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Enhanced human receptor binding by H5 haemagglutinins

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ABSTRACT

Mutant H5N1 influenza viruses have been isolated from humans that have increased human receptor avidity. We have compared the receptor binding properties of these mutants with those of wild-type viruses, and determined the structures of their haemagglutinins in complex with receptor analogues. Mutants from Vietnam bind tighter to human receptor by acquiring basic residues near the receptor binding site. They bind more weakly to avian receptor because they lack specific interactions between Asn-186 and Gln-226. In contrast, a double mutant, Δ133/Ile155Thr, isolated in Egypt has greater avidity for human receptor while retaining wild-type avidity for avian receptor. Despite these increases in human receptor binding, none of the mutants prefers human receptor, unlike aerosol transmissible H5N1 viruses. Nevertheless, mutants with high avidity for both human and avian receptors may be intermediates in the evolution of H5N1 viruses that could infect both humans and poultry.

Introduction

Since 1996, highly pathogenic avian influenza viruses (H5N1) have become widespread in poultry and wild birds in Eurasia and Africa and have caused more than 600 zoonotic human infections with a death rate approaching 60% (WHO, 2013). Sporadic human infections continue to occur in countries where H5N1 viruses are endemic, creating a persistent threat to public health and the possibility of virus evolution towards efficient transmission in the human population.

The molecular mechanisms that enable avian influenza viruses to cross the species barrier and transmit efficiently in humans are incompletely understood. However, it has been established for the H1 (1918), H2 (1957) and H3 (1968) pandemic viruses that a change in the binding specificity of the virus membrane glycoprotein, Haemagglutinin (HA), of avian viruses from a preference for α-2,3-linked sialo-saccharides (avian receptor) to a preference for α-2,6-linked sialo-saccharides (human receptor) is a prerequisite for efficient transmission of avian viruses to humans (Connor et al., 1994; Matrosovich et al., 2000). Recent H5 transmission studies also found that a change in the binding preference of the H5 haemagglutinin (HA) from avian receptor to human receptor was necessary to gain aerosol transmissibility among ferrets, the widely accepted animal models for influenza in humans (Chen et al., 2012; Herfst et al., 2012; Imai et al., 2012; Xiong et al., 2013a; Zhang et al., 2013). The receptor binding specificity and transmissibility changes described in all these H5 transmission studies involved the substitution Gln226Leu in HA (in H3 HA numbering here and subsequently), a substitution previously correlated experimentally with the avian to human receptor binding change (Rogers et al., 1983). Furthermore, avian influenza viruses of the H9N2 (Lin et al., 2000) and H7N9 subtypes (Gao et al., 2013; Xiong et al., 2013b) that have also recently caused human infections have HAs with leucine at residue 226. An H5 virus in which the Gln226Leu substitution has occurred has not been isolated from either birds or from humans. However, surveillance of human H5 viruses has revealed a number of H5 mutants that show enhanced human receptor binding with HAs that contain other amino acid substitutions in the receptor binding sub-domain (Chutinimitkul et al., 2010; Gambaryan et al., 2006; Watanabe et al., 2011; Yamada et al., 2006). We have studied the mechanisms by which these mutations alter receptor binding. We have used biolayer interferometry (BLI) to quantitate receptor binding by HAs identified in Southeast Asian clade 1 H5N1 human isolates (Gambaryan et al., 2006; Yamada et al., 2006) and in mutant clade 2.2 viruses that are endemic in Egypt. (Watanabe et al., 2011). The results indicate that two substitutions in H5 clade 1 HA Asn186Lys and Ser227Asn

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significantly decreased affinity for avian receptor and that the former mutation, which introduces a positive charge, enhanced human receptor binding. The clade 2.2 H5 double mutant Δ133/Ile155Thr HA also had increased affinity for human receptor but had little changed affinity for avian receptor. Based on these results, the human and avian receptor binding modes of H5 HAs bearing the Ser227Asn/Gln196Arg, Asn186Lys, and Δ133/Ile155Thr substitutions were determined by X-ray crystallography. Our observations shed light on the molecular basis of the receptor binding specificity of the mutants and suggest mechanisms for the evolution of HA receptor binding properties during H5 virus infection of humans.

Results

Recombinant H5N1 viruses were generated by replacing the HA and NA genes of A/Puerto Rico/8/34 (H1N1) (PR8) with corresponding H5N1 HA and NA genes using a previously described reverse genetics system (Hoffmann et al., 2000). cDNA clones of HA genes from A/Vietnam/1194/2004 (H5N1) (VN1194) and A/turkey/Turkey/1/2005 (H5N1) (tyTy) were subcloned into pHW2000 plasmid and their multibasic cleavage sites (QRERRRKKR) were replaced (QRETR). Mutant VN1194 viruses containing HA substitutions – Ser227Asn, Gln196Arg, Asn186Lys, Gly143Arg and selected combinations – Ser227Asn/Gln196Arg and Asn186Lys/Gly143Arg (H3 numbering is used throughout the manuscript unless otherwise stated) and mutant A/turkey/Turkey/1/2005 (H5N1) (tyTy) viruses containing Δ133, Ile155Thr, Ser125Asn and combinations of them were generated by reverse genetics following PCR mutagenesis of their respective wild-type HA cDNAs. All recombinant viruses in this study were propagated and purified from embryonated chicken eggs.

