Biosensors and Bioelectronics 87 (2017) 164–170

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

High sensitive and selective electrochemical biosensor: Label-free detection of human norovirus using affinity peptide as molecular binder

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ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
1 August 2016
Accepted 11 August 2016
Available online 12 August 2016

Keywords:
Human norovirus
Affinity peptide
Molecular binder
Limit of detection
Sensitivity
Biosensor

ABSTRACT

Norovirus is known as the major cause of highly infection for gastrointestinal tracts. In this study, robust and highly sensitive biosensors for detecting human norovirus by employing a recognition affinity peptide-based electrochemical platform were described. A series of amino acid-substituted and cysteine-incorporated recognition peptides isolated from evolutionary phage display technique was chemically synthesized and immobilized to a gold sensor layer, the detection performance of the gold-immobilized synthetic peptide-based sensor system was assessed using QCM, CV and EIS. Using EIS, the limit of detection with Noro-1 as a molecular binder was found to be 99.8 nM for recombinant noroviral capsid proteins (rP2) and 7.8 copies/mL for human norovirus, thereby demonstrating a high degree of sensitivity for their corresponding targets. These results suggest that a biosensor which consists of affinity peptides as a molecular binder and miniaturized microdevices as diagnostic tool could be served as a new type of biosensing platform for point-of-care testing.

1. Introduction

Norovirus is the most common cause of foodborne-disease outbreaks worldwide. Norovirus exposure occurs mainly from contaminated water or polluted food, and infection can rapidly spread from person to person through fecal-oral routes (Beier et al., 2014; Bu et al., 2008; Chung et al., 2015). Infection with norovirus often occurs with relatively low amounts of virus, as low as 1×10^2 copies/mL (Yakes et al., 2013), however, there are no reports of development of vaccines or tremendous anti-noroviral agents. Considering these issues, a platform that yields rapid, sensitive, and accurate detection of norovirus is desirable (Han et al., 2014; Kim et al., 2015). Currently, there are several methods utilized for efficient detection of the relative levels of norovirus in a sample, such as nucleic acid amplification (Ishida et al., 2008), antibody-based immunoassay (Hong et al., 2015; Yoda et al., 2000), real-time PCR (Suffredini et al., 2011), and other methods (Kwon et al., 2010; Yakes et al., 2013). However, these methods require multiple steps, a long time for sample preparation, and relatively expensive analytical instruments (Park et al., 2010). One of the major issues with these methods is that they are not ready-to-use with high sensitivity and are not suitable for point-of-care clinical applications.

Many studies have focused on providing promising alternative methods for more sensitive and accurate detection of norovirus, such as aptamer-based electrochemical biosensing platforms (Beier et al., 2014; Giamberardino et al., 2013) and miniaturized microfluidic biochips (Chung et al., 2015). In fact, the miniaturized electrochemical detection technique has been widely used for the creation of straightforward label-free diagnostic biosensors capable of real-time monitoring (Wu et al., 2010a, 2010b, 2011; Zhang et al., 2014). The advantages of using an electrochemical method are that it is cost-effective, produces a fast response, is label-free, and is easy to integrate into miniaturized microdevices like portable biosensors (Hong et al., 2015; Shin et al., 2011; Wu et al., 2011; Yea et al., 2016). For example, quartz crystal microbalance (QCM) has been widely used in biosensor development (Geng et al., 2008; Ogi et al., 2011; Wu et al., 2011). With this system, two parameters including frequency (related to mass and thickness)

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http://dx.doi.org/10.1016/j.bios.2016.08.031
0956-5663/

and dissipation (related to rigidity) are measured simultaneously in real time on the sensor surface (Wu et al., 2011). In addition, both cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) are widely used to measure either the current or the impedance obtained by varying the applied potential (Geng et al., 2008; Wu et al., 2011) upon the binding of targets. Therefore, there is no doubt that electrochemical-based bioanalytical techniques are powerful tools for studying molecular binding events such as peptide-protein interactions on a gold surface.

In aforementioned above, current antibody-based immunoassay has been used for clinical applications. This method relies on antibodies as affinity element and is relatively expensive and multi-step processing for sample preparation. Polyclonal antibodies are relatively cheaper, but heterogeneous to binding properties, while monoclonal antibodies are expensive to produce, but homogeneous. In modern clinical practice, more rapid, sensitive and cost-effective diagnoses for norovirus are crucial. Although the progress has been made in ready-to-use applications, improved biosensing platforms are still needed. Several studies have addressed these pitfalls on the affinity element with the use of peptide (Park et al., 2010; Wu et al., 2010a, 2010b), peptide nucleic acid (Mateo-Marti et al., 2007; Wang et al., 2009), aptamer (Kwon et al., 2010) and nanobody (Chen et al., 2016). In comparison to the molecular weight or size of antibodies, peptides are relatively small, and it can be cost-effectively synthesized. The molecular structures of antibodies are standard units with different isotypes, however, peptides are linear or cyclic, indicating that peptides are more amenable over antibodies to engineering at the molecular level (Hwang et al., 2015). As we previously described (Hwang et al., 2015), unique and short linear peptides specific for recombinant noroviral capsid proteins (rP2) was identified with evolutionary phage display and characterized by ELISA assay. As we previously described (Hwang et al., 2015), P-domain in noroviral capsid proteins connects with the internal S-domain, and it is exposed on the outermost viral surface. In addition, P-domain is involved in norovirus-host cell binding and possesses immunogenic activity (Bereszczak et al., 2012; Bu et al., 2008), indicating that P2 domain has enough potential to be a good candidate for recognizing and detection of norovirus. Based on the our results, the binding affinity of the peptide-displayed phage particles was found to be a nanomolar range against rP2 proteins. Therefore, we have chosen the best peptides specific for noroviral proteins and further explored the performance of the biosensor for electrochemical detection for human norovirus. In this study, we demonstrate an effective approach by creating a novel peptide-based small-molecule electrochemical biosensor for the detection of human norovirus.

