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Detection and differentiation of influenza viruses with glycan-functionalized gold nanoparticles

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ABSTRACT

Accurate diagnosis of influenza viruses is difficult and generally requires a complex process because of viral diversity and rapid mutability. In this study, we report a simple and rapid strategy for the detection and differentiation of influenza viruses using glycan-functionalized gold nanoparticles (gGNPs). This method is based on the aggregation of gGNP probes on the viral surface, which is mediated by the specific binding of the virus to the glycans. Using a set of gGNPs bearing different glycan structures, fourteen influenza virus strains, including the major subtypes currently circulating in human and avian populations, were readily differentiated from each other and from a human respiratory syncytial virus in a single-step colorimetric procedure. The results presented here demonstrate the potential of this gGNP-based system in the development of convenient and portable sensors for the clinical diagnosis and surveillance of influenza viruses.

1. Introduction

Influenza viruses are classified into three serologically separate groups: A, B, and C, of which only types A and B can cause serious illness in humans. Both A and B viruses are coated with two major envelope proteins: hemagglutinin (HA) and neuraminidase (NA). According to the antigenicity of HA and NA, the A viruses are further divided into several subtypes such as H1N1 and H3N2. Although seasonal influenza epidemics are caused by strains belonging to human A and B viruses, the A viruses are of particular concern because they have a broader host range and, through genetic reassortment between human and animal viruses, can develop into pandemic strains such as H1N1 in 1918 and 2009, H2N2 in 1957, and H3N2 in 1968 (Imai and Kawaoka, 2012). The recent emergence of pathogenic avian H5N1, H7N9, and H5N6 A viruses, which can infect humans and result in high morbidity and mortality, poses additional challenges to public health (Shi et al., 2013; Stevens et al., 2006c; Yang et al., 2015). Given the continuing threat of these evolving pathogens, fast and accurate diagnosis is critical, especially with tools that can differentiate between and identify specific strains, to facilitate early and targeted response in both clinical settings and medical administration.

Conventional methods for the detection of influenza virus are

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typically dependent on immunoanalytical or molecular biology techniques (Kumar and Henrickson, 2012; Landry, 2011). Rapid diagnostic tests based on antigen–antibody reactions can produce results in a few minutes, but face limitations such as the instability of specific biological components. PCR-based detection may provide advantages in terms of sensitivity and selectivity, but suffers from several drawbacks such as the requirement for costly equipment and reagents, and the time required to obtain results. Recently, approaches employing mass spectrometry (Schwahn et al., 2009, 2010), oligonucleotide microarray (Dawson et al., 2006, 2007; Ryabinin et al., 2011; Zhao et al., 2010), and Raman spectroscopy (Lim et al., 2015) have been explored; however, these methods are still in their nascent stages and their utility needs to be verified with more serotypes and strains.

An emerging technique for the simple detection of influenza viruses is to use the distance-dependent optical property of gold nanoparticles (GNPs) (Quinten et al., 1985; Elghanian et al., 1997), which has also been exploited for developing improved assays of biomolecules and cells (Boisselier and Astruc, 2009; Marín et al., 2015; Marradi et al., 2013; Rosi and Mirkin, 2005; Saha et al., 2012). Following conjugation of multiple copies of influenza virus-specific elements on their surface, the GNPs are able to bind and assemble on the viral surface, resulting in color and plasmon resonance shifts that can be measured visually and with spectroscopy. So far, a number of influenza virus-binding molecules have been used for conjugation with the GNPs and include antibodies (Chen and Neethirajan, 2015; Driskell et al., 2011; Lai et al., 2015; Lee et al., 2015; Liu et al., 2015), aptamers (Le et al., 2014), the NA inhibitor oseltamivir (Stanley et al., 2012), and glycans containing terminal sialic acid (SA) (Lee et al., 2013; Marín et al., 2013). The latter in particular, serves as the virus receptor on host cells and is critical in determining the host range and transmission. Binding of a particular strain to the host cells relies greatly on interactions between the viral HA and specific structural features of the cell-surface glycans. In general, human viruses prefer the α2,6-linked SA receptors, whereas avian viruses preferentially bind to receptors with an α2,3-configuration (Imai and Kawaoka, 2012). The HA-binding preferences are further heavily influenced by fine modifications in the internal sugars of SA receptors such as *N*-acetylation, sulfation, and fucosylation (Stevens et al., 2006a).

