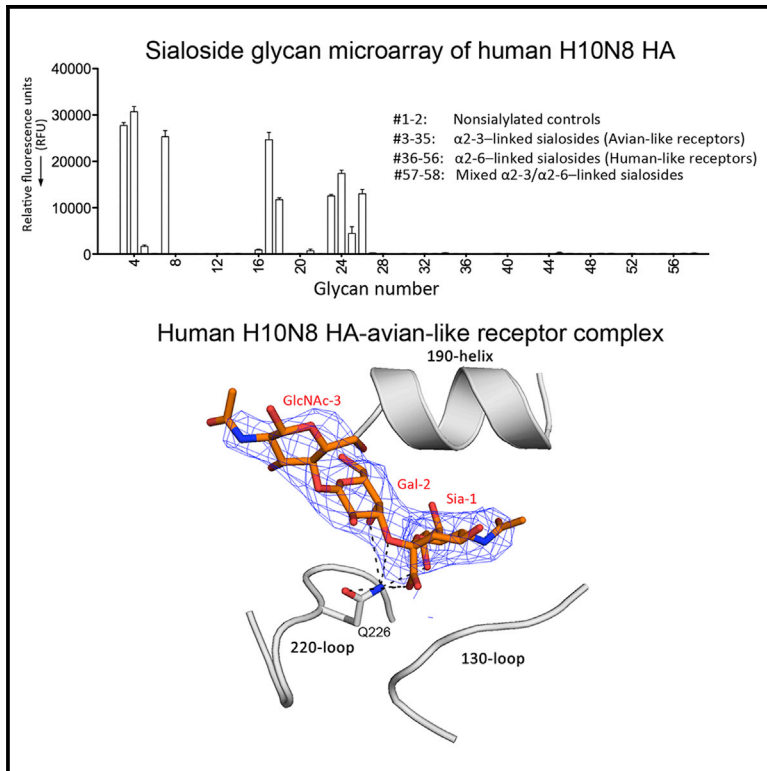


Cell Host & Microbe

A Human-Infecting H10N8 Influenza Virus Retains a Strong Preference for Avian-type Receptors

Graphical Abstract



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In Brief

The recently identified avian-origin human H10N8 influenza A virus poses a potential pandemic threat. By performing structural studies and examining receptor specificity, Zhang et al. reveal that H10N8 hemagglutinin retains a strong preference for avian receptors and suggest that the current human H10N8 is poorly adapted for efficient human-to-human transmission.

Highlights

- Human influenza H10N8 HA has negligible binding to human-like receptors
- Human influenza H10N8 HA retains strong binding to avian-like receptors
- The human receptor orientation in H10 HA differs from most human HA complexes
- Mutations that switch specificity in pandemic viruses do not alter H10 specificity

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A Human-Infecting H10N8 Influenza Virus Retains a Strong Preference for Avian-type Receptors

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SUMMARY

Recent avian-origin H10N8 influenza A viruses that have infected humans pose a potential pandemic threat. Alterations in the viral surface glycoprotein, hemagglutinin (HA), typically are required for influenza A viruses to cross the species barrier for adaptation to a new host, but whether H10N8 contains adaptations supporting human infection remains incompletely understood. We investigated whether H10N8 HA can bind human receptors. Sialoside glycan microarray analysis showed that the H10 HA retains a strong preference for avian receptor analogs and negligible binding to human receptor analogs. Crystal structures of H10 HA with avian and human receptor analogs revealed the basis for preferential recognition of avian-like receptors. Furthermore, introduction of mutations into the H10 receptor-binding site (RBS) known to convert other HA subtypes from avian to human receptor specificity failed to switch preference to human receptors. Collectively, these findings suggest that the current H10N8 human isolates are poorly adapted for efficient human-to-human transmission.

INTRODUCTION

In December 2013, a reassortant H10N8 influenza A virus was identified in Jiangxi province, China (A/Jiangxi-Donghu/346/2013 [H10N8]) (Chen et al., 2014). Three further cases of human infection with H10N8 virus (AIV) have been confirmed, leading to two deaths (Report of Health and Family Planning Commission of Jiangxi Province, 2014). In May 2014, this H10N8 AIV was reported to be infectious among feral dogs in live poultry markets in Guangdong Province, China (Su et al., 2014). Thus, it is of major public health interest to understand the extent to which the current circulating H10N8 viruses have evolved any capability to

bind human receptors and thus facilitate human-to-human transmission (García-Sastre and Schmolke, 2014).

