Versatile aptasensor for electrochemical quantification of cell surface glycan and naked-eye tracking glycolytic inhibition in living cells

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\textbf{ABSTRACT}

Quantifying the glycan expression status on cell surfaces is of vital importance for insight into the glycan function in biological processes and related diseases. Here we developed a versatile aptasensor for electrochemical quantification of cell surface glycan by taking advantage of the cell-specific aptamer, and the lectin-functionalized gold nanoparticles acting as both a glycan recognition unit and a signal amplification probe. To construct the aptasensor, amine-functionalized mucin 1 protein (MUC1) aptamer was first covalently conjugated to carboxylated-magnetic beads (MBs) using the succinimide coupling (EDC-NHS) method. On the basis of the specific recognition between aptamer and MUC1 protein that overexpressed on the surface of MCF-7 cells, the aptamer conjugated MBs showed a predominant capability for cell capture with high selectivity. Moreover, a lectin-based nanoprobe was designed by noncovalent assembly of concanavalin A (ConA) on gold nanoparticles (AuNPs). This nanoprobe incorporated the abilities of both the specific carbohydrate recognition and the signal amplification based on the gold-promoted reduction of silver ions. By coupling with electrochemical stripping analysis, the proposed sandwich-type cytosensor showed an excellent analytical performance for the ultrasensitive detection of MCF-7 cells and quantification of cell surface glycan. More importantly, taking advantage of Con A-gold nanoprobe catalyzed silver enhancement, the proposed method was further used for naked-eye tracking glycolytic inhibition in living cells. This aptasensor holds great promise as a new point-of-care diagnostic tool for analyzing glycan expression on living cells and further helps cancer diagnosis and treatment.

1. Introduction

Cell surface glycan play important roles in many biological and pathological processes including cell differentiation, cell–cell communication, immune response, inflammation, and tumor cell metastasis (Cao et al., 2015b; Palecek et al., 2015). Changes in the expression of cell-type specific glycan are often associated with a variety of diseases, especially cancers (Rambaruth and Dwek, 2011). Thus, effective tools for the quantification of cell surface glycan expression are essential not only for understanding their roles in disease development but also for early diagnosis (Bertok et al., 2013).

Many analytical approaches have been developed for glycan analysis, including mass spectrometry (Zhang et al., 2016b), nuclear magnetic resonance (Barb and Prestegard, 2011), and flow cytometry (Robinson et al., 2015). However, these methods usually required relatively expensive equipments and specialized personnel, which are sample-consuming and labor intensive. To address these limitations, we and others have taken advantage of glycan-binding lectins (Ding et al., 2011; Li et al., 2015), such as concanavalin A (ConA), and integrated them with electrochemical (Liu et al., 2015a; Shao et al., 2010; Xue et al., 2010; Zhang et al., 2010a, 2010b, 2015b), electro-chemiluminescence (Chen et al., 2013; He et al., 2015; Wu et al., 2016; Zhou et al., 2014), surface enhanced raman scattering (Tabatabaei et al., 2016), and fluorescent techniques (Cao et al., 2012a, 2012b) to measure glycan expression in living cells. In addition, by coupling the nanostructured biointerfaces, these lectin-based biosensors offer attractive advantages of high sensitivity, convenient operation, and rapid detection (Cao et al., 2015a; Dechtrirat et al., 2014; Qian et al., 2012; Wang et al., 2013; Wu et al., 2015; Xia et al., 2013; Zhang et al., 2011c). Although promising, these techniques often suffer from low specificity and stability because of the issues of active site accessibility and low affinity of the lectin and cell surface glycan. This is especially

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true in the case of glycan analysis in cancer since lectins to cancer cells cross-react with normal cells, which may lead to non-specific test results. Therefore, it is necessary and remains a challenge to develop feasible biosensors with sufficient sensitivity and specificity to detect glycan on cancer cell surface (Su et al., 2015).

Isolated via a technique called systematic evolution of ligands by exponential enrichment (SELEX), aptamers are DNA or RNA sequences that can bind tightly and selectively to a wide range of small molecules (Zhang et al., 2016a), proteins (Lu and Liu, 2006), and cells (Liu et al., 2011a; Meng et al., 2015). In a comparison with lectins for cell recognition, aptamers have distinct advantages including a small size, a tunable structure, high resistance against denaturation, and programmable sequences which make them powerful specific recognition ligands for targeting numerous cancers (Jia et al., 2016; Mira et al., 2015; Shen et al., 2016; Sun et al., 2016; Wang et al., 2015). Moreover, by combining the inherent features of nanomaterials with the specific recognition ability of aptamers, a range of aptamer-conjugated nanomaterials have proven their utility in advanced cancer diagnostics (Chen et al., 2014; Dirkwazer et al., 2016).

