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

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Glyoxalase I, although classified as a lyase (GlxI; EC 4.4.1.5), catalyzes the isomerization of the hemimercaptal formed nonenzymatically from pyruvaldehyde and glutathione (GSH) to the thioester of D-lactate. This is the first step in the detoxification of cytotoxic pyruvaldehyde.[1,2] All GlxI enzymes studied to date require a metal for catalytic activity. However, the *Homo sapiens*, *Saccharomyces cerevisiae*, and *Pseudomonas putida* enzymes are active in the presence of Zn^{2+},[3−5] while the *Escherichia coli* GlxI is the first example of a Ni-dependent isomerase and is not active with Zn bound.[6,7] The exact role of the catalytic metal in GlxI is unknown, but suggestions include direct metal interaction with the oxygens of the enediolate intermediate[8] or the activation of bound water molecules.[9,10] We report here X-ray absorption spectroscopy (XAS) studies of the metal sites in Ni-containing and Zn-substituted *E. coli* GlxI that provide the first detailed structural information regarding the active site.

The samples used in the XAS studies were prepared as previously described by reconstitution of recombinant apoGlxI with 1.5−2.5 equiv of $NiCl_2$ or $ZnCl_2$.[7] X-ray fluorescence data on frozen samples at 50 K were collected on beam line X9B at the National Synchrotron Light Source (2.8 GeV, 170−300 mA) with a Si[220] double-crystal monochromator. The data were recorded using a 13-element Ge array detector (Canberra) and were calibrated to the first inflection points in metal foil spectra (Ni, 8331.6 eV; Zn, 9661.0 eV). The summed fluorescence data were then background-corrected and normalized, and the extended X-ray absorption fine structure (EXAFS) results were fit using the program WinXAS.[11] X-ray absorption near-edge structure (XANES) data were obtained and analyzed as previously described.[12] EXAFS data were analyzed employing k^3-weighted Fourier filtered data (FT limits = 2.0−12.5 Å^{-1}; back-transform limits = 1.1−4.0 Å, uncorrected for phase shifts). Theoretical phases and amplitudes used in fitting the EXAFS for single and multiple scattering pathways were obtained from FEFF 6 calculations of crystallographically characterized model compounds.[13] Edge spectra monitored as a function of exposure time did not indicate any changes in the redox state or ligand environment.

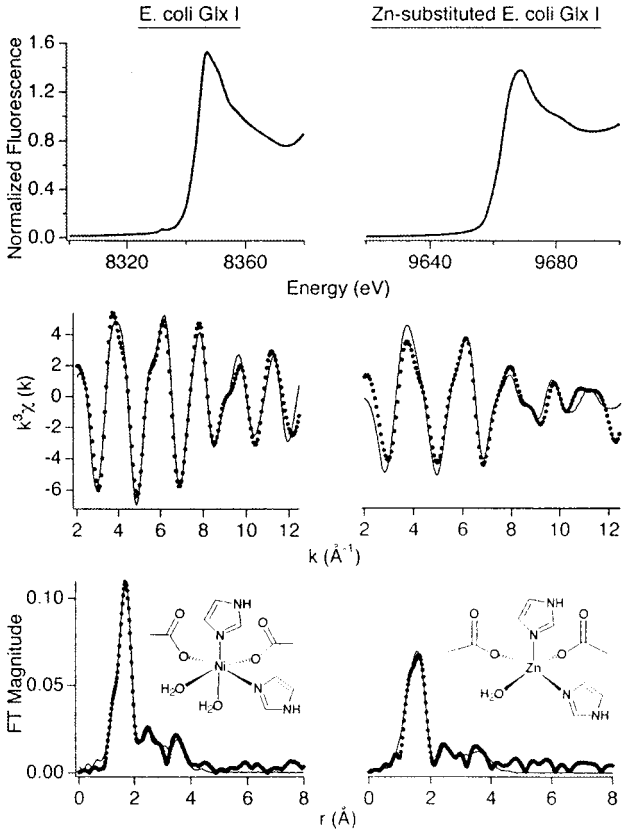

Figure 1. Comparison of the Ni and Zn-substituted *E. coli* GlxI metal sites: (top) normalized Ni (left) and Zn (right) K-edges; (middle) Fourier filtered (back-transform window, r = 1.1−4.0 Å) EXAFS; (bottom) Fourier transformed (k = 2−12.5 Å^{-1}) EXAFS. Data are shown as solid circles, best fits as solid lines. r is uncorrected for phase shifts.