Several H10 viruses cause disease in mammals. H10N7 viruses have caused conjunctivitis in humans in Egypt in 2004 and Australia in 2010 and 2012 (Arzey et al., 2012; Pan American Health Organization, 2004), whereas aerosol infection of mink with an H10N7 virus led to mild pulmonary lesions (Englund, 2000). Very recently, H10N7 virus was detected in dead seals and involved in mass mortality in Denmark, Sweden, Germany, and the Netherlands (Zohari et al., 2014). Relatively few studies have been carried out on avian H10N8 infections in humans and other species. An avian H10N8 strain (A/environment/Dongting Lake/Hunan/3-9/2007), isolated from water samples of Dongting Lake wetland, replicated efficiently in the mouse lung, and virulence increased rapidly during adaptation, indicating ability to adapt to a mammalian host (Zhang et al., 2011). Phylogenetic analysis shows that human H10N8 originated through the reassortment of H9N2 strains with other viruses circulating in poultry and in environmental samples (such as wild birds and water samples from their habitat in the wetlands) from Jiangxi Province; its hemagglutinin (HA) and neuraminidase (NA) genes originated from ducks and wild birds, respectively (Chen et al., 2014; Shi et al., 2014; Liu et al., 2015). This type of reassortment is similar to influenza A H5N1 and H7N9 viruses isolated from humans; the H10N8 virus also acquired six internal gene segments from an H9N2 virus (Chen et al., 2014).

HA is the viral surface glycoprotein responsible for viral entry into host cells through binding to sialylated receptors on the cell surface followed by pH-triggered membrane fusion in endosomal compartments. A switch in receptor-binding specificity from avian α 2-3 to human α 2-6 linked receptors is a major obstacle for influenza A viruses to cross the species barrier for adaptation to a new host. The Gly225-Gln226-Ser227-Gly228 (H3 numbering is used throughout) motif in the receptor-binding site (RBS) of the human H10N8 HA suggested avian-like receptor binding preference. Only one basic amino acid (arginine) in the cleavage site between HA1 and HA2 was consistent with its low pathogenicity in poultry (Chen et al., 2014). However, the H10N8 HA contained Ala135Thr and Ser138Ala substitutions that favor mammalian adaptation; M1 Asn30Asp and Thr215Ala

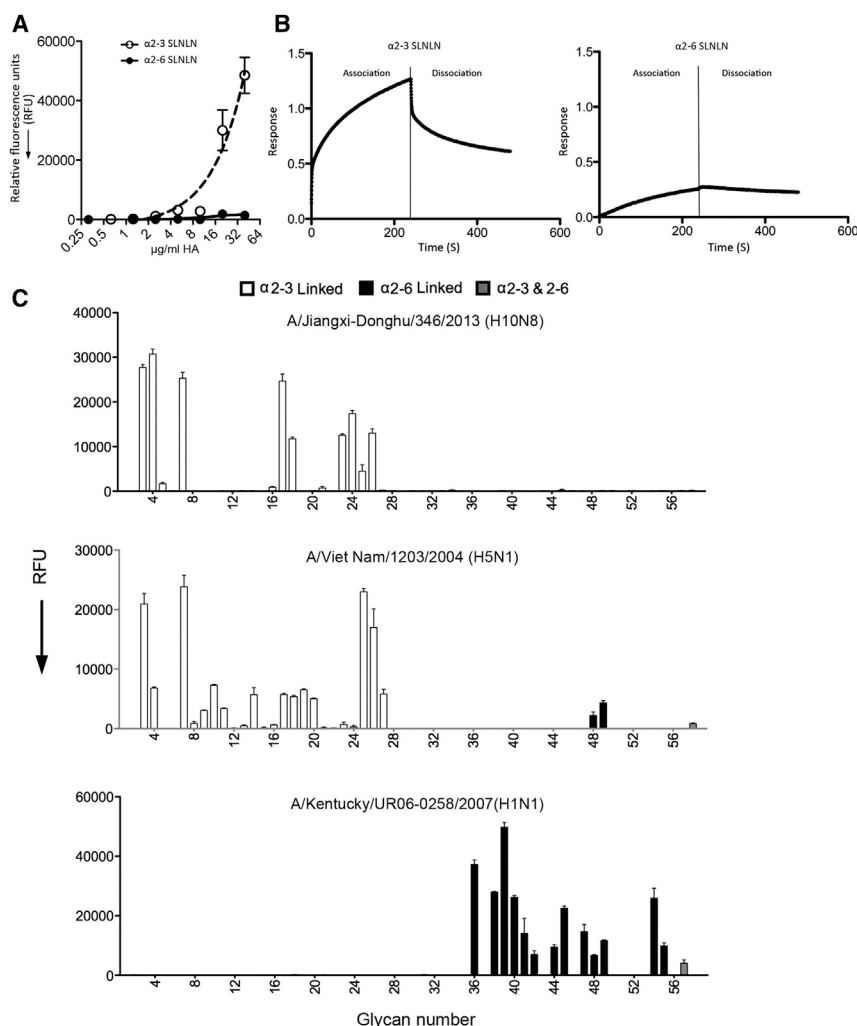


Figure 1. Receptor Binding Properties of Human H10N8 HA from the A/Jiangxi-Donghu/346/2013 Virus

(A) Glycan binding specificity of H10 HA by an ELISA-like assay. The micro-well glass slides were imprinted with either avian receptor α 2-3- or human receptor α 2-6-linked SLN LN-polyacrylamide (PAA) and probed with recombinant HAs produced in HEK293S GnT1[−] cells.

(B) Glycan binding specificity of H10 HA by biolayer interferometry on an Octet RED system (ForteBio) using recombinant HAs produced in insect cells. HA binding at 0.55 mg/ml was measured against immobilized biotinylated glycans α 2-3- and α 2-6-SLN LN. The binding curve for α 2-3-SLN LN was best fitted using the 2:1 heterogeneous ligand binding model (Concepcion et al., 2009), with apparent K_D values of 0.86 μM and 0.65 μM . See also Figure S1A.

