Studies on the Structure of the Influenza Virus Haemagglutinin at the pH of Membrane Fusion

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SUMMARY

At the pH required to trigger the membrane fusion activity of the influenza virus haemagglutinin (HA) the soluble ectodomain of the molecule, BHA, which is released from virus by bromelain digestion, aggregates into rosettes. Analyses of soluble proteolytic fragments derived from the rosettes indicated that aggregation is mediated by association of the conserved hydrophobic amino-terminal region of BHA2, the smaller glycopolypeptide component of each BHA subunit. Further analyses of the structure of the soluble fragments and of HA in its low pH conformation by electron microscopy, spectroscopy and in crosslinking experiments showed that, although the membrane distal globular domains lose their trimer structure at the pH of fusion, the central fibrous stem of the molecule remains trimeric and assumes a more stable conformation. The increase in length of BHA2 at low pH observed microscopically appears to result from movement of the amino-terminal region to the membrane proximal end of the molecule and in virus incubated at low pH the amino terminus may insert into the virus membrane. The consequences of these possibilities for the mechanism of membrane fusion are discussed.

INTRODUCTION

Haemagglutinin (HA) is the major membrane glycoprotein of influenza virus. It is a trimer of Mr 220K and each of the identical subunits consists of two disulphide-linked glycopolypeptides, HA1 and HA2. The ectodomain of the molecule which is released from virus by bromelain digestion has been crystallized and its structure is known to 0.3 nm resolution (Wilson et al., 1981). Infection by influenza virus involves binding of virus to cell surface receptors, cellular uptake by endocytosis and fusion of viral and endosomal membranes. Both receptor-binding and membrane fusion processes are mediated by HA. The latter is triggered at endosomal pH, between pH 5.0 and 6.0, and is accompanied by irreversible changes in the HA structure (for reviews see Wiley & Skehel, 1987; Wharton, 1987).

At the pH of fusion, the bromelain-released soluble HA molecule, BHA, acquires hydrophobic properties and aggregates into rosettes (Skehel et al., 1982; Doms et al., 1985). The region of the molecule that appears to be exposed to generate these properties is the conserved, hydrophobic amino terminus of HA2 which has been indirectly implicated in the fusion process (Skehel et al., 1982; Gething et al., 1986). We have reported before that digestion of low pH BHA rosettes with thermolysin results in removal of the HA2 amino terminus and restores the solubility of the aggregated molecules (Daniels et al., 1983). Here we confirm and extend these findings and report a detailed characterization of the products of thermolytic digestion using a number of biochemical and biophysical techniques. We present information on the orientation

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of BHA in low pH BHA rosettes, and on the morphological differences between low pH BHA and low pH HA on virus particles. We also show that, contrary to the membrane distal HA$_1$ domains which dissociate to monomers at low pH, HA$_2$ remains trimeric and is extremely stable.

**METHODS**

**Virus, BHA and BHA$_2$ aggregates.** X-31 (H3N2) influenza A virus was grown in the allantoic cavity of embryonated eggs and purified as described (Skehel & Schild, 1971). BHA (HA$_1$ plus HA$_2$ residues 1 to 175) was prepared by releasing HA from purified virus with bromelain (Sigma) and purified by sucrose density gradient centrifugation (Brand & Skehel, 1972).

Low pH BHA was prepared by incubating native BHA at pH 5.0 for 10 min at room temperature. After readjusting the pH to 6.0 with 1 M-Tris–HCl BHA$_2$ aggregates (HA$_1$ residues 1 to 27 plus HA$_2$ residues 1 to 175, see Fig. 8) were prepared by digesting low pH BHA with trypsin (Sigma) (100:1 w/w, BHA:trypsin) for 1 h at room temperature after which the reaction was stopped with an equal weight of soybean trypsin inhibitor (Sigma). The reaction products were separated on 5 to 25% sucrose gradients with a 60% sucrose cushion (16 h at 20°C, 150000 g). BHA$_2$ aggregates were recovered from the 25 to 60% sucrose interface.