2. Materials and methods

2.1. Chemicals

Fetal bovine serum (FBS) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The synthetic peptides modified with a C-terminal cysteine and a linker (-GGGGS-) (Noro-1 sequence: QHKMHKPHKNTKGGGGSC, Noro-2 sequence: QHIMHLPHINTLGGGGSC) were synthesized (> 95% purity) by Peptron (Daejeon, Korea). The quartz crystals deposited with gold was obtained from Biolin Scientific (Stockholm, Sweden). Phosphate-buffered saline (PBS, pH 7.4) and Tris-HCl buffer (pH 7.4) solutions were used to make all protein (1–10 μg/mL) and peptide (0.1 mM) solutions for the QCM, CV and EIS measurements.

2.2. Preparation of virus samples

Human norovirus from Chung-Ang University (Korea) was confirmed in patient stool by real-time reverse transcription polymerase chain reaction. Its genotype was determined as norovirus GII.4 subtype by sequence analysis. The patient stool was mixed with 1:10 ratio of serum-free Dulbecco's modified Eagles medium (DMEM) and homogenized using vertex shaker (Genius 3, IKA, USA) for 5 min. The homogenized sample was centrifuged at 10,000 × *g*, 4 °C for 15 min. The supernatant was collected and serially filtered with 0.8 μm, 0.45 μm, and 0.2 μm low-protein binding filter (Millipore Corporation, Billerica, MA, USA). The norovirus titer was determined as 1×10^7 virus particles/mL by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-qPCR). More detailed virus preparations including quality test by RT-qPCR were described in a previous report (Choi and Kingsley, 2016; Lee et al., 2015).

Human rotavirus strain Wa from Centers for Disease Control & Prevention (Osong, Korea) was cultured on MA-104 cells from American Type Culture and Collection (ATCC, Manassas, VA, USA) according to our previous report (Seo et al., 2014). After centrifuging the medium at 10,000 × *g*, 4 °C for 5 min, and the supernatant was aliquoted and used as a human rotavirus.

2.3. Circular dichroism (CD) spectroscopy

The CD spectra of all of peptides solutions with a concentration of 50 μM were recorded on a circular dichroism spectrometer (J-715, JASCO, Tokyo, Japan) using a UV cell with 0.1-cm of optical path length at 25 °C. In the CD experiments, PBS solution (pH 7.4) was used, and the CD spectra were added for 4 times, as previously reported (Greenfield, 2006; Sawada et al., 2013).

2.4. Preparation of affinity peptide-modified working electrode

Peptide-functionalized gold working electrode was prepared by the following steps. Firstly, the gold electrode was immersed into piranha solution (H_2SO_4: H_2O_2=4:1, v/v) for removing dusts and impurities on the surface of the gold electrode for about 10 min. Then, the electrode was rinsed with deionized (DI) water several times and sonicated in the water during 1 min. This pre-treated electrode was dried by blowing N_2 gas for several seconds. After polishing steps, the electrode and voltammetric cell were assembled together. 100 μL of thiol-modified peptide (50 μg/mL) was dropped onto the gold electrode and incubated in room temperature for overnight. In order to remove unbound peptides, this cell was washed with 1 × PBS (pH 7.4) and then DI water. The peptide-bound electrode was coated using a Pt using a sputter-coater (COXEM, KIC-1A, Seoul, Korea) to avoid charge-up effect of peptides on the surface. The surfaces of gold electrodes were observed using field-emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Tokyo, Japan). Next, 30 μL of serially-diluted norovirus samples (10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1 copies/mL) were loaded on the each assembled cell and then incubated at room temperature for 3 h. In order to remove the unbounded virus, these cells were sequentially washed with 1X PBS and DI water.

2.5. QCM measurements for determination of binding interactions on gold-immobilized peptide sensor layer

The QCM measurements were conducted using a Q-Sense E1 instrument (Biolin Scientific, Stockholm, Sweden). This instrument consists of a very thin quartz disc with gold electrode on both sides. The QCM measurements were conducted at room temperature, and a stable baseline was achieved 1 h after flow injection on a clean crystal at a flow rate of 1 mL/min. Briefly, the gold