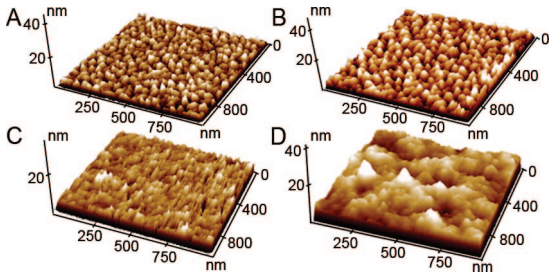

Figure 1. Topographic images of (A) MUA/Au, (B) ADH/MUA/Au, (C) mannan/ADH/MUA/Au, and (D) QD−Con A/BSA-blocked mannan/ADH/MUA/Au.

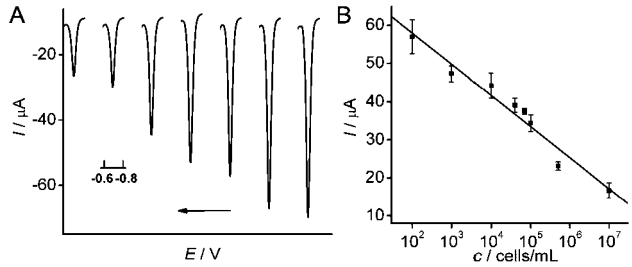

Figure 2. Competition between cells and mannan monolayer for binding to QD−Con A. (A) Anodic stripping voltammograms of QDs captured by mannan-modified gold substrates competing with 0, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 cells/mL cells (right to left). (B) Linear calibration plot. Square-wave stripping detection was performed by 4 min deposition at −1.1 V on a mercury film modified glassy carbon electrode (7 mm^2) and scanning from −0.9 to −0.2 V at an amplitude of 25 mV and a frequency of 15 Hz.

work used mannan to perform the detection for ensuring the effective competition. In spite of the exclusive existence of C-type lectins (Ca^{2+}-dependent lectins) on the K562 cell surface, these cell surface lectins did not affect the competitive recognition for detection of cell surface mannose moieties (Figure S6 in Supporting Information).

Ca^{2+} and Mn^{2+}, which were essential for lectin−carbohydrate interaction, led to aggregation of QDs at high concentrations. Their suitable concentrations were 0.1 mM, at which recognition reaction rate was fast enough for analytical purpose and no aggregation was observed (Figure S7 in Supporting Information). The optimized concentration of QD−Con A conjugates and recognition time were 0.5 μM and 50 min, respectively (Figure S8 in Supporting Information). Under constant gentle shaking, the conjugates in solution could effectively access all the active surface carbohydrate sites on the cell.

The proposed method exhibited sensitive response to K562 cell surface carbohydrates (Figure 2A). Upon addition of K562 cells in the recognition solution, the decrease of the stripping peak current was initially fast and then slow, which was attributed to the competition of the active carbohydrate sites on K562 cells with the surface-confined mannan to bind QD−Con A conjugates. The calibration curve showed a linear relationship between the peak current and the logarithm of cell concentration in a wide range down to 10^2 cells/mL (Figure 2B).

To quantify the ability of cell surface carbohydrates to bind lectin, mannan was employed to replace K562 cells for the same experiment, and the linear relationships between stripping peak current and the amounts of cells and mannan were analyzed. The appropriate amounts were in the ranges of 1000−8000 cells and 0.01−0.10 μg mannan, respectively (Figure S5 in Supporting Information). Assuming they had the same binding kinetics, the average Con A binding capacity of single K562 cell could be estimated to correspond to 6.9 pg or 2.3×10^{10} mannose moieties, which was slightly higher than $(4.9 \pm 0.6) \times 10^9$ mannose moieties obtained with an enzymatic method,[15] due to the inevitable loss of mannose in the destructive sample preparation for enzymatic analysis (see Supporting Information).

In conclusion, this work provides a novel protocol based on a surface-confined carbohydrate strategy for convenient in situ evaluation of cell surface carbohydrate sites of interest. This protocol is specific due to the specific interaction between lectin and the corresponding carbohydrate. This strategy integrates the advantages of surface assembly, nanotechnology, bioconjugate techniques, and electrochemical detection. In spite of the limit of available lectins, this technology could be expanded for other carbohydrates with the addition of more glycan/lectin(antibody) interaction pairs to the repertoire.[11a] Owing to the good stability, convenient labeling, and multiple coding capability of QDs, high-throughput profiling of cell surface carbohydrates could be achieved by using more pairs of QDs−lectins and carbohydrates under compromising salt and pH conditions, which could be optimized due to the not-so-narrow windows of salt concentration and pH values for favorable lectin−carbohydrate interactions.[16] We expect that this technique will contribute considerably to meeting the challenges in unraveling the complex mechanisms underlying biological processes related to carbohydrates.

Acknowledgment. We gratefully acknowledge the National Science Funds for Creative Research Groups (20521503), the Major Research Plan (90713015), and Key Program (20535010) from the National Natural Science Foundation of China.

Supporting Information Available: Detailed description of experiment, characterization of specific binding activity of conjugates and the carbohydrate monolayer, comparison of binding capability to QD−Con A conjugates, stability of QDs, and condition optimization. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA801468B