(C) Receptor binding analysis of H10 HA by a sialoside glycan microarray using recombinant HAs produced in HEK293S GnT1[−] cells. Recombinant HAs from H5N1 and human seasonal H1N1 viruses were used as controls. The mean signal and SE were calculated from four independent replicates. Non-sialylated controls are #1 and #2, α 2-3 sialosides are in white bars (#3–#35), α 2-6-linked sialosides in black bars (#36–#56), and mixed biantennary glycans in gray bars (#57 and #58). Glycans imprinted on the array are listed in Table S1. See also Figure S1B.

(apparent K_D of 0.86 μM and 0.65 μM [for K_{D1} and K_{D2}]) with no detectable binding to human analog α 2-6 SLN LN (Figures 1B and S1A). This binding affinity is similar to human H7N9 HA (A/Shanghai/2/2013) with apparent $K_D > 1 \mu\text{M}$ to α 2-3 SLN LN and no detectable binding to α 2-6 SLN LN (Xu et al., 2013). However, human H10N8 HA was recently reported to have similar

binding affinities to avian-like receptor 3'-SLN and human-like receptor 6'-SLN (1.81 and 1.39 mM, respectively) (Vachier et al., 2014).

We therefore further characterized H10 HA receptor binding on a custom influenza receptor glycan microarray comprised of diverse α 2-3 sialosides (#3–#35) and α 2-6 sialosides (#36–#56) that correspond to biologically relevant N- and O-linked glycans and linear fragments on mammalian glycoproteins and glycolipids (Table S1). Human H10 HA expressed in mammalian cells showed strong selective recognition of avian α 2-3 receptor analogs, with negligible binding to human-like α 2-6 linked receptors (Figure 1C), similar to H5N1 HA (A/Viet Nam/1203/2004) and in marked contrast to a human seasonal H1N1 virus (A/Kentucky/UR06-0258/2007) (Figure 1C). The bound α 2-3 sialosides include sulfated linear glycans (#3, #4, and #7), linear and short branched O-linked glycans (#17 and #18), and long branched biantennary O-linked and N-linked glycans (#23–#26). Additional avian α 2-3 receptor analogs can be bound by H10 HA expressed in insect cells (Figure S1B), which can be attributed to less hindrance from the smaller high-mannose glycans on the HA surface (Stevens et al., 2006b; Xu et al., 2013). Thus, human H10 HA exhibits strong avian-type receptor specificity, with no detectable binding

RESULTS

Receptor Binding of H10 HA

We analyzed binding of recombinant H10 HA to avian and human linear glycan receptor analogs, α 2-3-sialylated di-N-acetylglucosamine (SLN LN) and α 2-6 SLN LN, respectively. The ELISA-like binding assay showed that H10 HA has specific recognition for avian analog α 2-3 SLN LN, but no detectable binding to human analog α 2-6 SLN LN, even at high concentrations (up to 50 $\mu\text{g/ml}$; Figure 1A). Similarly, by biolayer interferometry, specific binding was observed to avian analog α 2-3 SLN LN

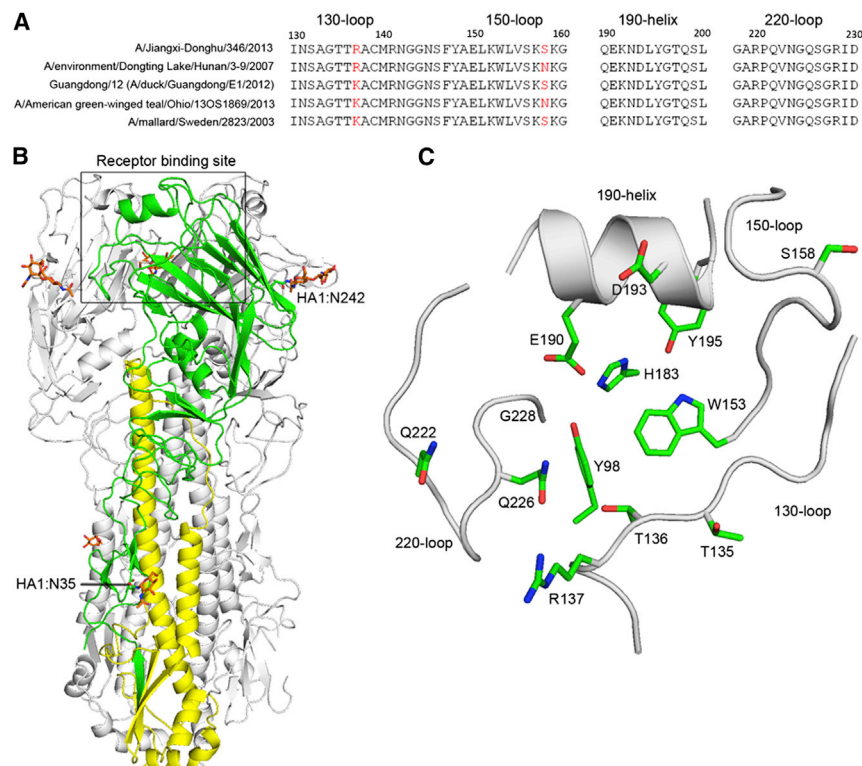


Figure 2. Crystal Structure of Human H10N8 HA from the A/Jiangxi-Donghu/346/2013 Virus

