Enzymatic synthesis of acyl-ACP substrates for Esal AHL synthase enzyme

Miranda Ghali, Dastagiri Dudekula*, Rajesh Nagarajan*, Ph.D.

Department of Chemistry, Stetson University, DeLand, Florida; *Department of Chemistry and Biochemistry, Boise State University, Boise, Idaho

Abstract

Bacterial infections have become more difficult to treat as communication between bacteria allows for growth and resistance. Bacteria use autoinducer molecules to take a census count of neighboring bacteria in the colony. The autoinducer molecules are synthesized inside the cell by a dedicated set of enzymes and are released outside to aid in intercellular communication. This mechanism of communication, known as quorum sensing, allows bacteria to coordinate their activities that include secretion of virulence factors and formation of biofilms. Esal is an AHL synthase enzyme in the plant pathogen Pantoea stewartii, which uses S-adenosyl-L-methionine and 3-octanoyl-ACP to make 3-octanoyl-HSL autoinducer molecules inside the cell. This project focused on purification of 3-octanoyl ACP and hexanoyl ACP as substrates for Esal enzyme. In this reaction, acyl-CoA was reacted with apo-ACP in presence of a phosphopantetheiny1 transferase enzyme to form acyl-ACP. The efficiency of Bacillus subtilis Sfp enzyme with E.coli holo-ACP synthase (apcs5) was compared in acyl-ACP substrate synthesis. HPLC was used to separate products from the reaction mixture and the identity of the product was confirmed using LC-MS. The activity of these substrates with Esal enzyme will be measured using a colorimetric assay. The Esal AHL synthase will be subjected to mechanistic studies using acyl-ACP substrates synthesized above, which will lead to inhibitor design for the enzyme.

Background

Bacteria can control their own population density by monitoring it with chemical signals. This mechanism, known as quorum sensing (QS), allows for biofilm development.1 QS is most studied in N-acyl-L-homoserine lactones (AHLs) in Gram-negative bacteria.2 AHLs are autoinducer molecules that help to establish cell-to-cell communication in bacteria. Pantoea stewartii, a Gram-negative bacteria, uses QS to create an exopolysaccharide that form the stalks with in sweet corn, rice, maize etc.2 The Esal AHL synthase enzyme uses S-adenosyl-L-methionine (SAM) and 3-octanoyl-ACP to make 3-octanoyl homoserine lactone autoinducer in P. stewartii bacteria. (Figure 1)

Figure 1. Proposed mechanism of AHL synthase in Esal

Because it is difficult to synthesize 3-octanoyl ACP substrate with good yields, mechanistic studies on Esal enzyme have been slow. This project focuses on optimizing synthesis of acyl-ACP substrates for Esal AHL synthase to enable mechanistic studies on this enzyme, which will inform inhibitor design. Esal inhibitors will disrupt quorum sensing and thus reduce virulence caused by P. stewartii bacteria.

Methods

Reaction assays of various concentrations of phosphopantetheiny1 transferase enzyme Sfp, E. Coli ACP, (C6, C10, C12)-Coenzyme A, MgCl2, and MES buffer were conducted for varying times up to six hours at 37°C. The reactions were quenched to 0.1% TFA after the time length needed. These solutions were then read on HPLC to confirm identity of compound created with reaction. The gradient setting on the HPLC was a twelve minute linear gradient from water plus 0.1% TFA to acetonitrile plus 0.1% TFA. A C-18 reverse phase HPLC column was used.

Results

Figure 2. Acyl-ACP Synthesis (Enzymatic Method).

The reactions were quenched to 0.1% TFA after the time length needed. These solutions were then read on HPLC to confirm identity of compound created with reaction. The gradient setting on the HPLC was a twelve minute linear gradient from water plus 0.1% TFA to acetonitrile plus 0.1% TFA. A C-18 reverse phase HPLC column was used.

Results Continued

Table 1. Percent Product of Various Acyl-ACPs

<table>
<thead>
<tr>
<th>Acyl-ACP</th>
<th>Time 3 hours (percent product)</th>
<th>Time 4 hours (percent product)</th>
</tr>
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<tbody>
<tr>
<td>C6-ACP</td>
<td>36.78%</td>
<td>50.12%</td>
</tr>
<tr>
<td>C10-ACP</td>
<td>39.13%</td>
<td>41.78%</td>
</tr>
<tr>
<td>C12-ACP</td>
<td>29.69%</td>
<td>36.65%</td>
</tr>
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Percent product was obtained by the equation: percent product = (peak area of product/peak area of product + peak area of reactant) x 100. From this data, we can see that C6-CoA is more readily reacted with Sfp.

Conclusion

We have succeeded in making C6, C10, and C12 acyl-ACP substrates. We have also determined that 0.1μM of Sfp enzyme is sufficient for turnover of acyl-ACP substrates.

Future Works

Use E.coli FabB to make 3-octanoyl ACP and hexanoyl-ACP substrates for Esal. Mass spectrophotometry will be run on collected peaks for confirmation of products. The K<sub>n</sub> and K<sub>p</sub> of these substrates with Esal enzyme will be measured using a spectrophotometric assay.

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References