On the other hand, due to the fact that most cancer cells exhibit increased glycolysis, glycolytic inhibition has been considered as an effective tool in cancer treatment (Beneteau et al., 2012; Xu et al., 2005). Many glycolytic inhibitors, such as 3-bromopyruvate and 2-deoxyglucose (2-DG), are expected to be candidate drugs for cancer treatment, and hence screening research on these inhibitors has potential for cancer therapy (Pelicano et al., 2006). Although these glycolytic inhibitors have been found to be inhibitors of key glycolytic enzymes, it remains a challenge to evaluate whether these glycolytic enzyme inhibitors actually modulate glycolysis in living cells. From this perspective, the establishment of a simple, sensitive and specific cell-based sensing platform to evaluate glycolytic inhibition is still an urgent demand.

The aim of the present investigation was to design a versatile aptasensor for electrochemical quantification of cell surface glycan by taking advantage of the cell-specific aptamer, and the lectin-functionalized gold nanoparticles acting as both a glycan recognition unit and a signal amplification probe. By coupling with electrochemical stripping analysis, the proposed sandwich-type cytosensor showed an excellent analytical performance for the ultrasensitive detection of cancer cells and quantification of cell surface glycan. More importantly, taking advantage of Con A-gold nanoprobe catalyzed silver enhancement, the proposed method also provided a reliable and convenient tool for naked-eye tracking glycolytic inhibition in living cells.

2. Experimental section

2.1. Materials and Reagents

Concanavalin A (Con A), fluorescein-labeled Con A, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS), 2-deoxyglucose (2-DG), silver enhancer solution A and B, tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (U.S.A.). Chloroauric acid (HAuCl₄) and trisodium citrate were obtained from Shanghai Chemical Reagent.
Co. Ltd. (Shanghai, China). Mannose with analytical grade was from Sinopharm Chemical Reagent Co. Ltd. (China). Phosphate buffer saline (PBS, pH 7.4) contained 137 mM NaCl, 2.7 mM KCl, 87 mM Na$_2$HPO$_4$ and 14 mM KH$_2$PO$_4$. All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Fig. 1. (A) SEM image of the prepared Fe$_3$O$_4$ nanoparticles. (B) FTIR spectra of Fe$_3$O$_4$ (curve a) and Fe$_3$O$_4$-Aptamer (curve b). (C) UV–vis spectra of 2.2 nM Au nanoparticles in water (curve a) and incubation buffer (curve c), and (curve b) 10.0 nM AuNPs-ConA nanoprobes in incubation buffer. Inset: optical image of Au nanoparticles in water (a) and incubation buffer (b), and AuNPs-Con A nanoprobes in water (c) and incubation buffer (d). (D) TEM images of AuNPs-Con A nanoprobes. Inset is the corresponding HRTEM image.

Fig. 2. The effect of the concentration of AuNPs-Con A nanoprobes (A) and the time of silver enhancement (B) on the electrochemical signal of the cytosensor.
MUC1 binding aptamer was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequence: 5′-NH2-GCA GTT GAT CCT TTG GAT ACC CTG G-3′.

2.2. Apparatus

Transmission electron microscopic (TEM) image was observed under a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Scanning electron micrographs (SEM) were obtained with a Hitachi S4800 scanning electron microscope. Confocal laser scanning microscopy (CLSM) studies were performed using a Leica TCS SP5 microscope (Germany) with excitation at 488 nm. Electrochemical measurements were performed on a CHI 660c workstation (Shanghai Chenhua Apparatus Corporation, China) with conventional three-electrode system comprised of a platinum wire as the auxiliary, a Ag/AgCl (saturated with KCl) as the reference, and a glassy carbon electrode (GCE) as the working electrode. Phase-contrast images were acquired using a Nikon TE2000-U inverted fluorescent microscope (Japan). Scanning electron micrographs (SEM) were obtained with a Leica TCS SP5 microscope. Optical images of sandwich format after silver enhancement was performed in the 96-well plate using gel image systems (Bio-Rad). The relative intensity of each well was scored using Quantity One (Bio-Rad), a quantitative analysis software program.

