

Oxidases and Oxygenases

(We'd die without 'em)

The Introduction

Oxidases:

- Oxidase activity is characterized by dioxygen reduction
- Oxidases couple one-, two-, or four-electron oxidation of substrates to the two- or four- electron reduction of dioxygen (i.e. O_2) to hydroperoxide or water.

Oxygenases:

- ◊ Oxygenase activity is characterized by activation for incorporation into organic substrates
- ◊ Oxygenases incorporate either one (monooxygenases) or two (dioxygenases) atoms of oxygen into an organic substrate
 - **Monooxygenases** - a nonincorporated oxygen atom is reduced to water either by an additional two-electron reductant, or by the substrate itself in the internal monooxygenases
 - **Dioxygenases** - either both oxygen atoms are incorporated into the substrate (intramolecular) or one oxygen atom is incorporated into substrate and the second into an additional organic cofactor (intermolecular)

Where are they found?

- ◊ Oxidases:
 - Found extensively in electron transfer processes, especially in ATP formation.
 - Used in cell membrane processes, such as in Proton Pumps
 - ◊ Cytochrome C Oxidases
 - ◊ Quinol Oxidases
- ◊ Oxygenases:
 - Heme oxygenase
 - ◊ oxidizes heme to CO, biliverdin, and ferrous iron
 - ◊ Required for proper iron distribution in the body

What metals are present at the active site?

- ◊ Non-heme iron
- ◊ Copper
- ◊ Copper-heme compounds

Oxidase and Oxygenase Reactions

Oxidases	2 Electron	$2RH + O_2 \rightarrow 2R + H_2O_2$
		$RH_2 + O_2 \rightarrow R + H_2O_2$
		$4RH + O_2 \rightarrow 4R + 2 H_2O$
	4 Electron	$2RH_2 + O_2 \rightarrow 2R + 2 H_2O$
		$RH_4 + O_2 \rightarrow R + 2 H_2O$
Monooxygenases	External	$S + RH_2 + O_2 \rightarrow SO + R + H_2O$
	(uncoupled)	$S' + RH_2 + O_2 \rightarrow S' + R' + H_2O_2$
Dioxygenases	Internal	$SH_2 + O_2 \rightarrow SO + H_2O$
	Intramolecular	$S + O_2 \rightarrow SO_2$
	Intermolecular	$S + C_0 + O_2 \rightarrow SO + C_0O$

*RH₂ = reductant; S = substrate; S' = poor substrate; C₀ = cofactor

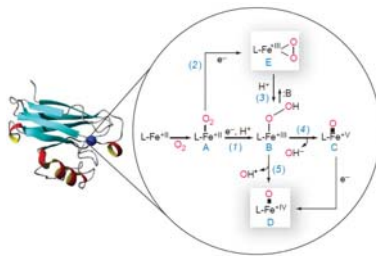
Non-heme enzymes

- ◊ Catalyze wide variety of dioxygen reactions
 - 1) Monooxygenation
 - 2) Dioxygenation
 - 3) Desaturation
 - 4) 4 e⁻ reduction of dioxygen to water
- ◊ Difficult to study → lack $\pi \rightarrow \pi^*$ transition found in porphyrin-based heme

Mononuclear non-heme enzymes

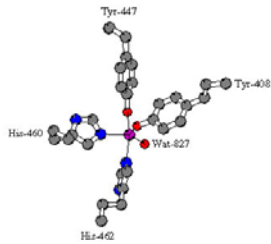
- ◊ Separated into 2 classes, based on oxidation state
 - Fe(III) in intradiol dioxygenases and lipooxygenases
 - Fe(II) in extradiol dioxygenases and all other mononuclear non-heme enzymes

Non-heme enzymes



- ◊ Mechanism compared to Fe heme enzymes
- ◊ Assumed similar

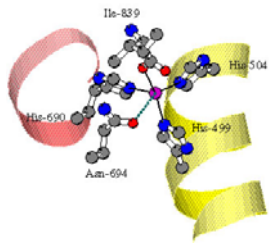
Mononuclear non-heme enzymes



Intradiol dioxygenase: protocatechuate 3,4-dioxygenase

- ◊ High-spin Fe(III): determines mode of reactivity
- ◊ Functions by substrate activation
- ◊ Sequential reactivity → substrate coordination required for dioxygen binding

