Plant Based Enzymatic Synthesis of Resolvin and Protectin Analogues

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Image: Arachidonate 15-lipoxygenase, Orientation of Proteins in Membranes database
What are Resolvins and Protectins?

- Molecules with anti-inflammatory and pro-resolution properties
- Potential for treatment of Cardiovascular disease, Asthma and Arthritis
- Di-hydroxy and tri-hydroxy derivatives of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)
Why Plant Based Enzymes?

• **Mammals**

  - Lipoxygenase (LOX)
  - Cyclooxygenase (COX)
  - EPA and DHA
  - $O_2$

  → Resolvins, Protectins

• **Plants**

  - Lipoxygenase (LOX)
  - Allene Oxide Synthase (AOS)
  - EPA and DHA
  - $O_2$

  → Analogues of Resolvins and Protectins
Which plant species?

- Literature review showed over 60 species of plants with LOX activity.

- However, most species have only been characterised with linoleic acid as substrate.

- Our aim was to determine what products are formed using different plant species with long chain polyunsaturated fatty acids (AA, EPA, DHA).
Chemistry of LOX catalysed reactions

\[
\begin{align*}
R_1 & \quad \text{LOX} \quad O_2 \\
\text{R}_1 \quad & \quad \text{OOH} \\
R_2 & \quad \text{NaBH}_4 \\
\end{align*}
\]

DHA
Methods

• Homogenise plant material in buffer in a simple blender
• The homogenate was diluted in buffer and stirred with 100 µM fatty acid for 15 minutes
• The solution was acidified, reduced with NaBH₄ (if necessary), and solvent extracted or solid phase extracted before analysis
Analysis

- UV-vis spectrophotometry (detect LOX activity)
- NP-HPLC (retention time, UV-vis spectrum of each peak)
- TOF-MS (molecular weight)
- GCMS (after hydrogenation and trimethylsilylation)
## Results

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Organ</th>
<th>Proposed monohydroxy-DHA products</th>
<th>Proposed dihydroxy-DHA products</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae</td>
<td>Soybean (Cayman)</td>
<td>Seed</td>
<td>17-HDHA (17-hydroxy DHA)</td>
<td>7,17-diHDHA 10,17-diHDHA</td>
<td>GCMS (Dobson 2013)*</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>Lupin</td>
<td>Seed</td>
<td>17-HDHA</td>
<td>7,17-diHDHA 10,17-diHDHA</td>
<td>HPLC</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>Broadbean</td>
<td>Germinated seed</td>
<td>17-HDHA</td>
<td>-</td>
<td>HPLC</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>Green lentil</td>
<td>Germinated seed</td>
<td>17-HDHA</td>
<td>7,17-diOHDHA 10,17-diHDHA</td>
<td>HPLC</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>Potato</td>
<td>Tuber</td>
<td>10-HDHA</td>
<td>10,20-diHDHA</td>
<td>GCMS</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>Eggplant</td>
<td>Fruit</td>
<td>10-HDHA</td>
<td>10,20-diHDHA</td>
<td>GCMS</td>
</tr>
<tr>
<td>Poaceae</td>
<td>Barley</td>
<td>Seed</td>
<td>10-HDHA</td>
<td>-</td>
<td>HPLC</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Sunflower</td>
<td>Germinated seed</td>
<td>17-HDHA (major) 7-HDHA (minor)</td>
<td>7-17-diHDHA</td>
<td>GCMS</td>
</tr>
</tbody>
</table>

Results

• Unusual results with germinated sunflower seeds

![Chemical reaction diagrams]

UV visible spectrophotometry

- Absorbance (234nm)
- Time (minutes)
Results: Sunflower + DHA (Non-reduced products)

NP-HPLC

TOF-MS
C_{22}H_{32}O_{4} \text{(M+Na)}^+ \text{ ion}
Expected (m/z) 383.21928
Actual (m/z) 383.21534

Proposed compound: 16-oxo, 17-hydroxy DPA
Results: Sunflower + DHA (Non-reduced products)

