Biosensors and Bioelectronics 32 (2012) 195–201

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Monitoring influenza hemagglutinin and glycan interactions using surface plasmon resonance

Emi Suenaga, Hiroshi Mizuno, Kumar K.R. Penmetcha*

RNA Processing Group, Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba City 305-8566, Ibaraki, Japan

ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form
18 November 2011
Accepted 2 December 2011
Available online 24 December 2011

Keywords:
Hemagglutinin
Glycans
Surface plasmon resonance
Human influenza virus
Biomolecular interactions

ABSTRACT

Hemagglutinin (HA) is a trimeric glycoprotein expressed on the influenza virus membrane. HA of influenza viruses binds to the host's cell surface complex glycans via a terminal sialic acid (Sia), as the first key step in the process of infection, transmission and virulence of influenza viruses. It is important to monitor and evaluate the receptor (glycan) binding preferences of the HAs derived from influenza A viruses, especially those originating from birds and swine, to understand their potential ability for interspecies transmission. From this viewpoint, in the present study, we have developed a protocol for analyzing the glycan–HA interactions efficiently and kinetically, based on surface plasmon resonance (SPR). Our results showed that glycan–HA binding analyses can be performed reliably and efficiently on Biacore-chips in the SPR system, using chemically synthesized biotinylated multivalent-glycans. Using the CAP-chip, we were able to regenerate the surface for multiple analyses, allowing us to derive, for the first time, the precise kinetic parameters for different HA–glycan complexes of newly emerging influenza viruses. These studies suggested that this SPR-based method is suitable for influenza surveillance to define the pandemic scenario as well as to screen of synthetic glycans and other compounds that may interfere with glycan–HA interactions.

1. Introduction

Glycan-binding proteins are often expressed by viruses, bacteria and protozoa on their surfaces to facilitate their attachment to the host cells, a requirement for establishing colonization and infection. Among the viral-glycan binding proteins, the most thoroughly studied example is the influenza virus hemagglutinin (HA). Influenza virus contains two major surface proteins, HA and neuraminidase (NA). HA mediates both receptor (glycan) binding and membrane fusion for cell entry, and NA functions as the receptor destroying enzyme in virus release (WHO, 1980). These proteins form the basis for classifying the sub-types of influenza viruses. HA is a homotrimer composed of disulfide-linked polypeptide chains, HA1 and HA2, formed after cleaving the host's enzymes at the proteolytic site; the former is the major component of the HA antigen. Currently, 16 subtypes of HAs have been identified in avian species (H1–H16) (Fouchier et al., 2005) and although there are differences in their primary sequences, they share similar three-dimensional structures. Among these 16-subtypes, only three HA-subtypes H1N1, H2N2, and H3N2, have successfully adapted to humans (Garten et al., 2009; Scholtissek et al., 1978; Kawaoka et al., 1989). However, the last four decades have witnessed a growing number of human cases of avian influenza virus infections, including H5N1, H7N2, H7N7, and H9N2 sub-types (De Jong et al., 1997; Taubenberger et al., 2007).

HA of influenza viruses binds to host cell surface complex glycans via a terminal sialic acid (Sia) with α2-3 and α2-6 linkages, and this is the first key step in the process of infection, transmission and virulence of influenza viruses (Skehel and Wiley, 2000; Russell et al., 2006). The receptor (glycan) binding domain is located within the HA1 globular domain. The crystal structures of the H1, H3, H5, H7, and H9 HA subtypes and their complexes with α2-3 Sia and/or α2-6 Sia glycans have been reported (Gamblin et al., 2004; Xu et al., 2010; Eisen et al., 1997; Ha et al., 2001, 2003; Yang et al., 2010). The HAs derived from avian influenza viruses are known to bind specifically to the α2-3 Sia glycan preferentially expressed in the intestinal tracts of waterfowl. On the other hand, human-adapted influenza viruses prefer to bind to the α2-6 Sia glycan extensively expressed on the epithelial cells of the human upper respiratory tract (Shinya et al., 2006). It was suggested that the HA of avian influenza viruses needs to adapt for binding to α2-6 Sia glycan, for efficient transmission to humans (Matrosovich et al., 2000). Further studies in this direction revealed mutations in HA that alter the glycan specificity, from α2-3 Sia to α2-6 Sia, for the pandemic sub-types of avian influenza viruses. For example, the H1 1918 pandemic virus was able to switch the HA glycan