(A) Sequence alignment of the RBS of HAs from representative H10N8 strains from Asia, North America, and Europe. Human A/Jiangxi-Donghu/346/2013 virus was isolated from a geographic area similar to that of avian A/environment/Dongting Lake/Hunan/3-9/2007 and Guangdong/12 (A/duck/Guangdong/E1/2012) in China. Different residues are shown in red. Residues between human and avian H10 HAs in RBSs are highly conserved. See also Figures S2A and S2B.

(B) HA trimer is shown in cartoon representation. For one protomer, HA1 is colored in green and HA2 in yellow. The other two protomers are in gray. N-glycosylation sites and N-linked glycans are highlighted in sticks and numbered at the Asn attachment site.

(C) The RBS of human H10N8 HA with a characteristic avian glutamine (Q226).

Structure of the Human H10 HA in Complex with an Avian Receptor Analog

To uncover the basis for preferential recognition of avian-like receptors, we determined the H10 HA structure with avian receptor analog 3'-SLN at 2.85 Å

to human-type receptors, similar to HAs from other recent human-infecting, avian-origin H7N9, H5N1, and H6N1 viruses analyzed by us and others (Paulson and de Vries, 2013; Shi et al., 2013; Xu et al., 2013; de Vries et al., 2014; Tzarum et al., 2015).

Structural Characterization of H10N8 HA and Its RBS

Sequence alignment of human H10N8 HA with those in avian H10N8 and H10 viruses isolated from mammals showed that the H10 HA RBS residues are highly conserved (Figure 2A), although many substitutions can be observed (Table S2). To explore the structural characteristics of human H10N8 HA, we first determined its crystal structure at 2.60 Å resolution (Figure 2B; Table S3) using human H7N9 HA (A/Shanghai/2/2013) (Protein Data Bank [PDB] entry 4N5J) as the molecular replacement search model. The H10 and H7 HA structures are similar with an overall C_{α} root-mean-square deviation (RMSD) of 1.3 Å and only 0.4 Å for the receptor binding subdomain (residues 117–265) (Ha et al., 2002). Superimposition of the H10 HA monomer on other avian and human HAs confirms that H10 is structurally closest to Group 2 H3 and H7 HAs (PDB entries 4FNK and 4N5J) with overall C_{α} RMSDs of 1.3–1.8 Å, compared to 2.6–2.9 Å for Group 1 H1, H2, and H5 HAs (PDB entries 3AL4, 3KU5, and 2FKO). Electron density for two N-linked glycosylation sites was observed at the conserved Asn35, as well as at Asn242, in each HA1 (Figure 2B).

The RBS base consists of highly conserved Tyr98, Trp153, His183, and Tyr195, and its sides are formed from three secondary elements: 130-loop, 190-helix, and 220-loop (Figure 2C). The main differences in the H10 RBS arise from Ala135Thr, Ser137Arg, and Leu226Gln substitutions compared to human H7 HA (A/Shanghai/2/2013) (Table S4).

(Table S3). Clear, interpretable electron density was observed for all sugars of 3'-SLN: sialic acid (Sia-1), galactose-2 (Gal-2), and N-acetylglucosamine-3 (GlcNAc-3) (Figure 3A).

Seven highly conserved hydrogen bonds are formed between Sia-1 and the 190-helix, 130-loop, and 220-loop (Figure 3B). The Gln226 side chain makes three direct contacts with the O1-hydroxyl and O3-carboxyl group of Sia-1 (2.4 Å, 2.8 Å, and 3.4 Å). This mode of recognition is conserved in avian HA subtypes (Xu and Wilson, 2012). The Tyr98 hydroxyl hydrogen bonds to Sia-1 8- and 9-hydroxyls, and Trp153 makes hydrophobic interactions with Sia-1 C7 and C8 (Figure 3B). A slightly stronger conserved hydrogen bond (2.9 Å) between His183 Ne-2 and Sia-1 9-hydroxyl can be observed compared to most HA-avian receptor structures (3.1–3.6 Å). The major RBS substitution between avian and human H10 HAs is Lys137Arg. In human H10 HA, Arg137 Ne-2 and its main-chain amide hydrogen bond with the 1-hydroxyl of Sia-1 (2.9 Å) and 2-hydroxyl of Gal-2 (3.1 Å), respectively. However, no contact was observed between Lys137 and the avian receptor in the recent avian H10N2 HA structure (PDB entry 4CZ0) (Vachieri et al., 2014).

