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4-Aminophenyl boronic acid modified gold platforms for influenza diagnosis

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ABSTRACT

As a potential pandemic threat to human health, there has been an urgent need for rapid, sensitive, simpler and less expensive detection method for the highly pathogenic influenza A virus. For this purpose, Quartz Crystal Microbalance (QCM) and Surface Plasmon Resonance (SPR) sensors have been developed for the recognition of hemagglutinin (HA) which is a major protein of influenza A virus. 4-Aminophenyl boronic acid (4-APBA) has been synthesized and used as a new ligand for binding of sialic acid (SA) via boronic acid–sugar interaction. SA has an important role in binding of HA. QCM and SPR sensor surfaces have been modified with thiol groups and then 4-APBA and SA have been immobilized on sensor surfaces, respectively. Sensor surfaces have been screened with AFM and used for the determination of HA from aqueous solution. The selective recognition of the QCM and SPR sensors toward Concanavalin A has been reported in this work. Also, the binding capacity and detection limits of QCM and SPR sensors have been calculated and detection limits were found to be 4.7×10^{-2} μM, (0.26 μg ml^{-1}) and 1.28×10^{-1} μM, (0.72 μg ml^{-1}) in the 95% confidence interval, respectively.

1. Introduction

Influenza, commonly called "the flu," is a highly contagious viral infection of the respiratory tract. Compared with most other viral respiratory infections, such as the common cold, influenza infection often causes a more severe illness. Influenza virus uses a receptor that binds to human erythrocytes, causing hemagglutination. This is the first step in infection. This attachment step is mediated by the interaction of the viral envelope glycoprotein hemagglutinin (HA) with cell-surface molecules containing sialic acid (SA) [1]. Influenza virus particles are spherical with a diameter of about 100 nm and present two components [2]. HA, which has the function to bind to SA and agglutinate the erythrocytes, and neuraminidase (NA), which catalyzes removal of terminal SA linked to glycoproteins and glycolipids and allows newly created viruses to leave the cell [3]. Sialic acids that are present on cellular surface structures (glycoproteins and glycolipids) represent the targets for binding by HA.

HA is the major component of the virus that has a SA binding site located in the distal top of the molecule and it is defined as a pocket of aminoacids that are highly conserved among influenza virus strains [4,5]. According to literature, it has been considered that the carboxylate anion in SA is indispensable to bind with HA on the influenza virus (Fig. 1) [6]. However, it does not bind to neuraminidase.

Quartz crystal microbalance (QCM) immunosensors have approved widespread applications in the analysis of clinical targets [7–9], the monitoring of environmental contaminants, such as pathogen and bacteria [10–12] and the detection of biomolecular interaction [13–15].

Surface plasmon resonance (SPR) biosensors have made great strides both in terms of technology and applications [16] such as characterizing and quantifying biomolecular interactions [17–19], determination of affinity and binding constants [20–22], monitoring [23,24], diagnosis [25–28] and DNA sensing [29,30].

There is much interest in developing new influenza sensors for quick and reliable testing for influenza virus. One of the strategies is to develop single step direct sensing methods that eliminate separation, incubation or use of any signal-reporting agents. In recent years, non-labeling techniques such as SPR and QCM have attracted a great deal of attention in detection of viral samples [31]. These methods have the advantage of simplifying the analytical method by excluding the labeling procedures [32–35].

Sato et al. used QCM to study the binding of influenza A virus to monosialoganglioside in membranes and explore the influence of membrane composition on receptor functions of gangliosides GM3 reconstituted in sphingomyelin (SM) and glucosylceramide (GlcCer) monolayers were used as the viral receptor [36]. On the other hand, SPR has been used for the detection of influenza virus and the study of interactions that involve viral proteins and receptors. The first use of SPR in influenza virus detection was reported by Schofield and Dimmock [33]. This study was followed by Critchley and Dimmock who studied the binding of influenza A virus to a neomembrane composed of bovine brain lipids that contains sialoglycolipids [37].

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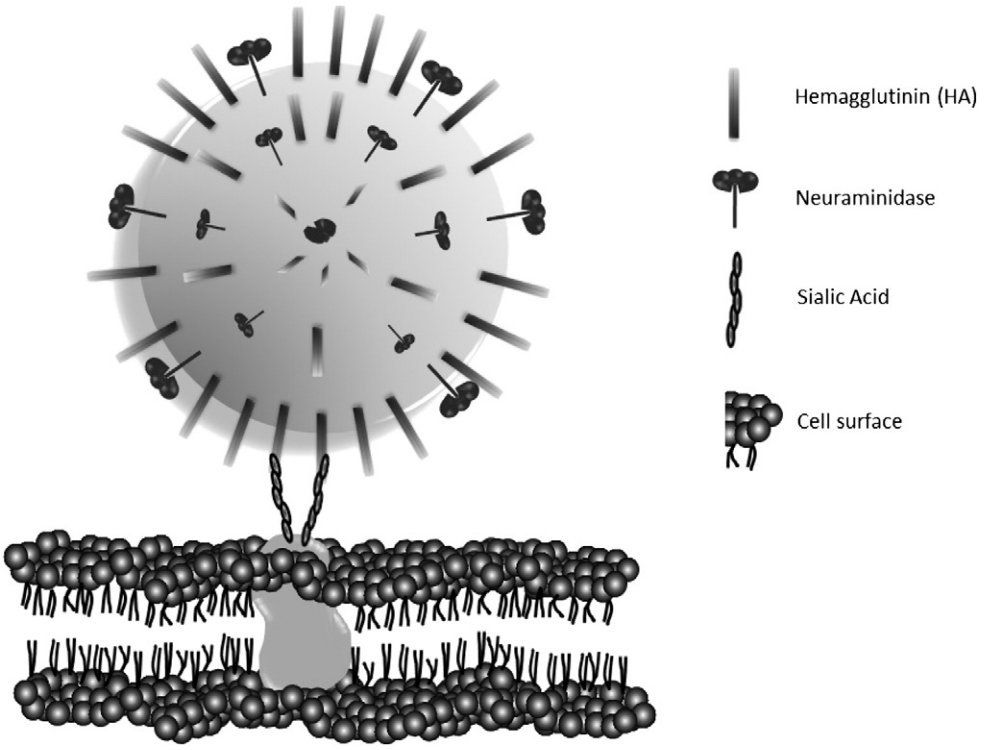

