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Self-enzyme chemiluminescence immunoassay capable of rapidly diagnosing the infection of influenza A (H1N1) virus

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ABSTRACT

A highly sensitive self-enzyme immunoassay with 1,1′-oxalyldiimidazole chemiluminescence (ODI-CL) detection was developed for the first time to quantify influenza A (H1N1) virus. A specific capture antibody, capable of capturing hemagglutinin (HA) subtypes of H1N1, was immobilized on the surface of the magnetic Au-Fe_3O_4 nanocomposite. Neuraminidase (NA) subtype of H1N1 was acts as an enzyme in the self-enzyme immunoassay. A sample mixed with HA antibodies immobilized on the surface of magnetic Au-Fe_3O_4 nanocomposites was incubated for 1 h at 37 °C. After the incubation, magnetic Au-Fe_3O_4 nanocomposites separated with a magnetic bar were washed 3 times using phosphate buffered saline with Tween-20 (PBST). Then, 4-Methylumbelliferyl-*N*-acetyl-α-D-neuraminic acid (MUNANA), a fluorogenic substrate of NA, was added and incubated for 10 min to produce 4-Methylumbelliferone (4-MU) from the enzyme reaction between MUNANA and NA of H1N1. After the incubation, the solution containing 4-MU emitted bright light with the addition of ODI-CL reagents (e.g., H_2O_2 and ODI). The relative CL intensity of 4-MU was proportionally enhanced with the increase of H1N1. Also, the brightness and color of 4-MU light emitted from the self-enzyme immunoassay was dependent on pH. The self-enzyme immunoassay was able to analyze trace levels of influenza A (H1N1) virus with good accuracy, precision, reproducibility and excellent selectivity. The limit of detection (LOD = 3σ/slope) was as low as 0.19 U/ml. We expect that the self-enzyme immunoassay can be applied as a cost-effective and rapid method capable of selectively quantifying a specific influenza A virus such as H1N1, H3N2, and H5N1.

1. Introduction

The flu, which is caused by various influenza viruses, is a contagious respiratory illness. Most people with an influenza virus infection have mild symptoms and can recover within two weeks. However, in serious cases, which are likely to occur in children under the age of 5, adults over the age of 65, pregnant women, and people who have medical conditions, patients are likely to either be hospitalized or even die.

The major types of influenza viruses that routinely spread among people are influenza A and B viruses [1]. Influenza A viruses are classified according to 15 different hemagglutinin (HA) subtypes and 9 different neuraminidase (NA) subtypes while influenza B virus is not divided into subtypes [1]. Various types of influenza A viruses created by many different combinations of HA and NA proteins have been reported [1,2].

It is difficult to diagnose a specific type of influenza A virus based on only the symptoms [2]. Thus, various methods for the diagnosis of specific influenza A viruses have been developed [3–6]. The sandwich enzyme immunoassays (EIA) with a capture antibody and a detection antibody are widely applied to analyze a specific type of influenza A virus in a sample. Currently, electrochemical [7] and optical sensors such as chemiluminescence [8], colorimetric [9], and fluorescence [10] are widely applied as a detection method of sandwich EIA operated with a capture antibody and a detection antibody conjugated with horseradish peroxidase (HRP) [11,12] or alkaline phosphatase (AP) [13,14]. In order to rapidly analyze influenza A viruses with high sensitivity and selectivity, EIAs using nanoparticles, such as gold [8,15], platinum [16], silver [15], and magnetic bead (MB) [17] have been developed. However, Most of EIAs using two specific antibodies reported so far are complicated, expensive, and time-consuming.

NA, a subtype of influenza A virus, can be applied as an enzyme to devise biosensors with fluorescence detection. 2′-(4-Methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA), a fluorogenic substrate, is converted to 4-methyllumbelliferone (4-MU) in

