6-Substituted Derivatives of Dopamine as Substrates of L-DOPA Dioxygenase: Understanding Steric and Electronic Substituent Effects

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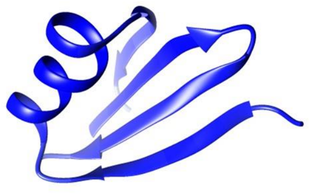
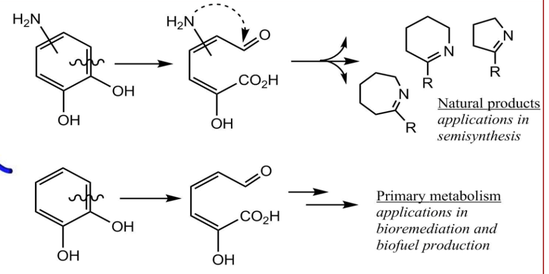
Abstract

Dioxygenase enzymes are nature's catalysts for the breakdown of catecholic rings, such as those found in the woody tissue of plants. This chemistry has tremendous potential not only in the degradation of plant material, but also in natural product biosynthesis. A suite of synthetic dopamines, derivatized at the 6-position and varied in substituent size and electron-withdrawing character, were examined as substrates of *S. lincolnensis* L-DOPA dioxygenase via *in-silico* docking and subsequent analysis of the enzyme catalyzed transformations by mass spectrometry and UV-Visible spectroscopy. 6-bromodopamine, 6-cyanodopamine and 6-carboxydopamine were robust substrates, while cyclicdopamine and 6-nitrodopamine exhibited limited turnover. Docking and kinetic experiments are used to explain these substrate preferences by L-DOPA dioxygenase.

The EDX Family

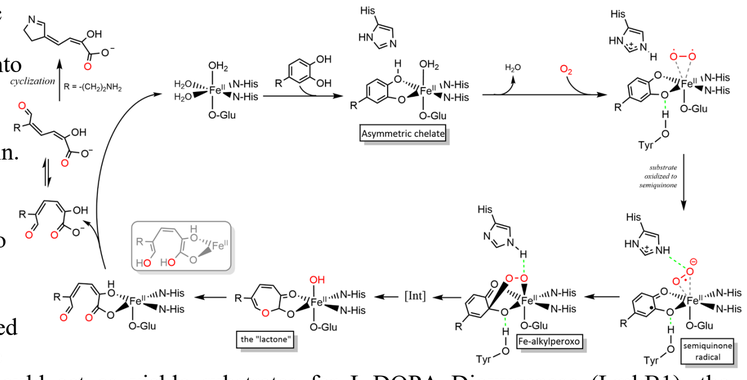




Extradiol Dioxygenases or EDX enzymes are a family of enzymes that cleave catecholic rings. All enzymes of the EDX family contain two $\beta\alpha\beta\beta\beta$ motif and maintain a divalent metal at the active site that chelates vicinal oxygens of the substrate. The substrate is cleaved within the aromatic ring outside of this pair of hydroxyls[1]

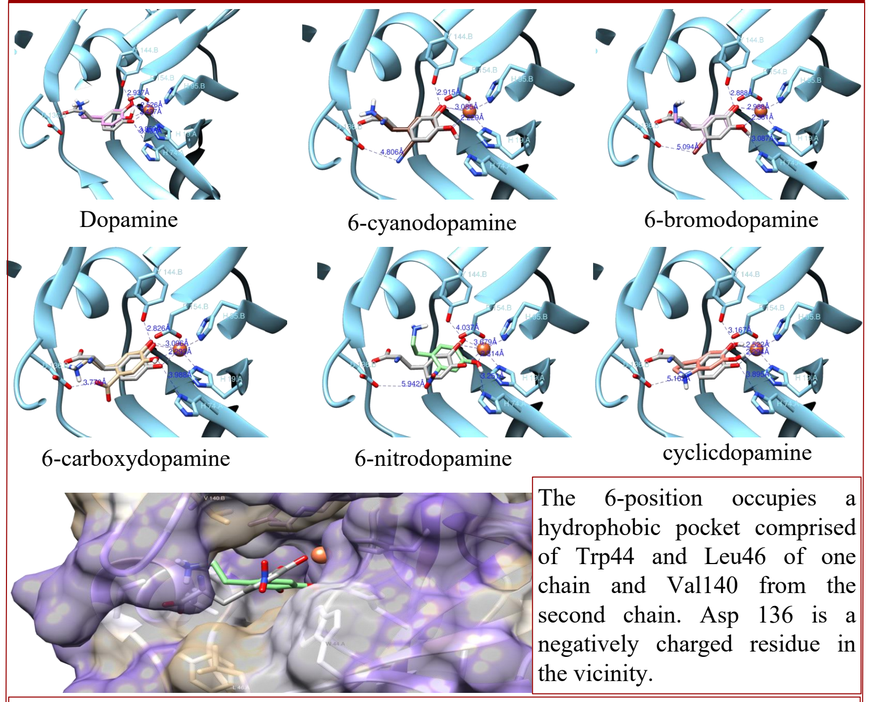
The Bigger Picture

EDX enzymes have the ability to break down catecholic rings, such as those derived from lignin, a molecule found in plant fibers. This linearization of the catecholic ring converts the plant material into raw materials for biosynthesis and biofeed stocks as well as accessing plant sugars that can provide a cost-effective route into viable biofuels[2]. L-DOPA dioxygenase is an EDX enzyme that participates in converting a catecholic ring (L-DOPA) into a natural product, lincomycin.