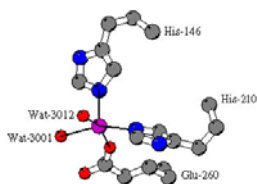
Mononuclear non-heme enzymes



Lipoxygenase

- ◊ 2 open *cis* positions
- ◊ Becomes coordinatively saturated when bound to fatty acid substrate
- ◊ Fatty acid reduces Fe(III)

Mononuclear non-heme enzymes

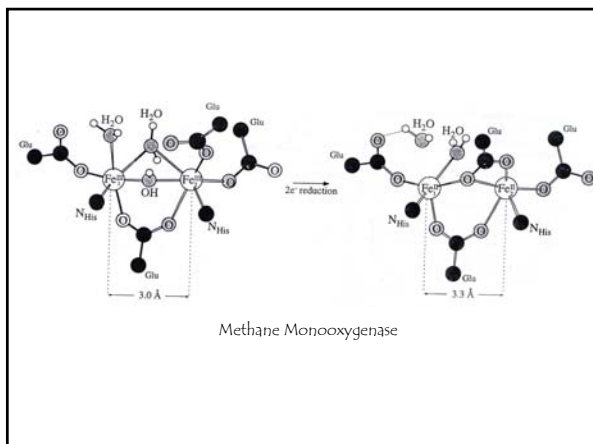


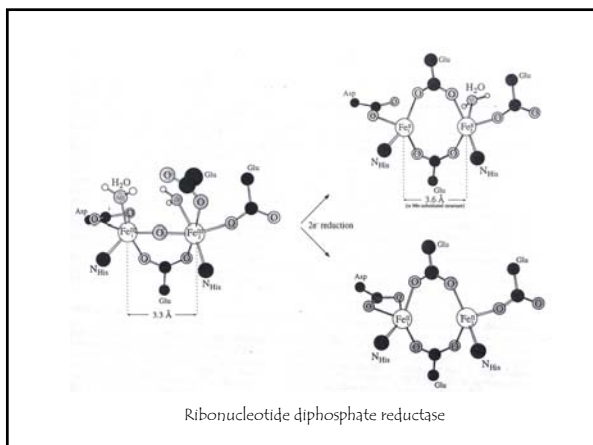
Extradiol dioxygenase: catechol 2,3-dioxygenase

- ◊ Equatorial H₂O replaced by substrate
- ◊ Functions by dioxygen activation
- ◊ Sequential reactivity

Binuclear Non-heme Enzymes

- ◊ 2 coordinatively saturated metal centers (4- and 5-coordinate)
- ◊ Several structures:
 - 1) Methane Monooxygenase
 - 2) Ribonucleotide diphosphate reductase
- ◊ Bridging H_2O is site of dioxygen activity



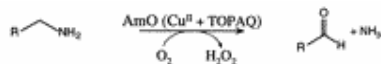


Mononuclear Copper Enzymes

- ◊ Amine Oxidase, (AmO), [Cu(II)(N His)₃(OH₂)₂]
- ◊ Isolated from *Escherichia coli*
- ◊ 1 copper center yet catalyzes the two electron reduction of molecular oxygen to hydrogen peroxide with the aid of organic cofactor.
- ◊ Unique in that copper is not directly involved in the reaction.

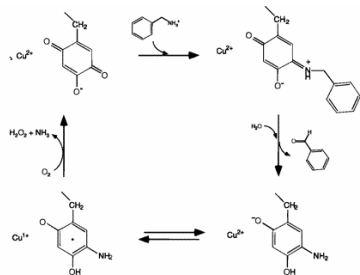
Mononuclear Copper Enzymes

- ◊ Generic Reaction of Amine Oxidase



Mononuclear Copper Enzyme

- ◊ Proposed Mechanism



Binuclear Copper Enzymes

- ◊ 2 basic types of binuclear copper enzymes

Coupled

-characterized by: antiferromagnetic interaction, superexchange pathway, direct bridging ligand
 $S=1/2 \text{ Cu(II)} \rightarrow S_{\text{total}}=0 \rightarrow \text{EPR silent!!}$

Uncoupled

- Metal centers greater than 6 angstrom apart
- No direct bridging ligand
- Usually classified as mononuclear copper enzymes

Binuclear Copper Enzymes

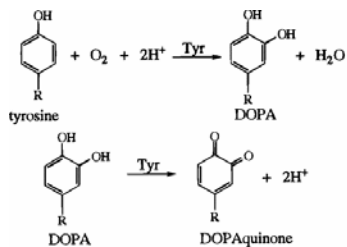
- ◊ Tyrosinase (flashback to bio 107)

- Very similar to hemocyanin

- Major difference is higher accessibility of exogenous ligands to active site.

