Receptor-binding properties of swine influenza viruses isolated and propagated in MDCK cells

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Abstract

To study the receptor specificities of H1 and H3 influenza viruses isolated recently from pigs, we employed the analogues of natural receptors, namely sialyloligosaccharides conjugated with polyacrylamide in biotinylated and label free forms. All Madin-Darby canine kidney (MDCK) cell-propagated viruses with human H3 or classical swine H1 hemagglutinins bound only to Neu5Ac2-6Gal1-bearing polymers, and not to Neu5Ac2-3Gal1-bearing polymers. This receptor-binding pattern is typical for human influenza viruses and it differs from the previously described receptor-binding specificity of egg-adapted swine influenza viruses. Swine virus isolates with avian-like H1 and H3 hemagglutinins displayed distinct receptor specificity by binding to both Neu5Ac2-6Gal- and Neu5Ac2-3Gal-containing receptors. These viruses, as well as egg-adapted swine and turkey viruses with a classical swine HA, differed from the related duck viruses by increased affinity to sulfated sialyloligosaccharide, Su-SiaLex. Except for avian-like H3 viruses, none of the studied swine viruses bound to Neu5Gc-containing sialoglycopolymers, suggesting that binding to these sialic acid species abundantly expressed in pigs may not be essential for virus replication in this host.

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1. Introduction

Influenza viruses isolated from North American pigs have originated from several independent genetic lineages, namely, classical swine H1N1 viruses, human H3N2 viruses, and avian viruses of several subtypes. Wholly swine, wholly human and wholly avian viruses, as well as reassortants of these lineages have been isolated from pigs in North America since 1998 (Olsen, 2002; Karasin et al., 2000a,b, 2004; Webby et al., 2000; Zhou et al., 1999). Genotype of the reassortant viruses was determined by complete genome sequencing and phylogenetic analyses. The most common genotype of H3N2 viruses isolated to date in the USA from pigs was a triple reassortant containing hemagglutinin (HA), neuraminidase (NA), and PB1 polymerase genes of human influenza virus origin, matrix (M), nonstructural (NS), and nucleoprotein (NP) genes of classical swine influenza virus origin, and PA and PB2 polymerase genes of avian influenza virus origin. Subsequent to the appearance of these H3N2 viruses, H1N2 viruses were isolated from these animals.
Phylogenetic analyses demonstrated that these H1N2 viruses appeared as a result of reassortment between a classical H1N1 swine influenza virus, which supplied its H1 HA gene segment, and one of the triple-reassortant swine H1N2 viruses, which supplied all of the remaining RNA segments. Finally, wholly avian viruses of H3N3 and H1N1 subtypes have been isolated most recently from pigs in Canada (Karasin et al., 2004).

Classical swine viruses and avian-like H1N1 swine viruses belong to distinct phylogenetic lineages (Brown et al., 1997; Olsen et al., 2000). Viruses with classical swine HA have also been isolated frequently from turkeys (Lipkind et al., 1984; Altmuller et al., 1992; Wright et al., 1992; Suarez et al., 2002). Comparison of HA sequences of turkey and swine viruses reveals a high degree of similarity between these viruses. Recently, H1N2 swine viruses with human-like HA were isolated from turkeys (Choi et al., 2004).

Influenza virus infection is initiated by interactions between the viral HA and sialic acid-containing molecules on target cells. Viruses from different host species usually display binding preference for either Neu5Acα2–3Gal or Neu5Acα2–6Gal natural disaccharide epitopes (reviewed by Paulson, 1985; see also Nobusawa et al., 1991; Connor et al., 1994; Matrosovich et al., 2000 and references therein). Human viruses of H1, H2, and H3 subtypes recognize α2–6-linked sialic acid, the major form found on cells of the human respiratory tract (Baum and Paulson, 1990; Couceiro et al., 1993). Avian viruses preferentially bind to Neu5Acα2–3Gal, the form that predominates in the duck enteric tract where these viruses replicate (Ito et al., 1999). The common receptor specificity of avian viruses with different HA subtypes is maintained by conserved amino acids in positions 190, 194, and 225. The Glu190Asp change alone was apparently sufficient to allow replication of 1918–19 viruses in the human respiratory tract. However, the existence of three strains with the additional Gly225Asp substitution demonstrates that both receptor-binding variants were co-circulating during the 1918 pandemic (Reid et al., 2003). The Gly225Asn change was also present in the two earliest avian-like swine viruses but is absent in the HAs of the later avian-like isolates as well as of the classical H1N1 swine and turkey virus strains (Matrosovich et al., 2000).