Fig. 1. Illustration of influenza A binding to sialic acids on cell surface.

In this study, novel detection methods based on boronic acid–sugar interaction to use in the medical field for diagnosis influenza virus have improved. For this purpose, surfaces of quartz crystals and SPR chips have modified with thiol groups for providing 4-APBA–SA interaction and SA has immobilized. Then, HA detection was achieved by using prepared QCM and SPR sensors.

2. Materials and methods

2.1. Materials

Boric acid, 11-mercaptoundecanoic acid, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide, *N*-hydroxysuccinimide, thionyl chloride, butyllithium, isopropyl alcohol, sodium carbonate and hemagglutinin were supplied by Aldrich (Milwaukee, WI, USA). All glassware was extensively washed with dilute HNO_3 before use. All other chemicals were of analytical grade purity and purchased from Merck AG (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANO pure® organic/colloid removal and ion exchange packed-bed system.

2.2. Synthesis of 4-aminophenyl boronic acid (4-APBA)

Firstly, tri-isopropyl borate was synthesized. For this purpose, boric acid was used as a starting compound. As seen in Fig. 2a, thionyl chloride ($SOCl_2$), (3), (105.29 g, 885 mmol) was slowly added to suspension of boric acid, 1, (9.12 g, 147 mmol) in excess of isopropyl alcohol, (2), under nitrogen atmosphere. After the all $SOCl_2$ was added, the reaction mixture was refluxed. Then, distillation apparatus built under nitrogen atmosphere and excess of isopropyl alcohol was distilled at 80–82 °C and distillation carried out at 110–120 °C as well. Finally, tri-isopropyl borate, (4), was obtained (colorless liquid product, 8.20 g, 93% yield), as a product. Then, in order to synthesize *N*,*N*-dibenzyl-4-bromo aniline, (7), sodium carbonate (4.62 g, 43.59 mmol) was added to 4-bromo aniline, (5), (5 g, 29.06 mmol) and benzyl bromide, (6), (10.31 g, 61.03 mmol) solutions in 150 ml of DMF under nitrogen atmosphere. Obtained suspension was stirred for 10 h at 100–110 °C. After the reaction completed, pieces of ice were added to suspension and stirred. The precipitate of *N*,*N*-dibenzyl-4-bromo aniline compound, (7), was filtered, washed with water and dried under vacuum (10 g product, 98% yield) (Fig. 2b). For the synthesis of trimeric-4-(*N*,*N*-dibenzylamino)-phenylboronic acid, (8), 2.5 M of butyllithium (BuLi) (13.27 ml, 33.19 mmol) in hexane was added dropwise to *N*,*N*-dibenzyl-4-bromo aniline, (7), (7.70 g, 22.12 mmol) in distilled THF (100 ml) at −78 °C under nitrogen atmosphere. Then, tri-isopropyl borate, (4), (8.30 g, 44.24 mmol) was added dropwise into reaction mixture. The reaction mixture was stirred for 1 h at −78 °C and 30 min at room temperature. The solvent was evaporated and obtained solid was dissolved in ethyl acetate (150 ml) and extracted by water. Organic phase was dried by $MgSO_4$ and filtered. Obtained white solid was suspended in hexane and filtered again. The precipitate was dried by vacuum and the product is trimeric-4-(*N*,*N*-dibenzylamino)-phenylboronic acid, (8), (10 g, 76% yield) (Fig. 2c). Catalytic amount of Pd/C (10%) and concentrated HCl were added to trimeric-4-(*N*,*N*-dibenzylamino)-phenylboronic acid (5 g, 5.76 mmol), (8), in dry methanol at the last step. Reaction mixture was stirred for 3 h under 14 bar H_2(g) atmosphere. At the end of the reaction, the catalyst was filtered, the solvent was evaporated and light brown 4-aminophenyl boronic acid (4-APBA) was obtained (2.27 g, 96% yield) (Fig. 2d). In this synthesis, benzyl groups which are protective for amine group have turned into toluene, trimeric structure has broken up and 4-APBA, (9), has synthesized.

2.3. Characterizations

Characterization of synthesized compounds was carried using ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz) and ^{11}B-NMR (166 MHz).