Aldehyde ferredoxin oxidoreductase (AOR)

- Catalyse the metabolism of aldehydes to carboxylic acids Irreversible
- Found in thermophilic anaerobic archaea (most well studied in pyrococcus) and bacteria (Clostridiales)
- Uses high oxidation state of tungsten(IV) to activate carbonyl
- Couples with NiFe hydrogenase to reduce protons no Q cycle involved no cofactors(FADH or NADH)
- Hydrogenase pumps protons outside the cell to create a proton gradient for ATP synthase
- Substrates = aliphatic and aromatic amino acids from peptide fermentation

\[
\text{RCHO} + \text{H}_2\text{O} \rightarrow \text{RCOOH} + 2\text{H}^+ + 2e^-
\]

- Homodimer of two chains, each containing three domains
- Tetrahedral Fe complex at dimer interface; Ligands = 2 × OGlu & 2 × NHis (only structural role)
- Trigonal prismatic W complex coordinated by 2 dithioles; Ligand = W-bis-MPT
- Magnesium octahedrally coordinated to both PO₄ of W-bis-MPT, 2 × H₂O, backbone carbonyl of Asn⁹³ & Ala¹⁸³
- e.t. path through:
  - [4Fe4S], Arg⁷⁶ &/or Cys⁴⁰⁴ and W-bis-MPT
Proposed Mechanism:
Galactose Oxidase: Samantha A. Bakker and James W. Favell

**They're pretty fungi's:**
- Genus Fusarium
- Responsible for a large range of crop diseases
- Produce mycotoxins
- Human pathogen
- Bacteriostatic

**Function:**
- Produce $H_2O_2$
- Strict regioselectivity to convert R-OH to R=O

**Residue rigidness and radical stability:**
- Delocalization over the aromatic Tyr ring and thioether bridge.
- Trp290 acts as a shield protecting the radical form reacting with external environment solvent.
- Literature mutation in Try272 lead to a lower stability of the active form of GAO.

- Thioether bond between Try272—Cys228 keeps the Try residue in place.
- The indol ring in Trp290 π-stacking with the Try272, holding it in place.

**Where have we seen this? Intradiol Catachol Dioxygenase!**

**Similarities:**
- 2x NHis
- 2x TyrO
- Small inorganic molecules ($O_2$ or $H_2O$)

**Differences:**
- Different metal atom and reaction mechanism

**Interesting thing! GAO Biosensors:**
Galactose and other sugars can be qualified and quantified through GAO-based biosensors. The production of $H_2O_2$ is measured electrochemically, and can be used to determine how much galactose is present.
Superoxide formation and $O_2$ reaction with the active site:\(^2,3\)

Overall reaction:\(^3\)

Mechanism:\(^3\)

References:
Isopenicillin N Synthase (IPNS)

Kelly Corley-Smith, Matt Siebert, Justin Zimmer

- Wide range of microorganisms including many Streptomyces, some unicellular bacteria, and several filamentous fungi
- Key enzyme in penicillin and cephalosporin biosynthetic pathway (β-lactam antibiotics) forming the precursor Isopenicillin N (IPN)

- IPNS is a non-heme iron dependent oxidase
- Active Site (with unbound substrate): Fe^{2+}, 2 x NHis, 2 x H₂O, OGln, OAsp
- Key reaction is the iron-dioxygen-mediated ring closures of the tripeptide δ-(L-α-AAA)-L-Cys-D-Val (ACV), forming a bicyclic β-lactam (4 member), and thiazolidine (5 member) ring within the product IPN

Active Site

Overall Reaction

R = L-α-amino-δ-adipoyl
Mechanism

General Information:
Mechanism adapted from:

Protein Structure:
Methyl-Coenzyme M Reductase
An enzyme that catalyzes the final step in the formation of methane

ENZYME INFO
- Multiprotein complex made up of two identical halves
- 3 subunits in each half: McrA, McrB, McrG
- Contains 2x F430 Active Site: 1 per subunit
- Active sites are buried in deep tunnels to protect from reaction with H₂O
- 2x Tyr, 1x Arg form binding pocket

ACTIVE SITE
- Nickel tetrahydrocorphinoid
- Glutamine amino acid coordinated to sidechain
- Active in Ni(I) oxidation state
- Extremely reactive including with O₂

OVERALL REACTION

PROPOSED MECHANISMS

STRENGTHS
Excellent source of methane production
Found in methanogenic archaea which are found in the bottom of lakes, swamps, and puddles
Responsible for methane production in the guts of animals

WEAKNESSES
Oxygen (needs an anaerobic environment)
Inhibitor: 3-nitrooxypropanol
Inhibitor has the potential to reduce greenhouse gas production in cows

