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Structural basis for the divergent evolution of influenza B virus hemagglutinin

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ABSTRACT

Influenza A and B viruses are responsible for the severe morbidity and mortality worldwide in annual influenza epidemics. Currently circulating influenza B virus belongs to the B/Victoria or B/Yamagata lineage that was diverged from each other about 30–40 years ago. However, a mechanistic understanding of their divergent evolution is still lacking. Here we report the crystal structures of influenza B/Yamanashi/166/1998 hemagglutinin (HA) belonging to B/Yamagata lineage and its complex with the avian-like receptor analogue. Comparison of these structures with those of undiverged and diverged influenza B virus HAs, in conjunction with sequence analysis, reveals the molecular basis for the divergent evolution of influenza B virus HAs. Furthermore, HAs of diverged influenza B virus strains display much stronger molecular interactions with terminal sialic acid of bound receptors, which may allow for a different tissue tropism for current influenza B viruses, for which further investigation is required.

Introduction

Influenza A and B viruses of the *Orthomyxoviridae* family are responsible for seasonal influenza epidemics with severe morbidity and mortality worldwide (Palese and Shaw, 2007; Taubenberger and Morens, 2008; Wright et al., 2007). Annual influenza vaccine has been conventionally used to reduce human suffering and economic burden caused by the viruses. However, the constant genetic and antigenic changes of influenza viruses render them the ability to evade host immune system (Nelson and Holmes, 2007), thus limiting the vaccine effectiveness.

Influenza B virus was first isolated in 1940 and has diverged into two genetically and antigenically distinct lineages since 1983 or earlier (Chen et al., 2007; Shen et al., 2009): the B/Victoria lineage represented by the reference strain B/Victoria/2/87, and the B/Yamagata lineage represented by the B/Yamagata/16/88 strain, respectively (Kanegae et al., 1990; Rota et al., 1990; Shaw et al., 2002). The B/Victoria lineage predominated during the 1980s, while the B/Yamagata lineage predominated in most part of the world during the 1990s (Lin et al., 2004). The B/Victoria lineage re-emerged in Europe and United States in 2001, and the two lineages have co-circulated ever since (Ikonen et al., 2005; Shaw et al., 2002).

As one of the two major surface glycoproteins of influenza virus, hemagglutinin (HA) mediates host entry of the virus and is a primary target for host neutralizing antibodies (Han and Marasco, 2011). The precursor of HA, HA_0, is synthesized as a single-stranded polypeptide, which is then cleaved into two disulfide-bonded subunits: HA_1 and HA_2 (Copeland et al., 1986). HA_1 contains the receptor-binding site and harbors the majority of antigenic sites that undergo constant antigenic variations (Knossow and Skehel, 2006). On the other hand, HA_2, which contains the fusion peptide at its N-terminus and is responsible for inducing fusion of viral envelope and endosomal membrane, is the most conserved (Vareckova et al., 2003). In an infection, HA first binds to the sialic-acid receptors on the host cell surface, thus triggering the internalization of the virus by endocytosis (Matlin et al., 1981; Skehel and Wiley, 2000). The low-pH environment in the late endosome results in the protonation of multiple negative charged residues located at the HA_1–HA_1 and HA_1–HA_2 interfaces, thereby dissociating HA_1 and HA_2 (Korte et al., 2007; Rachakonda et al., 2007; Wang et al., 2008). The subsequent large-scale conformational change in HA_2 (Bullough et al., 1994; Chen et al., 1999) fuses the viral and endosomal membranes and allows the delivery of viral genetic materials into cellular cytosol.

The determination of the first crystal structure of the ectodomain of influenza virus B/Hongkong/8/1973 (B/HK/73) HA has allowed the mapping of its antigenic structure (Wang et al., 2008). There are four major epitopes on the membrane-distal globular domain of influenza B virus HA: the 120-loop ($HA_1$116–137), the 150-loop ($HA_1$141–150), the 160-loop ($HA_1$162–167), and

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Table 1
Data collection and refinement statistics.

	B/Yamanashi/98 HA	B/Yamamashi/98 HA-LSTa
Data collection statistics		
Resolution range (Å)	43.6–3.54 (3.73–3.54)	39.37–2.50 (2.64–2.50)
Space group	*C* 1 2 1	*C* 1 2 1
Unit cell		
a, *b*, *c* (Å)	174.9, 101.3, 136.8	176.1, 101.5, 137.4
α, β, γ (°)	90, 115.2, 90	90, 115.2, 90
Unique reflections	26,547	75,849
Multiplicity	3.8 (3.8)	3.8 (3.8)
Completeness (%)	99.98 (100.00)	99.99 (100.00)
Mean I/sigma(I)	10.6 (2.3)	11.7 (2.3)
Wilson *B*-factor (Å^2)	109.1	46.7
R_{merge} (%)	9.8 (49.7)	9.1 (49.4)
Refinement statistics		
R_{cryst} (%)	19.8 (27.2)	18.8 (26.2)
R_{free} (%)	24.4 (30.9)	22.4 (32.0)
Number of atoms	12,115	12,754
Protein	11,695	11,695
Ligands	420	588
Water	0	471
RMSD bond length (Å)/bond angle (°)	0.004/1.03	0.007/0.87
Ramachandran plot		
Favored, allowed, disallowed (%)	96.3, 3.6, 0.1	96.5, 3.4, 0.1
Average *B*-factor (Å^2)	120.6	50.3

