A Tale of Two Dioxygenases: Non-heme Fe(II) Dioxygenases with Sulfur-containing Substrates

Sergio C. Chai and Michael J. Maroney

Department of Chemistry, University of Massachusetts, Amherst, MA 01003

Cysteine dioxygenase (CDO) catalyzes the oxidation of cysteine to cysteine sulfinic acid (CSA), which is the first major step in cysteine catabolism in mammalian tissues. Rat liver CDO was cloned and expressed in E. coli as a 26.8 kDa N-terminal fusion protein bearing a poly-histidine tag. As compared to existing purification protocols for native CDO, the milder conditions used in the isolation of the recombinant CDO by immobilized-metal affinity chromatography allowed a more controlled study of the properties and activity of CDO, clarifying conflicting findings in the literature (Chai et al. *J. Biol. Chem.* **2005**, *280*, 9865). Apo-protein was inactive in catalysis and was only activated by Fe. Metal analysis of purified recombinant protein indicated that only 10% of the protein contained Fe, and that the Fe was loosely bound to the protein. Kinetic studies showed that the recombinant enzyme displayed a Km value of 2.5 ± 0.4 mM at pH 7.5 and 37 °C. Kinetics studies, characterization of the Fe-active site by X-ray absorption spectroscopy (XAS) and the effect of cysteine analogs on CDO activity are reported.

Acireductone dioxygenases (ARDs) are enzymes involved in the methionine recycle pathway, which regulate aspects of the cell cycle. Klebsiella pneumoniae produces two enzymes that share a common polypeptide sequence and differ only in the metal ion present. Reaction of acireductone (1,2-dihydroxy-3-keto-5-methylthiopentene) with Fe-ARD and dioxygen produces formate and 2-keto-4-methylthiobutanoic acid, the -ketoacid precursor of methionine. Ni-ARD reacts with acireductone and dioxygen to produce methylthiopropionate, CO, and formate and does not lie on the methionine recycle pathway. An X-ray absorption spectroscopy (XAS) study of the structure of the catalytic Fe center in resting Fe-ARD enzyme and in the enzyme-substrate complex is reported, providing insight into the mechanism of catalysis employed by a Fe-containing dioxygenase. The metal center of Fe-ARD is compared with our previous studies of the Ni-ARD active site (T. C. Pochapsky et al. *Nature Struct. Biol.* **2002**, *9*, 966).