Callaghan Innovation

Kelp phospholipids

M. Vyssotski, A. MacKenzie, D. Scott, K. Lagutin
Edible brown algae are considered a healthy food because:

- They are a low fat source of minerals and fibre, vitamins and amino acids
- Some species are rich in iodine
- They are a source of beneficial allenic carotenoids (e.g., fucoxanthin)
- They contain glyco- and phospholipids enriched in polyunsaturated fatty acids
- They are good in removing heavy metals, and possess radio protective components.
When our routinely used technique of $^{31}\text{P}-\text{NMR}$ analysis of phospholipids was applied to the samples of locally harvested brown algae, a number of signals corresponding to unidentified phosphorus-containing compounds were observed in total lipid extracts.

Kelp *Undaria pinnatifida*, an unwanted organism under the Biosecurity Act 1993
Unlike green algae, brown algae contain no or little phosphatidylserine, having an unusual major aminophospholipid instead.

In 1994 the structure of this novel phospholipid was suggested as N-(1-carboxy-3-aminopropyl-3)-1,2-diacyl-sn-3-glycerophosphoryl ethanolamine, abbreviated as N-CAPE (Shmid et al. J.Plant Physiol. 143:570-574, 1994)
Or is it?

A year later a different structure of that lipid has been published, namely phosphatidyl-O-[N-(2-hydroxyethyl)glycine], abbreviated as PHEG (Eichenberger et al. J.Plant Physiol. 146:398-404, 1995)

While the PHEG lipid is more commonly referred to in the recent literature, N-CAPE structure is still occasionally mentioned.
*Undaria* phospholipids – where’s PHEG?
Undaria phospholipids – where’s PHEG?

pH 8.1
Some common brown algae in NZ

Neptune’s necklace - *Hormosira banksii*

Kelp *Ecklonia radiata*

“Tree” - *Landsburgia quercifolia*

“Grass” - *Scytothamnus australis*
$^{31}\text{P-NMR}$ of deacylated lipids

pH 7.0
Betaine lipid - DGTA

Order *Fucales*: PC completely or mostly replaced with DGTA:

![Chemical structure of DGTA](image)

Diacylglycerol hydroxymethyltrimethyl-β-alanine, DGTA

Order *Laminariales*: PC > 40% of phospholipids, no DGTA
Confirmation – let’s make some PHEG!

PC + Actinomadura PLD → PHEG
Confirmed!

2hr reaction

pH 7.0

Mix

Kelp

GPI

GPG

GPC

GPHEG

GPE

[Graph with chemical peaks labeled GPE, GPHEG, GPC, GPG, and GPI at specific ppm values]
PHEG: NI ESI MS
Kelp: PHEG + ?
No M-32 pattern observed
No M-32 pattern observed
M-32!
An unexpected fragmentation pattern (M-32) was found to be identical to that of phosphatidyl hydroxyethyl methylcarbamate, PECM:

PECM was reported to be an artefact formed from PE during chloroform-methanol extraction of *E.coli* (Garrett *et al.* 2011).
An unexpected outcome was a finding of ceramide phosphoinositol, CPI, (mostly d18:0/18:0 and d18:1/18:0 species), of all macro algae previously reported for red algae only:

Possibly, a contribution from epiphytes
Kelp deacylated

Fraction 60:40 C:M deacylated
X is... (3-((4-((dimethylarsoryl)methyl)-3,5-dihydroxytetrahydrofuran-2-yl)oxy) phosphatidylglycerol, conveniently referred to as “arsenosugar phospholipid”
Unknowns?
Knowns! (mostly...)
TLC of Kelp phospholipids
## Phospholipid profiles by $^{31}$P-NMR

<table>
<thead>
<tr>
<th></th>
<th>Hormosira banksii</th>
<th>Ecklonia radiata</th>
<th>Scytothamnus australis</th>
<th>Landsburgia quercifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>0.0</td>
<td>30.7</td>
<td>52.0</td>
<td>1.3</td>
</tr>
<tr>
<td>PI</td>
<td>8.7</td>
<td>8.9</td>
<td>8.3</td>
<td>10.0</td>
</tr>
<tr>
<td>PHEG</td>
<td>8.4</td>
<td>5.4</td>
<td>4.9</td>
<td>9.3</td>
</tr>
<tr>
<td>PE</td>
<td>47.7</td>
<td>24.8</td>
<td>17.6</td>
<td>31.0</td>
</tr>
<tr>
<td>AsPL</td>
<td>4.8</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>CL</td>
<td>3.6</td>
<td>1.3</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>PG</td>
<td>22.1</td>
<td>16.9</td>
<td>10.8</td>
<td>39.4</td>
</tr>
<tr>
<td>PA</td>
<td>4.7</td>
<td>4.3</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Total PL wt% in lipid</strong></td>
<td><strong>3.4</strong></td>
<td><strong>37.2</strong></td>
<td><strong>21.4</strong></td>
<td><strong>3.6</strong></td>
</tr>
<tr>
<td><strong>Lipid yield</strong></td>
<td>0.32</td>
<td>0.61</td>
<td>1.34</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Total PL wt% in wet sample</strong></td>
<td><strong>0.01</strong></td>
<td><strong>0.23</strong></td>
<td><strong>0.29</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>

Minor phospholipids (e.g., CPI) were not quantified.
Conclusions

- Kelps contain uncommon phospholipids with bioactivities yet to be established
- $^{31}$P-NMR is a convenient way to qualitatively and quantitatively analyse kelp phospholipids (including arsenic-containing phospholipids)
- Novel phospholipids were synthesised and their TLC, MS and NMR characteristics were determined
Acknowledgements:

The authors are grateful to Callaghan Innovation for funding, to Dr Stephen Tallon’s Process Engineering team for supercritical/near critical fluid extraction experiments, Drs Kevin Mitchell and Yinrong Lu for help with MS support (all - Callaghan Innovation), and to Dr Shigeyuki Imamura (Imamura Enzyme Technologies Corporation, Shizuoka, Japan) for a gift of Actinomadura PLD.
0800 4 CALLAGHAN
(0800 422 552)
callaghaninnovation.govt.nz