L-DOPA Dioxygenases from Diverse Natural Product Pathways

Kameron L. Klugh[1], Paige Jones[2], Riri Yoza[2], Larryn W. Peterson[1], Keri L. Colabroy [2]

[1]Department of Chemistry, Rhodes College, Memphis, TN, United States
[2]Department of Chemistry, Muhlenberg College, Allentown, PA , United States
Email: klukl-22@rhodes.edu and kericolabroy@muhlenberg.edu

Abstract

L-DOPA dioxygenase is part of a mini-pathway to the synthon 3-vinyl-2,3-pyrroline-5-carboxylic acid (VPCA) that is elaborated and embedded within the final product structures of lincomycin, anthramycin, sibiromycin, tomaymycin and hormaomycin. Using the VPCA mini-pathway as a starting point, we searched sequence space to identify novel natural product pathways containing a VPCA synthon. From among these novel natural product pathways, representative L-DOPA dioxygenase gene products from *Streptomyces hygroscopicus* subsp.*jinggangensis* and *Nocardia arthritidis* were studied as pure proteins for their stability and activity on L-DOPA and related catechols in steady-state assays for catechol cleavage. These results were analyzed in comparison to characterized L-DOPA dioxygenases from *Streptomyces lincolnensis* and *Streptomyces sclerotialus*.

Background and Motivation

- Vicinal oxygen chelate dioxygenases (VOCs) are enzymes that cleave the aromatic ring of catechols. The products of this cleavage could pave the way for development of biofuels or commercial products[1] (**Figure 1**). For example, the cell of of many plants – lignin – is composed of a primarily catecholic structure **(Figure 2).** The breakdown of this structure is integral for tapping into plants for biofuel[2].

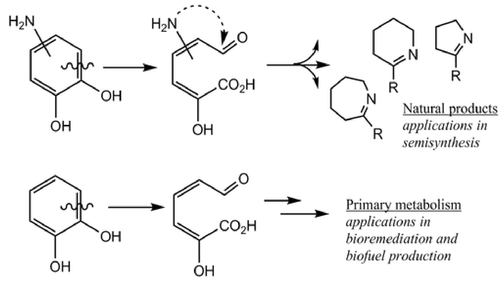

Fig. 1. General scheme of extradiol cleavage with both degradative and biosynthetic potential.

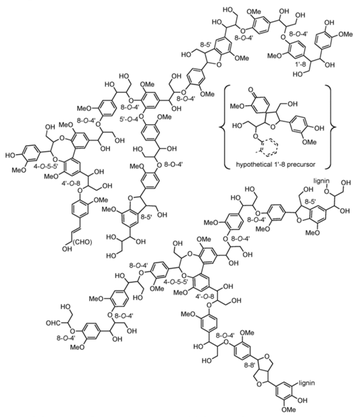

Fig. 2. The structure of lignin contains a multitude of catecholic rings with potential for biofuel.

- Within the structural family of VOCs exits a family of L-3,4-dihydroxyphenylalanine (L-DOPA) 3,4-dioxygenase enzymes that include sequences from the bacteria *Streptomyces hygroscopicus* subsp. *jinggangensis* (JING) and *Nocardia arthritidis*. (NocArt).

- Investigating the reaction mechanism of JING and NocArt in comparison to known L-DOPA dioxygenases will provide a better understanding of the mechanisms of L-DOPA dioxygenase enzymes.

- In this study, JING and NocArt were tested with L-DOPA, dopamine, and 3,4-dihydroxycinnamic acid (DHHCA).

Steady State Kinetics – Methods

- NocArt and JING were grown in lab through overexpression and purification methods
- UV-Visible Spectrometry was used to measure the steady state kinetics by identifying the product of steady-state turnover (**Figure 3**).
- Each assay was run using a varied concentration of substrate on a constant concentration of enzyme. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer and a steady concentration of oxygenated HEPES were used for each assay.

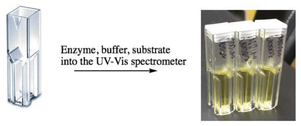

Fig. 3. Primary methodology for obtaining steady state kinetic assays. Steady amounts of HEPES buffer (oxygenated and unoxygenated) and enzyme were added to a cuvette. Varied amounts of substrate were added over the course of multiple trials. The cuvettes were placed in the UV-Visible spectrometer for results. The reactions turned yellow when they reached completion.

