Hemagglutinin structure, membrane fusion and virus entry

Petr Michalek [1], Ludmila Krejcova [1], Vojtech Adam [1,2] and Rene Kizek [1,2*]

[1] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic, European Union;

[2] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic, European Union;

* Author to whom correspondence should be addressed; E-Mail: kizek@sci.muni.cz;

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Hemagglutinin (HA) is an antigenic glycoprotein, which is placed on the surface of the influenza viruses. It is responsible for binding the virus to the host cell, that is being infected. The name „hemagglutinin" comes from the ability of protein to cause erythrocytes to agglutinate („clump together"). The process is like this: Hemagglutinin (HA) binds to the monosaccharide sialic acid which is present on the surface of its target host cells. The cell membrane then engulfs the virus through endocytosis and followed by formation of endosome. The cell then attempts to begin digesting the contents of the endosome by acidifying its interior and transforming it into a lysosome. When the pH decrease to 6.0, the HA molecule becomes partially unfold, and release a hydrophobic portion of peptide chain that was previously hidden. This so-called „fusion peptide" acts like a molecular grapple hook for lock on the endosomal membrane. The rest of the HA molecule refolds into a new structure and pulls the endosomal membrane right up next to the viral membrane, causing the two to fuse together. When it happened, the viral RNA genome enters into the cell's cytoplasm.

1. Introduction

Influenza A viruses belong to the order Orthomyxoviridae and are responsible for significant annual morbidity and mortality. They are classified serologically based on the antigenic properties of their surface glycoproteins: the hemagglutinin (HA) and the neuraminidase (NA) [1]. To date, 18 HA subtypes, caused by antigenic shift, have been determined [2, 3]. These subtypes can be divided into 6 clades and two groups and this variability makes it difficult to effectively aim any drug against this structure. Furthermore, also antigenic drift can strengthen the ability to escape the virus from effective blockage [4].

2. Structure and function of hemagglutinin

HA is a type I transmembrane glycoprotein with a signal sequence that is removed post-translationally, a membrane anchor domain near the C terminus, and a short cytoplasmic tail [5]. Its size si about 13.5 nm and a molecular weight of about 76 kDa [6]. HA is a target molecule for neutralizing antibodies, and therefore is considered as the major surface antigen [7]. Primary function of HA is the initiation of infection, HA also involved in the host cell recognition and binding of the virus to host cell receptor, which is composed of sialic acid [6, 8]. The virus-receptor binding is followed by virus entry into the host cell and release of viral RNA from the virion, which allows subsequent replication. Nowadays, H1 (H1N1) and H3 (H3N2) are the most widespread HA influenza subtypes in human population. Also other subtypes, which are typically occured in waterfowl, may cause human infection or deaths such as H5 [9, 10], H7 [11], and H9 [12].

HA monomers are synthesized are assembled noncovalently to trimer in the endoplasmic reticulum, where also glycosylation occurs, and transported through the Golgi complex to the cell surface of infected cells as an uncleaved,

fusionin-incompetent precursor HA0, which is proteolytically cleaved into two smaller subunits (HA1 and HA2) [13-16]. Disulphide bridges, which linkage HA1 and HA2 subunits, which were formed in viral replication in the HA0 folding proces, are cleaved [17, 18]. HA0 cleavage can take place either in the Golgi apparatus or extracellularly (using enzymes produced by cells of the respiratory system), the process HA0 cleavage is essential for infectivity of the virus particles [19, 20]. After cleavage of HA0 precursors, three HA structures form a trimer, which has a mushroom shape [18]. Mushroom consists of antiparallel beta-sheet region HA1 subunits and elongated membrane-proximal domain (stem region) dominated by intertwined and interconnecting α-helices (HA2) [21]. Large membrane-distal, globular HA1 subunit, or the receptor binding region enables the binding of the virus to glycan receptors on host cells, which are formed by the sialic acid bounded by galactose [18, 22, 23].

3. Membrane fusion and virus entry

Sialic acid, linked to complex glycans on either glycoproteins or glycolipids, is the receptor for influenza binding. No significant conformational change of HA appears during receptor binding and virion is just attached to the cell surface. Fusion-inducing conformational change is activated by binding of one or more of protons, as the pH in the endosome goes lower [24]. Enveloped viruses enter cells through fusion of their viral membrane with a host cell membrane. This fusion process is thermodynamically favorable but kinetically very slow [25].

Influenza infection is initiated by the viral HA binding to sialic acid receptors on the surface of the host cell. It is widely accepted that the human-adapted HA subtypes preferentially bind to the α(2,6)-sialic acid linkage, whereas the avian-adapted HA subtypes preferentially bind to the α(2,3)-sialic acid linkage [26, 27]. Membrane fusion between host cell and influenza virus is a thermodynamically favorable process, but a high kinetic barrier is crossed as the two bilayers approach each other [28]. When bound to the specific sialic acid receptor on the target cell, the influenza virion is endocytosed in coated pits and vesicles, and delivered to endosomes [17]. This process is cell-type dependent and influenza virus can enter the host cell using either clathrin-dependent and clathrin-independent endocytosis or by macropinocytosis [29-31]. Proton pumps in the membranes of endocytic vesicles induce an accumulation in protons and therefore lowering of the pH between 5 and 6, which is essential for HA cleavage and causes the HA1 'head' to separate from the HA2 'stem' and enables a set of HA2 conformational transformations [13, 32]. This change causes the exposure of the N terminus of HA2, known as the fusion peptide and is required to promote fusion between the viral envelope and the target membrane and therefore is essential for virus infection [33-35].

4. Conclusions

The infection process of HA has been well documented throughout the years. But, the understanding of the influenza viral attack mechanism is still crucial for designing of new antiviral therapeutics such as protease and fusion inhibitors and cross-neutralizing antibodies that interfere with the fusion process.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements" for biomedical papers.

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