Similar to most avian HA structures, including avian wild-type H5 (Liu et al., 2009; Lin et al., 2009; Xiong et al., 2013; Xu et al., 2013), 3'-SLN binds in a *trans* conformation (Figures 3B and S3A). The second (Gal) and the third (GlcNAc) sugars in 3'-SLN exit above the 220-loop. This conformation is stabilized by hydrogen bonding of Gal-2 to Gln226 in most avian HA-receptor analogs (Liu et al., 2009; Lin et al., 2009; Xiong et al., 2013). Similarly, the *trans* conformation in H10 complex is mediated by hydrogen bonding of Gal-2 (O3 and O4) to Gln226 and also from Gal-2 (O2) to Arg137 (Figure 3B), which has only been reported for an avian analog with a human-transmissible H5

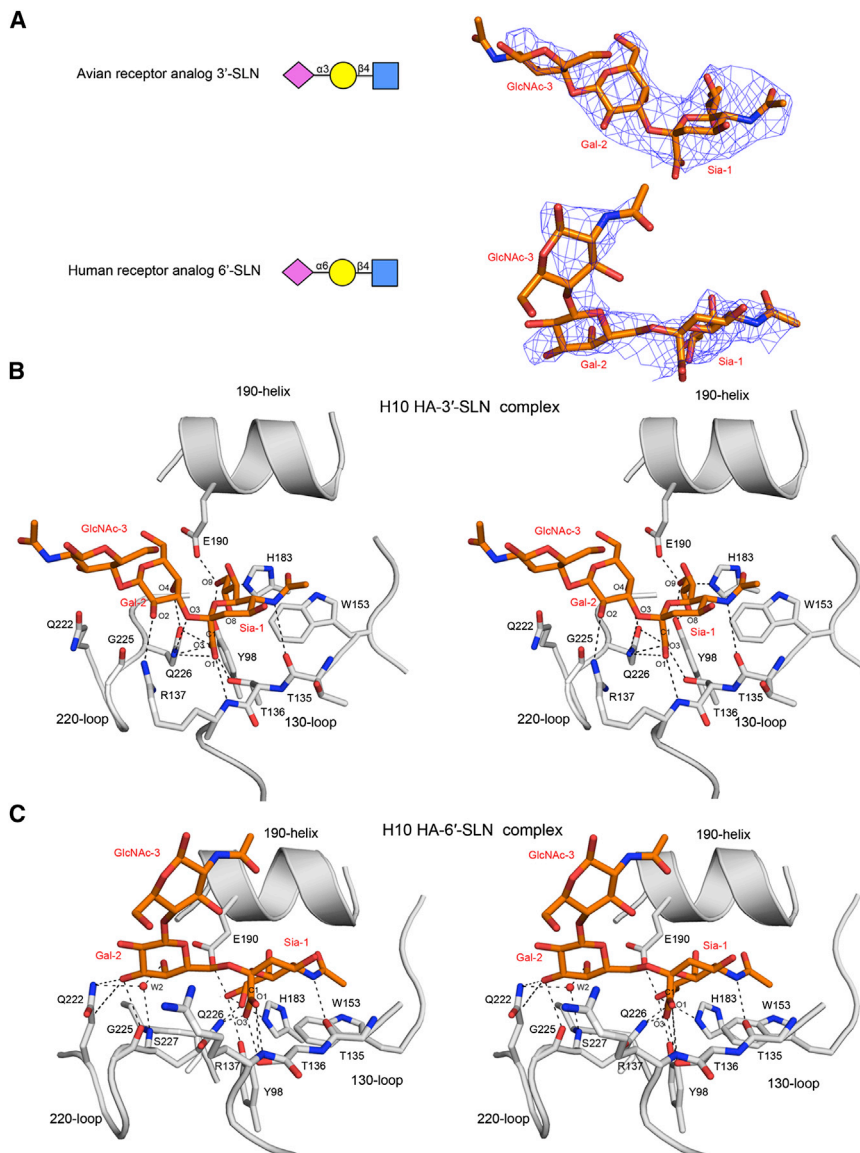


Figure 3. Crystal Structures of Human H10N8 HA in Complex with Avian and Human Receptor Analogs

(A) Glycan structures of avian receptor analog 3'-SLN and human receptor analog 6'-SLN in human H10 HA complexes and unbiased omit electron density map for the ligands contoured at a 1 σ level. In the glycan structures, purple diamonds represent sialic acid (Sia), yellow circles represent galactose (Gal), and blue squares represent glucose N-acetylglucosamine (GlcNAc). In the 3'-SLN complex structure, electron density for all three sugars is well defined in chains A and C, while Sia-1 and Gal-2 are visible in chain E. In the 6'-SLN complex structure, electron density for all three sugars is well defined in chain A, while Sia-1 and Gal-2 are interpretable in chain C, and no sugar is observed in chain E (RBS may be partially occluded).

(B and C) Interactions between the H10 HA RBS and avian and human receptor analogs. Conserved secondary elements of the RBS (130-loop, 190-helix, and 220-loop) are labeled and shown in cartoon representation. Selected residues and receptor analogs are labeled and shown in sticks. The RBS is colored in gray and receptor analogs in orange. Hydrogen bond interactions of Sia-1, Gal-2, and GlcNAc-3 of 3'-SLN (B) and 6'-SLN (C) with the H10 HA RBS are shown. See also [Figures S3 and S4](#).

mutant ([Zhang et al., 2013](#)). The Q226 interactions with the avian receptor resemble those of avian H1 and are distinct from those in human H1 HAs ([Lin et al., 2009](#); [Xu et al., 2012](#)) ([Figure S3B](#)). The preference of human H10 for avian receptors therefore correlates with the presence of glutamine 226 as in avian H1N1 HAs.

