Elucidating the Role of Reactive Oxygen Species on Dopamine β-Hydroxylase Activity and Aggregation

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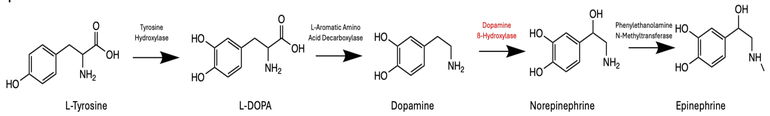
Background and Significance

Dopamine β-Hydroxylase (DBH) is a copper containing monooxygenase that catalyzes the hydroxylation of dopamine to norepinephrine.[1] Deficiency of norepinephrine, a neurotransmitter important for the modulation of nervous system functions such as attention, arousal, and cognition, learning, and memory has ties to the progression of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD).[2] Evidence shows that AD and PD both may be caused through DBH dysfunction through either degeneration of locus coeruleus (LC) noradrenergic neurons, neuroinflammation, or dysregulation of metals such as copper and iron.[2, 3, 4] Overproduction of reactive oxygen species can lead to the irreversible oxidation of proteins which can affect cell homeostasis.[5]



Scheme 1. Catecholamine Synthesis Pathway. Catecholamine neurotransmitters are synthesized from L-tyrosine. There are various enzymes that catalyze each step of the pathway. Dopamine β-Hydroxylase, highlighted in red, is a key enzyme that catalyzes the hydroxylation of dopamine to norepinephrine.

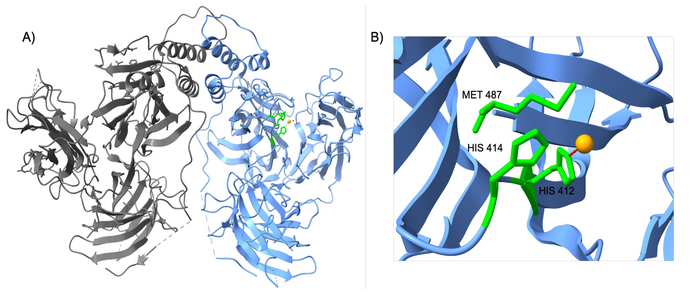


Figure 1. Crystallographic model of Dopamine ß-Hydroxylase. A) The dimeric structure of DBH is shown with a ribbon model. B) The copper coordination residues (green) are shown with copper (orange). Adapted from PDB 4ZEL.

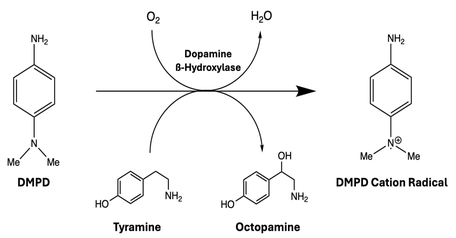
Copper is a redox-active metal and plays an essential role in O_2 activation for reduction. DBH has two copper-binding sites so there is potential for dysfunction in which this activation and reduction are uncontrolled, leading to oxidative stress and cellular damage.[6] Oxidative stress leads to oxidative modification, irreversible in nature, which goes hand in hand with the loss of protein function. Given this, DBH potentially undergoes Fenton-like chemistry in which copper is oxidized by H_2O_2, resulting in the formation of •OH.[7]

Research Question

What is the connection between exposure to reactive oxygen species and DBH activity and aggregation?

Specific Aims:

- Acquire enough functional enzyme to test activity by utilizing a colorimetric DMPD assay.
- Investigate activity changes of DBH when exposed to ROS and excess iron via a colorimetric DMPD assay.
- Identify sites of oxidation using mass spectrophotometry (HPLC-MS/MS).



Scheme 2. DMPD continuous spectrophotometric assay.

Klinman Mechanism[8]

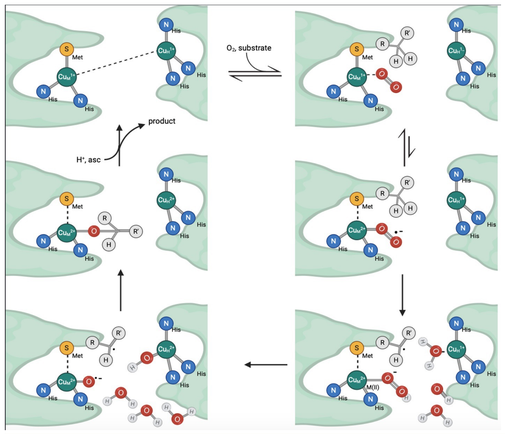


Figure 2: Dopamine hydroxylation via the Klinman mechanism.

- Substrate and O_2 bind, forming the ternary complex.
- Cu_M site is involved in the initiation of oxygen activation through electron transfer.
- O2 activation occurs forming a copper-superoxo intermediate
- Reactivity in the active site is based on charge transfer and binding strength of the superoxide anion to Cu(II).
- CuH site provides the second electron for the reaction mechanism.