Table 1

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<thead>
<tr>
<th>Oligosaccharide parts of sialosylglycos polymers</th>
<th>Abbreviation</th>
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<tr>
<td>Biotinylated polymers (1000 kDa)</td>
<td>SL</td>
</tr>
<tr>
<td>Neu5Acα2–3Galβ1–4Glcβ1–3(Fucα1–2)Galβ1–3GlcNAc</td>
<td>SLN</td>
</tr>
<tr>
<td>Neu5Acα2–3Galβ1–4Glcβ1–3(Fucα1–2)Galβ1–3GlcNAc-6S</td>
<td>SLN</td>
</tr>
<tr>
<td>Neu5Acα2–3Galβ1–4Glcβ1–3(Fucα1–2)Galβ1–3GlcNAc-6S-6S</td>
<td>SLN</td>
</tr>
<tr>
<td>Neu5Acα2–3Galβ1–4Glcβ1–3(Fucα1–2)Galβ1–3GlcNAc-6S-6S-6S</td>
<td>SLN</td>
</tr>
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Swine influenza viruses were reported to bind sialic acids with both α2–6 and α2–3 linkages (Rogers and D’Souza, 1989; Gambaryan et al., 1997; Matrosovich et al., 1997, 2000; Marinina et al., 2004), consistent with the presence of either linkage type in swine tracheal tissue (Ito et al., 1998). In addition to N-acetyl neuraminic acid (Neu5Ac), its N-glycolyl analog (Neu5Gc) was shown to be abundantly expressed in pigs (Suzuki et al., 1997). It was speculated, therefore, that swine viruses might have increased affinity for Neu5Gc (Suzuki et al., 1997; Matrosovich et al., 2000). Recently, increased affinity for sulfated forms of sialyloligosaccharide receptors was reported for swine viruses belonging to three evolutionary lineages and also for chicken viruses (Gambaryan et al., 2004; Marinina et al., 2004).

All the strains of three independent swine H1N1 virus lineages carry the mutator Gln190Asp/Asn, suggesting that this is essential for the adaptation of viruses to replication in pigs (Matrosovich et al., 2000; Reid et al., 1999; Olsen et al., 2000). A Gln190Asp substitution was found in all the five sequenced human H1N1 1918 influenza strains (Reid et al., 2003). Two of these strains (A/New York/1/1918 and A/London/1/1919) had amino acids responsible for receptor-binding activity identical to those of A/sw/Iowa/1976/31 (a classical swine virus). Comparing to the avian HA gene consensus, other 1918 viruses had an additional change at the amino acid 225. The Gln190Asp change alone was apparently sufficient to allow replication of 1918–19 viruses in the human respiratory tract. However, the existence of three strains with the additional Gly225Asp substitution demonstrates that both receptor-binding variants were co-circulating during the 1918 pandemic (Reid et al., 2003). The Gly225Asn change was also present in the two earliest avian-like swine viruses but is absent in the HAs of the later avian-like isolates as well as of the classical H1N1 swine and turkey virus strains (Matrosovich et al., 2000).

A pitfall of the previous studies on swine and turkey viruses was that these viruses were isolated in embryonated hen eggs. It is well demonstrated, however, that isolation and propagation of human influenza viruses in eggs always alters their receptor specificity. In particular, egg-adaptation always increases the affinity of human viruses for α2–3 linked sialic acid (Ito et al., 1997; Gambaryan et al., 1997, 1999). The Asp225Gly change in the HA molecule often accounts for this effect in the case of human H1 viruses (Robertson, 1993). Furthermore, as we found recently, the HA of H1N1 pig viruses isolated in MDCK cells harbor Asp225, thus differing at this key amino acid position from the egg-adapted viruses of the same evolutionary lineage (Karasin et al., 2000a,b, 2002). These observations prompted us to test the receptor-binding characteristics of non-egg adapted viruses that were isolated and propagated solely in MDCK cells. To determine the receptor binding preference of swine viruses, we used a large panel of synthetic sialylglycos polymers (Table 1).

