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Design of carbohydrate/electron-transfer peptides for human histocytic lymphoma cell sensing

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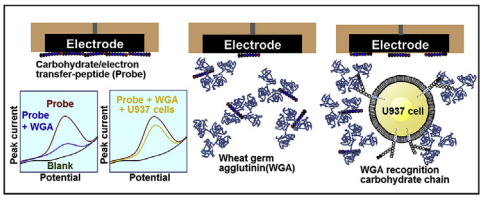
HIGHLIGHTS

- Carbohydrate/electron-transfer peptides were fabricated for electrochemical cell sensing.
- Cells were measured via a competitive reaction to a protein between carbohydrate chains on the cell surface and peptides.
- The concentrations obtained by this method agreed with those established via ELISA.

GRAPHICAL ABSTRACT



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ABSTRACT

A carbohydrate/electro-transfer peptide probe was fabricated to perform cell sensing. The probe consisted of a cello-oligosaccharide that was created by the conjugation of an electron-transfer peptide (Y_5C) and a carbohydrate via a Schiff base. An oxidation wave due to a phenolic hydroxyl group was obtained by scanning with a glassy carbon electrode. This cell-sensing system was based on a competitive reaction between carbohydrates on a cell surface and the probe as each reacted to a protein that recognized the carbohydrate. When amounts of the protein and probe were constant, the peak current of the probe was changed as the number of cells increased. A human histocytic lymphoma cell (U937 cell) with carbohydrates such as glucose and *N*-acetylglucosamine on its surface was selected as the target cell. Wheat germ agglutinin (WGA) binded to both the probe and the carbohydrates on U937 cells, which resulted in a linear peak current of the cellobiose/electron-transfer peptide at concentrations that ranged from 100 to 3500 cells/ml. The values of the cell sensing using this electrochemical method were consistent with those established via ELSIA. The sensitivity of this procedure, however, was two-fold superior to that of ELISA. Consequently, this carbohydrate/electron-transfer peptide could be a powerful tool for cell sensing and searching for carbohydrate chains on a cell surface.

1. Introduction

The carbohydrate chains that exist on cell surfaces are essential to life, which equates to their involvement in a variety of functions. Protein-carbohydrate interaction stimulates communication among the cells of these chains [1], and is controlled by thermodynamics and structural factors. Accordingly, there have been many investigations into how these changes can influence the ligand specificity of protein-carbohydrate binding [2–4]. In addition, cytosensing has been performed using interactions between

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carbohydrates and proteins because the carbohydrate chains of a cell surface are significant biomarkers in the diagnosis of cancer and disease [5–7]. For example, sensing systems based on electrogenerated chemiluminescence (ECL) have been reported. A biosensor for cells was developed using integration of the peptide-modified interface for highly specific carbohydrate recognition and sodium alginate-loaded glucose oxidase [8]. A measurement method for lectin-carbohydrate profiling and *in situ* cell surface carbohydrate expression was also proposed using gold nanoparticle catalyzed luminol ECL with a nanocarrier [9]. Chen et al. proposed a biosensor wherein concanavalin A is integrated with a gold-nanoparticle-modified ruthenium complex doped silica probe with monitoring of the cell surface via N-glycan expression [10]. In contrast, a scanometric strategy for the detection of mannose groups on living cells has also been carried out [11]. Xie et al. constructed a ratiometric graphene sensor for selective detection of live cells and pathogens that express mannose-binding proteins [12]. On the other hand, electrochemical procedures have been stablished to monitor cells using the binding between protein and carbohydrate. A strategy for the *in situ* analysis of cell surface carbohydrates by integrating carbohydrate layers and promoting competitive recognition in a one-molecule, two-surfaces format, and then measuring the QDs has been attempted [13]. Maltez-da Costa et al. suggested a cancer assay system using the electrocatalytic properties of gold nanoparticles for hydrogen evolution reactions [14]. For sensitive detection of K562 cells, sensing has been performed using the specific interaction between concanavalin A with a label and mannan [15]. Qian et al. reported monitoring cells via the measurements of sialic acids using a labeling technique and a dual-functionalized nanohorn probe [16].