Fenton Chemistry[9]

Fenton-Chemistry:

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \bullet OH + OH^-$

$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + \bullet OOH + H^+$

$2H_2O_2 \longrightarrow \bullet OH + \bullet OOH + H_2O$

Fenton-like Chemistry:

$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + \bullet OH + OH^-$

$Cu^{2+} + H_2O_2 \longrightarrow Cu^{+} + \bullet OH + H_2O$

Predicted Peptide Cleavage Sites Via Trypsin

Position of Cleavage Site	Resulting Peptide Sequence	Peptide Length [aa]	Peptide Mass [Da]
6	**M**PALSR	6	673.828
16	WASLPGPS**M**R	10	1101.288
43	EAAF**M**YSTAVAIFLVILVAALQGSAPR	27	2810.347
79	ESPLPY**H**IPLDPEGSLELSWNVSYTQEAI**H**FQLLVR	36	4179.698
92	AGVLFG**M**SDR	10	1052.213
138	GQI**H**LDPQQDYQLLQVQR	18	2179.420
178	DYLIEDGTV**H**LVYGILEEPFR	21	2478.784
194	SLEAINGSGLQ**M**GLQR	16	1673.905
235	VQLLKPNIPEPELPSDACT**M**EVQAPNIQIPSQETTYWCYIK	41	4689.390
248	**HH**IIK	5	646.790
286	GNEALV**HH**MEVFQCAPE**M**DSVP**H**FSGPCDSK*	31	3399.790
291	**M**KPDR	5	645.775
307	**H**VLAAWALGAK	11	1136.362
343	LEV**H**Y**H**NPLVIEGR*	14	1675.908
380	FNAGI**M**ELGLVYTPV**M**AIPPR	21	2289.779
418	CTQLALPPSGI**H**IFASQL**H**T**H**LTGR*	25	2699.126
448	EWEIVNQDN**H**YSP**H**FQEIR	19	2441.601
473	VVSV**H**PGDVLITSCTYNTEDR	21	2305.545
504	ELATVGGFGILEE**M**CVNYV**H**YYPQTQLELCK*	31	3549.089
521	YF**H**LINR	7	962.119
567	ALYSFAPIS**MH**CNK	14	1581.868

Table 1. Predicted Trypsin Cleavage Sites via Expasy PeptideCutter. The position of cleavage, resulting peptide sequence, peptide length (aa), and peptide mass (Da) are given. Histidine and methionine residues are bolded with the copper coordination residues (M487, H262, H263, H333, H412, H414) highlighted in yellow. Peptide fragments with a copper coordination residue are denoted by an asterisk (*).

Mass Spectrophotometry

LC-MS Samples	
With Guanidination	Without Guanidination (control)
1. DBH	2. DBH
3. DBH + H_2O_2	4. DBH + H_2O_2
5. DBH + Fe^{2+}	6. DBH + Fe^{2+}
7. DBH + Fe^{2+} + H_2O_2	8. DBH + Fe^{2+} + H_2O_2

Table 2. LC-MS Samples with and without guanidination.

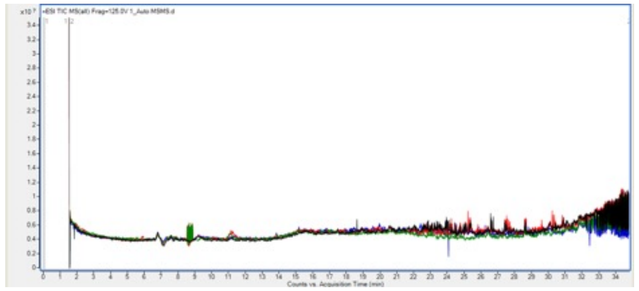


Figure 3. Total Ion Chromatogram from trypsin-digested and guanidinated samples. DBH only is in black, DBH + hydrogen peroxide is in red, DBH + Fe(II) is in green, and DBH + Fe(II) + hydrogen peroxide is in blue.

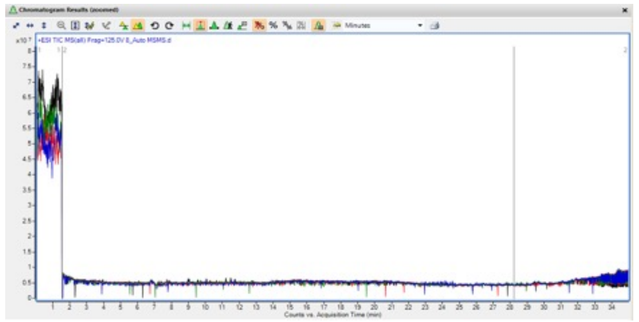


Figure 4. Total Ion Chromatogram from trypsin-digested and non-guanidinated samples. DBH only is in black, DBH + hydrogen peroxide is in red, DBH + Fe(II) is in green, and DBH + Fe(II) + hydrogen peroxide is in blue.

Conclusions

- DBH was found to be inactive under our conditions
- A total of 21 predicted peptide fragments formed via trypsin digestion contain histidine and methionine residues.
- The HPLC-MS/MS chromatograms did not yield any significant peaks associated with the mass values of interest.

Future Work

- The role of ROS exposure on DBH activity and aggregation needs further exploration.
- Experiments should be redone with enzymatically-active DBH.
- Using more concentrated and less dilute samples may yield more noticeable peaks associated with the mass values of interest.
- Emphasis on tyrosine hydroxylase and conducting similar experiments on it may be a valuable comparison point as it is the only other metalloenzyme in this biosynthetic pathway.

References

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