Structure of Human H10 HA in Complex with a Human Receptor Analog

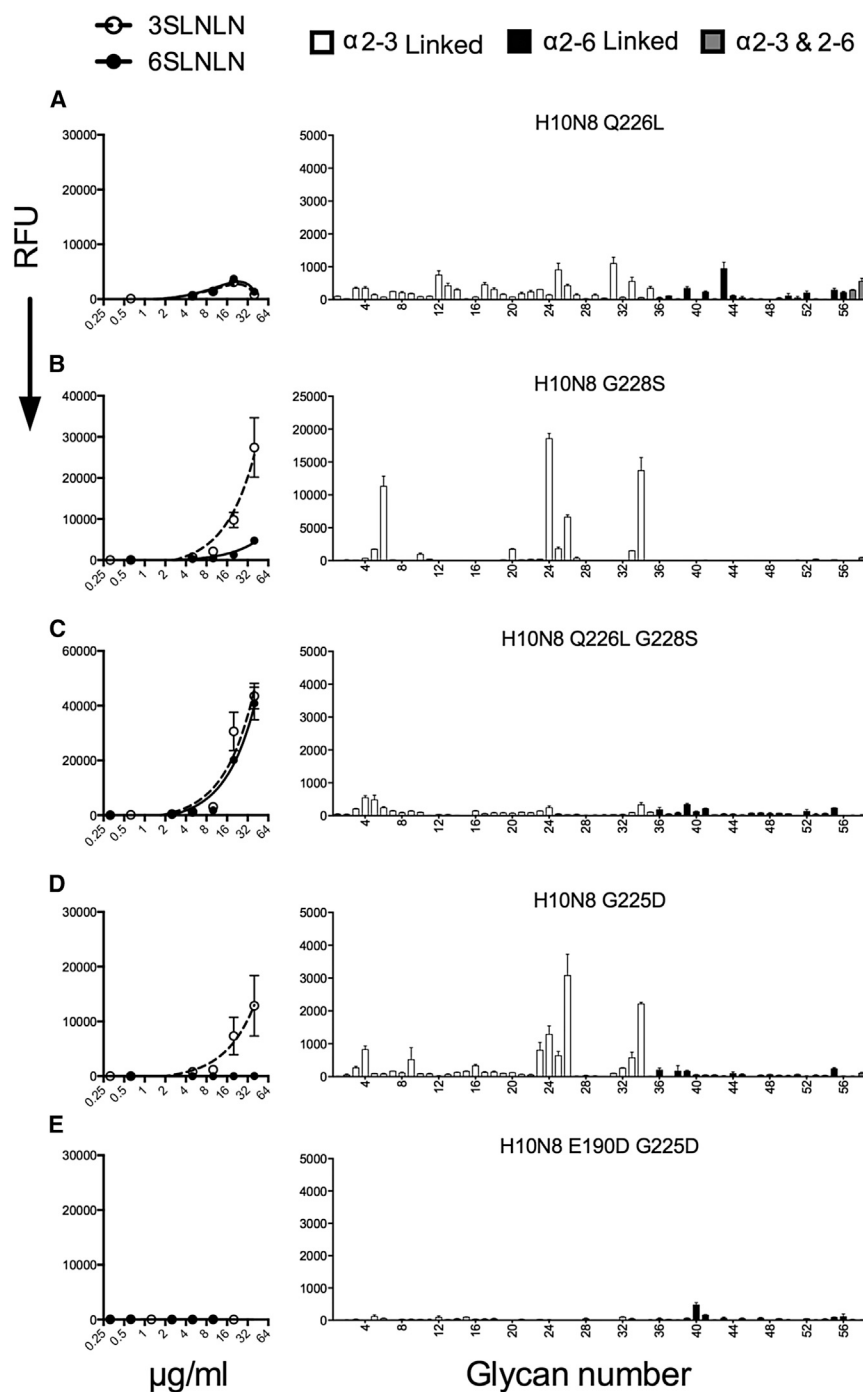
Despite no detectable binding of α 2-6 SLN to H10 HA in the glycan array ([Figures 1A and 1B](#)), soaking of HA crystals with very high concentrations (5 mM; HA:ligand stoichiometry of 1:55) of human receptor analog 6'-SLN enabled visualization of this weakly bound ligand at 3.31 Å resolution ([Table S3](#)). Electron density for all three sugars was fairly well defined with weaker density for GlcNAc-3 ([Figure 3A](#)). The 6'-SLN binds in a *cis* conformation ([Figure 3C](#)), similar to other human HA-6'-SLN structures ([Lin et al., 2009, 2012](#); [Shi et al., 2013](#); [Xu et al., 2012](#); [Zhang et al., 2013](#)), but with a different orientation due to rotation around the

Gal-2 C6-C5 bond compared to human H1/H3 and ferret-transmissible H5 complexes, and similar to the human H7 complex ([Figures S4A–S4D](#)). The receptor also has slightly different phi angles ($O6_{Sia}-C2_{Sia}-O-C6_{Gal}$) from those in H1/H3/H5 complexes (41° in H10 to 60° – 70° in H1/H3/H5/H7; [Figures S4A–S4D](#)). This conformation differs from the low-energy solution conformation of α 2-6 sialosides ([Sabesan et al., 1991](#)) or the equivalent umbrella-like topology observed in HA-human-like (α 2-6) receptor structures ([Chandrasekaran et al., 2008](#)). Thus, the H10 HA RBS is not configured for binding of human α 2-6 receptors.

