Expression, Purification and Enzymatic Characterization of D-Phenylglycine Aminotransferase

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Abstract

Pseudomonas stutzeri ST-201 isolated D-phenylglycine aminotransferase (D-PhgAT) was expressed in competent BL21 (DE3) Escherichia coli, purified via the Bio-Rad Profina Purification system (automated affinity chromatography with subsequent desalting), analyzed by SDS-PAGE and functionally characterized. The purified protein yield was ~324.7ug/mL (with a total protein mass of ~171.4mg) and the enzyme activity was found to be 0.12 UmL⁻¹, which was reproducibly achieved three times. The specific enzyme activity was found to be 0.49 Umg⁻¹.

Introduction

Aminotransferases, also known as transaminases, catalyze the transfer of an amino group to the α-keto acid of an acceptor molecule, generating a chiral amine product. The stereoselective nature of these reactions has potential industrial use for the synthesis of chiral amines, which are frequently used as intermediates in the production of various pharmaceuticals. However, strict substrate specificity and enzyme instability limits the application of transaminase reactions for commercial purposes. Thus, enzymatic expression and subsequent characterization provides valuable insight for prospective goals of enhanced transaminase function via engineered polypeptides.

D-phenylglycine aminotransferase (~49kDa per monomer) has stereocverting transaminase activity, catalyzing the reversible transamination of D-phenylglycine to L-glutamate with 2-oxoglutarate as the amino-group acceptor. Its expression, purification and activity assay will be further applied for comparison with engineered mutants used for the asymmetric synthesis of D-amino acids.

Materials and Methods

Minpreparation XL1 Blue Escherichia coli pET11a D-phenylglycine aminotransferase recombinant plasmid DNA.

Transformation of chemically competent BL21 (DE3) Escherichia coli with recombinant pET11a.

IPTG-induced expression of D-Phenynglycine Aminotransferase.

Purification of D-Phenynglycine Aminotransferase via BioRad Profina Protein Purification System.

SDS-PAGE analysis of purified D-Phenynglycine Aminotransferase.

Bradford Assay of Protein Sample.

D-Phenynglycine Aminotransferase - Glutamate Dehydrogenase Coupled Enzyme Assay.

Coupled Enzyme Assay Reaction (at 37 C):

\[
\begin{align*}
\text{D-phenylglycine} & \xrightarrow{\text{D-phenylglycine aminotransferase}} \text{D-2-oxoglutarate} \\
\text{L-glutamate} & \xrightarrow{\text{glutamate dehydrogenase}} \text{2-oxoglutarate} + \text{NADH} + \text{H}^+ \\
\text{2-oxoglutarate} & \xrightarrow{\text{glutamate dehydrogenase}} \text{L-glutamate} + \text{NAD}^+ \\
\text{NH}_3 & \xrightarrow{\text{2-Hydroxyacid}} \text{NH}_4^+ + \text{HCO}_3^-
\end{align*}
\]

Assay Reaction Mixture: 20µl 100mM 2-oxoglutarate 10ul 25mM D-phenylglycine 20 ul 10mM Pyruvate 20ul 1.6mM PLP 20ul NAD+ 1ul GDH 70ul phosphate buffer (pH 7.6) 20ul of purified protein sample 200ul Total Volume

Results

Table 1. Absorbance and associated concentration (in ug/mL) of BSA standards and DPhgAT protein purification samples. The values correspond to the standard curve in Fig. 2.

<table>
<thead>
<tr>
<th>Protein Sample</th>
<th>Concentration (ug/mL)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA Standard 1</td>
<td>76</td>
<td>0.071</td>
</tr>
<tr>
<td>BSA Standard 2</td>
<td>100</td>
<td>0.078</td>
</tr>
<tr>
<td>BSA Standard 3</td>
<td>200</td>
<td>0.17</td>
</tr>
<tr>
<td>BSA Standard 4</td>
<td>300</td>
<td>0.131</td>
</tr>
<tr>
<td>BSA Standard 5</td>
<td>400</td>
<td>0.215</td>
</tr>
<tr>
<td>BSA Standard 6</td>
<td>500</td>
<td>0.228</td>
</tr>
<tr>
<td>BSA Standard 7</td>
<td>600</td>
<td>0.408</td>
</tr>
<tr>
<td>BSA Standard 8</td>
<td>750</td>
<td>0.494</td>
</tr>
<tr>
<td>BSA Standard 9</td>
<td>1000</td>
<td>0.501</td>
</tr>
<tr>
<td>DPhgAT</td>
<td>836.8</td>
<td>0.414</td>
</tr>
</tbody>
</table>

Table 2. Summary of DPhgAT enzymatic characterization experimental results. Protein concentration, yield, purity and specific activity are displayed.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Experimental Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Yield</td>
<td>342.72 ug/mL</td>
</tr>
<tr>
<td>Protein Purity</td>
<td>98%</td>
</tr>
<tr>
<td>Specific Activity</td>
<td>0.49U/mg</td>
</tr>
</tbody>
</table>

Conclusions

SDS-PAGE assessment (Fig. 1) of the purified enzyme gave a strong protein band at a molecular weight of approximately 49 kDa, corresponding to a total purified protein concentration of 42.84g/L in 4mL of elution as determined via Bradford assay.

The glutamate dehydrogenase-coupled spectrophotometric assay was used to determine D-PhgAT activity in the direction of L-glutamate synthesis, monitoring formation of NADH by increase in absorbance (340nm) over time. The enzyme activity was found to be 0.12 UmL⁻¹ in phosphate buffer (pH 7.6), corresponding to a specific activity of 0.49 Umg⁻¹. These results are comparable to those of literature, where the specific activity for Pseudomonas putida D-4-hydroxyphenyl transaminase was found to be 0.25 Umg⁻¹ (pH of 7.0 and 30 C).

Further study should examine the effects of pH and temperature on transaminase activity. Subsequently, upon computational assessment of protein catalysis and associated structural features, site-directed mutagenesis could be applied for protein engineering purposes.

Literature Cited


Acknowledgements

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