Synthesis and Characterization of Model Complexes for the Dioxygenase of Metalloenzymes

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Abstract

Our goal is to understand more about the active sites and catalytic mechanism of Cysteine Dioxygenase and Quercetin Dioxygenase, which are both important dioxygenase enzymes. Dioxygenase Enzymes catalyze critical reactions in the body and the environment.
Our research involves synthesis, structural and electronic characterization of model molecules that have one or more significant properties of a metalloenzyme active site.
The aim of this work is to synthesize Iron (II) Salen and Nickel (II) Salen coordination complexes and characterize them by using Mass Spectrometry, UV/Vis and IR Spectroscopy. Reactivity studies with cysteine and quercetin will be explored.

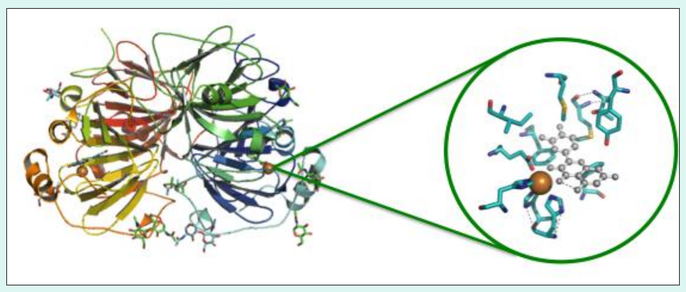
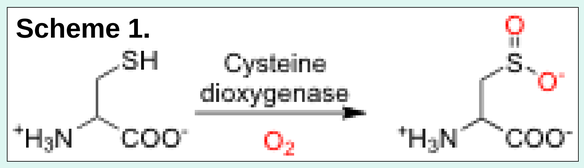


Fig 1. Ribbon illustration (left) and active site (right) of quercetin 2,3 dioxygenase from *Aspergillus japonicus* where quercetin is bound to the copper (II) center.[1]

Background

Cysteine dioxygenase (CDO) catalyzes the oxidation of L-cysteine to cysteine sulfinic acid (**Scheme 1**), an important biochemical pathway used for oxidation of cysteine to sulfate as well as an important step in taurine biosynthesis. Toxic bioaccumulation of cysteine has been associated with neurological disorders such as motor neuron disease, Alzheimer's and Parkinson's disease, as well as rheumatoid arthritis and liver disease. The CDO enzyme is considered to be a potential drug target in therapies addressing imbalances in the metabolism of cysteine.
The active site of cysteine dioxygenase (**Figure 2**) from *Mus musculus* contains an iron(II) center with His-86, -88 and -140 as the coordinating amino acid residues as well as a cysteinyl-tyrosine linkage in the vicinity of the active site.[4]

Scheme 1.



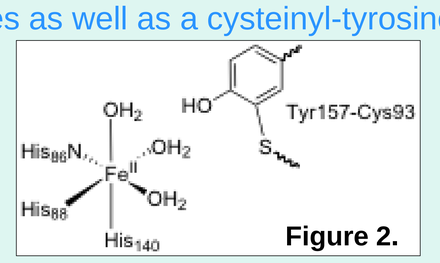


Figure 2.

Quercetin dioxygenase (QDO) catalyzes the oxidation of two carbon-carbon bonds of quercetin, with release of carbon monoxide and a corresponding depside (**Scheme 2)**. Bacteria use enzymes like QDO to degrade aromatic compounds in the environment and quercetin has been shown to exhibit anti-inflammatory and anti-carcinogenic properties in the human body.
The active site structures of *Aspergillus japonicus* quercetin 2,3 dioxygenase[1] contains a Cu(II) center with three histidine residues and one glutamate residue and from *YxaG*, from *Bacillus subtilis,* the copper(II) is replaced by iron(II), but has similar amino acid residues (**Figure 3**).

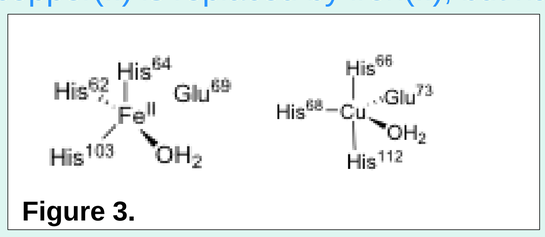
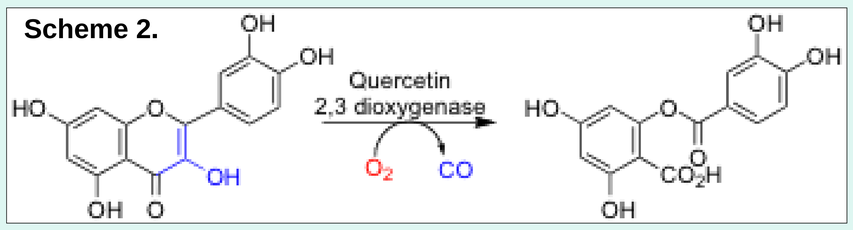


Figure 3.

Scheme 2.



