Expression, Purification and Enzymatic Characterization of D-Phenylglycine Aminotransferase

Undergraduate Research Opportunity Program, Winter 2011

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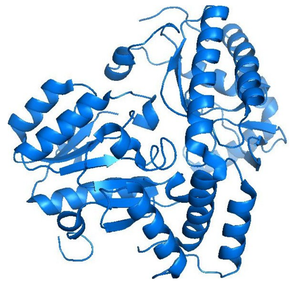
Abstract

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 ~342.7ug/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}.



Crystal structure of D-phenylglycine aminotransferase (D-PhgAT) from Pseudomonas strutzeri ST-201. RCSB PDB entry 2CY8.

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 stereo-converting 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.

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

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IPTG-induced expression of D-Phenylglycine Aminotransferase.

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

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SDS-PAGE analysis of purified D-Phenylglycine Aminotransferase.

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Bradford Assay of Protein Sample.

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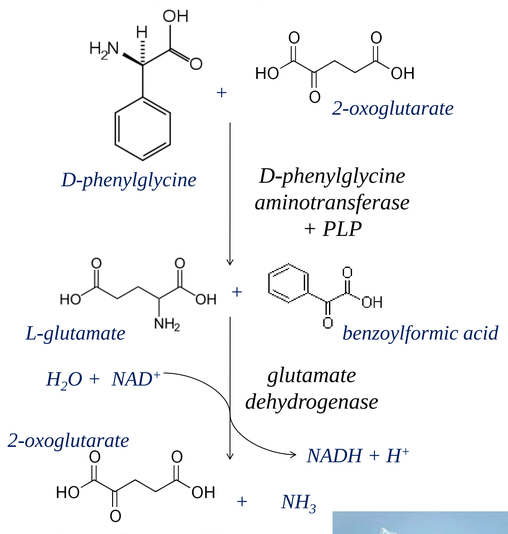
D-Phenylglycine Aminotransferase - Glutamate Dehydrogenase Coupled Enzyme Assay.

Coupled Enzyme Assay Reaction (at 37 C):



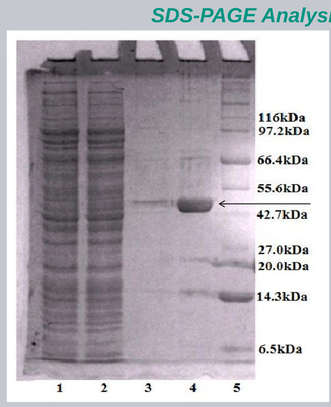
Assay Reaction Mixture:

20ul 100mM 2-oxoglutarate
10ul 25mM D-phenylglycine
20 ul 10mM Pyruvate
20ul 1.6mM PLP
20ul NAD+
1ul GDH
79ul phosphate buffer (pH 7.6)
20ul of purified protein sample
200ul Total Volume

Greiner 96 Flat Bottom Transparent Polystyrol microplates used for assay (Greiner Bio-One Product Catalogue).

Results

SDS-PAGE Analysis:



Legend:
1- Flowthrough
2- Wash 1
3- Wash 2
4- Eluted Sample
5- Bio Labs Protein Molecular Weight Markers

Figure 1. SDS-PAGE image of Profina Purification System protein fractions.

Bradford Assay of Protein Sample:

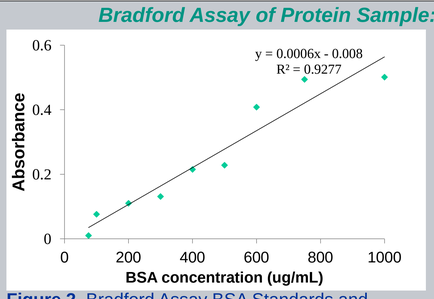


Figure 2. Bradford Assay BSA Standards and corresponding Absorbance at 595nm via OmniPur® Bradford Assay Kit.

Protein Sample	Concentration (ug/mL)	Absorbance
BSA Standard 1	75	0.01
BSA Standard 2	100	0.076
BSA Standard 3	200	0.11
BSA Standard 4	300	0.131
BSA Standard 5	400	0.215
BSA Standard 6	500	0.228
BSA Standard 7	600	0.408
BSA Standard 8	750	0.494
BSA Standard 9	1000	0.501
DPhgAT	856.8	0.414
1/10 dil. DPhgAT	78.4	0.012

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**.

NADH Enzyme Coupled Assay:

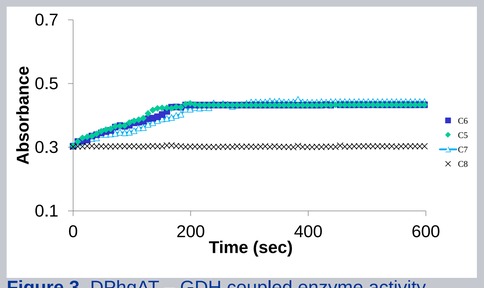
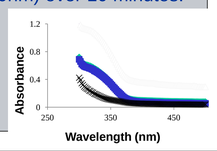


Figure 3. DPhgAT – GDH coupled enzyme activity absorbance measurements (at 340nm) over 10 minutes.

Figure 4. Absorbance as function of wavelength from 250nm to 500nm for the DPhgAT – GDH coupled enzyme.



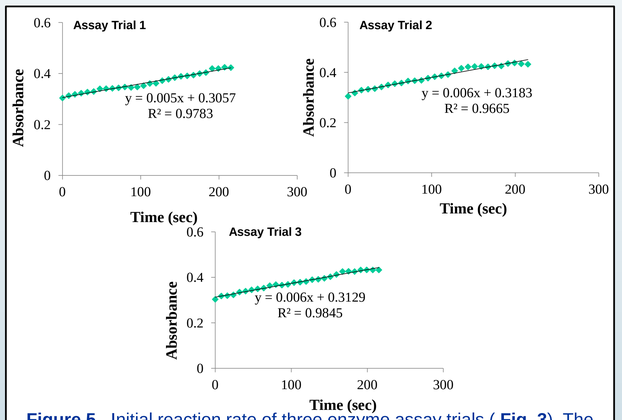


Figure 5. Initial reaction rate of three enzyme assay trials (**Fig. 3**). The samples correspond to identical reaction conditions at 37 C. The fit and regression equation are present for all three reactions. The average slope was applied for specific activity calculations.

	DPhgAT Experimental Results
[Protein]	42.84 g/L
Protein Yield	342.72 ug/mL
Protein Purity	98%
Specific Activity	0.49U/mg

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

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 spectrophometric 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

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Acknowledgements

Special thanks to Curtis Walton for his assistance throughout the project and the Dr. Chica Lab group.