The hydrogen bonding interactions of Sia-1 with the RBS are conserved and similar to the 3'-SLN complex ([Figures 3B and 3C](#)), but the Gal-2 interactions differ. Gal-2 3-hydroxyl hydrogen bonds to Gln222 $N\epsilon$ -2 and $O\epsilon$ -1 and Gly225 main-chain carbonyl. Interactions between Gal-2 4-hydroxyl and Gln222 $N\epsilon$ -2 and Ser227 main-chain amide mediated via a well-ordered water molecule (W2) ([Figure 3C](#)) also help stabilize the Gal-2 conformation. Overall, our results suggest that the current H10 has not yet evolved to preferentially bind human receptors, and Arg137 has no direct contact with the human receptor ([Figure 3C](#)) in comparison to another study of human H10 ([Figures S4E–S4G](#)).

Mutation of Key Residues in the RBS of the Human H10 HA

The potential for H10 HA to acquire human type specificity was assed using mutations associated with the switch in human



pandemic viruses, namely Q226L/G228S in H2N2/H3N2 and E190D/G225D in H1N1. Neither pair of mutations causes a significant switch in receptor specificity (Figure 4). The Q226L substitution reduces affinity to α 2-3 sialosides but gains only very weak binding to human-type α 2-6 receptors (Figure 4A). The G228S mutant does not affect binding avidity or overall specificity compared to wild-type HA (Figure 4B); however, several fucosylated structures (#32–#34) are now bound. The double-mutant Q226L/G228S shows increased binding to PAA-linked 6'-SLNLN, but with no reduction in binding to PAA-linked 3'-

receptor binding by avian (mallard) H10N2 viruses was recently reported to exhibit a preference for avian-type receptors, but with binding to human receptors almost as strong as that of pandemic H1 (1918 Spanish influenza) and H3 (1968 Hong Kong influenza) (Vachieri et al., 2014). This apparent discrepancy in binding specificity is likely a reflection of a difference in the assays in the two studies in this study, such as the use of whole influenza viruses instead of hemagglutinin, and the use of lower avidity short receptor analogs that are not bound in the glycan microarray (α 2-3 and α 2-6 linked SLN; Figure 1C, glycans #8 and #38).

Figure 4. Receptor Binding Specificities of Human H10 HA RBS Mutants by ELISA-like Glycan Binding and Glycan Microarray Assays

(A–E) Micro-well glass slides imprinted with either α 2-3- or α 2-6-linked SLNLN-PAA (left), and glycan arrays for assessment of receptor binding specificity (right), as in Figure 1. The slides were probed with recombinant HA mutants in RBS, including Q226L (A), G228S (B), Q226L/G228S (C), E190D (D), and E190D/G225D (E), produced in HEK293S GnTI⁻ cells. The mean signal and SE were calculated from six independent replicates. The mutations associated with a switch from avian to human receptor binding in pandemic H1/H2/H3 viruses did not generate human receptor binding for human H10 HA.

SLNLN in the ELISA type assay. However, the double mutant largely abrogates binding to both types of receptors in the glycan array (Figure 4C). Such differential binding results obtained by ELISA-like assay and glycan array (presumably due to higher density and, hence, avidity effects with PAA polymers in the ELISA-like assay) have been observed for human H7 HA (A/Shanghai/2/2013) and receptor mutants of human H5N1 (A/Vietnam/1203/2004) (Chen et al., 2012; Xu et al., 2013). The G225D mutation on the H10 HA framework retains specificity for α 2-3-sialylated glycans (Figure 4D), but with reduced avidity to a different subset of glycan receptors relative to wild-type (Figure 1C). The double E190D/G225D mutant exhibits dramatically reduced avidity in both assays (Figure 4E), as observed previously for A/Vietnam/1203/2004 (Stevens et al., 2006c).