The Ni K-edge XANES spectrum (Figure 1) of *E. coli* GlxI reveals a small peak near 8332 eV assigned to a 1s → 3d transition with an intensity (2.8(5) × 10^{-2} eV) that indicates a symmetric ligand environment. The absence of features associated with a 1s → 4p_z transition rules out a four-coordinate planar geometry, indicating that the active site Ni is six-coordinate.[12] Analyses of the Ni K-edge EXAFS spectra are shown in Table 1 and Figure 1. The first coordination sphere of the active site Ni is adequately

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(7) Clugston, S. L.; Barnard, J. F. J.; Kinach, R.; Miedema, D.; Ruman, R.; Daub, E.; Honek, J. F. *Biochemistry* **1998**, *37*, 8754. All enzyme samples were concentrated to 1.2−2.7 mM in dimeric enzyme using an Amicon Centricon concentrator (YM10; Millipore, Bedford, MA), the buffer changed to 50 mM MOPS and 25% glycerol adjusted to pH 7.0 with tetraethylammonium hydroxide (10% solution in water), prior to loading into sample holders and freezing on dry ice. Enzymatic activity was monitored throughout each sample preparation, with enzyme concentrations determined by the Bradford method. To confirm enzyme activity following X-ray exposure, amino acid analysis to verify protein concentrations (Nebraska Medical Center, Omaha) and Ni analysis by graphite furnace atomic absorption spectroscopy (University of Massachusetts Environmental Analysis Laboratory) were performed on the sample used for XAS. *E. coli* GlxI becomes fully active upon binding one Ni^{2+} per homodimeric (29 838 Da) protein. Amino acid and Ni analysis of the enzyme sample used in this study showed that the sample contained 1.1 Ni atoms/protein.
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fit by a single shell of O(N) scattering atoms at an average distance of 2.07(2) Å (fit Ni01). Thus, the data extend the trend observed in Ni metalloenzymes, wherein S-donor ligands are excluded from sites that are not involved in redox catalysis.[14,15] Modeling the first coordination sphere as a six-coordinate site composed of two shells of O/N donors (1−2 at 1.91(2) Å and 4−5 at 2.08(2) Å) slightly improves the fit (Ni02). Multiple scattering analyses of features near 3 and 4 Å are consistent with the coordination of two histidine ligands (fit Ni03),[13] a result that is in agreement with the presence of two histidines in the amino acid sequence of the enzyme and our predictions based on sequence analyses (vide infra).[7] Addition of a shell of C atoms with multiple scattering parameters in the second coordination sphere at 2.98(2) Å, as might be expected from carboxylate donors, also improves the fit (Ni04). The best fits are obtained with 1−2 C atoms in this shell, indicating that at least one of the O/N donors is a carboxylate ligand.

The Zn-substituted enzyme offers a structural comparison between the Zn site in human ZnGlxI and *E. coli* GlxI. The EXAFS is consistent with a five-coordinate Zn site with two shells of O/N donor ligands (2 at 1.90 Å and 3 at 2.05 Å, fit Zn02). Addition of one well-ordered histidine ligand, or two histidines with significant disorder (fit Zn03) in the atoms in the third coordination sphere, greatly improves the fit. No additional C atoms can be in the second coordination sphere.

