Investigation of Potential Nitrogen Mustard Hydrolysis Assays

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Introduction

Nitrogen mustards are highly toxic blistering agents, a feature characteristic of most compounds within the mustard family. These toxic mustard agents, including sulfur mustard, were developed during the early Twentieth Century for use as chemical weapons. The sulfur mustards were used extensively for this purpose during World War I. The Chemical Weapons Convention outlawed the use of these compounds as chemical warfare agents (Convention 1997), but they are still utilized in specialized cases of chemotherapy (Cummings et al. 1991). Mustard agents are being replaced as chemotherapeutic agents, but they are considered prime components for terrorist weapons because they are easily synthesized and capable of inflicting great suffering on localized populations (Department of Health 2008).

Numerous studies have investigated the ability of bacterial enzymes to degrade xenobiotics and several bacterial enzymes have been identified with the fortuitous ability to degrade organophosphorus nerve agents despite the fact that these agents have never been available in appreciable concentrations in the environment (Walker et al. 2007, Dawson et al. 2008). Strategies are currently being investigated to use these enzymes to produce prophylactic products for treatment after exposure. Such enzymes may be discovered for mustard agents but an effective assay must be developed to permit the simultaneous screening of large numbers of samples for mustardase activity.

Purpose

The purpose of this research was to investigate several potential assays for use in screening bacteria and plants for enzymes with a fortuitous ability to degrade nitrogen mustards. The assays evaluated include refractometry, gas chromatography, and ultraviolet light analysis.

Materials and Methods

Nitrogen mustards HN1 [bis-(2-chlorethyl)-ethylamine], HN2 [bis-(2-chlorethyl)-methylamine, and HN3 [tris-(2-chlorethyl)-amine] were obtained along with their known hydrolysis products N-ethyldiethanolamine (EDEA), N-methyldiethanolamine (MDEA), and triethanolamine (TEA) in aqueous form from Sigma-Aldrich Chemical Company.

Refractometry was conducted on a Fischer Scientific Portable Refractometer. Crystalline nitrogen mustards were solubilized in a 0.5 M potassium phosphate buffer (pH=7.6) to concentrations of 0.5 grams per 3 mL of buffer and analyzed through refractometry. The refractive indices obtained were compared with those of associated mustard hydrolysis analogs to determine any significant differences between products and reactants.

Gas chromatography (GC) was conducted on a GOW-MAC Gas Chromatograph Thermal Conductivity Detector running at initial injection port, detector, and column temperatures of 60ºC. Crystalline mustards were solubilized in a 0.5 M potassium phosphate buffer. Samples were injected and temperatures were increased gradually to near maximum (100ºC) for sample elution and analysis.

Nitrogen compounds and respective hydrolysis products were incorporated into LB agar for analysis under ultraviolet light. Three bi-compartment agar plates were created containing mustard components at concentrations of 7%. Each nitrogen mustard was poured next to its corresponding hydrolysis product for differences that could be detected through ultraviolet light exposure at 1.5 second intervals (Fig. 1).

Results

<table>
<thead>
<tr>
<th>Chemical Compound</th>
<th>Mean Refractive Index</th>
<th>Standard Deviation</th>
<th>Hydrolysis Product</th>
<th>Mean Refractive Index</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN1</td>
<td>1.580</td>
<td>0.0017</td>
<td>EDEA</td>
<td>1.407</td>
<td>0.0012</td>
</tr>
<tr>
<td>HN2</td>
<td>1.542</td>
<td>0.0023</td>
<td>MDEA</td>
<td>1.475</td>
<td>0.0019</td>
</tr>
<tr>
<td>HN3</td>
<td>1.558</td>
<td>0.0021</td>
<td>TEA</td>
<td>1.485</td>
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Table 1. Mean refractive indices of solubilized nitrogen mustards and respective hydrolysis products are shown. Standard deviations and p-values are presented for mustardase/hydrolysis pairs.

Conclusions

Refractometry was determined to be a successful assay for detecting nitrogen mustard hydrolysis. The refractive indices obtained for each mustard/hydrolysis pair were found to have p-values less than 0.05, indicating significant refractive differences between mustards and degradation products.

Gas chromatography failed to distinguish between these compounds as they did not elute as discernable peaks on the chromatograph. The likely cause of this may be attributed to the relatively high boiling points of the chemicals under investigation and the GOW-MAC Gas Chromatograph Thermal Conductivity Detector’s inability to reach the necessary temperatures for proper sample elution.

Ultraviolet light analysis of nitrogen mustards exhibited fluorescent differences between HN2 and HN3 and their respective hydrolysis products MDEA and TEA. Exposing HN1 to ultraviolet light yielded no such disparity to accurately distinguish it from its hydrolysis analog EDEA.

References


Mentor: Shane Gold
Randy Day
Gary Frederick
BYU-Hawaii Biology Faculty
BYU-Hawaii FAST Program

Acknowledgements