2. Materials and methods

2.1. Materials

For receptor-binding assays, 96-well plates from Costar, USA, and horseradish peroxidase-streptavidin conjugate
and o-phenylenediamine from Boehringer Mannheim, Germany were used. Synthesis of label-free sialylglycopolymers (Glyc-PAA), 30 kDa, was described earlier (Bovin et al., 1993; Parzynina et al., 2003). Synthesis of biotinylated polymers, Glyc-PAA-biot, 1000 kDa, was described by Shilova et al. (2005). See Table 1 for the structure and abbreviations of receptor analogs.

2.2. Isolation and propagation of viruses

Viruses were isolated from lung tissue or nasal swabs of pigs. MDCK cells were obtained from the American Type Culture Collection. Homogenates (10%, w/v) of pooled lung tissues were prepared and inoculated into MDCK cell cultures with 1.5/μg ml of TPCK-treated trypsin per ml (Karasin et al., 2000a,b, 2002, 2004). Samples of nasal secretions were collected using Dacron swabs and placed in viral transport media (2000 U/ml Penicillin G, 4 mg/ml Streptomycin, 16 μg/ml Gentamicin, 1000 U/ml Nystatin and 0.5% BSA in PBS). Samples were propagated in MDCK cells in MEM medium at 37°C.

2.3. Direct binding assays

The assay was performed as described previously (Matrosovich et al., 2000). In brief, virus-containing fluids (0.1 ml) were added to wells of the plates precoated with fetuin. After overnight incubation at 4°C, the plates were washed with PBS containing 0.01% of Tween-20 (washing solution). After addition of Glyc-PAA-biot (30 μg/well) in the working buffer (PBS with 0.01% Tween-20; 0.1% BSA and 0.1 μM neuramidase inhibitor oseltamivir carboxylate), plates were incubated for 2 h at 4°C. The starting concentration of Glyc-PAA-biot was 0.5 μM with respect to sialic acid; serial two-fold dilutions were used. Plates were washed with cold washing solution and incubated with streptavidin-peroxidase in the working buffer at 4°C for 1 h. After washing, peroxidase activity in the wells was assayed using o-phenylenediamine substrate solution. The absorbencies (490 nm) were determined and the data were converted to the Scatchard plots A490/C versus A490, where C is concentration of sialic acid, A490 is absorbency in the corresponding well (see Fig. 1). The dissociation constants of virus complexes with sialylglycopolymers (Kdiss) were determined from the slopes of Scatchard plots and expressed in μM Neu5Ac. The data presented in Tables 2 and 3 are averaged values of two to three independent experiments.

2.4. Competitive binding assay

The dissociation constants of the virus complexes with low molecular weight (30 kDa) Glyc-PAA were determined in a fetuin binding inhibition assay as previously described (Gambaryan and Matrosovich, 1992). The assay is based on the competition between the receptor analog under study and standard preparation of peroxidase-labeled fetuin for the binding sites on a solid-phase immobilized virus. The competitive assay was performed for 1 h at 2–4°C in the working buffer.

2.5. Analysis of HA amino acid sequences

The HA amino acid sequences were obtained from GenBank and from the Influenza Sequence Database (Macken et al., 2001). The sequences were managed by using GeneDoc 2.3. The H3 numbering system in accordance with the alignment of Nobusawa et al. (1991) is used throughout the paper.

3. Results and discussion

3.1. Virus recognition of the type of the sialic acid linkage (α2–3 versus α2–6)

First, we tested virus binding to biotin-labeled probes, Glyc-PAA-biot (Glyc = 3′SL, 6′SL, or 6′SLN), using the
Table 2