GCMS

- Hydrogenated, methoxyaminated, trimethylsilylated
**Summary of Non-reduced products (HPLC and TOF-MS)**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Proposed product</th>
<th>Formula</th>
<th>TOF-MS Expected (M+Na)$^+$ (m/z)</th>
<th>TOF-MS Actual (m/z)</th>
<th>UV-vis $\lambda_{max}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>14-oxo, 15-HETrA</td>
<td>C$<em>{20}$H$</em>{32}$O$_{4}$</td>
<td>359.21928</td>
<td>359.21583</td>
<td>207</td>
</tr>
<tr>
<td>EPA</td>
<td>14-oxo, 15-HETA</td>
<td>C$<em>{20}$H$</em>{30}$O$_{4}$</td>
<td>357.20363</td>
<td>357.20120</td>
<td>207</td>
</tr>
<tr>
<td>DHA</td>
<td>16-oxo, 17-HDPA</td>
<td>C$<em>{22}$H$</em>{32}$O$_{4}$</td>
<td>383.21928</td>
<td>383.21534</td>
<td>206</td>
</tr>
</tbody>
</table>

HETrA: Hydroxy eicosatrienoic acid  
HETA: Hydroxyeicosatetraenoic acid  
HDPA: Hydroxydocosapentaenoic acid
Results: Sunflower + DHA (Reduced products)

NP-HPLC

GCMS (hydrogenation, trimethylsilylation)

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>TIC Peak Area (x10^6 units)</th>
<th>Fragments (m/z)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.432</td>
<td>155.2</td>
<td>73, 117, 129, 145, 397, 412</td>
<td>DHA</td>
</tr>
<tr>
<td>26.648</td>
<td>151.7</td>
<td>73, 289, 313, 469, 485</td>
<td>7-HDHA (7-hydroxy DHA)</td>
</tr>
<tr>
<td>26.860</td>
<td>7.2</td>
<td>73, 187, 415, 469, 485</td>
<td>16-HDHA</td>
</tr>
<tr>
<td>28.988</td>
<td>123.0</td>
<td>73, 173, 429, 469, 485</td>
<td>17-HDHA</td>
</tr>
<tr>
<td>27.805</td>
<td>7.8</td>
<td>73, 131, 204, 217, 471, 485</td>
<td>20-HDHA</td>
</tr>
<tr>
<td>28.374</td>
<td>144.8</td>
<td>73, 173, 415, 573</td>
<td>16,17-diHDP (16,17-dihydroxy DPA)</td>
</tr>
<tr>
<td>28.586</td>
<td>72.5</td>
<td>73, 173, 415, 573</td>
<td>16,17-diHDP</td>
</tr>
<tr>
<td>28.889</td>
<td>57.6</td>
<td>73, 173, 289, 311, 427, 517, 573</td>
<td>7,17-diHDHA (7,17-dihydroxy DHA)</td>
</tr>
</tbody>
</table>
### Summary of reduced products (HPLC and GCMS)

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monohydroxy products</strong></td>
<td>5-HETA 8-HETA 14-HETA 15-HETA</td>
<td>5-HEPA 8-HEPA 14-HEPA 15-HEPA</td>
<td>7-HDHA 16-HDHA 17-HDHA 20-HDHA</td>
</tr>
<tr>
<td>(single LOX reaction)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dihydroxy products</strong></td>
<td>8,15-diHETA 5,15-diHETA</td>
<td>5,15-diHEPA 5,18-diHEPA</td>
<td>7,17-diHDHA</td>
</tr>
<tr>
<td>(double LOX reaction)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vicinal diol products</strong></td>
<td>14,15-diHETrA</td>
<td>14,15-diHETA</td>
<td>16,17-diHDPA</td>
</tr>
<tr>
<td>(LOX and AOS reaction)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HETrA: Hydroxyeicosatrienoic acid  
HETA: Hydroxyeicosatetraenoic acid  
HEPA: Hydroxyeicosapentaenoic acid  
HDHA: Hydroxydocosahexaenoic acid
Conclusions

• Inexpensive crude plant extracts contain active LOX and AOS enzymes

• Multiple enzyme catalysed reactions

• A number of potentially useful analogues of Resolvins and Protectins were formed
Further work

• Investigate specificity of AOS enzyme towards different positional isomers of hydroperoxides

• Investigate biological activity of AOS products

• Investigate other hydroperoxide metabolising enzymes (e.g. Allene Oxide Cyclase)

Image: Allene Oxide Synthase, Orientation of Proteins in Membranes (OPM) database
Acknowledgements

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