Interactions of allelochemicals with plant plasma membrane: a case study with alkaloids from barley

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Background
Allelopathy is defined as “any direct or indirect harmful effect by one plant on another through production of chemical compounds that escape into the environment” (Rice, 1974). This phenomenon is seen as a potential tool for weeds management within the framework of sustainable agriculture. While many studies investigated the mode of action of various allelochemicals (molecules emitted by allelopathic plants), little attention was given to their initial contact with the plant plasma membrane. In our work, this key step is explored for two alkaloids, gramine and hordenine, that are allelochemicals produced by barley.

Structures

Phytotoxicity assays

Principles
- 75 chamomile (Matricaria recutita L.) seeds on a filter paper wetted with alkaloid solution or buffer alone (control) grown for 7 days
- Mean root length = phytotoxicity marker
- 7 reps for each treatment, 14 reps for control

Observations
Both alkaloids are phytotoxic towards chamomile seedlings, with gramine being more potent than hordenine. No synergy is observed.

Biophysical assays

Principles
- Biophysical techniques used in order to study the interactions between the alkaloids and plant plasma membranes
- Liposomes used as model membranes (in vitro experiments)
- Isothermal titration calorimetry: measuring heat absorption or production upon addition of liposomes to an alkaloid solution; enables thermodynamic characterization of interactions
- Fourier-transformed infrared spectroscopy: investigating the impact of alkaloids on lipid phase transition temperature (Tm)
- Molecular dynamics: computer simulations of lipid bilayers with alkaloid molecules; gives details at the molecular level

Infrared spectroscopy: do they affect lipid properties?

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>Alkaloid</th>
<th>Phase transition temperature (°C)</th>
<th>ΔTm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>/</td>
<td>23.69 (± 0.15)</td>
<td>-1.09</td>
</tr>
<tr>
<td>DMPC</td>
<td>Gramine</td>
<td>22.6 (± 0.18)</td>
<td>-0.04</td>
</tr>
<tr>
<td>DMPC</td>
<td>Hordenine</td>
<td>23.65 (± 0.13)</td>
<td>-0.04</td>
</tr>
<tr>
<td>DMPC</td>
<td>/</td>
<td>23.67 (± 0.15)</td>
<td>-0.04</td>
</tr>
<tr>
<td>DMPC</td>
<td>Gramine</td>
<td>16.54 (± 0.17)</td>
<td>-7.13</td>
</tr>
<tr>
<td>DMPC</td>
<td>Hordenine</td>
<td>20.52 (± 0.25)</td>
<td>-3.15</td>
</tr>
<tr>
<td>DMPC:DMPC (4:1)</td>
<td>/</td>
<td>24.03 (± 0.21)</td>
<td>-3.86</td>
</tr>
<tr>
<td>DMPC:DMPC (4:1)</td>
<td>Gramine</td>
<td>20.17 (± 0.21)</td>
<td>-3.86</td>
</tr>
<tr>
<td>DMPC:DMPC (4:1)</td>
<td>Hordenine</td>
<td>22.81 (± 0.17)</td>
<td>-2.22</td>
</tr>
</tbody>
</table>

Observations
Lipid phase transition Tm decreases when gramine is present, which indicates membrane destabilization. Hordenine impact on lipids Tm is always lower than that of gramine, regardless of lipid composition.

Isothermal titration calorimetry: do they interact with lipids?

Molecular dynamics simulations: molecular details

(Illustration: 32 gramine molecules (blue-colored) with lipid bilayer, water and ions omitted for clarity)

Observations
Both alkaloids spontaneously bind to liposomes. Gramine affinity for lipids (k) is higher than hordenine affinity for lipids.

0 ns 50 ns 500 ns

Conclusion
Both alkaloids are proven to be phytotoxic and to interact with model membranes. Moreover, a good correlation between phytotoxicity and ability to interact with- and to disturb lipid bilayers is found. This suggests that interactions of these allelochemicals with plant plasma membrane could be linked to their mode of action.

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