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Direct and label-free influenza virus detection based on multisite binding to sialic acid receptors

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A B S T R A C T

A system to discriminate human or avian influenza A remains a highly sought-after tool for prevention of influenza pandemics in humans. Selective binding of the influenza A viral hemagglutinin (HA) to specific sialic acid (SA) receptors (Neu5Acα(2-6)Gal in humans, Neu5Acα(2-3)Gal in birds) is determined by the genotype of the HA and neuraminidase (NA) segments, making it one of the key characteristics that distinguishes human or avian influenza A virus. Here we demonstrate the direct detection of whole H1N1 influenza A virus using 6′-sialyllactose (Neu5Acα(2-6)Galβ(1-4)Glc, 6SL)-immobilized gold electrodes as biosensing surfaces. The sensitivity was higher than that of conventional immunochromatographic technique (ICT) for influenza virus and not restricted by genetic drift. The label-free detection technology via direct attachment of a whole virus using a chemically modified electrode is a promising means to provide a simple and rapid diagnostic system for viral infections.

1. Introduction

Protection against infectious diseases such as influenza virus is a sought-after goal, which may be more attainable if also addressed from a viewpoint of diagnostic performance. For example, highly pathogenic avian influenza (HPAI) infections in humans, such as the H5N1 subtype, have been identified as emerging infectious diseases with a high mortality rate compared with human influenza viruses (Peiris et al., 2007; Subbarao et al., 1998; Yuen et al., 1998). There are two main infection mechanisms. One mechanism is the expression of avian influenza receptors in human cells such as type II pneumocytes, which promotes infection with the predominantly avian-restricted H5N1 subtype (Kogure et al., 2006; Yao et al., 2008). The other is mutation of HA, which results in the ability to bind to human SA (Neu5Acα(2-6) Gal). It has been reported that mutations in several key amino acids in the antigenic epitope of HA create an interphyletic type of HA that can bind both avian SA (Neu5Acα(2-3)Gal) and human SA moieties (Sawada et al., 2010; Yamada et al., 2006). This genetic drift can lead to species jump and human-to-human transmission in a previously avian-restricted virus, and may ultimately cause a serious influenza pandemic. For example, despite preferentially binding to avian SA, such rapid mutation of the HA gene has resulted in subtypes H7N7 and H9N2 being capable of binding to the human form, albeit frequently with reduced efficacy, and thus represent emerging pandemic threats. Therefore, the ability to rapidly detect the host restriction of emerging viruses with high sensitivity may enhance surveillance and indicate imminent species jump events.

To identify the subtype of a given influenza virus, biosensing systems bearing specific antibodies (Rowe et al., 1999; Yuen et al., 1998) and nucleic acid aptamers (Gopinath and Kumar, 2013; Jeon et al., 2004; Wang et al., 2013) for HA, which plays a role in the process of virus attachment to the cell membrane during infection (Hamilton et al., 2012; Skehel and Wiley, 2000; Wiley and Skehel, 1987), have been reported. However, antigenic drift poses a problem to the recognition of HA (Carrat and Flahault, 2007), which may cause serious false negative results because of the escape from specific binding. On the other hand, human SA and avian SA are essential for infection with human and avian influenza viruses, respectively, and therefore represent suitable moieties to identify the subtype of virus (Watanabe et al., 2015). However, the detection sensitivity of conventional rapid assays such as ICT is over 5 hemagglutinating units (HAU) (Watanabe et al., 2015; Yeo et al., 2014), which occasionally leads to false negative results in the early stage of infection. If the sensitivity of diagnostic techniques could be improved using SA receptors, it may help prevent the occurrence of a serious influenza pandemic.

To realize this detection system, we demonstrate the label-free

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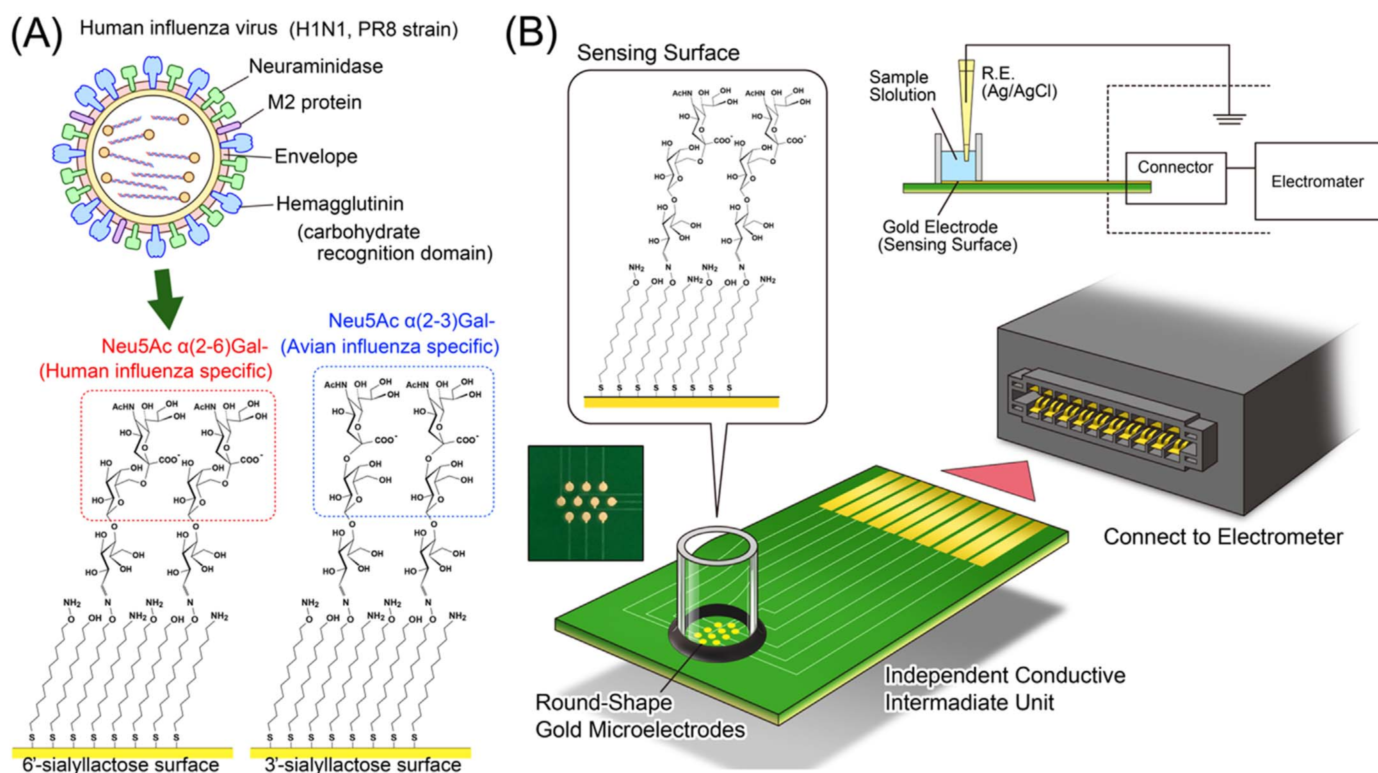

