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Carbohydrate derivative-functionalized biosensing toward highly sensitive electrochemical detection of cell surface glycan expression as cancer biomarker

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ABSTRACT

Accurate and highly sensitive detection of glycan expression on cell surface is extremely important for cancer diagnosis and therapy. Herein, a carbohydrate derivative-functionalized biosensor was developed for electrochemical detection of the expression level of cell surface glycan (mannose used as model). Thiomannosyl dimer was synthesized to design the thiomannosyl-functionalized biosensor by direct and rapid one-step protocols. The biosensing surface-confined mannose could effectively mimic the presentation of cell surface mannose and was responsible for competing with mannose on cancer cells in incubation solution. Greatly enhanced sensitivity was achieved by exploiting the excellent conductivity of multiwalled carbon nanotube/Au nanoparticle (MWNT/AuNP), the amplification effect of MWNTs, and the favorable catalytic ability of horseradish peroxidase (HRP). Using competitive strategy, the developed biosensor exhibits attractive performances for the analysis of mannose expression with rapid response, high sensitivity and accuracy, and possesses great promise for evaluation of cell surface glycan expression by using a greater variety of lectins.

1. Introduction

Glycans, the key components of cell surface glycolipids and glycoproteins, play critical roles in fundamental biological events including bacterial pathogenesis, inflammation, and cancer cell metastasis (Ohtsubo and Marth, 2006; Helenius and Aebi, 2001; Haslam et al., 2006; Zhang et al., 2011; Matsumoto et al., 2009). According to research, the expression levels of glycans can be acted as therapeutic targets or clinical biomarkers to convey information about the physiological state of cells (Ding et al., 2009; Zhang et al., 2010; Hollingsworth and Swanson, 2004; Gorelik et al., 2001). Therefore, it is urgent to develop simple technology for sensitive and accurate assay of glycan expression on cell surface.

Consequently, many kinds of biosensors were developed for glycan analysis based on optical, electrochemical, and piezoelectric transducers (Park et al., 2009; Dai et al., 2006; Lyu et al., 2008; Svarovsky and Joshi, 2014). Among them, electrochemical biosensors have been well recognized to be a promising solution for signal transduction due to the fact that electrochemical detectors are simple, portable and inexpensive (Zhang et al., 2009; Peng et al., 2014; Zheng et al., 2014). Combining with nanotechnology in materials science, electrochemical biosensor opens up new horizons for the highly sensitive determination of biological and environmental analytes because of the nanomaterial-based signal amplification platform (Zhang et al., 2014; Ma and Nakazato, 2014). For example, a high sensitive biosensor based on FePt/CNTs nanocomposite/N-(4-hydroxyphenyl)-3,5-dinitrobenzamide modified carbon paste electrode was developed for simultaneous determination of glutathione and piroxicam (Karimi-Maleh et al., 2014). The 2,2′-[1,2-ethanediylbis(nitriloethylidyne)]-bis-hydroquinone double-wall carbon nanotube paste electrode was reported for simultaneous determination of epinephrine, uric acid and folic acid (Beitollahi et al., 2008). A highly sensitive nanostructure-based electrochemical sensor was described for electrocatalytic determination of norepinephrine in the presence of acetaminophen and tryptophan (Mazloum-Ardakani et al., 2011). Carbon paste electrode modified with cobalt porphyrin and TiO_2 nanoparticles was used for the determination of levodopa in the presence of carbidopa (Mazloum-Ardakani et al., 2012).

However, regarding the assay of cell surface glycans, many researchers are enthusiastic in looking for effective ways to improve

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the analysis performance due to the lack of electroactive groups in glycans. In this respect, various methods based on labeling technologies with electroactive moieties, enzymes, interactive substances, and nanomaterials have been developed to enhance the electrochemical signal (Singh et al., 2013; Zhang et al., 2015; Lotierzo et al., 2012; Scavetta et al., 2014). Among these labels, enzymes have attracted broad attentions because of their easy labeling and especially signal amplification for sensitivity enhancement (Zhao et al., 2014; Jeong et al., 2013).

With the aim of designing a successful biosensor, the applied biomaterials for sensing interface are crucial for improving biosensor performance. Inspired by the concept of DNA and protein biosensors, carbohydrate-functionalized biosensors, which are composed of carbohydrate attached to a solid surface, have recently been developed as reliable and efficient tools for the assay of biological analytes (Pussak et al., 2013; Shin et al., 2005; Martin et al., 2013). This technology not only facilitates fast and sensitive analysis of a large number of biomolecules, but also effectively mimics the presentation of glycans on living cell surface to exhibit multivalent interactions with receptors (Bian et al., 2014; Mader et al., 2012). Regarding the construction of the carbohydrate-functionalized biosensor, the efficient immobilization of carbohydrates is an important factor because carbohydrates do not have functional groups (Yamada et al., 2013). Most of the attempted strategies for carbohydrate immobilization so far rely on multistep protocols aimed at indirect immobilization. However, the utilization of these methods might be hindered owing to the biosensor requirement for one-step protocols with direct and rapid immobilization (Seo et al., 2007). Alternatively, the development of carbohydrate derivatives containing anchor groups (e.g., thiols, disulfides, amines, selenols) to functionalize electrode surfaces provides a simple and rapid avenue for the biosensor preparation (Zou et al., 2008; Deng et al., 2011).

