Sensors and Actuators B 186 (2013) 571–579

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Lectin-based optical sensing for quantitative analysis of cancer antigen CA15-3 as a breast cancer marker

Yoo Min Park[a], Su Jin Kim[a], Kangil Kim[b], Yong Duk Han[a], Sang Sik Yang[b], Hyun C. Yoon[a,*]

[a] Department of Molecular Science & Technology, Ajou University, Suwon 443749, Republic of Korea
[b] Department of Electrical & Computer Engineering, Ajou University, Suwon 443749, Republic of Korea

ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form 11 June 2013
Accepted 19 June 2013
Available online 28 June 2013

Keywords:
Fluoro-microbead guiding chip
Cancer antigen 15-3
Antibody–lectin sandwich assay
Affinity biosensor

ABSTRACT

Accurate detection of tumor-associated biomarkers remains a challenge in the diagnosis of cancer. To address the issue, we developed a sensitive biosensing system using lectins for breast cancer diagnosis based on the analysis of tumor-associated *O*-linked glycoprotein such as cancer antigen 15-3 (CA15-3). In cancerous cells, aberrant short-terminated glycan moieties such as TF-antigen and sialic acid are expressed on cell surfaces, becoming cancer antigens. Based on the difference between the glycan moieties in normal and cancer cells, CA15-3 was biospecifically detected using *Sambucus nigra* agglutinin (SNA) and peanut agglutinin (PNA) lectins. These lectins were conjugated with fluoro-microbeads and used as a detection molecule for an antibody–lectin sandwich assay. A fluoro-microbead guiding chip (FMGC) containing multiple sensing and fluidic channels was designed to measure CA15-3 proteins using the lectin-based assay. A capture antibody against CA15-3 that binds to the peptide backbone was immobilized on the gold-patterned sensing surface. On the modified FMGC, antibody–lectin sandwich assay for target CA15-3 was conducted. The developed PNA- and SNA-based assays, which exhibited a detection limit of 1.2 and 0.4 U/mL, worked well over the clinically important range from 1.25 to 25 U/mL CA15-3. The developed biosensor showed good sensitivity, fast response and reproducibility with a small volume of sample. This lectin-based sandwich assay provides a promising tool for the quantitative diagnosis of CA15-3 and glycated markers in clinical applications.

1. Introduction

Cancer-associated protein markers are present in the blood, and are important for early diagnosis of cancer [1]. Cancer marker proteins may be produced by cancer cells, by the body's reaction to cancer, or by other various conditions [2]. There are many types of breast cancer markers that can be used to obtain an early diagnosis [3,4]. Cancer antigen 15-3 (CA15-3) is one of the most accepted markers for the early detection of breast cancer [5,6]. Its concentration in healthy human blood is maintained usually less than 30 U/mL. However, as breast cancer progresses, CA15-3 is overexpressed on cell surfaces and secreted into the blood. Previous studies have shown that CA15-3 levels in the blood of patients with breast cancer were significantly higher than in healthy person [6–8]. A number of optical and electrochemical methods have been developed to measure CA15-3 [9–12]. However, these methods require complex treatments to generate the electrochemical reaction or the secondary detection molecules to induce an optical signal. The goal of the present study was to develop a simple and rapid biosensing system for CA15-3 using its molecularly distinct glycan properties without additional enzymes or complex treatments.

CA15-3 is a glycoprotein and a member of the mucin family. Mucins, the main class of membrane bound *O*-linked glycoproteins, contain a repeating amino acid sequence that is rich in serine and threonine [13]. In healthy humans, *O*-linked glycans have a linear polylactosaminic chain that can be fucosylated [14]. In contrast, breast cancer cells express incomplete *O*-linked glycans, resulting in the aberrant expression of short *O*-linked glycan chains with dense Thomsen–Friedenreich (T/TF) antigens or sialylated TF-antigens [13,15–17]. The truncated TF-antigen on the breast cancer-associated protein includes the sugar N-acetylgalactosamine (GalNAc) or sialylated GalNAc. In particular, GalNAc is linked to galactose on the surfaces of cancer-associated mucins [18,19] (Fig. 1). Thus, this study employed specific lectins to target the cancer-specific *O*-linked glycan moiety on the CA15-3 antigen.

Lectins are of natural non-immune origin and are glyco-binding proteins that recognize and bind to specific carbohydrate epitopes [20]. For this reason, lectins are frequently used for carbohydrate,

* Corresponding author. Tel.: +82 31 219 2512; fax: +82 31 219 2394.
E-mail address: hcyoon@ajou.ac.kr (H.C. Yoon).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.06.060