Binding affinity constants of virus complexes with HRP-labeled fetuin and Glyc-PAA-biot, 1000 kDa, μM SA

| Viruses Subtype | Fetuin 3′SL | Sugar determinants 6′SLN
<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Su-3′SLN SiaLe x Su-SiaLe c 6′SLN</td>
</tr>
<tr>
<td>With classical swine HA, MDCK grown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sw/Wisconsin/H03/03</td>
<td>H1N1</td>
<td>0.5</td>
</tr>
<tr>
<td>Sw/Wisconsin/H03G1/03</td>
<td>H1N1</td>
<td>0.5</td>
</tr>
<tr>
<td>Sw/Wisconsin/R33F01</td>
<td>H1N2</td>
<td>1</td>
</tr>
<tr>
<td>Sw/Wisconsin/R46F01</td>
<td>H1N2</td>
<td>0.5</td>
</tr>
<tr>
<td>Sw/North Carolina/93231/01</td>
<td>H1N2</td>
<td>1</td>
</tr>
<tr>
<td>Sw/North Carolina/93225/01</td>
<td>H1N2</td>
<td>1</td>
</tr>
<tr>
<td>Sw/Wisconsin/H03H04/03</td>
<td>H1N2</td>
<td>1</td>
</tr>
<tr>
<td>Sw/Iowa/930/01</td>
<td>H1N2</td>
<td>1</td>
</tr>
<tr>
<td>With classical swine HA, egg grown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sw/Iowa/1976/31</td>
<td>H1N1</td>
<td>0.03</td>
</tr>
<tr>
<td>Sw/Indiana/1726/88</td>
<td>H1N1</td>
<td>0.05</td>
</tr>
<tr>
<td>Turkey/Kansas/4480/09</td>
<td>H1N1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* The data were averaged from three sets of experiments. Standard deviations did not exceed 50% of the mean values. Higher values of \( K_{\text{diss}} \) correspond to lower affinities.

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direct binding assay. Fig. 1 demonstrates the examples of primary binding data after their transformation into Scatchard plots. A/Sw/Saskatchewan/18789/02 (H1N1) is a wholly avian virus isolated from a pig (Karasin et al., 2004). This isolate bound strongly to all of the three probes. By contrast, viruses with classical swine HA (A/Sw/Wisconsin/H03H04/03) and human H3 HA (A/Sw/Wisconsin/R7C/01) did not appreciably bind to the 3′SL probe, and bound to 6′SLN with much higher affinity than to 6′SL.

In the next series of experiments, we used five labeled receptor analogs: peroxidase-labeled fetuin and four Glyc-PAA-biot probes: the three mentioned above and 6′(Neu5Gc)LN probe. Four distinct groups of swine viruses were tested, namely: (1) MDCK-isolated; (2) egg-isolated viruses with classical swine H1 HA; (3) MDCK-isolated viruses with human H3 HA; and (4) MDCK-isolated viruses with avian H1 or H3 HA. A/Duck/Hong Kong/717/79 (H1N3) and

<table>
<thead>
<tr>
<th>Viruses Subtype</th>
<th>3′SL</th>
<th>6′SL</th>
<th>6′SLN</th>
<th>6′(Neu5Gc)LN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sw/Saskatchewan/18789/02</td>
<td>H1N1</td>
<td>&gt;1000</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Sw/Ontario/K0477/01</td>
<td>H3N3</td>
<td>50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Sw/Ontario/42720/01</td>
<td>H3N3</td>
<td>50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Sw/Indiana/1726/88</td>
<td>H1N1</td>
<td>&gt;1000</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Sw/Iowa/1976/31</td>
<td>H1N1</td>
<td>&gt;1000</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Turkey/Kansas/881480</td>
<td>H1N1</td>
<td>&gt;1000</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>Duck/Alberta/357/66</td>
<td>H3N6</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* The data were averaged from three sets of experiments. Standard deviation did not exceed 50% of the mean values. Higher values of \( K_{\text{diss}} \) correspond to lower affinities.
MDCK-grown classical swine viruses had low affinity for fetuin. Viruses with human H3 HA did not bind this receptor analog, whereas MDCK-grown avian-like viruses bound fetuin with high affinity. Thus, assessment of virus affinity for fetuin may represent a simple assay for preliminary discrimination between viruses of avian and mammalian origin. All viruses tested except A/Duck/Hong Kong/717/79 and A/Duck/Alberta/3576 demonstrated high affinity for 6′SLN. Only avian-like H3N3 viruses displayed low affinity for 6′Neu5GcSLN, which harbors the N-glycolyl sialic acid species instead of Neu5Ac. The other viruses tested did not bind to 6′Neu5GcSLN (Table 2).