**Preparation of TBHA$_2$ by digestion of BHA$_2$ aggregates with thermolysin.** Digestion of BHA$_2$ aggregates with thermolysin (thermophilic bacterial protease Type X, Sigma) (50:1 w/w, BHA$_2$:thermolysin) was at pH 5.0, 37°C in PBS (10 mM-phosphate buffer, 150 mM-NaCl, 0.01% azide, pH adjusted to 5.0 with 0.1 M-citric acid) containing 1 mM-CaCl$_2$. Digestion was stopped by adding EDTA to a final concentration of 10 mM and was complete after 6 h (see Results). The digestion products were separated on 2.5 to 15% sucrose gradients with 60% sucrose cushions (20 h at 20°C, 150000 g) in PBS pH 5.0 containing 1 mM-EDTA.

**Crosslinking experiments.** BHA$_2$ aggregates solubilized by digestion with thermolysin (TBHA$_2$) were crosslinked using dimethyl suberimidate (DMS, Sigma) as described (Davies & Stark, 1970; Wiley et al., 1977). DMS was dissolved in PBS (made pH 11 by adding NaOH) by quickly warming the solution and was then immediately added to the TBHA$_2$ solution. The pH of the dissolved DMS solution was 9.5 and after mixing with TBHA$_2$ the pH was 9.0. Crosslinked samples were analysed by PAGE. HA$_1$ residues 28 to 328, containing a secondary cleavage site at residue 224, were crosslinked using the same method.

**Other techniques.** Thermal denaturation, circular dichroism (CD) and tryptophan fluorescence experiments were done as described (Ruigrok et al., 1986a), in PBS pH 5.0 unless otherwise stated. Protein concentrations were calculated from their absorbance at 280 nm and from the content of Trp and Tyr residues as described (Ruigrok et al., 1986a). Electron microscopy (EM) was as described in Ruigrok et al. (1986b). Samples were negatively stained with 1% neutral sodium silicotungstate (SST) and examined using the minimum dose and accurate defocusing technique (Wrigley et al., 1983) in a Jeol JEM-1200EX microscope.

Automated amino acid sequencing was done on an Applied Biosystems 477 pulsed-liquid phase sequencer with an on-line PTH analyser. Amino acid compositions were determined using an Applied Biosystems 420A derivatizer equipped with an on-line 130A analyser.

**RESULTS**

**The subunit composition of HA$_1$ and HA$_2$ in the low pH conformation**

When low pH BHA rosettes are digested with trypsin at pH 6 the membrane distal globular domain, HA$_1$ residues 28 to 328, is released by cleavage at HA$_1$ Lys 27 to leave only HA$_1$ residues 1 to 27 disulphide-linked to the intact BHA$_2$ (Skehel et al., 1982; see Fig. 8). BHA$_2$ remains as an aggregate and can be separated from the released HA$_1$ components by sucrose density gradient centrifugation during which it accumulates at the 25 to 60% sucrose interface (Fig. 1a). Treatment of BHA$_2$ aggregates with thermolysin results in their dissociation and the soluble structure produced, TBHA$_2$, was isolated in 2.5 to 15% sucrose density gradients (Fig. 1b, 2a). In these gradients soluble TBHA$_2$ sediments as a discrete peak of sedimentation coefficient approximately 3S which suggests that TBHA$_2$ is oligomeric. This was confirmed in crosslinking experiments in which increasing concentrations of DMS produce increasing amounts of dimer and trimer crosslinked species (Fig. 2b) in a similar fashion to the crosslinking of native BHA observed by Wiley et al. (1977). The HA$_1$ 28 to 328 components are cleaved secondarily by trypsin at residue 224 and the two glycopeptides produced, HA$_1$ 28 to 224 and HA$_1$ 225 to 328 are linked via a disulphide bond (Skehel et al., 1982). These two glycopeptide chains can be crosslinked but without formation of oligomers (Fig. 2c).
Low pH structure of influenza virus HA