Statistics for the highest-resolution shell are shown in parentheses. The coordinates and structure factors of B/Yamanashi/98 HA and B/Yamanashi/98 HA-LSTa have been deposited into Protein Data Bank under accession codes of 4M40 and 4M44, respectively.

the 190-helix ($HA_1$194–202) and their respective surrounding regions. All these four epitopes have been demonstrated to be under positive selective pressure in the course of evolution (Nunes et al., 2008; Pechirra et al., 2005; Shen et al., 2009). However, the mechanism by which amino-acid substitutions change the antigenic property of HA remains elusive.

Here we report the crystal structure of influenza B/Yamanashi/166/1998 (B/Yamanashi/98) HA determined to 3.54-Å resolution (Table 1), a strain belonging to the B/Yamagata lineage. Together with the recently determined structure of influenza B/Brisbane/60/2008 (B/Brisbane/08) HA (B/Victoria lineage) and the complex structure of the membrane-distal globular domain of influenza B/Florida/4/2006 (B/Florida/06) HA (B/Yamagata lineage) in the region of $HA_1$33–324 with its antibody (Dreyfus et al., 2012), we performed a systematic structural comparison to characterize the evolution of HA antigenicity at the molecular level. In addition, we have also determined the crystal structure of B/Yamanashi/98 HA in complex with avian-like receptor analogue to 2.50-Å resolution. This, in comparison with the structure of B/HK/73 HA complexed with avian-like receptor analogue (Wang et al., 2007), reveals that the diverged influenza B virus HAs have developed a distinct and much more efficient receptor-binding site than early strains.

Results

The overall structure of B/Yamanashi/98 HA

The structure of unliganded B/Yamanashi/HA is determined to 3.54-Å resolution (Table 1). There is one HA trimer in an asymmetric unit (Fig. 1a). Each subunit of B/Yamanashi/98 HA has the same seven disulfide bridges as found in B/HK/73 HA (Wang et al., 2008): $Cys4_1$–$Cys137_2$, $Cys54_1$–$Cys57_1$, $Cys60_1$–$Cys72_1$, $Cys94_1$–$Cys143_1$, $Cys178_1$–$Cys272_1$, $Cys292_1$–$Cys318_1$ and $Cys144_2$–$Cys148_2$ (Fig. 1b).

B/Yamanashi/98 HA has nine predicted glycosylation sites (six on HA_1: 25, 59, 145, 163, 301, 330; and three on HA_2: 145, 171, and 184) on each subunit. All glycans are built in the final structure (Fig. 1a and b) with the exception of $HA_2$171 for which no clear density is observed and $HA_2$184 that was not present in the construct used for crystallization. The glycosylation at $HA_1$145 that first appeared in B/Great Lakes/54 HA has been maintained up to today. On the other hand, the glycosylation at $HA_1$123 as found in B/HK/73 HA (Wang et al., 2008) is completely abolished in HAs of both B/Victoria and B/Yamagata lineages due to the predominant presence of Ile-125 instead of Thr-125 (Table 2). It is not clear why the glycosylation site at $HA_1$123 is so short-lived. One explanation is that since the 120-loop is a strong epitope of influenza B virus HA, different mutational strategies have been used to evade recognition by host immunity. The glycosylation site at $HA_1$123 in B/HK/73 is just one such strategy and did not get populated in subsequent influenza B virus strains. However, the majority of B/Victoria lineage strains have established a new glycosylation site at $HA_1$230, which is present in 50% of early influenza B virus strains, and not present in the majority of B/Yamagata lineage strains (Table 2).

Similar to B/HK/73 HA (Wang et al., 2008), the fusion peptide of B/Yamanashi/98 HA located at the N-terminus of HA_2 points away from its own helix A and helix B to interact with those of a neighbouring subunit instead (Fig. 1c).

Antigenic structure of influenza B virus HAs

Known influenza B virus HA structures are of B/HK/73 representing undiverged early strains (PDB codes: 3BT6, 2RFT and 2RFU) (Wang et al., 2008, 2007), B/Brisbane/08 representing the B/Victoria lineage (PDB code: 4FQM) (Dreyfus et al., 2012), B/Florida/06 (in the region of $HA_1$33–324, PDB code: 4FQJ) (Dreyfus et al., 2012) and B/Yamanashi/98 belonging to the B/Yamagata lineage. We performed a structure-based analysis of the divergent evolution of these HAs. Since the B/Florida/06 HA is in complex with human monoclonal antibody CR8071 in the region of residues $HA_1$37–41, 52–62, 85–90, and 282–287 (Dreyfus et al., 2012), we will ignore the structural variations in these regions in the following comparison.