Such differences in binding affinities provide a unique opportunity to design glycan-based assay systems for influenza viruses. Indeed, glycan microarrays comprising multiple SA receptors printed on glass chips have been developed to analyze the receptor specificity of various HAs and the whole viruses (Blixt et al., 2004; Childs et al., 2009; Dinh et al., 2014; Huang et al., 2015; Liao et al., 2010; Narla and Sun, 2012; Stevens et al., 2006a, 2006b). *S*-sialosides have been immobilized on the surface of a waveguide biosensor to differentiate between the H1N1 and H3N2 viruses (Kale et al., 2008). Glycan-functionalized gold nanoparticles (gGNPs) displaying a monovalent SA (Lee et al., 2013) and a trivalent *S*-sialoside (Marín et al., 2013) have demonstrated the ability to identify the B and H3N2 viruses, respectively. Our group previously developed a gGNP-based system (Wei et al., 2014) featuring seven structurally distinct SA receptors (Fig. 1), which represent natural glycan sequences commonly found in cell surface glycoproteins and glycolipids (Blixt et al., 2004). Using this assay platform, we successfully screened the receptor specificity of eight representative human and avian viral HAs. Furthermore, a preliminary test with three selected viruses, containing clearly different HAs and NAs, revealed their affinities for the seven gGNPs, with binding patterns largely similar to those of their corresponding recombinant HAs.

Although the gGNP assay has proved useful in inspecting the viral receptor specificity (Wei et al., 2014), the development of this method to detect virus is an important goal. The binding of influenza virus to host glycans is diverse and complex, which depends primarily on the viral HAs but can also be influenced by NAs, a sialidase that removes SA from the glycan receptors (Liu et al., 1995). This can make the glycan-based assays with whole viruses much more challenging than the exploration of receptor preference solely using recombinant HAs (Stevens et al., 2006b). In this study, we report an important advancement for the use of gGNP assay to detect and discriminate between influenza viruses. Fourteen viral strains belonging to current major serotypes were tested and readily identified in a single-step colorimetric process. The results presented here confirm the potential of gGNP technology for the rapid and simple differentiation of influenza viruses at the strain, subtype, and origin (human vs avian) levels, which together with our previous work on the analysis of viral receptor specificity (Wei et al., 2014) represents a step forward in the development of tools for the facile detection and surveillance of influenza viruses.

2. Materials and methods

2.1. gGNPs and viruses

All gGNPs were synthesized and fully characterized as described previously (Wei et al., 2014). The morphology and optical property of gGNPs were identified by transmission electron microscopy (TEM) and UV–Vis spectroscopy, respectively. The diameter of gGNPs (12–15 nm) was measured by TEM and dynamic light scattering (DLS) technique. The glycan number per particle was determined by X-ray photoelectron spectroscopy (XPS) and thermogravimetric analysis (TGA). The concentration of gGNPs in solution was quantified using UV–Vis spectroscopy (Ghosh and Pal, 2007). The human respiratory syncytial virus (hRSV; ACTT VR-26) was a clinical isolate stored at our laboratory (Zhang et al., 2015). This virus was propagated in Vero cells grown in Dulbecco's modified Eagle's medium (DMEM) before being used in the assays. All the influenza viruses were generated from the genes of their respective natural isolates through a reverse genetics method, as described in prior studies (Bi et al., 2015; Shi et al., 2013; Wei et al., 2014). The method used for the culture of influenza virus has also been detailed in those studies. The HA titer of influenza virus was determined with hemagglutination assays using 1% chicken red blood cells. The 50% tissue culture infective dose ($TCID_{50}$) of hRSV was quantified in Vero cells. The rescued influenza viruses were sequenced to exclude any mutations, and inactivated by treatment with an aqueous solution of *β*-propiolactone (0.5‰) before being used in gGNP assays. All experiments with the viruses before their inactivation were performed in approved biosafety level (BSL-3) containment laboratories.

2.2. Glycan-based enzyme-linked immunosorbent assay (ELISA)

The binding specificities of vieH5N1 and shaH1N1 were analyzed with an glycan-based ELISA method (also referred to as a solid-phase binding assay) (Shi et al., 2013) using biotinylated 3′SLNLN and 6′SLNLN (for the sequences of these glycans, see the legend of Fig. 2) obtained from the Consortium for Functional Glycomics (Scripps Research Institute, Department of Molecular Biology, La Jolla, CA, USA). In brief, the glycans were immobilized onto streptavidin-coated 96-well polystyrene plates (Corning, NY, USA) by overnight incubation of the plate with the solutions of glycan in PBS (3.5 μg/mL, pH 7.4). After adding serial dilutions of the viruses in PBS (0–128 HA titer) to the wells, incubations were performed for 12 h followed by washing with PBST. Primary antibodies (rabbit antisera against vieH5N1 and shaH1N1; 3 μg/mL in PBS containing 1% BSA; Sino Biological Inc., Beijing, China) were then added to the wells followed by incubation for 4 h and washing with ice-cold PBST. Subsequently, horse radish peroxidase-linked secondary antibody (goat anti-rabbit IgG; 0.13 μg/mL in PBS containing 1% BSA; Sino Biological Inc.) was added and incubated for 2 h. After washing the plate with ice-cold PBST, the bound antibody was detected using *O*-phenylenediamine substrate (in PBS containing 0.01% H_2O_2) following incubation for 10 min at room temperature. The enzymatic reaction was quenched