In order to verify whether synthesized dopamine analogs could act as viable substrates for L-DOPA Dioxygenase (LmbB1), the known crystal structure was used to predict the reactivity of certain dopamine analogs. Then the substrates were tested in vitro.

Substrate Docking

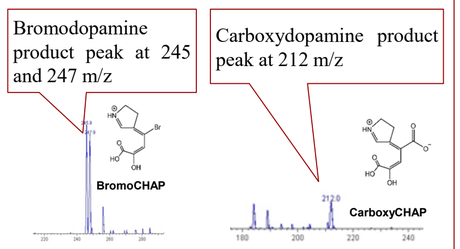


The 6-position occupies a hydrophobic pocket comprised of Trp44 and Leu46 of one chain and Val140 from the second chain. Asp 136 is a negatively charged residue in the vicinity.

Dopamine is able to dock with distances to the Fe^{II}, His74 and Tyr144 that are consistent with the native substrate, while 6-bromo- and 6-cyanodopamine dock with only slightly elongated Fe^{II}-O_3 distances. Less favorable docks were observed for 6-carboxydopamine and 6-nitrodopamine; these two compounds share a large, negatively charged substituent at the 6-position, which likely interacts unfavorably with the hydrophobic pocket.

Proving the Product

3-(3-carboxy-3-hydroxy-allylidene)-4,5-dihydro-2H-pyrrole (CHAP) and derivative CHAP products were obtained from reactions with Dopamine, 6-bromodopamine, 6-carboxydopamine, 6-cyanodopamine and 6-nitrodopamine with L-DOPA dioxygenase at pH 8 in 10 mM HPLC-grade ammonium bicarbonate buffer. L-DOPA dioxygenase was removed by ultrafiltration and the isolated product solution was adjusted to pH 6 before ESI-MS.



Kinetics

Compound	$K_{M\ DOPA/DA/6\text{-}X\text{-}DA}$ (μM)	k_{cat} (sec^{-1})	$K_M O_2$ (μM)	$k_{SP} O_2$ ($\mu M^{-1}\ min^{-1}$)[h]	λ_{max} of steady state product	Extinction Coefficient at λ_{max} ($M^{-1}\ cm^{-1}$)
L-DOPA	27.3 ± 2.1[f] 35.8 ± 1.8[g]	1.03 ± 0.12[f] 1.27 ± 0.11[g]	53.2 ± 1.6[e] 3.1 ± 7.4[d]	0.84 ± 0.02[e] 27.2 ± 64.7[d]	414 nm	47,500[a] 44,560[b]
Dopamine	120 ± 27[f] 571 ± 144[g]	8.7 ± 1.6[f] 1.49 ± 0.3[g]	982 ± 198[e] 499 ± 31[d]	0.051 ± 0.007[e] 0.20 ± 0.01[d]	433 nm	29,690 ± 520
6-bromoDA	118 ± 31[f] ---	1.32 ± 0.25[f] ---	296 ± 35[e] 48.2 ± 17.2[d]	0.18 ± 0.02[e] 2.5 ± 0.9[d]	429 nm	27,030 ± 360
6-carboxyDA	3640 ± 1510[f] ---	0.77 ± 0.20[f] ---	240 ± 11[e]	0.071 ±0.003[e]	425 nm	30,150 ± 940
cyclicDA	n.d.	n.d.	n.d.	n.d.	429 nm	n.d.
6-cyanoDA	264 ± 44[f] 438 ± 49[g]	1.00 ± 0.06[f] 0.59 ± 0.03[g]	845 ± 114[d]	0.135 ± 0.012[d]	435 nm	23,840 ± 160
6-nitroDA	n.d.	n.d.	n.d.	n.d.	365 nm	n.d.

[e] these constants were determined at dopamine concentrations that were at $k_{M,DA/6\text{-}X\text{-}DA}$
[d] determined in the excess of k_M for L-DOPA (10x), DA(10x) or 6-X-DA (5-10x)
[f] determined with 100% O_2 saturation
[g] determined with air/21% O_2 saturation

Conclusions

In this study, we have demonstrated that L-DOPA dioxygenase is competent to cleave dopamine analogs with steric and electronic diversity. In the case of electron rich, but neutral substituents like 6-bromo- and 6-cyanodopamine, the better substrate was the molecule that was more easily oxidized, but effective catalysis with oxygen was influenced by substituent size. Secondly, the cost of binding a large, negatively charged substituent at the 6-position outweighs the challenge of oxidation potential in the case of 6-carboxydopamine versus 6-cyanodopamine. Lastly, 6-nitrodopamine was still a weak substrate despite a large, negatively charged 6-substituent. Despite favorable docking modes, cyclic dopamine was likely a weak substrate due to unfavorable oxidation potential. When comparing dopamine to 6-bromodopamine, the two molecules are very competitive substrates. Most strikingly, 6-bromodopamine has oxygen dependent kinetics that are most comparable to L-DOPA. These results challenge the assumption that oxidation potential dictates the effectiveness of dioxygenase substrates of similar size. 6-Bromodopamine is more difficult to oxidize than dopamine, but it is also larger, and the neutral substituent at the 6-position does not interact unfavorably with the hydrophobic pocket and with Asp[136]. Finally, the larger 6-position substituent may in-part compensate for the extra room created at the active site from the missing carboxylic acid of L-DOPA.[3]

Acknowledgements

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3. Goldberg, A. M. et al (2021) L-DOPA dioxygenase activity on 6-substituted dopamine analogs *Biochemistry* Accepted.