AWARDS & ACCOMPLISHMENTS
Protein Data Bank’s “Molecule of the Month” in November 2014
Produces over one billion tons of methane per year across the world
Voted “Best Enzyme” in methanogenesis pathway

Presented by Jesse Lafontaine & Chelsey Brien
REFERENCES


Nitric Oxide Synthase
David Bakker, Nicholas Pinette, & Martin von Dach

- Enzymes found in life forms from bacteria to mammals
- Synthesizes nitric oxide (•NO) from L-arginine
- Fe heme with cysteine bound, and empty axial position for substrate
- Structural Zn holds together two monomers into functional homodimer with 4 cysteines (2 from each monomer)
- ET: NADPH → FAD → FMN

Overall Reaction:

Sub-Categories:
- Bacterial NOS (bNOS)
  o Prevents oxidative damage, does lots of other things
- Endothelial NOS (eNOS)
  o Vascular dilation, and lots of other things
- Neuronal NOS (nNOS)
  o Regulates neurotransmission and muscle contraction, implicated in lots of other things
- Inducible NOS (iNOS)
  o Immune response to kill bacteria and viruses, suggested as responsible for lots of other things
References:

[1] Dennis J. Stuehr, Jerome Santolini, Zhi-Qiang Wang, Chin-Chuan Wei, and Subrata Adak; Update on Mechanism and Catalytic Regulation in the NO Synthases; J. Biol. Chem. 2004 279: 36167-.
doi:10.1074/jbc.R400017200


Cu/Zn Superoxide Dismutase (SOD1)

Overview
- Converts two molecules of superoxide into hydrogen peroxide and dioxygen.
- Found in all aerobic organisms.
- SOD1- Located on chromosome 21 in humans.

Overall Reaction

\[
\begin{align*}
(SOD1)^{Cu^2+} + O_2^- & \rightarrow (SOD1)^{Cu^2+} + O_2 \\
(SOD1)^{Cu^2+} + O_2^- + 2H^+ & \rightarrow (SOD1)^{Cu^2+} + H_2O_2 \\
2O_2^- + 2H^+ & \rightarrow O_2 + H_2O_2
\end{align*}
\]

Cu/Zn Active Site
- Cu: Two-step redox reaction to catalyze the conversion of superoxide.
  - Distorted d⁹/d¹⁰ tetrahedral; 4NHis residues.
- Zn: Stabilizes the bridging His61 residue.
  - d¹⁰ tetrahedral; 3NHis & OAsp
- Arg141: Promotes binding of superoxide to copper via IMFs.
Mechanism

Key Features

- Superoxide donates a single electron to Cu$^{2+}$ forming one molecule of dioxygen.
- Bridging histidine residue dissociates and is protonated by surrounding acid.
- Cu$^{+}$ donates a single electron to second superoxide molecule.
- A series of acid base steps to produce hydrogen peroxide and regenerate initial state.

Applications

- Increased SOD expression in Down’s syndrome/Alzheimer’s by accelerating H$_2$O$_2$ production in the brain.
- Mutations in SOD enzymes have been correlated to patients with ALS resulting muscle degeneration due to mutations of motor neurons.

References

[6] De La Torre, R; Casado, A; Lopez-Fernandez, E; Carrascosa, D; Ramirez, V; Saez, J. Experientia 1996, 52(9), 871-873.
Superoxide (O2•−) is a potent oxidizing agent. Excessive amounts lead to a cascade of reactions causing damage to cell or even apoptosis. Excess superoxide plays a role in the pathogenesis of many disease states including type 2 diabetes, Parkinson’s disease, and hypertension. To protect cells from harmful amounts of superoxide, SODs convert two superoxide anions to oxygen and hydrogen peroxide using a cyclic reduction and oxidation reaction of the active site meta.

\[
\begin{align*}
\text{Mn}^{3+} + O_2^{•−} & \leftrightarrow \text{Mn}^{2+} + O_2 \\
\text{Mn}^{2+} + O_2^{•−} + 2H^+ & \leftrightarrow \text{Mn}^{3+} + H_2O_2
\end{align*}
\]

Dismutation: a type of redox reaction. A compound of intermediate oxidation state (eg. superoxide) is converted to products of higher and lower oxidation state (eg. peroxide and dioxygen).

Aerobic organisms contain MnSOD in their mitochondria, many bacteria and plant chloroplasts contain FeSOD. Mn and Fe ions can be used interchangeably since both have the same active site, alpha helices, and arrangement of inner and outer sphere residues.

Active site, both hydrophobic interactions and hydrogen bond exists.

Substrate and Product Diffusion: binding site is positively charged, attracts anion substrates. Arg 173 and Glu 162 guide and align the superoxide.