With increasing time of digestion of BHA₂ aggregates with thermolysin there was a gradual reduction in the size of BHA₂ with an intermediate size most prominent after 40 min incubation and a final digestion product after 6 h (Fig. 3a). Soluble samples obtained after 40 min and 6 h digestion were purified by sucrose density gradient centrifugation and then subjected to amino-terminal sequence analysis. The 40 min sample showed mainly a sequence from BHA₂ residue 24 onwards and a minority of sequence from 38 onwards whereas the 6 h sample only showed the sequence with amino-terminal residue 38. The amino-terminal residue of HA₁ is blocked by pyroglutamic acid (Ward, 1981) and thus the amino terminus of the HA₁ 1 to 27 fragment was not detected in these experiments. However, electrophoretic analysis under reducing and non-reducing conditions indicates that the HA₁ 1 to 27 fragment remains associated by the disulphide bond between HA₁ 14 and HA₂ 137 since soluble TBHA₂ migrates more slowly when not reduced. Amino acid analysis confirmed this conclusion indicating the presence of proline residues in TBHA₂ which are absent in HA₂ but present in HA₁ (positions 4 and 21).

Fig. 1. (a) Separation of BHA₂ aggregates from HA₁ residues 28 to 328 on a 9 ml 5 to 25% sucrose gradient with a 2 ml 60% sucrose cushion (fractions 1 to 4). Centrifugation was in a Beckman SW41 rotor, 35000 r.p.m., 20 °C for 16 h. BHA₂ aggregates sediment to fraction 4, the 25 to 60% sucrose interface, and the HA₁ components remain at the top of the gradient at fraction 19. (b) Sedimentation of TBHA₂ (●; left ordinate) and HA₁ (○; right ordinate) residues 28 to 328 on separate 9 ml 2.5 to 15% sucrose gradients with 2 ml 60% cushions. Centrifugation was as above for 20 h.
Fig. 2. Polyacrylamide gel electrophoresis under reducing conditions on 16% gels containing 2-5 M-urea, 0-1% SDS (Skehel et al., 1982). (a) Fractions from the sucrose gradient of Fig. 1(b). Lanes 1 to 10, alternate fractions from 2 to 20, showing TBHA$_2$ in fractions 10, 12 and 14 (lanes 5, 6 and 7). Lanes 11 and 12 show as markers BHA$_2$ aggregates and intact BHA (HA$_1$ plus BHA$_2$) respectively. (b) Crosslinking of TBHA$_2$ with DMS. Lane 1, Mr markers, HA$_1$ residues 28 to 328 (40K), HA$_2$ 28 to 224 (25K) and HA$_2$ 225 to 328 (15K). Lane 2, non-crosslinked TBHA$_2$. Lanes 3 to 5 show TBHA$_2$ crosslinked using increasing concentrations of DMS (4, 7 and 9 mg DMS/ml). Crosslinked species have apparent Mr values of 17K (monomer), 34K (dimer) and 56K (trimer) (denoted by arrowheads). (c) Crosslinking of HA$_2$ 28 to 328 with an additional cleavage at residue 224. Lane 1, crosslinked at 7 mg DMS/ml and lane 2 not crosslinked, as detailed for lane 1 in.

Fig. 3. Electrophoretic analysis of thermolytic digestion products of BHA$_2$ aggregates after different times of digestion. (a) Digestion with thermolysin at pH 5.0. Lanes 1 to 9 show products after 0, 5, 10, 20, 40, 90, 180, 270 and 360 min of digestion. Lane 10, intact BHA. (b) Digestion with trypsin at pH 8.0. Lanes 1 to 8 show products after 0, 15, 30, 60, 120, 180, 270 and 360 min of digestion.

Incubation of BHA$_2$ aggregates with trypsin at pH 6 does not result in digestion but incubation at pH 8 leads to a reduction in size of BHA$_2$ and in solubilization of the aggregates; see Fig. 3(b). In this case the amino-terminal sequences of the intermediate and final digestion products start at His 26 and Ser 40 indicating cleavage at Arg 25 and Lys 39. The similarity of the positions of cleavage by thermolysin and trypsin suggest that those obtained with thermolysin do not result from sequential removal of residues from the amino terminus.
Low pH structure of influenza virus HA