Results

- The results from the experiment are preliminaries for future studies with novel substrates. The enzymes were docked with Dopamine, L-DOPA, and DHHCA to determine the baseline activity of both enzymes (**Figures 4a, 4b).**

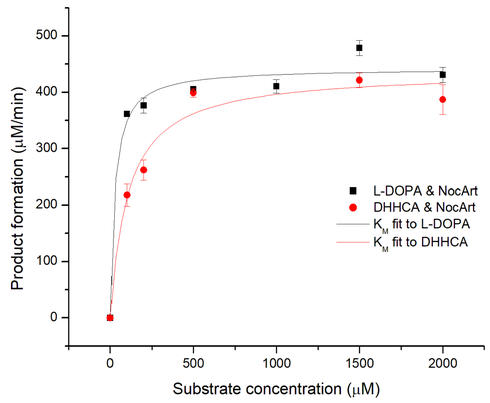

Fig. 4a. Varied substrate activity on NocArt. L-DOPA displays the highest affinity for the enzyme and dopamine has the lowest affinity for the enzyme.

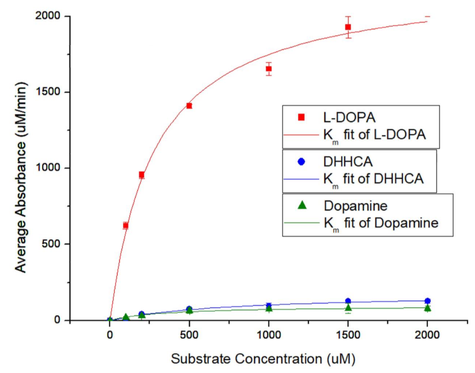

Fig. 4b. Varied substrate activity on JING. L-DOPA displays the highest affinity for the enzyme and dopamine displays the lowest affinity for the enzyme.

- When the catalytic efficiency of the most affective dioxygenase enzyme – SsDDO – was compared to the new enzymes, NocArt and JING both show promising activity as dioxygenase enzymes (**Table 1**).

Table 1. Comparison of steady state kinetics of NocArt, JING, and SsDDO. SsDDO is known to function well as a dioxygenase enzyme. When compared to NocArt and JING, it shows that JING has an equal or greater affinity for its substrates as SsDDO, whereas NocArt displays a moderate affinity for L-DOPA and DHHCA but little to no affinity for dopamine.

	NocArt	JING	SsDDO
k_{cat} L-DOPA	14.27 ± 0.65 sec^{-1}	79 ± 20sec^{-1}	4.48 ± 0.12 sec^{-1}
k_{cat} DHHCA	14.11 ± 0.87sec^{-1}	6.35 ± 0.58sec^{-1}	1.39 ± 0.12 sec^{-1}
k_{cat} Dopamine	-------	10.5 ± 2.50sec^{-1}	5.42 ± 0.95 sec^{-1}
K_m L-DOPA	26.7 ± 9.9 μM	281 ± 29 μM	11.4 ± 1.7 μM
K_m DHHCA	104.8 ± 26.3 μM	769 ± 173μM	162.8 ± 46.2μM
K_m Dopamine	-------	1792 ± 758 μM	386.4 ± 165.8μM

Conclusions and Future Implications

- In conclusion, both JING and NocArt provide potential as L-DOPA Dioxygenases. JING shows greater potential in that it can cleave L-DOPA, DHHCA, and Dopamine efficiently.
- Future directions will continue to investigate how the dioxygenase activity of NocArt can be increased on all given substrates.
- Future directions will be to synthesize novel L-DOPA, DHHCA, and dopamine (**Figure 5**) analogs substituted with electron withdrawing and electron donating groups at the 6th position of the ring. These analogs will be tested in the active sites of NocArt and JING to fully understand the mechanism of action of the enzymes.

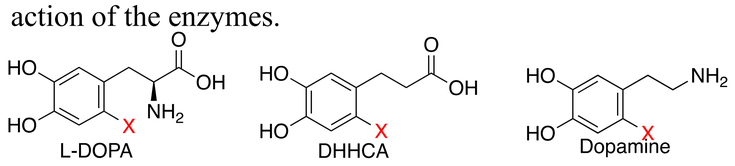

Fig. 5. Structures of 6-substituted L-DOPA, DHHCA, and dopamine.

Acknowledgements

- Thank you to Dr. Keri Colabroy and Muhlenberg for hosting and mentoring me this summer for research.
- Thank you to Dr. Larryn Peterson and Rhodes College for providing research opportunities.
- I would like to acknowledge the National Science Foundation grants CHE1708234 (LWP) and CHE1708237 (KLC) for providing us with the resources to do our research.
- Thank you to Rhodes college for financially supporting the travel to ASBMB.

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