IRON AND NICKEL EDGE MAD EXPERIMENTS ON NATIVE *R. rubrum* CARBON MONOXIDE DEHYDROGENASE

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INTRODUCTION

Carbon monoxide dehydrogenases (CODHs) play an important role in reducing the levels of toxic CO gas in our environment. An estimated 1 x 10^8 tons of CO are removed from the lower atmosphere and earth by bacteria annually [1]. A better understanding of the mechanism of carbon monoxide dehydrogenases could lead to the development of biomimetic catalysts for large scale use to lower CO concentration in heavily polluted areas [2].

There are three main classes of carbon monoxide dehydrogenases: those that oxidize CO exclusively (e.g. *Rhodospirillum rubrum*), those that synthesize acetyl-CoA from CO_2 (e.g. *Clostridium thermoaceticum*), and those that breakdown acetyl-CoA (e.g. *Methanosarcina thermophila*). All members of the CODH family utilize Ni/4Fe-4S clusters to catalyze their reactions. One Ni/4Fe-4S cluster, called the C-cluster, is responsible for the oxidation of CO. The other, the A-cluster, is the site of acetyl-CoA synthesis. Although these clusters have been studied extensively by spectroscopic techniques, their exact 3-dimensional structures remain unknown (Figure 1). The crystallographic studies of *R. rubrum* CODH will reveal the structure of a novel nickel center and will provide insight into the mechanism of these intriguing enzymes.

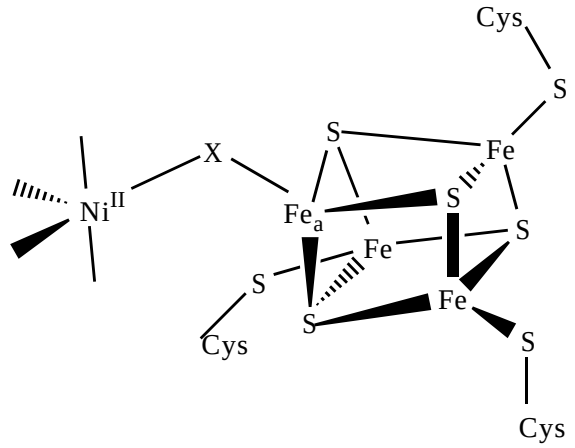

Figure 1. Model of A and C-clusters based on spectroscopic data. Although the chemistry performed by both clusters is different, the spectroscopic data suggests that the overall architecture of the A and C clusters is the same (reviewed in 2). The different reactivities may result from differences in the nature of the ligands to the nickel and in the amino acid residues surrounding the cluster. X-ray structures will reveal the identity of ligands to the nickel, the nature of a bridging ligand designated X, and the identity of surrounding amino acid residues.

RESULTS

To determine the crystal structure of *R. rubrum* CODH, a four wavelength Fe MAD (Multi-wavelength Anomalous Dispersion) phasing experiment was carried out at beamline 5.0.2 at ALS. A native data set and a data set at the nickel peak were also collected (Table 1). Data were processed in DENZO and SCALEPACK [3]. The Fe-sites were located using SOLVE [4], and refined in SHARP [5]. Using four data sets at the Fe-edge and one at the Ni-edge, the figure-of-merit (FOM) was 0.41 for acentric reflections between 20.0 - 4.0 Å resolution. The resulting electron density maps, after non-crystallographic averaging and phase extension, were of sufficient quality to trace the polypeptide chain. The model is currently being refined to 3.3 Å resolution.

Table 1: Data Collection Statistics

	Wavelength (Å)	Resolution (Å)	Rsym (%)	Completeness (%)	Redundancy
Native	1.2982	3.3	8.3	99.7	3.4
Fe-peak	1.7394	3.8	9.2	92.3	1.9
Fe-inflection	1.7419	5.0	13.7	88.3	2.0
Fe-high remote	1.6469	5.0	8.8	83.1	2.1
Fe-low remote	1.8456	4.0	9.6	96.6	2.2
Ni-peak	1.4846	4.0	8.6	95.5	2.3

CONCLUSION

The MAD data collected at ALS beamline 5.0.2 has made possible the structure determination of *R. rubrum* carbon monoxide dehydrogenase. This is the first structure of this class of nickel-iron-sulfur proteins. When the refinement of the model is complete, the nature of the unique nickel center (Figure 1) will be revealed.

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