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The Influence of pH Variation on CooA Activity

Brian R. Weaver
Valparaiso University, brian.weaver1@valpo.edu

Rachael DeVries
Valparaiso University

Amy Gunter
Valparaiso University

Robert W. Clark
Valparaiso University

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Brian R. Weaver, Rachael M. DeVries, Amy Gunter, and Robert W. Clark

Valparaiso University Department of Chemistry

ABSTRACT

CooA, a CO-sensing heme protein, acts as a transcriptional activator of CO-metabolizing proteins in bacteria such as *Rhodospirillum rubrum* and *Carboxydothermus hydrogenoformans* through sequence-specific DNA binding. Previous research indicated a reduced iron center and CO gas were necessary for CooA to achieve its active conformation and bind DNA. To determine if other reaction conditions facilitate CooA activation, the role of pH on CooA function was tested. Specifically, a fluorescence anisotropy assay was employed to measure possible Fe(III) CooA DNA binding from pH 3 - 12. Interestingly, CooA was observed to bind DNA without CO at acidic conditions, with optimal binding observed at pH ~3. These results are discussed in light of the normal CO-dependent activation mechanism of CooA proteins.

BACKGROUND

- **What is CooA?**
  - A CO-sensing heme protein that acts as a transcriptional activator of CO metabolizing proteins
- **CooA Heme Coordination Structure:**
  - CooA has a dynamic heme coordination structure that responds to changes in local environment

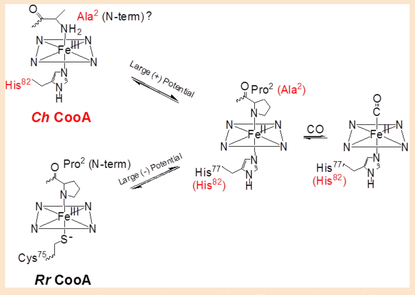


Figure 1. CooA has three different heme states in which two protein-derived ligands bind to the heme Fe.

Youn, H., et al.; *J. Bacteriol.* **2004**, 1320–1329.
Inagaki, S., et al.; *J. Biol. Chem.*, **2005** 3269-3274.

- ***In vivo* CooA DNA-Binding Requires CO Binding**

"Inactive" state + gas → "Active" state

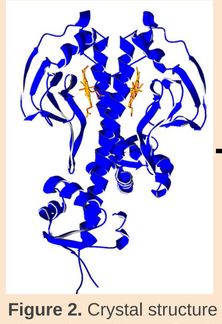


Figure 2. Crystal structure of inactive wild-type CooA.

Lanzilotta,W., et al.; *Nat. Struct. Biol.* **2000**, 876-883.

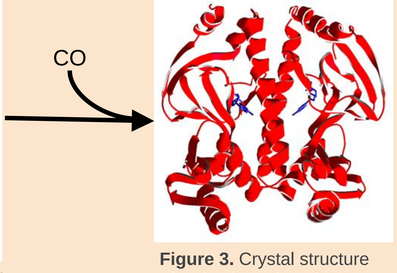


Figure 3. Crystal structure of active homolog, CRP

Passner J., et al.; *J. Biol. Chem.* **2000**, 847-859.

HYPOTHESIS & MOTIVATION

All published literature has indicated that CO binding to the heme is critical to forming an active CooA structure. Although this allosteric step is important, we hypothesize it may be bypassed entirely if other key interactions that stabilize the active state can be identified. In this study, we tested the role of pH on the *in vitro* activation of CooA.

METHODS

1. Protein Purification: Isolated recombinantly-expressed WT CooA from *E. coli*

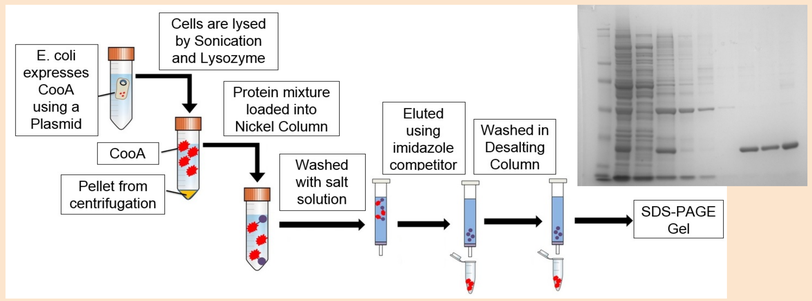


Figure 4. Diagram of Protein Purification including results from SDS-PAGE gel analysis

2. DNA Binding Assay: Probed CooA DNA-binding using fluorescence anisotropy.
- **Step 1:** Prepared CooA solutions (1 - 2000 nM)
- **Step 2:** Added DNA oligonucleotide with fluorophore attached
- **Step 3:** Measured fluorescence anisotropy using fluorimeter

Fluorescence Anisotropy: measure of the difference of parallel- and perpendicularly-polarized fluorescence intensity

