Expanding the Use of Tyrosine Phenol Lyase in the Enzymatic Synthesis of Naphthol Unnatural Amino Acids

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**Abstract**
Tyrosine phenol lyase (TPL), an enzyme biologically involved in the degradation of tyrosine, has been used to synthesize a variety of substituted tyrosines including fluorotyrosines for biochemical studies. In addition to fluorotyrosines, expanding the scope of substrates to naphthol-based compounds offers an attractive target for enzymatic synthesis of complex molecules, as substituted naphthols can be used as biochemical probes and as building blocks in potential pharmaceuticals. Using Citrobacter freundii, we enzymatically synthesized the naphthol-analog of tyrosine, but the unnatural amino acid was generated in low yield. The simplest model for the low conversion is that the larger substrate is poorly accommodated by the enzyme active site and this poor binding leads to the low yield in the enzymatic reaction. To test this hypothesis, we mutated several bulky residues near the substrate-binding site (M288, M397, F448) to open space for substrate binding and facilitate synthesis of the unnatural amino acid. A series of mutant plasmids were generated using site-directed mutagenesis and sequenced to confirm the mutation. The wild type and mutant enzymes were recombinantly expressed in E. coli, purified by affinity and ion-exchange chromatography. Tyrosine is used to make Dopamine, Norepinephrine and Epinephrine as well as a few other anti-cancer compounds. Tyrosine phenol lyase (TPL), an enzyme biologically involved in the degradation of tyrosine, has been used to synthesize a variety of substituted tyrosines including fluorotyrosines for biochemical studies.

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**Methodology**

**Expression and Purification of TPL**
- Both wild-type (WT) and mutants were expressed using E. coli bacteria
- Purified using a Ni²⁺ Affinity Column, followed by an Ion Exchange Column (IEC)
- Fractions collected using Fast Protein Liquid Chromatography (FPLC)

**Naphthol Analog of Tyrosine; Other Substituents**

**HPLC Results for the Naphthol Analog**

**What will these mutants test?**
- These mutants were made to replace larger, bulky residues with smaller ones.
- Smaller substituents tend to inhibit enzyme activity and result in lower yield of that particular tyrosine analog. Larger substituents, after the enzyme has been mutated, have higher yields of that tyrosine analog.
- Broadening the space of the active site may allow for larger substituents.
- Mutations may cause issues with protein folding and activity assays will be conducted on these mutated TPL.
- Active mutant enzymes will be used to create different analogs of tyrosine, some of which include using o-fluorophenol, o-bromophenol, and o-methoxy.

**Why Tyrosine Phenol Lyase in this study?**
- A PLP-dependent enzyme that catalyzes degradation of tyrosine into phenol and ammonium pyruvate.
- Previously used to generate unnatural amino acids.

**What is Tyrosine Phenol Lyase (TPL)?**
- A PLP-dependent enzyme that catalyzes degradation of tyrosine into phenol and ammonium pyruvate.
- Previously used to generate unnatural amino acids.

**What are the benefits of Tyrosine derivatives?**
- Used as fluorescent markers for cancer cells.
- Tyrosine is used to make Dopamine, Noradrenaline and Epinephrine as well as a few other anti-cancer compounds.
- m-tyrosine and its analogues have been used to treat Alzheimer’s, Parkinson’s disease and arthritis.

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**Introduction**

**Purification Challenges**
- Initial attempts to purify the WT and mutants suggested the enzymes may elute under different conditions.
- Currently optimizing conditions for purification.

**Future Work**
- More of these mutants will be purified and tested using activity assays.
- The synthesis of the Naphthol analog will be performed with a mutated enzyme.
- Tyrosine derivatives will be synthesized with mutant TPL.
- Western blot attempted with antibody used for Tryptophan Indole Lysase.

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