Exploring the Catalytic Cycle of Carbonic Anhydrase through Synthetic Modeling

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Abstract

Metalloenzymes perform and carry out numerous biological functions of varying importance. One such enzyme is carbonic anhydrase (CA), which is present in all living organisms. The primary biological function of CA is the reversible hydration of carbon dioxide (CO₂) to form bicarbonate (HCO₃⁻). Surprisingly, CA has also been determined to be the enzyme in plants that reacts with atmospheric carbonyl sulfide (COS) and carbon disulfide (CS₂).

Research directed at understanding the mechanism of COS and CS₂ activation by CA has been very limited and is the impetus for this research. Synthetic models, which mimic the active site of CA, have been synthesized and their reactivity with COS explored.

Details of the reactivity and characterization of complexes relevant to intermediates in the catalytic cycle of CA will be presented.

Introduction to Carbonic Anhydrase

Description

• A metalloenzyme common to animals, plants, and bacteria.
• The active site consists of a Zn(II) ion coordinated to three histidines and an aqua ligand.
• Both Zn and Cd forms found naturally.
• Active site requires hydrogen bonding residues for catalytic efficiency.

Functions

• Has a major role in CO₂ transport and regulation of blood pH levels
• Plays a crucial role in COS (carbonyl sulfide) sequestration by plants
• Has a high catalytic efficiency that approaches the diffusion-control limit

Research Question

What is the catalytic cycle of carbonic anhydrase and what factors are necessary for desulfurization of the Zn-SH intermediate?

Proposed Mechanism

Intercalation of COS and CS₂ into Zn-OH bond followed by solvent esterification. 1 and 2 were characterized by both ¹H NMR and X-ray crystallography.

Synthesis

Insertion of COS and CS₂ into Cd-OH bond followed by solvent esterification. 1 and 2 were characterized by both ¹H NMR and X-ray crystallography.

Reasoning

• When TPA is complexed with Zn it has been shown to interact with heterocumulenes under basic conditions.
• TPA is a neutral ligand that further replicates the electronics of the active site.

Research Objectives

• Isolate and characterize Zn and Cd intermediates pertinent to the mechanism proposed.
• Explain the chemistry fundamental to the formation of M⁺SH (M = Zn and Cd) complexes.
• Evaluate the effect that hydrogen bond donors have on the formation of M⁺SH complexes and their desulfurization.
• Develop a catalytic cycle based on carbonic anhydrase.

Zinc Chemistry: Reaction with CS₂ and COS

Hydrosulfide Complex Synthesis

The reaction with COS under aprotic conditions resulted in the isolation of a cationic zinc hydrosulfide complex.

Characterization of Hydrosulfide Complex

Crystal structure of cationic portion of 3.

Conclusions

• We have been successful at characterizing many compounds relevant to the catalytic cycle of carbonic anhydrase
• Obtained crystallographic data of these important intermediates in the catalytic cycle
• Developed and characterized the first mononuclear cationic zinc hydrosulfide complex

Future Studies

Alternative Ligand Structures

We are now pursuing a new anionic ligand system in an attempt to isolate a Cd-SH complex. The synthesis of the anionic KHB(3,5-I-P₂)₂ ligand is shown below.

Construction of the Ligand

Acknowledgements

This project was funded and a summer fellowship provided to PJE by NSF REU Grant # 1005159