Electron microscopy

The 10 min, 40 min and 6 h thermolytic digestion products were examined by EM. BHA2 aggregates are rosettes of 8 ± 1 thin stalks with a rosette radius of 15 nm (BHA2 trimers) which often have a small terminal knob (Ruigrok et al., 1986a, b). After 10 min digestion with thermolysin, some of these rosettes begin to dissociate and single thin molecules, 58% with knobs, can be observed (Fig. 4a). A histogram of the length distribution of these free molecules (Fig. 4a) shows a peak at 15 to 16 nm and a minor peak at 13 nm. After 40 min incubation there are hardly any aggregates left (Fig. 4b). The thin stalks produced have variable lengths most frequently of 13 nm, and 48% have a knob. After extensive digestion only thin rods with a length of 10-5 nm are detected, 39% with a knob (Fig. 4c). The final products of seven different

Fig. 4. Electron micrographs and length distribution histograms of the observed free molecules of the thermolytic digestion products of BHA2 aggregates after 10 min (a), 40 min (b) and 360 min (c) of digestion. Some of the free molecules in the micrographs are encircled. The bar marker represents 40 nm.
digestions were measured (in total 770 particles) which gave an average length of 10.5 ± 0.7 nm. Some TBHA2 preparations were readjusted to pH 7.2 after which native BHA was intermixed to serve as an internal standard. In these cases the TBHA2 length remained 10.5 nm and the average BHA length was 13.6 ± 0.5 nm (120 measurements) compared to 13.5 nm derived from the crystal structure (Wilson et al., 1981). Fig. 5 shows galleries of native BHA (top) which is clearly much thicker and longer than TBHA2 (middle row without knob, bottom row with knob). The knob remaining on TBHA2 was variable in appearance, sometimes merely a bright dot without obvious extra width, and the percentage of knobs varied with the staining conditions. The molecules with knobs had the same length as those without knobs.

Electron microscopy of the purified products of the digestion of BHA2 aggregates by trypsin at pH 8 also showed free thin molecules, some with knobs. Their length distribution also showed peaks at 10.5 and 13 nm.
Low pH structure of influenza virus HA

Fig. 7. (a) Electron micrograph of virus incubated at pH 4.9 and then digested with trypsin at pH 6.0. This treatment results in the removal of HA₁ residues 28 to 328. (b) Micrograph of virus as in (a) subsequently treated with thermolysin at pH 5.0 for 6 h. This treatment results in the removal of HA₂ residues 1 to 38. Arrows indicate neuraminidase molecules which are apparently unaffected by the treatments. The bar marker represents 100 nm.

We also studied the effects of thermolytic digestion of virus which had been incubated at pH 4.9 and digested with trypsin to remove HA₁ residues 28 to 328. The results in Fig. 6 show that digestion with thermolysin reduces the size of virus-associated HA₂ by about 3K. EM of virus preparations before and after digestion, however, showed that the appearances and the lengths of the HA₂ stalks were unchanged (11 nm). Both preparations showed knobs distal on HA₂ (Fig. 7).

CD analysis and thermostability of TBHA₂

Estimates of the secondary structure of BHA₂ aggregates and TBHA₂ by CD are given in Table 1. TBHA₂ is largely helical and comparison of the secondary structure of TBHA₂, predicted from CD measurements, with the secondary structure of the same residues in the native BHA molecule, as derived from the crystal structure (Wilson et al., 1981), indicates that
Table 1. Percentages of secondary structure derived from CD and X-ray crystallography

<table>
<thead>
<tr>
<th>Molecule</th>
<th>α-Helix (%)</th>
<th>β-Sheet (%)</th>
<th>Other (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHA₂ aggregates: CD*</td>
<td>51 ± 3 (9)†</td>
<td>14 ± 4</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>BHA₂ (1 to 175) + HA₁ (1 to 27): X-ray‡</td>
<td>46</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>TBHA₂: CD*</td>
<td>54 ± 3 (7)‡</td>
<td>18 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>BHA₂ (38 to 175) + HA₁ (1 to 27): X-ray‡</td>
<td>58</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

* Secondary structures were derived from CD using the contin procedure of Provencher & Glockner (1981).
† Averages with S.D. of 9 (BHA₂ aggregates) and 7 (TBHA₂) independent preparations.
‡ Values derived from the crystal structure of native BHA (Wilson et al., 1981).

neither the low pH conformational change, nor the protease treatments result in significant changes in secondary structure (see Skehel et al., 1982).

