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Does polysaccharide is an idea template selection for glycosyl imprinting?

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ABSTRACT

A novel glycoprotein imprinting strategy was proposed and was applied to the detection of the carbohydrate antigen 19-9. The glycosylated complex of glycoprotein was used as template to construct a glycosyl imprinted sensor. The eluted imprinted cavities showed good affinity for template glycosyls and glycoproteins carrying template glycosyl. The effect of template saccharide structure on glycosyl imprinted sensors is further discussed. More complex template structures can lead to better sensor performance including selectivity and sensitivity. As a result, the polysaccharide imprinted sensor showed preeminent linear response to CA19-9 in the range of 0.1–5 U/mL, with a detection limit of 0.028 U/mL (3δ/K), while the linear of the monosaccharide imprinted sensor was 1–60 U/mL and the detection limit was 0.17 U/mL. The complex structure on the template surface provides more possibilities for the recognition of the template molecules, consequently, led to the significant anti-interference capability of the polysaccharide imprinted sensor. Furthermore, recoveries ranging from 93.0% to 103.5% were achieved when human serum samples were assayed using the polysaccharide imprinted sensor.

1. Introduction

Pancreatic adenocarcinoma (PAC) is one of the most deadly cancers in the world. Biomarkers for the early detection of pancreatic adenocarcinoma are greatly important to increase survival. As the only biomarker used routinely in the management of PAC, carbohydrate antigen 19-9 (CA19-9) plays a significant role in the early detection of malignant tumors since it has 79–81% sensitivity and 82–90% specificity for diagnosis (O'Brien et al., 2015). The conventional methods, such as enzyme-linked immunoassay (ELISA) (Goonetilleke and Siriwardena, 2007), radio-immunoassay (Del Villano et al., 1983; Ker et al., 1991), and chemiluminescent immunoassay (Shi et al., 2014; Wei et al., 2011), are always limited by the long response time, expensive reagents, and high false-positive results. Therefore, the establishment of a high-sensitivity and high-accuracy detection assay for CA19-9 has good potential in tumor detection.

The molecular imprinting technique (MIT) (Chen et al., 2011; Whitcombe et al., 2014), which has been called the construction of a "plastic antibody", is a powerful tool in analytical science because of its high recognition specificity and high sensitivity. Nevertheless, the application of MIT in the macromolecules, such as protein, still faces major challenges because the greater mass transfer resistance during the imprinting of these large molecules as compared with small molecules. The imprinting of carbohydrates and glycoproteins is also one of the hotspots of molecular imprinting (Li et al., 2013; Shekarchizadeh et al., 2013; Wang et al., 2014; Ye et al., 2014). Glycosylation is a common feature of tumor cells (Carlin et al., 2007; Kodar et al., 2012; Terraneo et al., 2013); some types of structures are recognized as markers of tumor development. The study of Lewis expression in different tumor samples showed that the structure of sialyl Lewis^A (SLe^a) is often over-expressed in the serum of tumor patients; the expression of SLe^a was confirmed on the surface of tumor antigen CA19-9 (Kannagi, 2003; Tempero et al., 1987). As a precursor of CA19-9, SLe^a is a tetrasaccharide carbohydrate attached to O-glycans on the surface of cells. It plays a vital role in cell-to-cell recognition processes. The structure of SLe^a can be expressed as α-NeuAc-(2→3)-β-D-Gal-(1→3)-(α-L-Fuc-[1→4])-D-GlcNAc3′-SLe^a. On the other hand, as a terminal monosaccharide of SLe^a, sialic acid is the basic component of several glycoproteins, glycopeptides, and glycolipids, which exists in naturally-occurring carbohydrates with biological functions. *N*-Acetylneuraminic acid (NeuAc) is one of the most widely distributed forms of sialic acid. Herein, we established an efficient and simple CA19-9 detection sensor via glycosyl imprinting. Compared with the epitope imprinting (Li et al., 2015), glycosyl imprinted polymers (GIPs) can be prepared with short glycosyl fragments as

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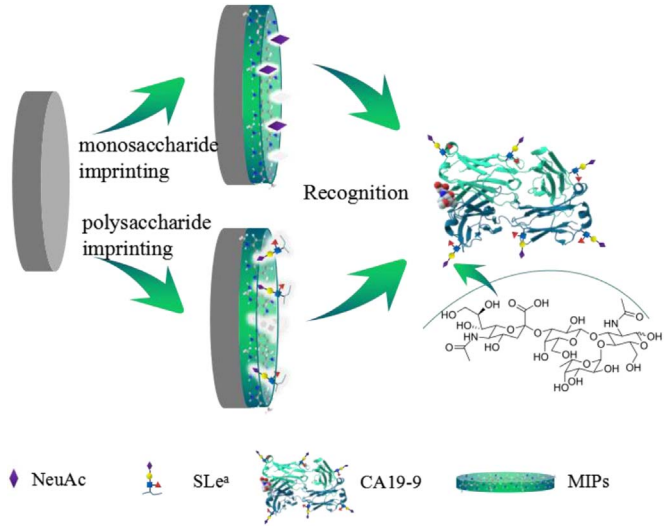

Scheme 1. Construction of a CA19-9 sensor based on glycosyl imprinting.

template to identify specific glycoproteins with the same glycosyl fragment, so glycosyl imprinting do not require confirmation of the specific structure of the target protein. The sensor constructed by glycosyl imprinting was shown in Scheme 1. First, the GIPs were prepared via co-electropolymerzation with functional monomer and the glycosyl template. Then, the detection of CA19-9 was completed by detecting the current response to the ferricyanide probe after the elution and rebinding steps described in our previous work (Li et al., 2012b).