Receptor binding properties of H5 mutants evaluated by biolayer interferometry (BLI)

BLI experiments with mutant clade 1 VN1194 viruses indicated that Ser227Asn (Fig. 1b) and Asn186Lys (Fig. 1f) mutations, located in close proximity to the receptor binding site, reduce avian receptor binding (red curves) by ~1200- and ~80-fold respectively, compared to the wild-type VN1194 (Fig. 1a). For the Ser227Asn mutant, binding to human receptor (blue curves) is weak with an avidity slightly less than wild-type VN1194. In contrast, human receptor binding by the Asn186Lys mutant increases 120-fold. Gln196Arg and Gly143Arg substitutions, located further away from the receptor binding site, modify receptor binding of the VN1194 HA in very similar ways (Fig. 1c and g). Both substitutions in isolation increase virus avidity for human receptor by 120- to 180-fold. However, avidities for avian receptor are almost the same as those of wild-type VN1194. Introduction of the double substitutions Ser227Asn/Gln196Arg and Asn186Lys/Gly143Arg, identified in viruses isolated from humans (Yamada et al., 2006), indicated that the weakened avian receptor binding that resulted from Ser227Asn or Asn186Lys mutations alone can be partially restored by the second substitutions. In the case of the Ser227Asn/Gln196Arg combination, avian receptor binding decreases ~50-fold relative to the

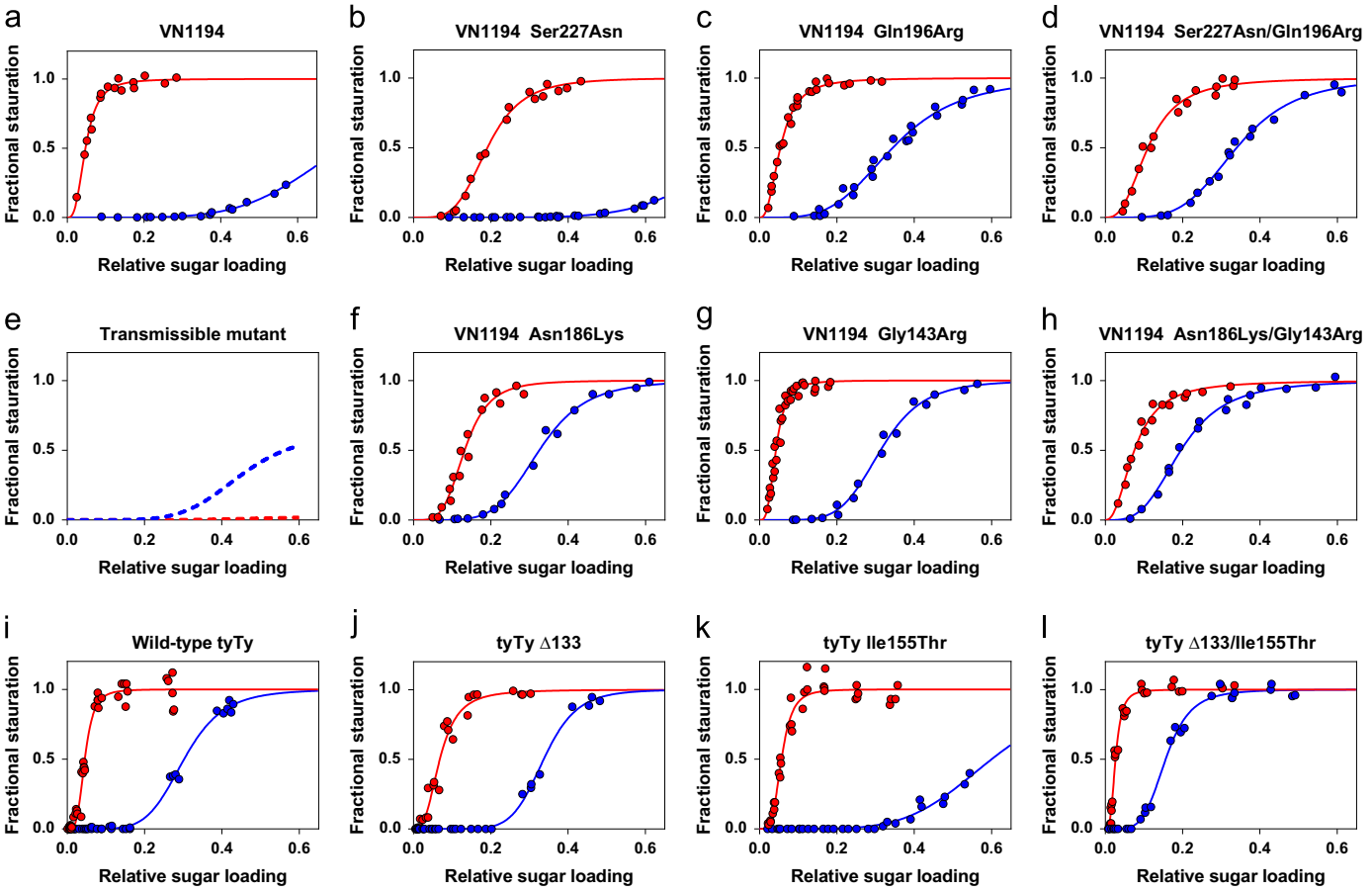

Fig. 1. Estimates of the affinity and specificity of receptor binding by mutant H5 influenza viruses. Binding of sialylglycopolymers containing α-2,3-sialolactosamine (3SLN, avian receptor analogue, red) and sialylglycopolymers containing α-2,6-sialolactosamine (6SLN, human receptor analogue, blue) by VN1194 mutants (b–d, f–h) and tyTy mutants (j–l) was characterised by biolayer interferometry (BLI) as detailed before (Lin et al., 2012). For comparison, data for wild-type VN1194 (a) and tyTy (i) viruses and theoretical binding curves for an aerosol transmissible H5 mutant HA (e) (Xiong et al., 2013a) are included.