To study thermostability, TBHA₂ was heated in PBS pH 7.2 or pH 5.0. As with low pH BHA rosettes and BHA₂ aggregates, TBHA₂ did not denature when heated to 95 °C in PBS pH 5.0 as determined by tryptophan fluorescence, CD and trypsin sensitivity. At pH 7.2, TBHA₂ denatures at 76 °C compared with 65 °C for native BHA, 76 °C for BHA₂ in low pH BHA and 80 °C for BHA₁ aggregates as previously reported (Ruigrok et al., 1986a). Thermal denaturation of TBHA₂ was accompanied by a sharp and irreversible decrease (85%) in tryptophan fluorescence and an irreversible loss of α-helix with only 4% helix remaining after the transition.

DISCUSSION

When BHA₂ aggregates are incubated with thermolysin, removal of the amino-terminal 23 residues results in solubilization of the aggregates in agreement with previous results (Daniels et al., 1983) and illustrates that the site of aggregation of low pH BHA and BHA₂ aggregates is the amino terminus of HA₂. The cleavage between Gly 23 and Phe 24 and between Asp 37 and Leu 38 is consistent with the reported broad specificity of thermolysin (Matsubara, 1970) and the absence of further digestion, even after 15 h incubation, indicates a stable and ordered structure of TBHA₂. This is confirmed by the crosslinking, CD and EM experiments which show that TBHA₂ is trimeric and suggest that the central α-helical coiled coil is intact.

Others have reported that low pH treatment of BHA results in monomerization of both HA₁ and BHA₂ (Doms & Helenius, 1986; Nestorowicz et al., 1985). However, these experiments involved very low concentrations of iodinated BHA, 1 μg/ml compared to 1 to 10 mg/ml in this study. When concentrations of 20 μg/ml were used, a decreased dissociation of the BHA₂ trimers was observed and with intact, detergent-isolated HA or with virus, on which HA is close packed, no dissociation of HA₂ was found upon low pH treatment (Doms & Helenius, 1986) in agreement with the result presented here.

BHA₂ aggregates are rosettes of 8 ± 1 thin rods with a terminal knob and a rosette radius of 15-0 nm (Ruigrok et al., 1986b). Thermolysin sequentially removes peptides from the amino terminus of BHA₂ which first results in disaggregation and then in reduction of the length of the isolated thin rods. The terminal knob is detectable on a large proportion of the rods at all stages of digestion. After 10 min digestion a small number of free molecules with a length of 15 to 16 nm are observed but because of their low yield we have not determined how many residues have been removed from the BHA₂ amino termini of these components. After 40 min incubation a major digestion product is formed with Phe 24 as the amino terminal residue and a length of 13 nm. The final digestion product with Leu 38 at its amino terminus is 10-5 nm long. The length decrease of low pH BHA₂ upon thermolytic digestion therefore is a result of the removal of the hydrophobic amino-terminal residues 1 to 23 and the adjoining β-strands between residues 24 and 30 and residues 31 and 38 up to the bottom of the shorter helix of HA₂ at residue 40 (see Fig. 8).

In a previous publication (Ruigrok et al., 1986b) electron microscopic observations on virions and HA rosettes suggested that the amino terminus of HA₂ is either extruded sideways or towards the membrane proximal end of the molecule. The results presented here indicating that
removal of the amino-terminal region by thermolytic digestion results in the shortening of BHA2 suggest that the amino terminus of HA2 does in fact move to the membrane proximal region of the molecule. This displacement could destabilize the adjoining β-strands which become susceptible to thermolysin. These changes could result in the increase in length of BHA2 from about 9.5 to 15 nm. In this process, Trp 92 in the longer helices of HA2 may be covered by HA2 residues either from the extended chains connecting the longer and shorter helices of HA2 or by a twist in the coiled coil burying Trp 92 in the trimer interface. The intrinsic tryptophan fluorescence of TBHA2 (described in Wharton et al., 1988a) shows that the only tryptophan residue in TBHA2, Trp 92 of HA2 (see Fig. 8), is highly fluorescent and inaccessible to solvent even though in the native BHA conformation Trp 92 is covered by residues of HA1 and if the same orientation is preserved in TBHA2 Trp 92 would be exposed when HA1 is removed by proteolysis.

