

An XAS Investigation of the Nickel Active Site Structure in *Escherichia coli* Glyoxalase I¹

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Beamline(s): X9B

Introduction: Glyoxalase I, (GlxI; S-D-lactoylglutathione methylglyoxal lyase (isomerizing), EC 4.4.1.5), catalyzes the isomerization of the hemimercaptal formed non-enzymatically from pyruvaldehyde and glutathione (GSH) to the thioester of D-lactate. This reaction is the first step in the detoxification of cytotoxic pyruvaldehyde. All GlxI enzymes studied to date require a metal for catalytic activity. However, unlike the *Homo sapiens*, *Saccharomyces cerevisiae*, and *Pseudomonas putida* GlxI enzymes, which are active in the presence of Zn²⁺, the *Escherichia coli* enzyme is not active with Zn bound and is maximally activated by Ni²⁺.² As such, *E. coli* GlxI constitutes the first example of a Ni-dependent isomerase. The XAS study of the Ni site in *E. coli* GlxI and in the Zn-substituted enzyme reported here provided the first detailed structural information regarding the active site of *E. coli* GlxI. The study was specifically aimed at examining structural differences between the Ni and Zn sites with the goal of understanding the origin of the metal specificity.

Methods and Materials: Ni K-edge and Zn -edge XAS was performed on beamline X9B using frozen samples held near 50K in a dispex unit. X-ray fluorescence data was collected using a 13-element Canberra detector. Data analysis was carried out using the program WinXAS. The integrity of the samples upon exposure was determined by a combination of three methods. First the K-edges of the samples were monitored on sequential scans to determine if the redox state or s metal site structure of the sample was changing upon exposure. Second, the activity of the NiGlxI samples was measured before and after exposure. There was no evidence for radiation damage to the samples.

Results: and Conclusions: *Escherichia coli* Glyoxalase I, GlxI, is the first example of an isomerase that is maximally activated by Ni²⁺. In contrast with GlxI enzymes from other sources that are Zn enzymes, *E. coli* GlxI is not active with Zn bound. Structural details from X-ray absorption spectroscopic analyses were determined for the active site Ni center and the Zn-substituted enzyme. The structure of the active Ni site is consistent with a Ni(Glu)₂(His)₂ site in the enzyme with the addition of two other O/N-donor ligands to form a six-coordinate active Ni site. The Zn-substituted enzyme has a spectrum consistent with a five-coordinate Zn(Glu)₂(His)₂ site with one additional O/N-donor ligand. Based on comparisons with human GlxI, which is a Zn enzyme with two aqua ligands, the additional O/N-donors are assigned to water molecules. The main difference in the two structures is therefore not due to a change in protein ligand environment, but rather a change in the number of water molecules, which implicates both water molecules in the enzyme mechanism.

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References: ¹ G. Davidson, S. Clugston, J. Honek, M. Maroney, *Inorg. Chem.* **39**, 2962-2963 (2000),
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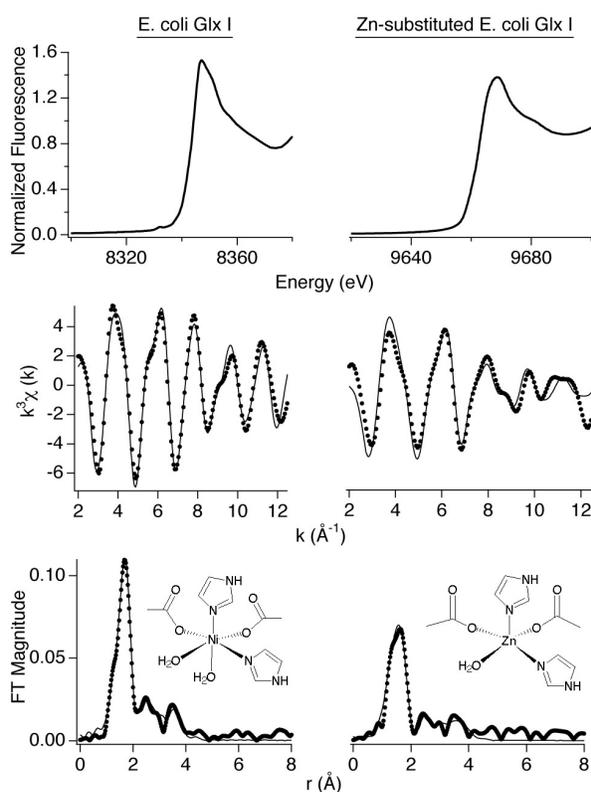


Figure 1. A comparison of the Ni active site of *E. coli* GlxI and the Zn-substituted site. Top: Normalized Ni K-edge (left) and Zn K-edge (right) XAS spectra in the edge and XANES regions. Middle: Fourier-filtered (backtransform window = 1.1 – 4.0 Å, uncorrected for phase shifts) EXAFS data (solid circles) and fits (solid lines).