The structure of the Ni site from XAS analysis is consistent with that obtained from single-crystal X-ray diffraction[16] and previous EXAFS studies[17] of the human GlxI Zn site, except for the substitution of the Gln residue by His in the *E. coli* enzyme. A six-coordinate structure is also supported by EPR studies of Co^{2+} [18] and Mn^{2+} [9] substituted human GlxI that conclude that the metal site is pseudooctahedral with 1−2 water ligands and by EPR spectra obtained from Mn^{2+} substituted *E. coli* GlxI that also indicate a six-coordinate metal site.[19] The human GlxI crystal structure identified four protein residues (Gln 33 Zn−O = 2.0 Å, Glu99 Zn−O = 2.0 Å, His126 Zn−N = 2.1 Å, and Glu172 Zn−O = 2.0 Å) and one aqua ligand (Zn−OH_2 = 2.1 Å) coordinated to the Zn^{2+} in each of two active sites.[16] In addition, there is a second water molecule with a Zn−O distance of 2.8 Å.[8,20] The *E. coli* GlxI sequence has only 36% identity to the *H. sapiens*. Nonetheless, protein sequence alignments between the *E. coli* and *H. sapiens* GlxI enzymes suggest the conservation of three of the four metal ligands in the *E. coli* enzyme (Glu56, His74, and Glu122), with the fourth ligand tentatively corresponding to a histidine (His5) (see Supporting Information).[7] When viewed in the context of the human crystal structure, sequence homologies, and available EPR data, the XAS data on *E. coli* GlxI support a six-coordinate $Ni(Glu)_2(His)_2(OH_2)_2$ site in the enzyme (Figure 1). The EXAFS analysis for the Zn-substituted *E. coli* GlxI is consistent with a $[Zn(His)_2(Glu)_2OH_2]$ site, which is similar to the human enzyme but differs in coordination number (5 instead of 6) and in the replacement of the Gln by a His ligand. However, the *S. cerevisiae* and *P. putida* enzymes are active with Zn bound[3,5] and also feature the replacement of Gln by His.[21] Thus, this change in ligation is not responsible for the critical difference in the activity. It is plausible that a six-coordinate structure with two aqua/hydroxo ligands is required and that the Zn-substituted *E. coli* enzyme is unable to bind a second aqua ligand.

Table 1. Selected Curve-Fitting Results of Filtered EXAFS Spectra for *Escherichia coli* Glyoxalase I[a]

fit	*N* Ni−X	*R* (Å)	σ^2 ($\times 10^3$ Å)[b]	GOF
Ni01	4 Ni−O	2.0691(2)	3.1	34.8
Ni02	1 Ni−O	1.9145(5)	0.2	29.2
	5 Ni−O	2.0827(2)	2.9	
Ni03	1 Ni−O	1.9138(5)	0.3	7.0
	5 Ni−O	2.0826(2)	2.9	
	2 [Ni−C(His)]	2.9540(7)	(5.4)	
		3.276(2)		
	2 [Ni−C/N(His)]	4.2052(9)	(4.5)	
		4.299(2)		
Ni04	1 Ni−O	1.9127(6)	1.5	5.8
	5 Ni−O	2.0813(2)	3.3	
	1 Ni−C	2.976(2)	6.1	
	2 [Ni−2C(His)]	2.9540(7)	(5.4)	
		3.277(2)		
	2 [Ni−2C/N(His)]	4.2052(9)	(4.4)	
		4.299(2)		
Zn01	4 Zn−O	1.9988(1)	8.5	38.5
Zn02	2 Zn−O	1.9022(2)	4.9	31.1
	3 Zn−O	2.0535(1)	4.0	
Zn03	2 Zn−O	1.9028(2)	5.2	8.9
	3 Zn−O	2.0523(1)	4.2	
	2 [Zn−2C(His)]	2.9962(4)	(8.2)	
		3.325(1)		
	2 [Zn−2C/N(His)]	4.2186(5)	(*11.4*)	
		4.521(2)		

[a] X is the scattering atom for each shell. *R* is the Ni−X distance. σ^2 is the root-mean-square disorder in the Ni−X distance. GOF = $1/\sigma^2\sum_{i=1}^{N}[y_{exp}(i) - y_{theo}(i)]^2$ (see ref 14). Accuracy of distances determined = ±0.02 Å for atoms in the first coordination sphere of the meal and ±0.05 Å for second and third coordination sphere atoms. Precisions (indicated) are <0.02 Å for well-ordered shells; thus, differences are more accurate than the absolute distances. [b] Italicized values are approaching physical insignificance. Large values of σ^2 indicate a coordination number that is too large or a badly disordered shell. Parentheses indicate that σ^2 for all atoms in that shell were constrained to a single value.

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Supporting Information Available: Tables S1 and S2 containing a compilation of fits for Ni and Zn K-edge Fourier filtered EXAFS data for the native and Zn-substituted *E. coli* GlxI samples [Table S3 of models used in FEFF 6 calculations] and Figure S1 containing sequence alignments for *E. coli* and *H. sapiens* GlxI enzymes. This material is available free of charge via the Internet at http://pubs.acs.org.

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