2.3. Synthesis of aptamer functionalized Fe3O4 nanoparticles (Apt-MNPs)

The aptamer functionalized Fe3O4 nanoparticles were synthesized by conjugate Amine-modified aptamer with carboxyl-functionalized Fe3O4 nanoparticles (MNPs) using the EDC/NHS reaction. Briefly, MNPs were first prepared via a microwave-assisted heating method as previously described (Liu et al., 2011b). Then, 0.5 mg of as-prepared MNPs was suspended in 1 mL of PBS, and mixed with 200 μL of 10 mg mL−1 EDC and 200 μL of 10 mg mL−1 NHS for 40 min to activate the terminal carboxyl group on the surface of MNPs. After that, 30 μL of 1 mM amine-aptamer was added, and incubated for 12 h with gentle shaking. The formed Apt-MNPs conjugate was separated from the incubation solution and washed three times with PBS, and finally suspended in 0.5 mL of PBS with a concentration of 1.0 mg mL−1.

2.4. Preparation of concanavalin A labeled gold nanoprobe (Con A-AuNPs)

AuNPs with a diameter of 15 nm were prepared according to the literature by adding a sodium citrate solution to a boiling HAuCl4 solution (Zhang et al., 2011a). The prepared AuNPs solution (2.2 nM) was stored at 4 °C before use. For Con A labeling, 5 mL of 2.2 nM AuNPs were mixed with 50 μL of Con A, and the pH of the mixture solution was adjusted to 9.0. Then, the mixture was incubated for 12 h at 25 °C with gentle stirring. Finally, the mixture was concentrated to 1 mL by centrifugation (15 000 g, 20 min, 4 °C), washing, and resuspension with incubation buffer (10.0 nM).

2.5. Cell culture and treatment

MCF-7 cells (human breast adenocarcinoma) were cultured in a flask in DMEM medium ( Gibco, Grand Island, NY, supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL−1), and streptomycin (100 μg mL−1) in an incubator (5% CO2, 37 °C). At the logarithmic growth phase, the cells were trypsinized and washed twice with sterile pH 7.4 PBS by centrifugation at 1000 rpm for 10 min. The sediment was then resuspended in 0.1 M pH 7.4 PBS containing 1 mM Ca2+ and 1 mM Mn2+ to obtain a homogeneous cell suspension. Here, the divalent cations Ca2+ and Mn2+ were used to ensure the effective binding between cell surface carbohydrate and ConA-AuNPs nanorobes. The cell number was determined using a Petroff-Hauser cell counter. 2-DG treated MCF-7 cells were obtained by incubating the cells in a culture medium containing different concentration of 2-DG. The incubation time varies from 0 to 48 h.

2.6. Preparation of the Cytosensor and electrochemical/optical analysis

In a clear-bottomed 96-well plate (Corning Costar), 50 μL of 1.0 mg mL−1 Apt-MNPs suspension was mixed with 150 μL of PBS buffer containing MCF-7 cells of a certain concentration, and was incubated at 37 °C for 30 min. After three times of magnetic separation and washing with PBS, the Apt-MNPs and MCF-7 complex (Apt-MNPs/MCF-7) was further mixed with 150 μL of PBS buffer and 50 μL of 10 nM ConA-AuNPs, and incubated at 37 °C for 30 min. After repeatedly separated by magnetic field and washed with PBS for three times, the sandwich format of Apt-MNPs/MCF-7/ConA-AuNPs were obtained. Finally, the silver enhancer was performed on each well by reaction with 20 μL 1:1 mixture of silver enhancer solutions A and B for 5 min. All silver enhancement experiments were performed under dark conditions at room temperature, and light exposure was avoided. After rinsed with ultrapure water and dried under a stream of nitrogen, the resulting 96-well plate was scanned using gel image systems (Bio-Rad). For electrochemical analysis, the deposited Ag was dissolved in 50 μL of 0.1 M HNO3 solution, and the resulting solution was mixed with 450 μL of 0.1 M KNO3 (pH 7.0) to perform differential pulse voltammetry (DPV) detection with a positive scan from 0.05 to 0.4 V (vs. Ag/AgCl saturated with KC) at 50 mV amplitude.

2.7. Flow cytometric analysis

Untreated and 2-DG treated MCF-7 cells were collected by centrifugation, washed with sterile cold PBS, and re-suspended in 500 μL of binding buffer. The cell concentration was determined. Then, 50 μL of 1×104 cell mL−1 suspension and 445 μL of PBS were mixed with 5 μL of 1 mg mL−1 fluorescein-labeled Con A, and incubated at room temperature for 30 min. The cells were collected by centrifugation, washed with PBS, and immediately analyzed on the FACS Sort flow cytometer (Becton Dickinson, USA) and CLSM with excitation at 488 nm. Fluorescent intensity of unlabeled MCF-7 cells was used for estimation of autofluorescence.