Heat denaturation experiments show that isolated TBHA2 is more stable than the equivalent region of the native BHA (Ruigrok et al., 1986a). The increase in stability of BHA2 observed after the low pH conformational change with low pH BHA rosettes and BHA2 aggregates is thus not due to aggregation. In the case of TBHA2, however, heat denaturation results in total and irreversible loss of α-helical content in contrast with low pH BHA and BHA2 where the loss is partly reversible (Ruigrok et al., 1986a). Apparently the presence of the amino-terminal aggregation site influences refolding of the α-helices upon lowering the temperature. The
denaturation temperature and the accompanying very sharp loss in fluorescence of TBHA\textsubscript{2} are very similar to those observed on heating low pH BHA and BHA\textsubscript{2} aggregates. This indicates that in all these structures quenching of Trp 92 is causing the fluorescence decrease. The substantial differences in stability of BHA\textsubscript{2} when denaturation experiments were done at pH 5.0 rather than at pH 7.2 were observed with TBHA\textsubscript{2} as well as with intact low pH BHA and BHA\textsubscript{2} aggregates (Ruigrok \textit{et al.}, 1986a) and are therefore not dependent on the presence of the HA\textsubscript{2} amino terminus.

BHA\textsubscript{2} in low pH BHA is much longer than BHA\textsubscript{2} in native BHA and this difference in length is removed upon thermolysin treatment. Low pH HA\textsubscript{2} on virus is not much longer than native HA\textsubscript{2} and no length reduction occurs on digestion with thermolysin. This suggests two main possibilities for the location of the amino terminus of low pH HA\textsubscript{2} on virus. First, the amino termini are extruded sideways and interact with the amino termini of other HA molecules. This could assist fusion by pulling HA molecules together and exposing parts of the viral membrane. Doms \& Helenius (1986) observed a rearrangement of HA on low pH-treated virus by negative stain electron microscopy consistent with such interactions. However, in these studies phosphotungstic acid (PTA), which is a membrane-destabilizing agent, was used as a negative stain (Booy \textit{et al.}, 1985). When we used PTA, we observed similar structures but when the less disruptive stain SST was used (Fig. 7), or the membrane-stabilizing stain uranyl acetate (not shown), we observed an even distribution of projections on the virus. This even distribution did not change upon treatment with thermolysin (Fig. 7). Second, the amino terminus inserts into the viral membrane. This would not result in a length increase since the HA\textsubscript{2} carboxy terminus is membrane-anchored. On the basis of the information available at present this possibility is favoured mainly because the protein on the virus would change in a similar fashion to the isolated ectodomain and because the sharply defined membrane edge observed in negative stain EM becomes poorly defined after incubation at low pH and stain penetration occurs more frequently.

The amino terminus of HA\textsubscript{2} is formed by cleavage of precursor HA\textsubscript{0} (Skehel \& Waterfield, 1975) which renders the virus infectious and fusogenic (Klenk \textit{et al.}, 1975; Lazarowitz \& Choppin, 1975; Maeda \textit{et al.}, 1981; White \textit{et al.}, 1982). Modification of the conserved hydrophobic amino-terminal amino acid sequence alters the fusion properties of HA (Daniels \textit{et al.}, 1985; Gething \textit{et al.}, 1986) and synthetic peptide analogues of this region fuse membranes (Lear \& DeGrado, 1987; Wharton \textit{et al.}, 1988b). From the results presented here we conclude that the amino terminus of HA\textsubscript{2} is also responsible for the aggregation of BHA specifically induced at the pH of fusion. Removal of this amino terminus results in rigid, highly stable trimeric molecules that closely resemble analogous structures on virus particles treated similarly. There is no indication from these observations of how the fusion of viral and endosomal membranes could be accomplished by bringing these membranes into close proximity since the rigid HA\textsubscript{2} structures appear more likely to keep them 11 nm apart. Alternatively such a rigid structure may itself be involved in the fusion process by interacting with the membrane of the endosome.

These observations are, however, made on isolated HA and virus particles and it is clear that, to relate them directly to the mechanism of membrane fusion, experiments need to be done in which virus is bound to a receptor and the pH subsequently lowered.

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REFERENCES


Low pH structure of influenza virus HA


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