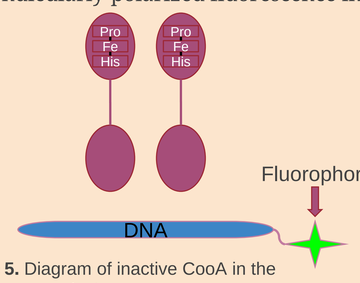


Figure 5. Diagram of inactive CooA in the fluorescence anisotropy assay.
- Unbound DNA, faster tumbling, perpendicular≅parallel fluorescence
- lower anisotropy value

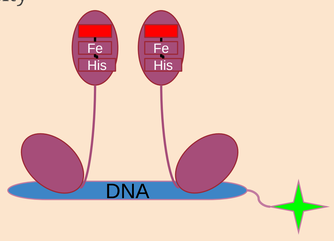


Figure 6. Diagram of active CooA in the fluorescence anisotropy assay.
- Bound DNA, slower tumbling, parallel>perpendicular fluorescence
- higher anisotropy value

J Biological Chemistry, 275, **39332-39338.**

RESULTS

1. DNA-Binding Studies Performed at Acidic pH Values

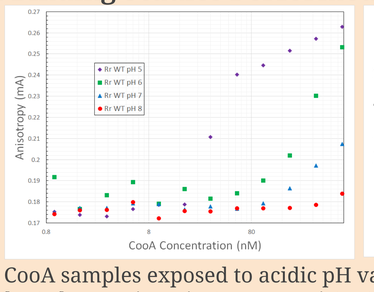


Figure 7. Anisotropy curves of WT CooA activation at different pH values.

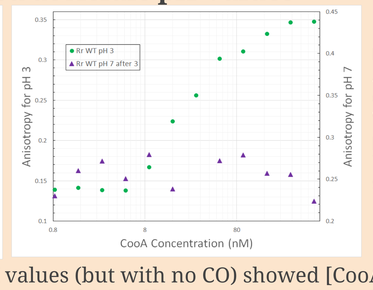


Figure 8. Anisotropy curves of WT CooA pH 3 to pH 7 cycling studies.

- Fe(III) CooA samples exposed to acidic pH values (but with no CO) showed [CooA]-dependent changes in anisotropy consistent with high-affinity DNA binding; behavior was like that observed in CO-containing assays performed at pH 8
- pH cycling: 1) tight binding, pH <7; 2) weak binding, pH ~7
- Addition of CO gas at low pH values did not improve DNA binding (data not shown)

RESULTS & DISCUSSION

2. DNA-Binding Studies Performed at Basic pH

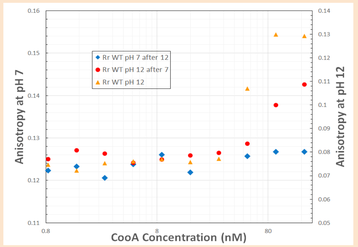


Figure 9. Anisotropy curves of CooA pH 12 to pH 7 cycling studies.

- Although Fe(III) CooA did not bind DNA tightly at pH 8, reversible binding was observed at pH 12 after an induction period

3. Link Between Activity & Heme Coordination

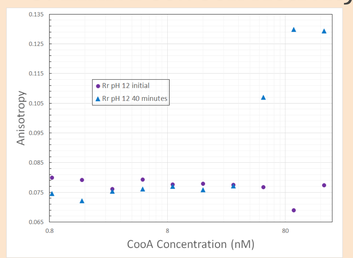


Figure 10. Anisotropy curves showing time-dependent activation at pH 12.

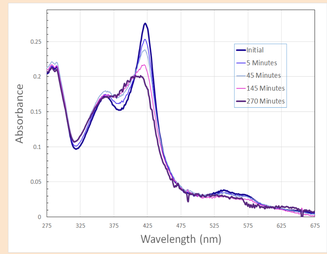


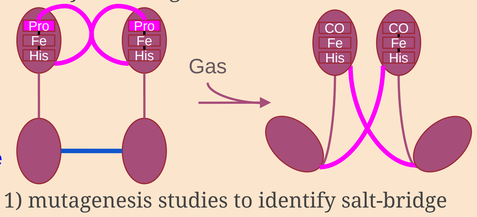
Figure 11. UV-Vis Spectra showing loss of ligand over time at pH 12.

- Based on Fig. 10 & 11, DNA-binding at pH 12 required an induction period likely correlated with the loss of the N-terminal heme ligand (where CO normally binds)

CONCLUSIONS & FUTURE WORK

- Fe(III) CooA exhibits tight DNA binding at pH<7 and modest binding at elevated pH = 12 (both in the absence of CO)
- pH cycling studies suggest DNA-binding does not result from protein denaturation
- All observed pH-dependent DNA binding is:
  - correlated with loss of N-terminal heme ligand
  - consistent with an activation mechanism which requires disruption of a key salt bridge that stabilizes inactive state

Rethinking CooA Activation:
1) loss of N-terminal ligand 2) CO binding
3) break salt bridge



- Future work: 1) mutagenesis studies to identify salt-bridge AAs; 2) circular dichroism to rule out denaturation

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