None of the MDCK-derived swine viruses with human or classical swine virus HAs bound to 3′SL but they displayed strong binding to 6′SL and even stronger affinity to 6′SLN. This pattern is remarkably similar to that displayed by non-egg-adapted influenza viruses (Gambaryan et al., 1997, 1999, Mochalova et al., 2003). Avian-like swine viruses bound to 3′SL polymer stronger than to other Glyc-PAA, but also demonstrated binding to 6′SLN and 6′SL while control wild duck viruses did not bind to 6′SL and 6′SLN probes.

Unlike MDCK-grown isolates, egg-adapted viruses with classical swine HA, A/ Sw/Iowa/31, A/Turkey/Kansas/4880/80, and A/Sw/Indiana/1726/88, bound to 3′SL, and to 6′SLN with nearly equal affinity and less strongly to 6′SL. To identify substitutions in the HA molecule of swine influenza viruses that could be responsible for the different receptor-binding phenotypes of egg-derived and MDCK-derived H1 swine viruses, we compared the H1 HA sequences deposited in the Influenza Sequence Database. Fig. 2 shows that all swine and turkey strains have the Glu190Asp(Asn) substitution, in agreement with the previous suggestion that this substitution is essential for the adaptation of wild duck viruses to these new hosts (Rogers and D’Souza, 1989, Matrosovich et al., 2000).

Also Fig. 2 demonstrates that HA sequences of classical swine viruses isolated before the year 2000 harbored Gly225, whereas the majority of more recent classical swine viruses have Asp225. This difference is consistent with the different receptor specificity of egg-adapted swine viruses tested previously and MDCK-grown viruses studied here. Namely, all MDCK-derived viruses tested herein did not bind to 3′SLN, while egg-adapted viruses tested in this and in previous studies had Gly225 and bound to both o2–6-linked and o2–3-linked sialic acids (Gambaryan et al., 1997, Matrosovich et al., 1997, 2000; Marinina et al., 2004). Therefore, we can speculate that non-egg-adapted swine viruses have Asp225 (and strict preference for 6′SLN receptors) and that Gly225 in the HA of classical swine viruses is the result of their adaptation to eggs. On the other hand, it was shown that a virus with Gly225 is also able to infect pigs efficiently (Clavijo et al., 2002).

Contemporary human H1 viruses are strictly adapted to 6′SLN demonstrating lower affinity to 6′SL. It was shown that Asp225 and Asp190 are responsible for such receptor specificity (Gambaryan et al., 1999). A correlation between the amino acid in position 225 of HA and virus receptor specificity has been described by many investigators (Rogers and D’Souza, 1989; Matrosovich et al., 1997, 2000; Govorkova et al., 1999; Mochalova et al., 2003). Crystallographic analyses of human and swine H1 HA molecules disclosed hydrogen bonds between Asp225 and galactose, attached to sialic acid by 2–6 linkage, and between Asp190 and the amino nitrogen of the GlcNAc-3 group within the 6′SLN molecule (Gamblin et al., 2004).

MDCK-derived turkey viruses have not yet been studied. It remains to be determined whether non-egg-adapted H1N1 viruses isolated from turkeys harbor Asp225 and display the receptor specificity similar to that of human-like swine viruses, or Gly225 and display the A/Turkey/Kansas/4880/80-like dual receptor specificity.

### 3.2. Receptor specificity to Sia(2–3)Gal receptors with different sugar cores

In this part of the study, egg adapted classical swine and avian-like swine viruses as well as A/Turkey/Kansas/4880/80 were tested for binding to Sia(2–3)Gal receptors differing by their inner core. Two viruses, A/Duck/Alberta/3576 (H1N1) and A/Duck/Buryatia/652/88 (H1N8), were included as controls. All these viruses contain Gly225 in their HA molecules. Label-free sialylglycopolymers with low molecular weight (30 kDa) were used in the competitive assay (Gambaryan and Matrosovich, 1992).

Only avian-like swine viruses of the H3 subtype, A/Sw/Ontario/K047701 (H3N3), A/Sw/Ontario/4222901 (H3N3), and A/Duck/Buryatia/652/88 (H3N8), but not the H1 swine, turkey or duck viruses, bound to 3′(Neu5Gc)LN (Table 3). This result confirmed our previous observation that the ability to bind Neu5Gc-group depends on the virus subtype rather than on the virus host (Marinina et al., 2004).

