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Letter

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Fabrication of Electrochemical Model Influenza A Virus Biosensor Based on the Measurements of Neuroaminidase Enzyme Activity

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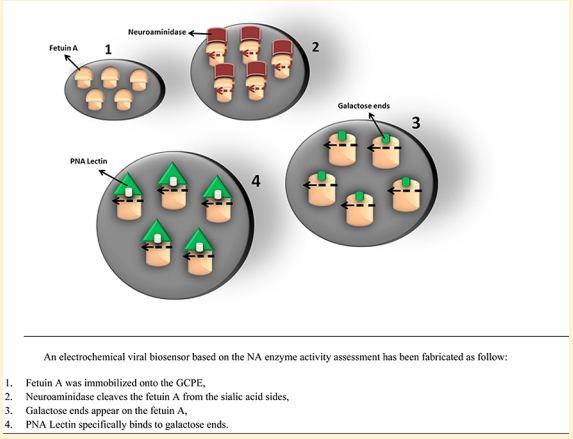
Supporting Information

ABSTRACT: Neuroaminidase (NA) enzyme is a kind of glycoprotein that is found on the influenza A virus. During infection, NA is important for the release of influenza virions from the host cell surface together with viral aggregates. It may also be involved in targeting the virus to respiratory epithelial cells. In this study, a model electrochemical influenza A viral biosensor in which receptor-binding properties have been based on NA was developed for the first time. The biosensor's working principle is based on monitoring the interactions between fetuin A and NA enzyme. The assay was monitored step by step by using electrochemical impedance spectroscopy.



Early diagnosis of influenza A virus is important for the recovery of patients as well as prevention of economic loss. Because of high variability at virus strains, every year there is a strong possibility about the emergency of a new pandemic, which can result with higher morbidity and mortality.[1−3] For this reason, rapid detection of this virus at early stages is mandatory. Since conventional methods are laborious and need specialists and expensive instruments, more practical, sensitive, and economical protocols are needed. In this sense, electrochemical influenza A biosensors offer portability together with higher accuracy and sensitivity.[4]

To date, only hemaglutinin (HA) based electrochemical biosensors including genosensors and immunosensors have been fabricated.[5−7] In this communication, a model electrochemical biosensor based on the neuroaminidase (NA) enzyme activity assessment has been fabricated for the first time.

Influenza, which is known as the flu, is a very contagious disease that affects millions of people every year.[6] Among the influenza viruses, influenza virus A is the most contagious one that causes serious pandemics each season. Influenza A virus can be described as negative stranded RNA virus which belongs to the family of *Orthomyxoviridae*. This virus is further classified into subtypes based on the antigenic properties of two surface glycoproteins, HA and NA. To date, 17 HA (1−17) and 10 NA (1−10) subtypes have been investigated.[1−3]

HA and NA recognize the same molecule (sialic acid) with conflicting activities. HA binds the host cells via sialic acid while NA cleavages the sialic acid for achieving the release of progeny virus. For this reason, drastic changes in HA or NA activity would affect the viral replication.[8] On the other hand, recently, it was demonstrated by Matrosovich et al.[9] that NA can initiate the influenza virus infection in the human air-way epithelium. Considering these two phenomena, designing a biosensor which monitors NA activity becomes more important.[9]

Herein we reported a NA activity based model electrochemical biosensor. For the fabrication of this biosensor, a composite electrode, glassy carbon paste electrode (GCPE) was used (see the Supporting Information for experimental details). After functionalizing this electrode with H_2SO_4, fetuin A was immobilized onto the surface. Then NA was attached and incubated for a proper period of time. Fetuin A is a kind of glycoprotein from fetal calf serum which contains terminal 12−14 sialic acid residues per molecule. Different influenza virus strains can be attached onto fetuin A. The enzymatic activity of the influenza NA proteins was monitored by following the removal of sialic acid residues.