* Corresponding author. Tel.: +81 298 61 6085; fax: +81 298 61 6738.
E-mail address: pkr-kumar@aist.go.jp (K.K.R. Penmetcha).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.12.003

specificity from α2-3 Sia to α2-6 Sia after acquiring two point mutations (E190D and G225D) (Glaser et al., 2005). Similarly, the H2 and H3 sub-type viruses switched their specificity after acquiring two point mutations (Q226L and G228S) (Naeve et al., 1984; Connor et al., 1994; Glaser et al., 2005). Therefore, it is, now believed that the main cause of the drift in infection transmission from avian influenza to human is due to the fact that the HAs of influenza viruses (H1, H2, and H3 in the past, and H5, H7, and H9 more recently) switched their binding preference from α2-3 Sia to α2-6 Sia (Maines et al., 2011; Jayaraman et al., 2011). However, in animal model studies using whole viruses, contradictory results were reported for influenza transmission, especially for viruses possessing binding ability to both types of glycans, α2-3 Sia and α2-6 Sia. For example, the A/New York/1/18 (NY18) and A/Texas/36/91 H1N1 viruses have dual specificity for α2-3 Sia and α2-6 Sia, but their transmission efficiencies are apparently different (Tumpey et al., 2007). Moreover, recent studies have shown that HA adaptation for "human" receptor binding alone is not sufficient for transmission among mammals, with the PB2 proteins of influenza viruses and other environmental factors also being required (Van Hoeven et al., 2009; Lowen et al., 2007, 2008). Even though these successive steps are important, the glycan–HA interactions seem to play a key role in determining the efficiency of influenza virus transmission. Therefore, it is important to monitor and evaluate the receptor (glycan) binding specificity and affinity of the HAs derived from influenza A viruses, especially those originating from birds and swine, to understand their potential ability for interspecies transmission clearly.

In the past, agglutinate and solid-phase fetuin capture assays were commonly used, but in the present forms both methods have shortcomings, in that the former defines only the sialic acid linkage and the latter is opposite to the physiological event (Chandrasekaran et al., 2008). Recent advancements in the chemical and enzymatic-based synthesis of glycans have lead to the development of two kinds of glycan array platforms, which are suitable for analyzing a wide range of glycan interactions with different HA subtypes (Childs et al., 2009; Consortium for Functional Glycomics, CFG). Although these array formats are suitable for high-throughput screening purposes, they have limitations in terms of kinetic analyses, as large amounts of HA are required for the analyses, due to their low sensitivity. As a consequence, the use of these arrays by different researchers may generate different conclusions in defining the glycan–HA specificity for some subtypes. For example, the HA derived from A/H1N1/California/04/2009 was reported to have dual specificity for binding, to both the α2-3 Sia and α2-6 Sia glycans (Childs et al., 2009), while another group reported that it had specificity for only the α2-6 Sia glycans (De Vries et al., 2011). Moreover, in these studies, it is important to consider that the glycan–HA interactions are multivalent in nature, especially when designing synthetic glycans for binding analyses, as mimics of cell surface glycans. These multivalent-glycans have higher binding affinity, as well as the ability to inhibit the glycan–HA interactions (Totani et al., 2003; Ogata et al., 2009). These studies suggested that such multivalent-glycans are more suitable for glycan–HA interaction analyses, as compared to the monomeric glycans.

