Introduction
Peptidyl-tRNA hydrolase (Pth) is a critical protein that recycles peptidyl-tRNAs formed as a result of premature termination of transcription. Accumulation of such peptidyl-tRNA decreases the ability of the cell to produce protein, quickly becoming fatal. The sequence for Pth is highly conserved across many bacteria. This makes Pth a promising target for antibiotic research. In order to cleave peptidyl-tRNA, Pth requires the presence of a divalent ion. It has been found that Magnesium serves this function. The purpose of the research conducted this summer was to gain a deeper understanding of the mechanism of Pth and how it would be effected in the presence of various ions and compounds other than Magnesium.

Screening for Pth Activity
To observe E. coli Pth activity under varying conditions, activity assays were conducted using acid-urea mini-gels (Fig. 1A).

The broad peptidyl-tRNA band (“un-cleaved”) reflects the heterogeneous population of peptide lengths attached to tRNAs. E. coli cells with compromised Pth function were used to produce the substrate.

Cleavage of the bulk peptidyl-tRNA by active Pth is illustrated by a population of peptide lengths attached to tRNAs.

To test the effect of various ions on the activity of Pth, a variation of the Pth activity assay was used (Table 1, Fig. 2).

Experimental set up for the screening. Each gel was run with cleaved and un-cleared controls. Conditions were varied by adding the appropriate ions to the final concentration of 5 mM. Reactions were quenched after 30 minutes by addition of 8 M urea.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Peptidyl-tRNA (14μg/mL)</th>
<th>Pth (36μM)</th>
<th>Ion (50mM)</th>
<th>Buffer</th>
<th>Reaction Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaved Control</td>
<td>1.5μL</td>
<td>1.5μL</td>
<td>0μL</td>
<td>17μL (10mM Tris, 10mM MgSO4, 10mM NH4SO4)</td>
<td>30 min</td>
</tr>
<tr>
<td>Un-cleared Control</td>
<td>1.5μL</td>
<td>0μL</td>
<td>0μL</td>
<td>18.5μL (10mM Tris, 10mM MgSO4, 10mM NH4SO4)</td>
<td>30 min</td>
</tr>
<tr>
<td>Variable Condition</td>
<td>1.5μL</td>
<td>1.5μL</td>
<td>2μL</td>
<td>15μL (10mM Tris, 10mM NH4SO4)</td>
<td>30 min</td>
</tr>
</tbody>
</table>

Results
To better visualize Pth activity as well as quantify the percent activity in varying conditions, the gels were scanned and analyzed to generate a graph of each band based on the changes in intensity throughout the band (Fig 1B).

As can be seen above, visualizing active versus inhibited Pth becomes much less difficult after this analysis. To gauge activity/inhibition of the various bands, the graphs were scaled to the control RNA bands (Fig 1B). These RNA bands are constant, allowing for normalization of the various bands (eliminating any scale variations resulting from incomplete destaining of the methylene blue dye or pipetting errors).

Figure 1 Assay of Pth activity. A, difference between the cleaved and un-cleared bands of the substrate as observed on the acid-urea mini-gel stained with methylene blue; B, computer processed data showing the intensity of the bands on the gel. Ctrl, control RNA band used for scaling.

Figure 2 Experimental data for screening of Pth activity under various conditions. A, acid-urea mini-gel with lanes corresponding to various conditions; B, intensity profiles for control reactions and reaction with 5 mM BaCl2. The profile of the band for reaction with the addition of BaCl2 corresponds to the cleaved control.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area of Curve(a.u.)</th>
<th>% Cleaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaved control</td>
<td>71529</td>
<td>100</td>
</tr>
<tr>
<td>BaCl2</td>
<td>66045</td>
<td>92</td>
</tr>
</tbody>
</table>

Conclusions
The screening of ions for Pth activity yielded several interesting findings. Of the ions screened, Ba2+ facilitated a notable level of Pth activity (listed below). However, none of the other divalent ions screened facilitated Pth activity.

Acknowledgments
This research was funded by the RCEU program with funds provided by the Presidents/Provosts office, funds provided by the Vice President for Research, funds provided by the Chemistry Department through their patent account, and external funding from the Alabama Space Grant Consortium.

I would also like to acknowledge Morgan Gilbert, Kasey Taylor, and other members of Dr. Robert McFeeters Lab for all the guidance and help on this project.

Future Work
- Conduct further investigation on the effect of Ba+2 on Pth activity.
- Collect structural information using NMR.
- Conduct further investigation on the effect of SO4-2 on Pth activity.

Another interesting finding was that Pth activity was accentuated in the presence of SO4-2 as well as when in the presence of NH4+. The experimental data illustrated a clear elevation of Pth activity in the presence MgSO4 in comparison to MgCl. While Pth activity was also elevated by the presence of NH4Cl, this effect was even greater when (NH4)2SO4 was present (Results not shown).

Future Work

- Conduct further investigation on the effect of Mg+2 on Pth activity.
- Collect structural information using NMR.
- Conduct further investigation on the effect of SO4-2 on Pth Activity.

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