Lastly, peanut agglutinin (PNA) lectin was added onto the electrode surface to observe the cleavage of fetuin by NA (Scheme 1). NA protein creates PNA specific binding sites, by masking the sugars from fetuin and then PNA lectin specifically binds to the *N*-acetylgalactosamine galactose-(Gal β1-3Gal-NAc[10] (Scheme 1).

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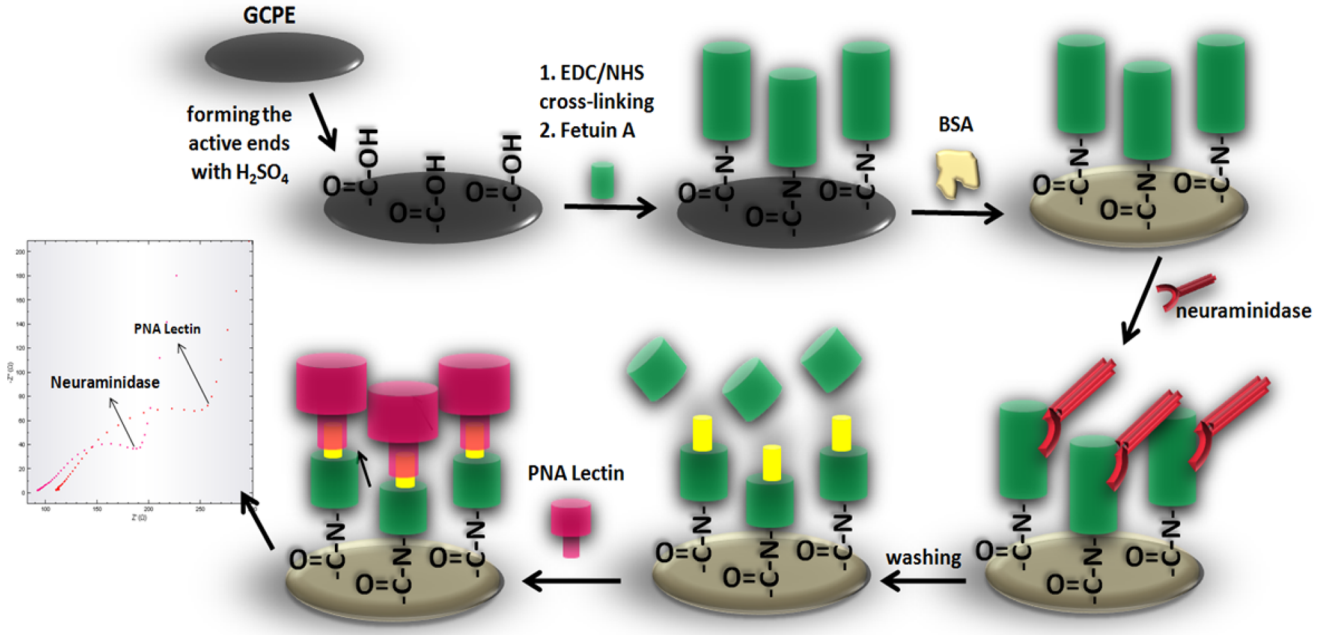
Scheme 1. Schematic Diagram Depicting the Experimental Procedures