To accurately define the glycan–HA binding specificity and affinity parameters for different complexes, an alternative and reliable method is required. In the present study, we have developed a protocol that allows us to analyze the glycan–HA interactions efficiently and kinetically by surface plasmon resonance (SPR), which is commonly used to assess various bio-molecular interactions (Kodoyianni, 2011; Cooper, 2003; Misono and Kumar, 2005), including carbohydrate–lectin interactions (Duverger et al., 2003; Dam et al., 2009). In our study, we showed that the glycan–HA binding analyses can be performed reliably and efficiently on Biacore-chips (either the SA-chip or the CAP-chip) in an SPR system, using chemically synthesized biotinylated multivalent-glycans. These analyses allowed us to determine the specificity as well as the global rate constants (association rate k_a, dissociation rate k_d, and equilibrium dissociation constant, K_D). When using the CAP-chip for these analyses, we were able to regenerate the surface for multiple analyses, allowing us to derive, for the first time, the precise kinetic parameters for different HA glycan complexes of newly emerging influenza viruses. These studies suggested that the SPR-based method is suitable for influenza surveillance to define the pandemic scenario, as well as for the screening of synthetic glycans and other compounds that may interfere with glycan–HA interactions.

2. Materials and methods

2.1. Proteins

Soluble forms of different HAs derived from newly emerging influenza viruses expressed in Baculovirus, were purchased from Prospec-Tany TechnoGene Ltd. (Tel Aviv, Israel). They are the A/H1N1/California/04/2009 (accession no. ACQ76318), A/H5N1/Vietnam/1203/2004 (accession no. AAW80717), and A/H5N1/Indonesia/05/2005 (accession no. ABP51969). The HA of A/H7N7/Netherlands/219/2003 (accession no. AAR02640) was obtained from SinoBiotechnology (China).

2.2. Glycans and buffers

Biotinylated multivalent-glycans were obtained from GlycoTech (MD, USA). The biotinylated multivalent-glycans used were: 01-077 [Neu5Acα2-3Galβ1-4GlcNAcβ1-PAA(polyacrylamide)-biotin]; 01-078 [Neu5Acα2-3Galβ1-3GlcNAcβ1-PAA-biotin]; 01-088 [Neu5Acα2-3Galβ1-3GalNAcα1-PAA-biotin]; 01-039a [Neu5Acα2-6Galβ1-4GlcNAcβ1-PAA-biotin]; and 01-000 [$HOCH_2(HOCH)_4CH_2NH$-PAA-biotin] (Fig. 1a). As a control 01-000 was used for binding to the spacer and biotin, without glycans. All HA glycan binding measurements were performed in HBS–P^+ buffer (0.01 M HEPES, 0.15 M NaCl, 0.05% (v/v) Surfactant P20, pH 7.4).

2.3. SPR analyses of glycan–HA interactions on the SA-sensor chip

To analyze the interactions between a multivalent-glycan and the HAs derived from newly emerging influenza A viruses, we performed SPR analyses on an SA-chip, using a Biacore T100 (GE Healthcare, USA), as illustrated in Fig. 1b. Initially, the biotinylated multivalent-glycans were dissolved in 0.3 M sodium phosphate buffer (pH 7.4). These glycans were then immobilized on the SA-chip (GE Healthcare, USA) by injecting a 100 nM concentration at a constant flow rate of 10 μl/min for 7 min, followed by washing with HBS–P^+ buffer. As a test case, the multivalent-glycan, 01-078, and the glycan-free compound, 01-000, were immobilized onto chips in flow cells 3 and 4, respectively. Once the glycan surfaces were prepared, we injected 60 μl of the HA protein (derived from A/H5N1/Vietnam/1203/2004 virus), as the analyte into the flow cell, at a flow rate of 30 μl/min for 2 min. All SPR analyses were performed at 25 °C. The binding data were collected for channels 3 and 4. Binding data sets from five different concentrations of HA (10–160 nM) were collected using a single-cycle kinetics mode (Karlsson et al., 2006). For each analyte concentration, cycles of analyte injection for 120 s, dissociation, and analyte-free buffer injection were performed. Between each cycle, a period of extended dissociation was carried out. The binding data were analyzed using the Biacore T100 Evaluation software, version 2.0.2 (GE Healthcare), and were fitted with a 1:1 binding model. This is the simplest model for the interaction between glycan and HA according to the