**ABSTRACT**

CooA, a CO-sensing heme protein, acts as a transcriptional activator of CO-metabolizing proteins in bacteria such as *Rhodopseudomonas 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 CO-DNA binding 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.

**METHODS**

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

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

**RESULTS & DISCUSSION**

1. **DNA-Binding Studies Performed at Acidic pH Values**

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**

**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

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**REFERENCES**

- [Valparaiso University Department of Chemistry](http://www.chem.valpo.edu/)
- [Phillip DeLassus Memorial Chemistry Fellowship](http://www.valpo.edu/chemistry/fellowships.php)
- [Josh Wagoner, Teryn Gehred, Jessica Lyyza & CHEM 317 students](http://www.chem.valpo.edu/)

**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.

**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

- **In vivo CooA DNA-Binding Requires CO Binding**
  - “Inactive” state + gas → “Active” state

**RESULTS**

- 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)

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