Wheat germ agglutinin (WGA) is a lectin that combines with chito-oligosaccharides and cello-oligosaccharides. WGA has two different sites, and the binding constants for chito-pentaose are 10^9 (strong binding site) and 10^4 l/mol (weak binding site) [17]. The specific interactions between WGA and monosaccharides were investigated on self-assembled monolayers of neoglycoconjugates using surface plasmon resonance (SPR) and atomic force microscopy (AFM) force measurements [18]. We reported the voltammetric behavior of chito-oligosaccharides and WGA by labeling an oligosaccharide with an electroactive compound [19] that was then covered with WGA and used to measure the change in the electrode response that corresponded to the level of binding between carbohydrates and proteins. Because the label was the anticancer agent daunomycin, the need for the development of labels with biocompatibility was apparent. Recently, our group has proposed an electro-transfer peptide for the detection of protein at a 10^{-12} M level [20]. The electron-transfer peptide was made up of a cysteine residue bound to the C-terminals of oligotyrosine (YYYY). When the peptide was conjugated to a molecular recognition peptide, the sequential peptide behaved as a sensing probe for the protein. Furthermore, the sensing of U937 cells based on a cell-penetrating/apoptosis-inducing/electron-transfer peptide probe was carried out. This system was predicated on the peak current of electron-transfer peptides that results from selective uptake into cells [21]. Because the electron-transfer peptide has an excellent function as an electrochemical label, the detection of a cell may be achieved using the carbohydrate chain recognition of a protein between a carbohydrate/electron-transfer peptide and a carbohydrate chain on a cell surface. A compound with an electron-transfer peptide bound to a carbohydrate is expected to be effective as a sensing probe for proteins and cells.

In the present study, a carbohydrate/electron-transfer peptide probe with biocompatibility was designed for cell sensing. To test this system, we selected the human histocytic lymphoma cell (U937 cell) that expresses WGA recognition carbohydrate chains on the surfaces of cells. Because WGA recognizes glucose, cellobiose, and cellotriose, those carbohydrates were bound to an electron-transfer peptide. Y_4C was used in a previous study [20], but Y_5C was chosen as the electron-transfer peptide for the present study because the sequential probe was fabricated via a Schiff base reaction. To confirm the effect of the reaction, the peak current of carbohydrate/Y_5C was compared with that of carbohydrate/Y_4C. The greatest advantage is that a carbohydrate/electron-transfer peptide can be rapidly prepared and separated using a spin column. The method does not require the immobilization of a probe and a protein on a basal plate, and the measurement step is easily carried out. Binding changed the tyrosine residue at the N-terminals of Y_5C from single to double bonds. The electrode response was decreased due to the covering of the electron-transfer peptide stimulus from the WGA-carbohydrate binding. The aim of this study was to detect cells using probes that consist of carbohydrates and peptides that have biocompatibility. The electrochemical sensing of a cell is achieved by changes in the peak current caused from the competitive reaction to WGA between peptide/cell-saccharides, as measured by electron-transfer (Fig. 1). The electrode response of the probe with the cell and WGA is greater than that with WGA. The peak current of the probe with the cells and WGA was decreased compared with the value of the probe alone (The peak current of probe > The peak current of probe with the cells and WGA > The peak current of probe with WGA).

The binding between WGA and chito-saccharide is stronger than that with cello-saccharide [17]. When WGA binds to a carbohydrate chain on the cell surface, the formation of a peptide probe/WGA complex has priority over the combination between a carbohydrate chain and WGA. Therefore, a cello-saccharide/electron-transfer peptide was constructed to sense the cells. Since the system is a model of cell sensing that uses a carbohydrate/electron-transfer peptide, this concept may be applied to the fabrication of other carbohydrate/electron-transfer peptides. Furthermore, this electrochemical method for cell sensing compared favorably to conventional ELISA, which confirmed that the probe recognizes the carbohydrate chains on a cell surface.

2. Experimental

2.1. Apparatus

Voltammetric measurements were carried out using an ALS electrochemical analyzer Model 610D. A glassy carbon electrode (diameter (3 mm), Cat. No. 002012, BAS) was used as the working electrode. The electrode was polished using 1.0-, 0.3- and 0.05-μm alumina (Baikowski International Corp.). An Ag/AgCl electrode (sat. NaCl, Model N Cat. No. 012167, BAS) and a platinum wire were applied as the reference electrode and the counter. All potentials were measured against an Ag/AgCl electrode. The absorption spectra of the peptide probes and the absorbance of the reaction solution in ELISA measurement were recorded using a UV 1280 Shimadzu visible spectrophotometer.

2.2. Reagents

Culture medium (RPMI 1640), Concanavalin A, lectin from arachis hypogaea (peanut), lectin from phaseolus vulgaris, lectin from pisum sativum (pea), lectin from glycine max (soybean), lectin from triticum vulgaris (wheat germ agglutinin), fetal bovine serum, penicillin-streptomycin, cellobiose, and cellotriose all were purchased from Sigma-Aldrich. Limax flavus lectin was acquired from Cosmo Bio Co., Ltd. Glucose was supplied from Wako Pure Chemical Industries, Ltd. Electron-transfer peptides (Y_4C and Y_5C) were fabricated from a cysteine residue coupled with the C-terminals of