In this work, an amplified carbohydrate derivative-functionalized electrochemical biosensor was developed for competitive assay of glycan expression on living cancer cells. Here, mannose present on cancer cells derived from human lung, liver, and prostate was used as a model glycan. A thiol-derivatized carbohydrate (thiomannosyl dimer, $[\text{mannose–S}]_2$), comprising of disulfide portion [S–S] and mannose portion, was synthesized to design the biosensor by direct and rapid one-step protocols, in which the disulfide part anchored the molecule and the mannose portion was responsible for competing with glycan on cell surface. The multiwalled carbon nanotube/Au nanoparticle (MWNT/AuNP)-modified glassy carbon electrode (GCE) was used as substrate to construct the thiomannosyl-functionalized biosensor (GCE/MWNT/Au–S–mannose). Owing to the large surface area and excellent conductivity of MWNT/AuNP, the substrate not only provided a highly suitable environment for thiomannosyl dimer assembling through Au–S bond but also played a significant role in signal enhancement. The {Con A–MWNT–HRP} bioconjugates, as recognition elements, were fabricated by exploiting the amplification effect of MWNTs for loading enormous horseradish peroxidase (HRP) labels and mannose-specific concanavalin A (Con A) (Fig. 1a). Unlike the two-step sandwich analysis, the present protocol relied on a rapid and one-step competitive assay, in which the biosensing surface-confined mannose competed with cell surface mannose to recognize the {Con A–MWNT–HRP} bioconjugates by the specific interaction between mannose and Con A. The analysis of cell surface mannose was performed based on the catalytic reaction of HRP toward the oxidation of hydroquinone (QH_2) by H_2O_2, which could introduce further signal enhancement (Fig. 1b). Under optimal conditions, the proposed biosensor exhibited attractive performances for the analysis of cancer cells with wide linear ranges and low detection limits. The average amount of mannose on single cell surface was also evaluated to be 1.3×10^{10} molecules for QGY-7703 (liver cancer cell), 5.8×10^{10} molecules for A549 (lung cancer cell), and 1.9×10^{10} molecules for LNCaP (prostate cancer cell). Therefore, the carbohydrate derivative-functionalized electrochemical biosensor gives a useful protocol for glycan assay with high sensitivity, accuracy and rapid response, and may help the medical diagnosis and treatment in the early process of cancer.

2. Experimental

2.1. Reagents and materials

Concanavalin A (Con A), horseradish peroxidase (HRP, MW

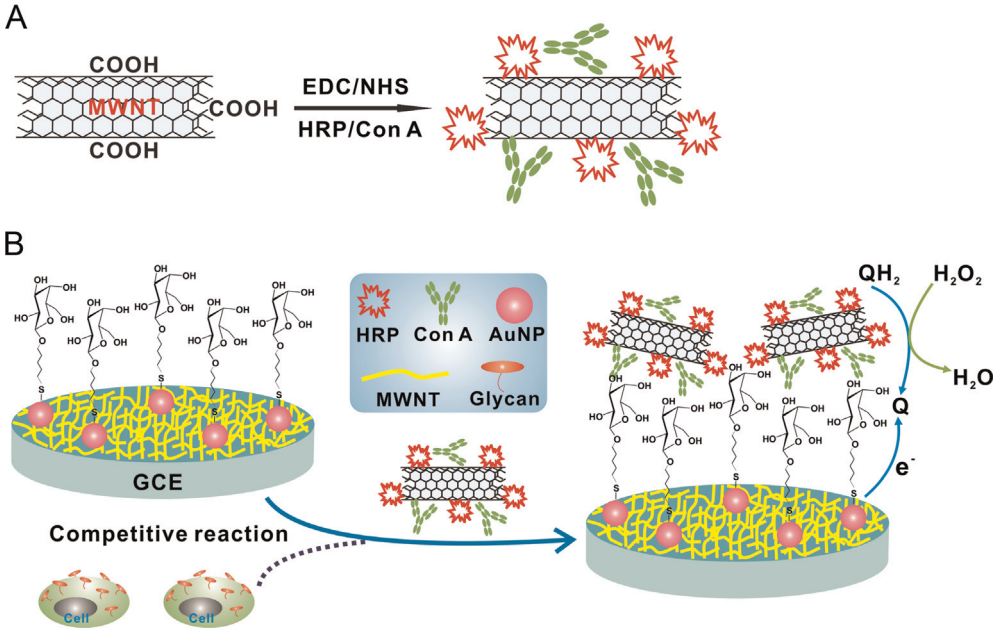

Fig. 1. Schematic illustration of (a) the preparation of the {Con A–MWNT–HRP} bioconjugates and (b) the thiomannosyl-functionalized electrochemical biosensor for competitive assay of mannose expression on cancer cells.