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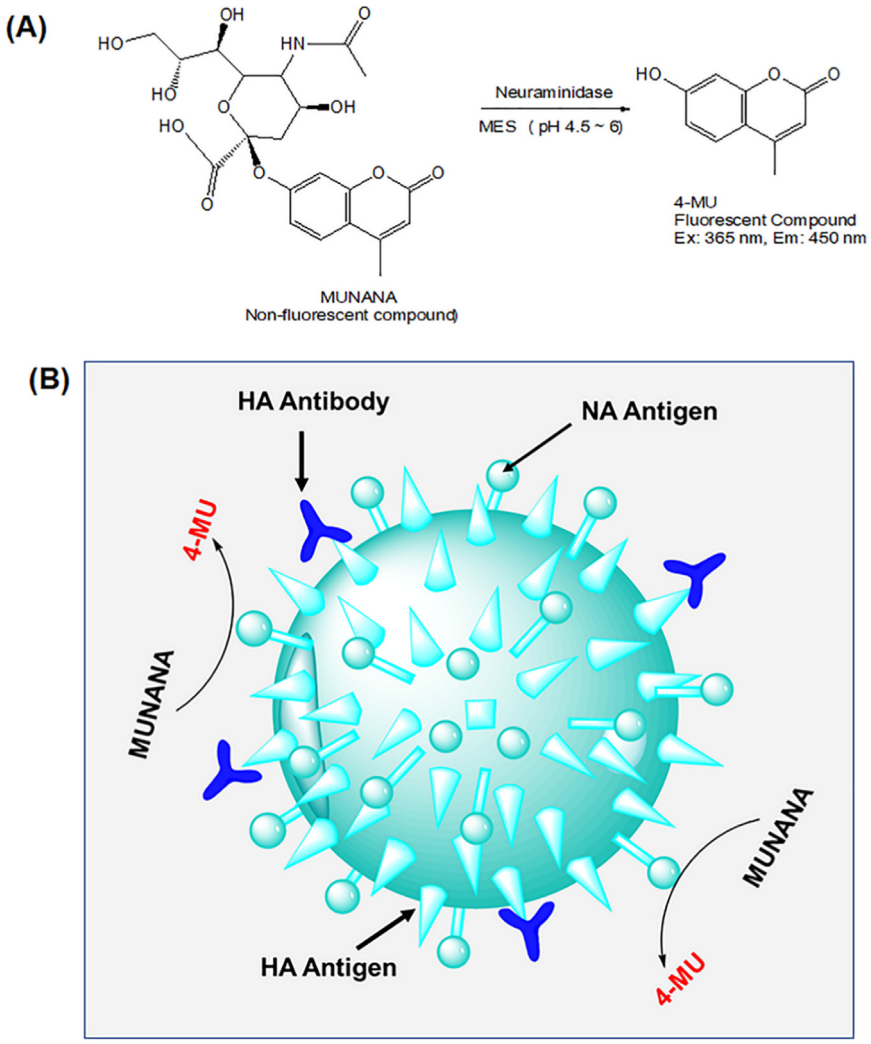

Fig. 1. (A) Reaction mechanism of MUNANA and NA to produce 4-MU. (B) Interaction between MUNANA and NA of influenza A virus to form 4-MU and binding of HA antigen of influenza A virus and a specific HA antibody.

the presence of NA at acidic condition (pH 4.5–6) [18,19]. The concentration of 4-MU formed from MUNANA in this reaction is determined on the concentration of NA in a sample. In other words, the fluorescence intensity of 4-MU formed from the reaction of NA and MUNANA as shown in Fig. 1(A) is proportionally enhanced with the increase of NA in a sample. 4-MU excited at 365 nm emits blue light at 450 nm.

Fig. 1(B) shows that 4-MU can be formed from the interaction of MUNANA and NA of an influenza A virus. Also, HA of the influenza A virus can bind with a specific HA antibody. The applications of NA and HA of the influenza A virus shown in Fig. 1(B) indicate that a new self-enzyme immunoassay with a specific HA capture antibody and MUNANA can be developed for the analysis of a influenza A virus without a detection antibody conjugated with a conventional enzyme (e.g., ALP, HRP). This is because NA of the influenza A virus is working as an enzyme of the self-enzyme immunoassay. The hypothesis shown in Fig. 1(B) indicate that a self-enzyme immunoassay with 1,1′-oxalyldiimidazole chemiluminescence (ODI-CL) detection or fluorescence detection can be developed for the quantification of a specific influenza A virus.

The high-energy intermediate (X) formed in ODI-CL reaction acts as a light source of fluorescence like a laser or Xenon lamp [20–23]. This is because X can transfer energy to a fluorescent compound based on the principle of internal chemiluminescence resonance energy transfer (inter-CRET). The fluorescent compound after the inter-CRET emits bright CL emission. It is well-known that ODI-CL with low background noise is more sensitive than fluorescence operated with high-voltage power supply to operate a laser or Xenon lamp [21,24]. Using the advantages of ODI-CL detection, various enzyme immunoassay operated with conventional enzymes have been developed for the early diagnosis of human diseases [20,25–27] or the rapid monitoring of a toxic material [28].

Based on the hypothesis shown in Fig. 1(B) and the advanced role of ODI-CL detection, we were able to design and develop for the first time a self-enzyme immunoassay with ODI-CL detection using NA instead of conventional enzymes. This paper is described in detail for the development of the self-enzyme immunoassay with ODI-CL detection.

2. Experimental

2.1. Chemical and materials

Recombinant proteins of influenza A H1N1 (100 U), H3N2 (80 U), and H5N1 (60 U) with NA activity were purchased from SINO Biological. Goat polyclonal influenza A antibody (H1N1) was purchased from Fitzgerald. 2′-(4-Methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA), 4-methyllumbelliferone (4-MU), Neuraminidase (6 U) from *Clostridium perfringens (C. welchii)*, bovine serum albumin (BSA), horseradish peroxidase (HRP), iron (III) chloride, iron(II) chloride, ammonium hydroxide (NH_3OH, 28% NH_3 in H_2O)) and 30% hydrogen