DISCUSSION

Analysis of the receptor specificity and X-ray structures of the H10 HA clearly show that the avian-origin human H10N8 influenza virus exhibits a clear preference for avian-type receptors, as for other avian influenza viruses. Using a different assay,

The nature of the HA receptor binding specificity is believed to be a critical factor for efficient virus transmission within and between species. Adaptation of avian HAs in group 1 (H1, H2, and H5) and in group 2 (H3 and H7) for human receptor specificity occurs by different mechanisms involving several key RBS mutations (Connor et al., 1994; Gamblin et al., 2004; Matrosovich et al., 2000; Nobusawa et al., 2000; Rogers et al., 1983; Stevens et al., 2006a, 2006c). In H1N1 pandemic influenza viruses, Glu190Asp and Gly225Asp substitutions switch specificity to human α 2-6 receptors, while avian Gln226 and Gly228 are maintained (Gamblin et al., 2004; Matrosovich et al., 2000; Nobusawa et al., 2000) (Table S4). However, Gln226Leu and Gly228Ser mutations completely change specificity to human receptors in pandemic H2N2 and H3N2 viruses, representing an adaptation that is essential for efficient human-to-human transmission (Connor et al., 1994; Rogers et al., 1983) (Table S4). Q226L and G228S are also known to affect the receptor binding preference of H5 HA A/Indonesia/5/05 (Chutinimitkul et al., 2010). Loss of glycosylation in H5 HA (A/Vietnam/1203/2004) in combination with these two substitutions enhances virus replication in ferrets (Wang et al., 2010), but not transmission between ferrets (Chen et al., 2012). Recently, E627K in PB2, H99Y in PB1, H107Y in HA, T160A (loss of a glycosylation site) in HA, and either HA-Q226L or HA-G228S (H5 numbering) in HA were found to constitute minimal sets of substitutions for airborne transmission of A/Indonesia/5/05 virus between ferrets (Herfst et al., 2012; Linster et al., 2014). Another recent study showed that four mutations, N158D (affecting the same N-glycosylation sequon as T160A), N224K, Q226L, and T318I in H5 HA (A/Vietnam/1203/2004), with the remaining seven gene segments from a 2009 pandemic H1N1 virus, enable efficient airborne transmission in ferrets (Imai et al., 2012). In our study, single or double mutations that occurred in human H1/H2/H3 HAs did not support preferential binding of human-type receptors, indicating that H10 HA adaptation to human specificity may differ from known pandemic viruses.

Distinct from the finding that H10 virus (avian H10N2) possesses significant avidity for human receptors (Vachieri et al., 2014), our studies at the structural and receptor specificity level revealed that the human H10N8 is a typical avian influenza virus with strong specificity for avian receptors. Our results are in agreement with the recent report from the Gao group (Wang et al., 2015) and suggest the present human H10N8 is poorly adapted for efficient human-to-human transmission, as is also true for human H7N9, H5N1, and H6N1 viruses.

EXPERIMENTAL PROCEDURES

Expression and Purification of HAs in Insect Cells and Mammalian Cells

Recombinant H10 HA was expressed in Hi5 insect cells using a pFastBac vector, purified by His-tag affinity purification, dialyzed, cleaved by trypsin and gel filtration chromatography, and used for crystallization and determination of binding affinities by biolayer interferometry. Uncleaved proteins were used for glycan microarray analyses. Recombinant HAs were also expressed in HEK293S GnT1^{-/-} cells using the pCD5 vector and purified from cell culture supernatants as described previously (de Vries et al., 2011; Xu et al., 2013) and used for ELISA-like glycan binding and glycan microarray ana-

lyses. Additional details can be found in the [Supplemental Experimental Procedures](#).

Crystallization and Structural Determination of the H10 HA

H10 HA crystals were obtained using vapor diffusion sitting drops at 4°C with 0.2 M NaSCN, 20% (w/v) PEG 3350. Complexes with receptor analogs were obtained by soaking HA crystals in reservoir solution that contained glycan ligands (5 mM). Diffraction data were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) (Table S3). The H10 *apo* structure was solved by molecular replacement and was used as the starting model for determination of the H10 HA-glycan structures.

Glycan Binding of HAs by Biolayer Interferometry

Recombinant HAs were buffer-exchanged into PBS buffer. Association of HAs was measured on an Octet Red (ForteBio) against immobilized biotinylated 3'-SLN and 6'-SLN (V-Labs) at 30°C.

ELISA-like Glycan Binding and Glycan Microarray Analyses of HAs

Recombinant HAs were pre-complexed with horseradish peroxidase (HRP)-linked mouse anti-Strep-tag antibody (IBA) and with Alexa647-linked anti-mouse IgG (Life Biosciences) prior to incubation. The mixtures were incubated on the micro-well slides that contained PAA-linked 3'-SLN and 6'-SLN (provided by the Consortium of Functional Glycomics) or on the microarray, followed by washing, scanning, and analyses.

ACCESSION NUMBERS

Atomic coordinates and structure factors have been deposited in the Protein Data Bank (PDB) under accession codes 4XQ5, 4XQU, and 4XQO for A/Jiangxi-Donghu/346/2013(H10N8) HA and complexes with 3'-SLN and 6'-SLN, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at <http://dx.doi.org/10.1016/j.chom.2015.02.006>.

AUTHOR CONTRIBUTIONS

Project design was done by H.Z., R.P.d.V., N.T., J.C.P., and I.A.W.; protein expression and production by H.Z., R.P.d.V., W.Y., and N.T.; X-ray work and analysis by H.Z., N.T., and X.Z.; glycan array work by R.P.d.V. and R.M.; and the manuscript was written by H.Z., R.P.d.V., N.T., J.C.P., and I.A.W. All authors were asked to comment on the manuscript. This is manuscript 29039 from The Scripps Research Institute.

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HA sequences obtained from GISAID's EpiFlu Database and from the influenza database of the NCBI.

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