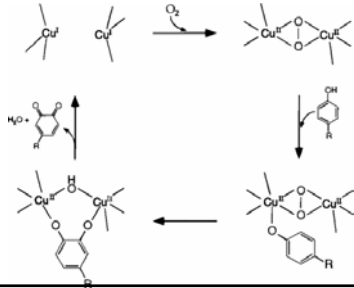
Binuclear Copper Enzymes

- ◊ Generic Reaction



Binuclear Copper Enzyme

- Proposed Mechanism



Trinuclear Copper Cluster Enzymes

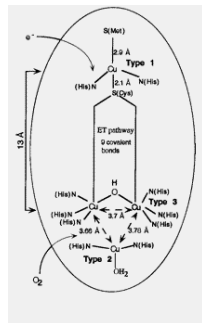


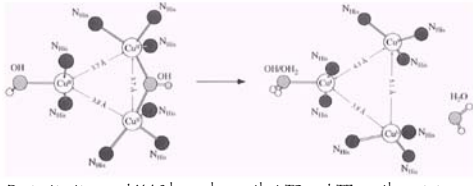
Laccase

- Most common: Laccase (LC), Ascorbate Oxidase (AO), and Ceruloplasmin (CEP)
- Three sites that together catalyze the 4 electron reduction of dioxygen.
- LC has the simplest stoichiometric ratio of each site type, as it contains one of each site type (4 Cu atoms).

Three types of active sites

- Type 1 (T1) site is a blue Cu center.
- Type 2 (T2) is considered a standard Cu(II) center.
- Type 3 (T3) is a binuclear, hydroxide-bridged, antiferromagnetically coupled Cu(II) center.
- The T1 site is responsible for electron scavenging from substrate
- Electrons are transferred $\approx 13 \text{ \AA}$ to the other two sites which are in close proximity ($\approx 5 \text{ \AA}$).
- T2 and T3 comprise the trinuclear Cu cluster, where dioxygen reduction takes place.





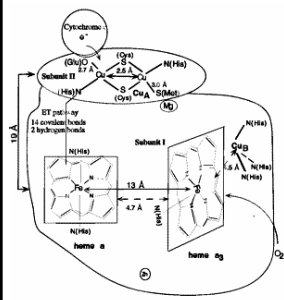
- ◊ Derivatization and XAS have shown that T2 and T3 are the minimum unit necessary for dioxygen reduction.
- ◊ All 3 Cu's have an open coordination site, oriented towards center.
- ◊ T2 site has 3-coordinate geometry unknown in Cu coordination chemistry.
- ◊ T3 site antiferromagnetically coupled \Rightarrow reduced form not active in MCD.
- ◊ T3 $d \rightarrow d$ transitions dominate the CD spectrum.

Copper-Heme Oxidases

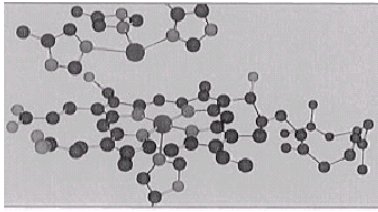
- ◊ Ubiquitous
- ◊ Catalyze 4 proton, 4 electron reduction of dioxygen to water.
- ◊ Use energy from reduction to "pump" protons for ATP synthesis
- ◊ Consume 80-90% of mammalian oxygen intake
- ◊ The heme and Cu-heme sites reside in trans-membrane subunit.



Cytochrome c Oxidase (CcO)



- ◊ Contains 3 active sites
 - Binuclear Cu center (substrate oxidation, Cu_A)
 - Standard Heme center
 - Cu-heme center (dioxygen reduction)
- ◊ Heme planes $\approx \perp$
- ◊ Mg²⁺ & Zn²⁺ co-factors
- ◊ Electrons are transferred 19 Å to the standard heme center
- ◊ Subsequent = 5 Å transfer to the Cu-heme center site.



- ◊ Cu-heme site is antiferromagnetically coupled
- ◊ X-Ray Crystallography fuzzy, probably a distorted water-derived ligand
- ◊ Open coordination sites on both the Cu and the heme in the Cu-heme site.

CcO Multicopper Comparison

- ◊ Cu_A parallels T1
- ◊ Long distance electron transfer
- ◊ Intermediate site in transfer (standard heme and T2)
- ◊ Cu-heme analogous to T3
- ◊ Antiferromagnetic coupling in reduced dioxygen reduction site.
- ◊ Similar intermediaries
- ◊ ET faster and farther in CcO
- ◊ Mechanisms differ

References

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