Substrate Binding to Active Site: at lower temperature for crystallization, the binding follows the 5-6-5 mechanism. At physiological temperatures the aspartic acid is displaced by the superoxide.

Proton Shuttling Pathway: transferring of protons to the active site is by outer sphere hydrogen bonding network. Change in the oxidation state of the metal, and in the electronic nature of the substrate can facilitate the transfer of protons through the residue pathway.
Proposed mechanism:

PDB file: 5vf9

Learn more about medical application of SOD
- J Leukoc Biol. 2004 Sep;76(3):537-44. Epub 2004 Jun 14

Get the ppt presentation on google drive:
https://drive.google.com/file/d/1JgL3ba_Gu
deLctf6Y99KY50DPqj50sp7/view?usp=sharing

References

*hint: this [6] should be especially useful if you’re another group doing SODs
Tryptophan 2,3-dioxygenase

- An enzyme that’s found mainly in liver cells but also present in cells throughout the body in animals.
- Converts L-Tryptophan to N-formyl-L-kynurenine (NFLK) via the insertion of O₂ which opens the pyrrole ring.
- This is the first step in the kynurenine pathway.

Active Site
- Heme protein with histidine occupying the axial coordination position to the heme iron
- There is a hydrophobic pocket which the rings fit into which is also seen in chymotrypsin.
- There are three amino acids which help to hold and align the carboxylic acid of the L-tryptophan, Arg117, Tyr113 and Thr254
- The iron atom is initially high spin d⁶ square pyramidal before the tryptophan, and O₂ coordinate then becomes low spin d⁶ after coordination.
**Proposed Mechanisms**

- Below are only three of many proposed mechanisms.
- It should be noted that the Criegee mechanism has a very high energy barrier, so it is most likely not the actual mechanism.
- It is believed that the two oxygen atoms are simultaneously inserted into the substrate.

- Computational models all point toward the radical addition reaction (right) as the most likely mechanism, given its low energy barrier.
**Tyrosinase and Catechol Oxidase**

**Active Site**

Before the active site is active it exists in a deoxy form. There are two Cu (I) atoms mirroring each other, both bonded to three separate histidines. The coppers both have a coordination number of 3 in the deoxy form. Once exposed to O₂ the deactivated site becomes activated. The active site is comprised of two coppers, both Cu (II), bonded to 3 histidines each, and bonded through a μ-η²:η² peroxide bridge. This is the same bonding structure seen in hemocyanin (goldenrod handout). The coppers are d⁹ and experience Jahn Teller distortion, to become a distorted octahedral with an open coordination site.

**Balanced Reaction:**

![Balanced Reaction Diagram](image)

**Monophenolase Mechanism:**

![Monophenolase Mechanism Diagram](image)

Kat Fraser and Queena Pan
Diphenolase Mechanism:

Reference:


Kat Fraser and Queena Pan
Urease:

- Natively found largely in bacteria and some plants (beans). The urease found in mammals originates from bacteria found in the digestive tract.
- Responsible for the decomposition/hydrolysis of urea into ammonia and carbon dioxide.

![Urease Reaction](image1)

Figure 1: Overall reaction of the decomposition of urea involving urease

- The most studied urease is from Klebsiella Aerogenes which has a trimer of trimer structure (common in ureases). There are three subunits within the protein strand; the active site is found within the large α subunit. Three proteins assemble into the quaternary structure.
- The active site is bi-nickel. Ni1 is coordinated with two NHis, an O from the carbamylated lysine, a bridging hydroxide, and a water (in the rest state). Ni2 is coordinated with two NHis, a monodentate OAsp, the other O from the carbamylated lysine, the bridging hydroxide, and another water (in the rest state).

![Urease Models](image2)

Figure 2: i) Model displaying the bi-nickel active site of urease, excluding water and bridging hydroxide. ii) Model of the active site of urease inhibited by diamidophosphate after the nucleophilic attack of the bridging hydroxide.

- The mechanism of the reaction, though not entirely agreed upon, has some consensus and experimental data to support the nucleophilic attack of the bridging hydroxide on the urea carbon.
Figure 3: Two proposed mechanisms for the decomposition of urea by urease

- First enzyme ever to be crystalized and won James B. Sumner the Nobel prize in chemistry in 1946. The first experimental proof that enzymes are indeed proteins.
- Found biologically in many bacteria, plants, and fungi. Also found in dirt as a soil enzyme which is only possible due to its remarkable stability.
- The optimal conditions of the enzyme are pH 7.4 and 60°C.

References:


Other papers used in the presentation:


* structures obtained from the Protein Data Bank and modeled using the UBCMol program.