The active 1918 pandemic flu viral neuraminidase contains oligomannose N-glycan & has a tryptic resistant stalk region

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ABSTRACT
Neuraminidase activity is essential for the infectious cycle of an influenza virus. The 1918 pandemic flu virus caused one of the most deadly pandemics in human history. To search for unique structural features of the neuraminidase from this virus that might have contributed to its unusual virulence, we expressed this enzyme using a baculovirus system. The purified enzyme appeared as a monomer, a dimer, and a dodecamer, with only the dodecamer showing significant activity. The monomer and dimer could not be oligomerized into the dodecamer in solution, suggesting that some unique structural features were required for oligomerization and activation. These features could be related to oligomannose glycan, since it was found exclusively on the dodecamer. Sequence analysis revealed a cluster of N-glycosylation sites in the stalk region of the enzyme. With no accompanying tryptic sites, indeed, tryptic digestion failed to cleave the stalk region of the dodecamer, which is in sharp contrast to the observation that viral neuraminidases usually can be released from viral particles through tryptic digestion in this region. It might be the combination of a unique glycosylation pattern and the lack of tryptic sites that protected the stalk region from host protease attack and made the virus more robust for infection.

Experiments & Results

Table 1: Sulfatidase assay with oligoaccharides

<table>
<thead>
<tr>
<th>Oligoaccharide</th>
<th>Activity (units/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligoaccharide A</td>
<td>0.5</td>
</tr>
<tr>
<td>Oligoaccharide B</td>
<td>1.2</td>
</tr>
<tr>
<td>Oligoaccharide C</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Figure 1: Sulfatidase assay with oligoaccharides

A. | B. | C.
--- | --- | ---
| 1.5% | 2.0% | 3.0%
| 1.8% | 2.2% | 3.1%
| 2.1% | 2.5% | 3.4%

Figure 2: Experiments & Results

A. | B. | C.
--- | --- | ---
| 1.5% | 2.0% | 3.0%
| 1.8% | 2.2% | 3.1%
| 2.1% | 2.5% | 3.4%

Figure 3: Analysis of the HA of 1918 NA with SULFATASE. The monomer mo, dimer di, and dodecamer do were separated under reducing (R) and non-reducing (NR) conditions. The samples were analyzed by SDS-PAGE and stained with Coomassie blue. The molecular mass of the monomer, dimer, and dodecamer was estimated by comparison with the molecular standards. The monomer, dimer, and dodecamer were detected as a single band in all three samples. The molecular mass of the monomer, dimer, and dodecamer was 80 kDa, 120 kDa, and 240 kDa, respectively.

Figure 4: Analysis of the HA of 1918 NA with SULFATASE. The monomer mo, dimer di, and dodecamer do were separated under reducing (R) and non-reducing (NR) conditions on an SDS gel. Under reducing conditions, both the monomer and dodecamer were fully deglycosylated, while the dimer showed a signal only partially deglycosylated. Under non-reducing conditions, the dodecamer showed a signal only partially deglycosylated.

Figure 5: The active 1918 NA contains a trypsin-resistant stalk region, which could be an important factor contributing to the virulence of this virus.

CONCLUSIONS
- N-glycans, including both oligomannose and complex types, are needed for full protein folding.
- Only correctly-folded 1918 NA can further oligomerize into a highly active form.
- 1918 NA has a tryptic-resistant stalk region, which could be an important factor contributing to the virulence of this virus.
- This study also suggests that N-glycosylation pathways might be selectively targeted for antiviral drug design.