Study of rabies virus by Differential Scanning Calorimetry (DSC)

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The rabies virus (RABV) continues to be a worldwide health problem.

- Rabies virus is a neurotropic virus and causes one of the most lethal zoonotic diseases – 100% of mortality in unvaccinated patients.
- Over 120 countries are still affected by canine rabies which kills someone every 9 minutes, mostly ages <15 years
- Over 55,000 people die of the disease annually, but this is probably a severe underestimation (2010)

Rabies virus

- Rhabdoviridae family
- Single-stranded RNA genome of about 12 kb which encodes five proteins
  - the nucleoprotein (N)
  - the viral RNA polymerase (L)
  - the phosphoprotein (P)
  - the matrix protein (M)
  - The lipid bilayer
  - the glycoprotein (G)

- Cryo-electron microscopy image of a frozen hydrated sample of RABV bullet-like morphology
  - 180nm length,
  - 80nm diameter


Glycoprotein G – The main antigen of the RABV vaccine

- Glycoprotein G is the only surface exposed viral protein
- This fusion protein (class III) is organized as a trimer anchored to the viral membrane
- There is a pH-dependent equilibrium between its pre- and post-fusion conformations

“*The major correlate of protection of rabies vaccine is the induction of neutralizing antibody against the unique transmembrane glycoprotein*” G (Wiktor TJ, 1973).

*This property mainly depends on the preservation of its three-dimensional structure* (Bunschoten H, 1989)


Bakker *et al.* (2005)

Roche *et al.* (2006) Science 313 p187

**Trimer of rabies glycoprotein G** per homology with VSV-G structure

Frédéric Gréco - DSC rabies virus
DSC of rabies virions

- Sanofi Pasteur as human vaccine manufacturer performs DSC analysis to obtain information on protein tertiary structure and lot to lot consistency
- The inactivated rabies virus unfolds in two transitions in DSC

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\begin{align*}
\text{Temperature (°C)} & \quad \text{Cp (Kcal/mole°C)} \\
-20 & \quad 0 \\
0 & \quad 20 \\
20 & \quad 40 \\
40 & \quad 60 \\
60 & \quad 80 \\
80 & \quad 100
\end{align*}
```

three different batches of inactivated rabies virus analysed two times independently

Sharpness of unfolding transitions and low SD of the Tm values:
DSC is a good analytical tool to monitor vaccine production process and lot to lot consistency

- Does it possible to assign viral protein to these events?
- Can we go deeper in the G-protein structure with DSC?
Viral sample are non inactivated or Beta-propiolactone (BPL)-inactivated bulk lots of strain Pitman-Moore

- Dialysis in PBS with 10kDa cutoff
- Protein 0.15-1.1mg/ml corresponding to $9 \times 10^{10}$-$7 \times 10^{11}$ viral particles
- Experiment performed on a VP-Capillary auto-CAP DSC
- Scan rate 200°C/h to help avoiding aggregation or precipitation
- Data were normalized using the concentration of the G-protein: approximately 25% of the viral proteins

Influence of Beta-propiolactone (BPL) inactivation

DSC of non inactivated and inactivated virus

Viral inactivation has a minor impact on protein unfolding

61-62°C  71-73°C

Reversibility of unfolding inactivated virus

Both unfolding events are entirely irreversible

rescan
Identification of the G-protein transition

Bromelain cleavage of G-protein ectodomain* suppresses first unfolding event

First unfolding event is primarily due to G unfolding

* Gaudin Y. et al. (1991)
Identification of the second transition

- Comparative DSC analysis of the entire inactivated rabies virus and recombinantly produced purified matrix protein.

The similarities in the Tm between M protein and the 2\textsuperscript{nd} event observed for the entire virus may suggest that this 2\textsuperscript{nd} event may be primarily due to the unfolding of the M protein.

Future studies are necessary to confirm this hypothesis.
Detection of conformational changes

- Lowering the pH, in order to mimic the acidic endosome, induces minor changes in the unfolding behavior of inactivated rabies virus.

From pre-fusion to post-fusion conformation some changes occur in the G-protein unfolding event

<table>
<thead>
<tr>
<th>pH</th>
<th>Tm</th>
<th>°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.3</td>
<td>1</td>
<td>61.4</td>
</tr>
<tr>
<td>6.3</td>
<td>1</td>
<td>59.6</td>
</tr>
<tr>
<td>5.8</td>
<td>1</td>
<td>62.2</td>
</tr>
</tbody>
</table>
Effect of reducing conditions on the G-protein conformation

- DSC shows large alteration in the unfolding behavior under reducing conditions.

![Graph showing temperature vs. heat capacity for inactivated rabies virus in PBS vs. PBS + 5mM DTT.]

**Inactivated rabies virus**
- in PBS
- in PBS + 5mM DTT

Reduction of G-protein disulfide bonds causes dramatic alterations to protein structure. They have significant effect on G structure.
Interactions of RABV with monoclonal antibodies

Equimolar mixtures of RABV + Mab

<table>
<thead>
<tr>
<th>Mab 50AD1</th>
<th>Mab D1-25</th>
<th>Mab TJU11-12</th>
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G-protein specific monoclonal antibodies cause an up-shift of the first unfolding event confirming the notion that it corresponds to a biologically active G-protein conformation.

Conclusion

- DSC of RABV virions exhibits two thermal transitions.
- The transition at 61°C has a major contribution from unfolding of G proteins, whereas the transition at 71°C may mainly correspond to unfolding of M proteins.
- Conformational changes as well as binding of monoclonal antibodies to the protein G can be detected by DSC.
- Reduction of G-protein disulfide bonds causes dramatic decrease in stability of G proteins.
- DSC can be used as sensitive analytical tool to monitor the vaccine production process and lot to lot consistency.
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