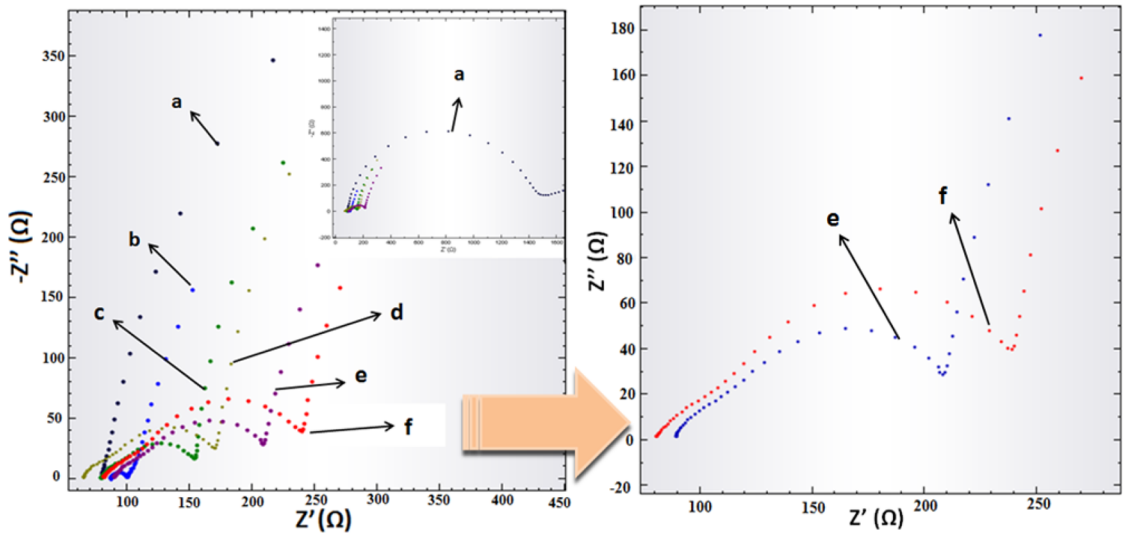


Figure 1. Electrochemical impedance spectra of influenza A model viral biosensor. (a) Plain GCPE, (b) carboxylic groups functionalized $GCPE_{COOH}$, (c) fetuin $A/GCPE_{COOH}$, (d) $BSA/fetuin\ A/GCPE_{COOH}$, (e) $NA/BSA/fetuin\ A/GCPE_{COOH}$, and (f) $PNA\text{-}Lectin/NA/BSA/fetuin\ A/GCPE_{COOH}$. The EIS procedure was set to measure the electron transfer resistance in frequency range of 0.1 Hz−10 kHz at a potential of 0.25 V and 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (in PBS) was used as a redox couple.

The layer by layer characterization of the electrode was done via electrochemical impedance spectroscopy (EIS) in the presence of $[Fe(CN)_6]^{3/4-}$ as the redox probe (Figure 1). EIS is usually preferred for the investigation of the changes in the electrode surface. As can be seen from the Nyquistic diagrams in Figure 1, the bare GCPE has the highest resistance (Figure 1a). After the activating of GCPE surface with H_2SO_4, the semicircle diameter decreases (Figure 1b). Next, fetuin A was immobilized onto the electrode surface with EDC/NHS. Since activated electrode surface was covered with fetuin A, GCPE surface resistance, hence the diameter of Nyquistic diagram increases (Figure 1c). Then, BSA was immobilized on to the electrode surface to block the unspecific binding, thus the blank side on the surface was filled and the resistance of the electrode surface increases (Figure 1d). As mentioned before, NA was used to cleave the fetuin A from the sialic acid sides. In this step, following the incubation of NA, the electrode surface was covered with NA and the charge transfer ability of the electrode was reduced resulting with increment in the semicircle diameter (Figure 1e). For the last step of modification, PNA lectin was used because of specificity to galactose molecule which appears after the cleaving of fetuin A with NA. When the PNA lectin binds onto the galactose molecules, electron transfer was getting even harder and semicircle diameter increases linked to the resistance increase on the electrode surface (Figure 1f).

In conclusion, we manage to fabricate a model electrochemical influenza A biosensor based on NA activity assessment for the first time. Besides the regular prevention strategies

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like vaccination, quarantine, personal precautions, etc., in an emerging pandemic, antiviral drug targeting NA enzyme will be important in terms of control strategies for preventing humans of getting the virus. Moreover, examination of the receptor-binding property of influenza virus, assessment of its NA activity and its response to anti-NA antiviral drug are crucial for controlling and preventing the passage of influenza viruses. In the future we will continue to improve the developed biosensor in terms of optimization of experimental parameters. Also on the basis of the main reaction, an electrochemical diagnostic kit could be designed that can be used for early detection of influenza A virus in humans.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01720.

Additional experimental details (PDF)

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Notes

The authors declare no competing financial interest.

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