2. Material and methods

2.1. Apparatus and reagents

Electroanalytical measurements, such as differential pulse voltammetry (DPV) and cyclic voltammetry (CV), were obtained on a CHI600C work station (Shanghai Chenhua Instrument Co., Ltd., Shanghai, China). Electrochemical impedance spectroscopy (EIS) was performed on an Autolab work station (Metrohm China Co., Ltd.). All electrochemical behaviors were completed with a standard three-electrode system, which consisted of a Ag/AgCl electrode containing a saturated KCl solution as the reference electrode, a platinum wire electrode as the auxiliary electrode, and a GIP-modified glass carbon electrode (GCE, d = 3 mm) as the working electrode. The buffer solution was configured by a PHS-3C model pH meter (Shanghai Leici Instruments). The atomic force microscope (AFM) (NT-MDT, Ntegra Prima SPM) and Scanning electron microscope (SEM) (S4800 Hitachi Corporation of Japan) were used to observe the surface morphology of the GIPs electrodes. All measurements were conducted at 25 °C. NeuAc, *o*-Phenylenediamine (*o*-PD), bovine serum protein (BSA), bovine hemoglobin (BHb) D-mannose (D-man), glucose, DL-cysteine (DL-Cys), ascorbic acid (vitamin C, Vc) and uric acid were purchased from Aladdin Reagent (China) Co., Ltd. CA19-9 and neural cell adhesion molecule 1 (NCAM-1) were obtained from Shanghai Linc-Bio Science Co. Ltd. Concanavalin A (ConA) and 3′-sialyl Lewis^A tetrasaccharide were provided by Sigma-Aldrich Co. Ltd. The 5 × 10^{-3} mol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution contained 0.1 mol/L KCl. The 0.01 mol/L phosphate buffer saline (PBS) was prepared with sodium dihydrogen phosphate and disodium hydrogen phosphate dodecahydrate containing 0.1 mol/L KCl. All conventional reagents were analytical grade and used without further purification. All solutions were prepared with ultra-pure water from a high-purity water system (Youpu Super Water Company, Ltd., Chengdu, China).

2.2. Preparation of GIP sensor and non-GIP (nGIP) sensor

Firstly, The GCE was polished by chamois leather with 1.0, 0.3, and 0.05 μm of aqueous slurry of alumina. The polished electrode was alternately washed with water, alcohol, and H_2SO_4 (1 mol/L). Finally, GIP was prepared on a polished GCE by electro-polymerization. The electrolyte contained 5×10^{-3} mol/L *o*-PD and 5 × 10^{-3} mol/L SLe^a solution, which was dissolved in 0.02 mol/L PBS buffer (pH = 7.4). Thirty cycles of CV were performed in the potential range from 0 V to + 0.8 V at a scan rate of 50 mV/s in the above mentioned solution to form a GIP membrane. The preparation of the nGIP film was identical to that of GIP, but SLe^a was not added.

The prepared GIP and nGIP sensors were washed with carbinol/acetic acid (8:1 in volume) to remove the templates of the internal cavities or the adsorbed substance on the surface of the membrane. Thus, the GIP sensors with specific recognition cavities in imprinted membranes were obtained. During rebinding, the prepared sensors were immersed in solutions with different concentrations of target to record the current of the probe on the electrode. After each measurement, the GIP sensors were washed with carbinol/acetic acid (8:1 in volume) to remove the templates.

2.3. Electrochemical measurements

All the electrochemical signals was measured with a 5 × 10^{-3} mol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution containing 0.1 mol/L KCl. CV was performed over a potential range from −0.2 V to + 0.6 V at a scan rate of 100 mV/s. DPV measurements were performed over a potential range from −0.2 V to + 0.6 V at a potential increment of 4 mV and a pulse amplitude of 50 mV. EIS was performed at a potential of 0.19 V over the frequency range from 100 mHz to 100 kHz, with an alternating voltage of 5 mV.

3. Results and discussion

3.1. Formation of glycosyl imprinted membrane

o-PD is often used as a functional monomer to prepare the MIP which offering hydrophilic, hydrophobic and basic recognition sites with template molecules via electrostatic interactions (Camurri et al., 2005; Li et al., 2012a; Liu et al., 2013). As shown in Fig. S1a, the electropolymerization of *o*-PD on the GCE is an irreversible process. The CV behavior indicated two distinct and irreversible oxidation peaks at 0.38 and 0.7 V in the pH = 7.4 PBS buffer solution. Along with the polymerization, the oxidation peak current of *o*-PD continuously and dramatically decreased. When the number of cycles increased to 30, the current density of the oxidation peak became smaller. Therefore, a compact non-conducting polymer film was successfully fabricated on the electrode surface. NeuAc and SLe^a are non electro-activity in the potential range from 0 V to + 0.8 V, in the imprinting process, they did not participate in the polymerization reaction (Fig. S1b). It is suggested that large amounts of hydrogen bonds or electrostatic interaction are formed between the hydroxyl and the amino groups in *o*-PD due to the presence of active groups such as hydroxyl groups in the carbohydrate structure.

3.2. AFM and SEM characterization of GIP and nGIP

In order to further confirm the imprinted process, the GIPs surface morphology was investigated with atomic force microscopy (AFM). Fig. S2 depicts the 3D morphological characteristics and typical cross-section lines of AFM profiles between GIP film and the control film (n-GIP) at the electrode surface, where a to f are polysaccharide imprinted film, polysaccharide imprinted film after elution, monosaccharide imprinted film, monosaccharide imprinted film after elution and the n-GIP film, respectively. The AFM images reveal a marked difference in