Fig. 1. (A) Conceptual rendering of the virus detection using saccharide recognition. (B) Illustration of the influenza virus detection system using electrical detection. The sensing surfaces are isolated from the measurement unit by a conductive intermediate, which can be used as a disposable sensor chip. The FET sensor is mounted in the electrometer with an operational amplifier, differential amplifier, amplifier circuit and capacitor for phase compensation.

detection of influenza A virus subtype H1N1 using 6SL immobilized on a self-assembled monolayer (SAM) on gold electrodes (Fig. 1A). To form the densely immobilized sialyllactose layer on the sensing surface, aminooxyundecyl disulfide (AOUD) was first immobilized as the SAM layer. Aminooxy groups on the SAM surface can react with hemiacetal (aldehyde) moieties of sialyllactose to form stable oxime bonds (Wu et al., 2010).

Recently, label-free immunosensing systems such as surface plasmon resonance sensors, quartz crystal microbalance (QCM) and shear horizontal surface acoustic wave sensors (Horiguchi et al., 2013) have been widely used as biosensors, which can detect specific interactions via direct attachment to the target without any pretreatment of the sample. In addition, biosensing systems using electrical detection, such as field effect transistors (FETs) have also attracted attention as label-free biosensors (Goda and Miyahara, 2010, 2013; Hideshima et al., 2013). In this report, the specific binding between human influenza A virus subtype H1N1 and 6SL-immobilized electrodes was first confirmed with QCM and then further confirmed using electrical detection techniques (Fig. 1B). Advantageously, the electrical detection techniques do not require optical devices or any other optional input transducers for biosensing. Therefore, the system can be miniaturized and the procedure simplified. In this report, a systematically fabricated SA receptor layer on a gold electrode for direct and label-free rapid detection of H1N1 influenza A virus was evaluated by QCM and electrical detection.

2. Materials and methods

2.1. Materials, reagents and equipment

11-Bromoundecane-1-thiol was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium hydrogen carbonate, magnesium sulfate, gelatin, ethyl acetate (EA), n-hexance, N,N-dimethylformamide (DMF) and dichloromethane (DCM) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). N-Hydroxyphthalimide, 6′-sialyllactose sodium salt and 3′-sialyllactose sodium salt were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All chemicals and reagents were used as received. Influenza A H1N1 (A/California/04/2009) hemagglutinin and influenza A virus subtype H5N1 (A/Vietnam/1194/2004) hemagglutinin were purchased from Sino Biological Inc. (Beijing, China). Though influenza A/Vietnam/1194/2004 was isolated from human, the specific interaction between HA and avian SA have been confirmed at previous report (Xiong et al., 2013). Human influenza A virus subtype H1N1 (A/PR/8/34) was cultivated in chicken embryos and then detoxified using 0.05% formalin solution. The size distribution of H1N1 influenza A virus particles in solution was measured using a qNano nanoparticle analyzer from Izon Science Ltd., Christchurch, New Zealand. Zeta potential measurement was performed using a Zetasizer Nano ZS from Malvern Instruments Ltd., (Worcestershire, UK).

2.2. Synthesis of 11-hydroxyundecyl 11-aminooxyundecyl disulfide, 11-aminoundecyl 11-aminooxyundecyl disulfide (aminooxyundecyl disulfide, AOUD)

Functional group transformation from a bromo moiety to oxylamine was reported previously (Park and Yousaf, 2008). Two hundred and fifty milligrams of 11-Bromoundecane-1-thiol (9.33×10^{-4} M) were dissolved in 5 mL of DMF. For the thiol oxidation reaction, the solution was refluxed for 24 h at 100 °C (solution 1). Separately, 396 mg of N-hydroxyphthalimide (2.43 mM) and 198 mg of sodium hydrogen carbonate (2.36 mM) were dissolved in 5 mL of DMF. The solution was heated to 80 °C (solution 2). After the mixture turned dark brown (15 min), solutions 1 and 2 were mixed and then refluxed for 17 h at 100 °C. After the reaction, the obtained product was extracted by water and EA. The solution was mixed with 20 mL water and then with 20 mL EA in a separating funnel. After collection of the EA product, another 20 mL of EA was added for further extraction. This extraction

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