Analysis of Quinone-Induced Protein Modifications using SDS-PAGE

Neethu Kurien
Quinones
PAHs

- Fuels
- Cigarette Smoke
- Vehicle Exhaust
- Cosmetics
- Dyes
- Food additives
- Medicines


http://ecigresearch.com/2016/02/03/conventional-cigarettes-contain-over-4000-chemicals


http://www.fashionlady.in/10-beautiful-punjabi-mehndi-designs/18612
Quinone Mechanism

Kim, J; Vaughn, A; Cho, C; Albu, T; Carver, E. *Bioorganic Chemistry*. **2012**, *40*, 92-98.
How to Read the Gel

RNase: 13.7 kDa
- Dimer: 27.4 kDa
- Trimer: 41.04 kDa
- Tetramer: 54.72 kDa

Lysozyme: 14.4 kDa
- Dimer: 28.8 kDa
- Trimer: 43.2 kDa
- Tetramer: 57.6 kDa

RNase + 1.0 mM CBQ

Kim, J; Albu, T; Vaughn, A; Kang, S; Carver, E; Stickle, D. Bioorganic Chemistry. 2015, 59, 106-116.
Previous Research:
Concentration dependent Rnase modifications by pBQ

Kim, J; Vaughn, A; Cho, C; Albu, T; Carver, E. *Bioorganic Chemistry*. 2012, 40, 92-98.
Previous Research: Rating Reactivity

MBQ<PBQ<CBQ

Kim, J; Albu, T; Vaughn, A; Kang, S; Carver, E; Stickle, D. Bioorganic Chemistry. 2015, 59, 106-116.
# RNase and Lysozyme

<table>
<thead>
<tr>
<th>RNaseA</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW: 13.7 kDa</td>
<td>MW: 14.4 kDa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>RNaseA</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (lysine)</td>
<td>10</td>
<td>KVFGREC</td>
</tr>
<tr>
<td>S (serine)</td>
<td>15</td>
<td>GLDNYRGYSLGNWVC</td>
</tr>
<tr>
<td>T (threonine)</td>
<td>10</td>
<td>AAKFESNFNTQATNRN</td>
</tr>
<tr>
<td>C (cysteine)</td>
<td>8</td>
<td>TDGSTDYGILQINSRWW</td>
</tr>
<tr>
<td>W (tryptophan)</td>
<td>0</td>
<td>WCNDGRTPGSRNLNCNI</td>
</tr>
<tr>
<td>Y (tyrosine)</td>
<td>6</td>
<td>PCSALLSSDITASVNCA</td>
</tr>
<tr>
<td>F (phenylalanine)</td>
<td>3</td>
<td>KKIVSDGNGMNAWVA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WRNRCKGTVDQAWIRGCRL</td>
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</table>
Quinones of Interest: Naphthoquinones

(PNQ) 1,4-naphthoquinone

(ONQ) 1,2-naphthoquinone

(HNQ) 2-hydroxy-1,4-naphthoquinone
Quinones of Interest:
Benzoquinones

1,4-benzoquinone (PBQ)
2-methyl-1,4-benzoquinone (MBQ)
2-phenyl-1,4-benzoquinone (PhBQ)
2-chloro-1,4-benzoquinone (CBQ)
Tetrachloro-1,4-benzoquinone (TCBQ)
Methods

Preparation for Incubation
- Weigh out Quinone and make stock solution
- Aliquot correct amounts

Incubation
- Take samples at specific time intervals

Denature
- Loading Dye
- Heat

Run gels

Staining

Destaining

Scanning and Analysis
Incubation Table

Reaction Details of Lysozyme (0.1 mM) with 1,2-naphthoquinone (3 mM)

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>FW (mg)</th>
<th>mmol</th>
<th>mg</th>
<th>solution</th>
<th>[Q]i</th>
<th>[Q]f</th>
<th>Incub Vol</th>
<th>Q vol</th>
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<tbody>
<tr>
<td>0.698 mM St Lyz (uL)</td>
<td>103.6</td>
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<tr>
<td>Protein (ug)</td>
<td>1035.9</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Phosphate buffer (uL)</td>
<td>508.4</td>
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<td></td>
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</tr>
<tr>
<td>Incub Time</td>
<td>0 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Incub Temp</td>
<td>37°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>20 mM Q stock, [Q]f 3 mM</td>
<td>108</td>
<td></td>
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<tr>
<td>Final Incubate Vol (uL)</td>
<td>720</td>
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<tr>
<td>Aliquot Taken (uL) X 4</td>
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<tr>
<td>LANE</td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
<td>L4</td>
<td>L5</td>
<td>L6</td>
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<tr>
<td>Loading Dye</td>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Incub Aliquot of Total</td>
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<td>15</td>
<td>15</td>
<td>15</td>
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<td>15</td>
<td>15</td>
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<tr>
<td>Loading Vol (uL)</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Protein loaded (ug)</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
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<tr>
<td>0.15 mM Lyz(uL).25ul</td>
<td>32</td>
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<td>0.10 mM Lyz(uL).25ul</td>
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<td>0.05 mM Lyz(uL).25ul</td>
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</tr>
</tbody>
</table>

Total FIN vol 480.0
Naphthoquinones pH 7.0

Thomas, Charles. “Fluorescence and UV-Vis Studies of Quinone-Induced Protein Modifications.” 2017
Benzoquinones pH 7.0

Thomas, Charles. “Fluorescence and UV-Vis Studies of Quinone-Induced Protein Modifications.” 2017
Benzoquinones pH 7.0

LYZ [0.1 mM] + MBQ [3 mM]
LYZ [0.1 mM] + PhBQ [3 mM]
LYZ [0.1 mM] + PBQ [3 mM]
LYZ [0.1 mM] + CBQ [3 mM]
LYZ [0.1 mM] + TCBQ [3 mM]
Future Prospects

- To look at pH dependence of quinone reactivity (pH 8.0, and pH 6.0)
  - Of naphthoquinones
  - Of benzoquinones
- To look at RNase with naphthoquinones
  - Also pH dependence
References


Kim, Jisook; Vaughn, Albert R.; Cho, Chris; Albu, Titus V.; Carver, Ethan A. Modifications of Ribonuclease A Induced by p-benzoquinone. *Bioorganic Chemistry*. **2012**, 40, 92-98.

Bolton, Judy L.; Trush, Michael A.; Penning, Trevor M.; Dryhurst, Glenn; Monks, Terrence J. Role of Quinones in Toxicology. *Chemical Research in Toxicology*. **2000**, 13, 135-160

Thomas, Charles. “Fluorescence and UV-Vis Studies of Quinone-Induced Protein Modifications.” **2017**
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