Ni(Salen) and Fe(Salen) Synthesis and Characterization

Synthesis of Metal Complexes

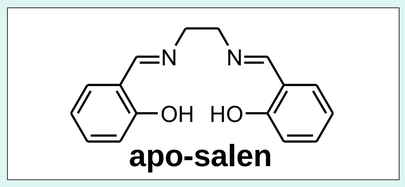
The nitrogen and oxygen-containing tetradentate chelating ligand SALEN (2,2'-Ethylenebis(nitrilomethylidene)diphenol, N,N'-Ethylenebis(salicylimine) was used to synthesize metal complexes with a square planar N_2O_2-donor coordination environment. We explored the first-row transition metal salts: Fe(II), Cu(II), Ni(II), Co(II), Mn(II) and Zn(II), which produced several stable metal complexes.

Characterization of Ni(II)-Salen and Fe(III)-Salen

The Ni(II)-salen afforded orange needles upon crystallization in dichloromethane and diethyl ether which agrees with a previous report of this compound.[6]
The Fe(III)-salen afforded a burgundy crayon-like solid after re-crystallization attempts.
Comparison of the spectroscopic, redox and magnetic properties of these metal-bound complexes will allow us to directly probe the effect of the nitrogen and oxygen containing amino acids in the active site as well as the 3-His structural constraints.

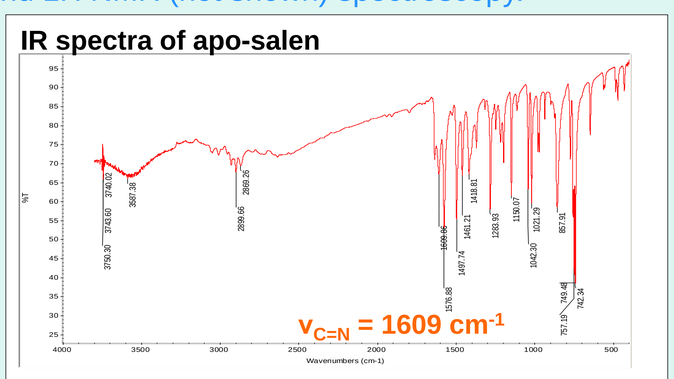
Synthesis of Ligand

Apo-salen was synthesized according to literature procedures and was characterized by IR (shown) and 1H NMR (not shown) spectroscopy.



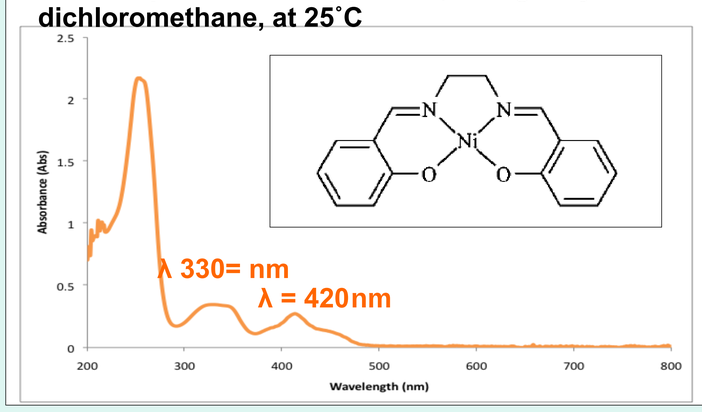
apo-salen

IR spectra of apo-salen

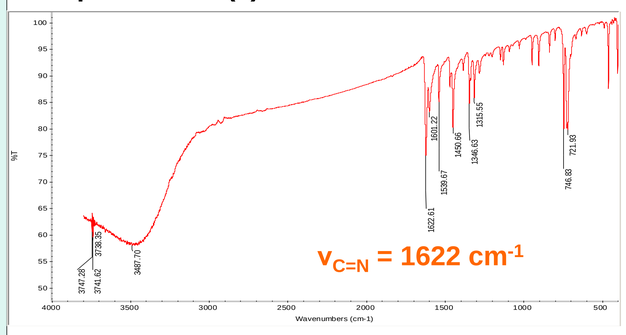


IR Spectra by Joe Heimann

UV/Vis Spectrum of Ni(II)-Salen, orange crystals in dichloromethane, at 25˚C

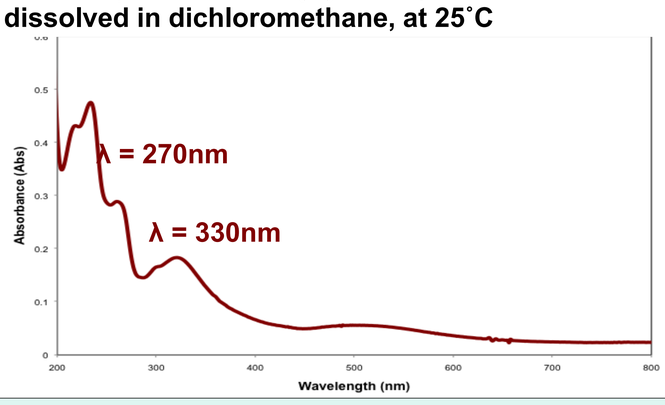


IR spectra of Ni(II)-Salen



IR Spectra by Joe Heimann

UV/Vis Spectrum of Fe(III)-Salen, brown solid dissolved in dichloromethane, at 25˚C



References (1) Steiner, R. A.; Kalk, K. H.; Dijkstra, B. W. Proc. Natl. Acad. Sci. U. S. A. 2002, 99, 16625-16630.
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