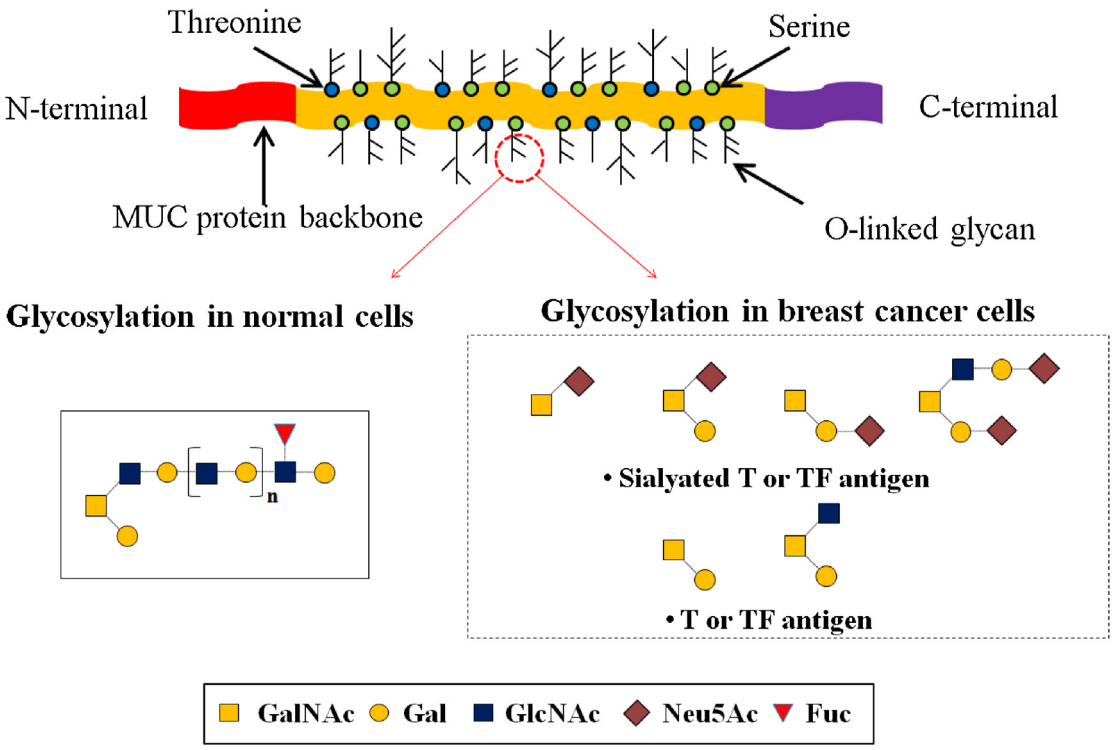

Fig. 1. The molecular structure of the CA15-3 antigen. Mucin (MUC) backbone and the differently expressed *O*-linked glycan chains found in normal and cancerous cells are schematically shown.

glycoprotein, and cell analysis [21–25]. In the current study, the *Arachis hypogeal*/peanut agglutinin (PNA) and *Sambucus nigra* agglutinin (SNA) were selected as signaling probes to materialize PNA-based and SNA-based assays that biospecifically bind with TF-antigen and sialic acid on CA15-3, respectively [14,26]. As a capturing molecule on the biosensing surface, a monoclonal antibody which specifically recognizes the peptide backbone of CA15-3 was utilized to sterically guarantee the required epitopes for lectin binding. By using these distinct molecular affinities for CA15-3, an antibody–lectin sandwich assay system could be designed [27].

Here, we fabricated an improved version of a fluoro-microbead guiding chip (FMGC) capable of simple and sensitive detection of CA15-3 using the antibody–lectin sandwich assay. We have previously reported a fluorescence biosensor for cardiac troponin I by employing an antibody–antibody sandwich format [28]. This immunosensing chip allowed for simultaneous measurement and quantitative analysis of various concentrations of biomarkers with a small volume of sample. However, the previous system has a limitation in terms of utility to the target marker proteins having antibody pair available for sandwich assay.

In the FMGC-based antibody–lectin sandwich assay, PNA and SNA lectins are conjugated with carboxylated fluoro-microbeads [29] and are used as detection molecules in a sandwich format. CA15-3 capture antibody was immobilized on the sensing area of FMGC using a self-assembled monolayer. Samples of various concentrations of CA15-3 were applied to the FMGC, and the optical signals from the FMGC were observed using a fluorescence microscope (Fig. 2). Quantitative analysis of various CA15-3 concentrations was conducted by counting the number of beads in the sensing area on the FMGC. By comparing the biospecific signal on the pattern in the FMGC with nonspecific signal (NSB) from the pattern outside, higher signal sensitivity was obtained. By employing the lectin–antibody pair and optimizing affinity sensing conditions, the determination of CA15-3 was effectively conducted, covering the required clinical detection range and signaling limit. Details are reported herein.

2. Experimental

2.1. Chemicals and apparatus

3-3′-Dithiobis-propionic acid N-hydroxysuccinimide ester, sodium periodate, L-lysine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Triton X-100, and ethanolamine, were purchased from Sigma–Aldrich. N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS) was from Fluka. Bovine serum albumin (fraction V) was obtained from Bioworld. CA15-3 protein antigen (Alpha diagnostic, CA153-N-10) and anti-CA15-3 mouse monoclonal IgG (Alpha diagnostic, CA1531-M) were used. CA15-3 ELISA was purchased from Fujirebio diagnostics. *S. nigra* agglutinin (SNA) and peanut agglutinin (PNA) lectins were purchased from Vector laboratories. FluoSpheres carboxylate-modified microspheres (excitation wavelength 540 nm and emission wavelength 560 nm, 2% solids, diameter = 200 nm) was purchased from Invitrogen. Polyethylene glycol (PEG, MW = 3.4 kDa) was purchased from Polyscience. Polydimethylsiloxane (PDMS) and its curing agent were from Dow Corning.

A phosphate-buffered saline solution containing 0.1 M phosphate and 0.15 M NaCl (pH 7.2), 50 mM 2-(N-morpholino) ethanesulfonic acid (MES, pH 6.0), and 10 mM HEPES containing 0.15 M NaCl and 0.1 mM $CaCl_2$ were prepared in doubly distilled and deionized water with a specific resistance greater than 18 MΩ-cm. PBST-1 (50 mM PBS, 150 mM NaCl, 0.1% Triton X-100, pH 7.4) and PBST-2 (50 mM PBS, 150 mM NaCl, 0.1% Triton X-100, 0.1% bovine serum albumin, pH 7.4) were used to wash the chip and dilute the fluoro-microbeads. Fluorescence images were taken with a charge-coupled device (CCD) camera connected to a fluorescence