The next series of experiments were performed based on our previous finding that some chicken and mammalian viruses demonstrated increased affinity to sulfated sialosides than to non-sulfated sialosides (Gambaryan et al., 2004). Our goal was to learn what effect the sulfatization of receptor analogs would have on the binding affinity of swine viruses. For this purpose, we tested four pairs of sialglycopolymers (see Table 1).

Four sugars differing in the type of Sia-Gal linkage (2–6 versus 2–3), type of Gal-GlcNAc linkage (1–3 versus 1–4), and presence or absence of fucose attached to glucosamine residue were examined in comparison to the same four compounds supplemented with the sulfo group at position O6 of glucosamine. The binding affinities of these receptor analogs are presented in Table 3.

Supplementation of sulfo-group does not affect the binding of duck viruses to the receptors (Table 3). At the same
time, all tested swine and the A/Turkey/Kansas/4880/80 viruses have increased affinity to sulfated 6′SLN, 3′SLN and SiaLe^x groups as compared with their unsulfated forms. This effect is especially pronounced for the sulfated SiaLe^x determinant.

To clarify the reason of high affinity of Gly225-containing swine viruses to Su-SiaLe^x, we simulated the docking of Su-SiaLe^x tetrasaccharide into the receptor-binding site of the A/Swine/Iowa/31 HA using Discovery Studio ViewerPro software (Fig. 3). Sialic acid of the simulated Su-SiaLe^x was superimposed over the sialic acid residue of LSTα, complexed with A/Swine/Iowa/31 HA (1RV0 structure, Brookhaven Protein Databank, Gamblin et al., 2004). The Su-SiaLe^x tetrasaccharide fitted perfectly within the receptor binding site (RBS) as shown in Fig. 3. Fucose is located near the amino-group of Lys222 and are capable to form hydrogen bonds with it. A sulfo-group located near Asp190 also seems to be favorable for the interaction. The presence of these additional molecular contacts can explain why the affinity of the A/Swine/Iowa/31 virus to Su-SiaLe^x is ten times higher than that to 3′SLN (Table 3). Fig. 3 shows that substitution of Gly225 with Asp225 (wire surface) would sterically impede fitting of Gal attached to sialic acid via 2–3 bond.

It was shown earlier that Su-SiaLe^x was a favorable receptor for H7 chicken virus (Gamaryan et al., 2004). Chicken viruses of the H5 HA subtype had also high affinity to the sulfated oligosaccharide Su-3′SLN (Gamaryan et al., 2004). It can be speculated that high affinity to Su-SiaLe^x arose as a result of adaptation of influenza viruses to gallinaceous hosts and that such receptor specificity of the HA of HI viruses may appear among avian viruses before their transmission into mammalian hosts. Importantly, viruses with Asp190 and...
**Fig. 3.** Putative position of the Su-SiaLe· in the receptor-binding site of the A/Swine/Iowa/31 HA. The molecular model of Su-SiaLe· was built based on SiaLe· (2 KMB structure, Brookhaven Protein Data Bank, Ng and weis, 1997). This analog was fitted into the RBS of the A/Swine/Iowa/31 HA complexed with LSTA (1RV0 structure, Brookhaven Protein Databank, Gamblin et al., 2004) by superimposing the sialic acid residue of the sulfated SiaLe· over the sialic acid residue of LSTA. The HA surface is shown in blue, except for the multicolored Asp190, Gly225 and Lys222 residues. The receptor is shown in green, except for the fucose (orange) and sulfo-group (yellow and red). Simulated surface of Asp225 shown in black wire. The modeling was performed using Discovery Studio ViewerPro.

Gly225 can efficiently infect both humans and pigs (Reid et al., 2003; Clavijo et al., 2002).

Many investigators (Lipkind et al., 1984; Altmuller et al., 1992; Wright et al., 1992; Suarez et al., 2002; Choi et al., 2004) have described interspecies transmission of viruses between pigs and turkeys. These observations allow us to speculate that dual receptor specificity of classical swine HA is not incidental, but could have evolved as a result of repeated transmission of viruses between pigs and turkeys. In general, such easy conversion of receptor specificity due to variability of amino acid 225 can provide an evolutionary advantage for maintaining influenza viruses in nature. It has to be established whether turkeys are the only birds that can participate in a similar virus exchange.

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