Zika virus NS2B-NS3 protease: Crystal structure, substrate specificity, inhibitors

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Organization of the Zika virus genome

NS2B-NS3\textsuperscript{pro} cleavage motif: \( \ldots (G/K/S) (K/R) R \downarrow X X X \ldots \)}
Topology of the ZIKV polyprotein

Cytoplasm

ER Lumen

ZIKV protease
Signalase
Furin
Recombinant production of ZIKV NS2B-NS3\textsuperscript{pro} yields disulfide-bonded dimers

**SDS-PAGE**
The ZIKV NS2B-NS3\textsuperscript{pro} appears to be hyperactive

Substrate: Bz-Nle-Lys-Lys-Arg-AMC

<table>
<thead>
<tr>
<th></th>
<th>ZIKV (Monomer)</th>
<th>ZIKV (SS-Dimer)</th>
<th>ZIKV (C80S,C143S)</th>
<th>WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{cat}}/K_m$ (M\textsuperscript{-1}s\textsuperscript{-1})</td>
<td>2440000 ± 215000</td>
<td>6650000 ± 3020000</td>
<td>5620000 ± 5460000</td>
<td>112000 ± 5000</td>
</tr>
<tr>
<td>$K_{\text{cat}}$ (min\textsuperscript{-1})</td>
<td>2678 ± 60.66</td>
<td>1348.4 ± 127.96</td>
<td>1724.8 ± 29.22</td>
<td>521 ± 7.50</td>
</tr>
<tr>
<td>$K_m$ (μM)</td>
<td>18.29 ± 1.59</td>
<td>3.378 ± 1.501</td>
<td>5.11 ± 0.49</td>
<td>77.41 ± 3.55</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (μM.min\textsuperscript{-1})</td>
<td>13.39 ± 0.30</td>
<td>3.371 ± 0.32</td>
<td>8.62 ± 0.15</td>
<td>10.42 ± 0.15</td>
</tr>
</tbody>
</table>

Values from literature (Vasudevan, Young, Lim, et al.)

WNV: $K_{\text{cat}}/K_m = 37000 ± 7000$ M\textsuperscript{-1}s\textsuperscript{-1}

DENV2: $K_{\text{cat}}/K_m = 30000 ± 7000$ M\textsuperscript{-1}s\textsuperscript{-1}

(Bz-Nle-Lys-Arg-Arg-AMC)
Overall structure of ZIKV NS2B-NS3\textsuperscript{pro} in complex with the peptidyl boronic-acid compound cn-716

Blue: ZIKV NS2B
Brown: ZIKV NS3\textsuperscript{pro}
Purple: cn-716
Green: Ser135-His51-Asp75

IC\textsubscript{50} = 0.25 ± 0.02 μM
K\textsubscript{i} = 0.040 ± 0.006 nM

Lei et al., Science 353, 503 – 505 (2016)
Details of the interaction between cn-716 and ZIKV NS2B-NS3<sup>pro</sup>

The boronic acid forms a cyclic diester with glycerol.

Yellow: ZIKV NS2B; cyan: ZIKV NS3\textsuperscript{pro}; purple: cn-716. K54 is from molecule B in the "tight dimer". Lei et al., Science \textbf{353}, 503 – 505 (2016)
<table>
<thead>
<tr>
<th>Protease</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
<th>$K_m$ (μM)</th>
<th>$k_{cat}/K_m$ (s$^{-1}$M$^{-1}$)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIKV NS2B-NS3 (wt)</td>
<td>6.04 ± 0.24</td>
<td>32.10 ± 4.64</td>
<td>2421.5 ± 383.3</td>
<td>100%</td>
</tr>
<tr>
<td>ZIKV NS2B-NS3 (D83*N)</td>
<td>3.90 ± 0.27</td>
<td>37.12 ± 5.47</td>
<td>1749.73 ± 285.02</td>
<td>72.25%</td>
</tr>
<tr>
<td>WNV NS2B-NS3</td>
<td>0.24 ± 0.02</td>
<td>46.45 ± 8.87</td>
<td>442.8 ± 94.8</td>
<td>18.29%</td>
</tr>
</tbody>
</table>

NS2B residues are labeled with an asterisk (*).

The FRET substrate (Dabcyl-KTGKR↓SGAL-E(Edans)-amide), corresponding to the NS2B/NS3 cleavage site, was used for the measurements.

In the crystal, there are "tight dimers" of ZIKV NS2B-NS3\textsuperscript{pro} linked by disulfide bonds

The "tight dimer" features pronounced shape complimentarity

Conclusions (I)

- The recombinant ZIKV NS2B-NS3pro is more active than the enzymes of WNV and DENV2
- The crystal structure and mutational analysis suggests that part of this hyperactivity is due to the Asp83* residue of NS2B
- The crystal structure with a boronic-acid inhibitor reveals the formation of a covalent adduct with S135 of the enzyme and of a cyclic diester with glycerol
Conclusions (II)

- In the crystal, a "tight dimer" of the ZIKV protease complex with the boronic acid is found, which is linked through disulfide bonds to neighboring tight dimers.
- The tight dimer shows a pronounced shape complementarity, but we cannot detect it in solution up to a concentration of 144 μM.
- However, in mass spectra, the tight dimer is detected.
- This dimer could be a model for the protease structure at the high concentrations existing at the ER membrane.
Acknowledgements

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