Tetrathiolate Ligation of Cd^{2+}−Desulforedoxin

Frank Rusnak* and Christopher Czaja

Department of Biochemistry and Molecular Biology, Mayo Clinic & Foundation, Rochester, Minnesota 55905

Lazaros T. Kakalis and Ian M. Armitage*

Departments of Pharmacology and Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut 06520

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Desulforedoxin (Dx) and desulfoferrodoxin (Dfx) are nonheme iron proteins isolated from sulfate-reducing *Desulfovibrio* bacteria.[1−4] Both proteins share a common domain of 37 amino acid residues, referred to as the Dx site, that binds a single iron atom with unique spectroscopic properties. Numerous studies including optical,[1,4−6] electron paramagnetic resonance (EPR),[3−6] Mössbauer,[4−6] and Resonance Raman[7] spectroscopies have suggested that the iron atom of the Dx site is coordinated by four cysteinyl sulfur ligands, analogous to the iron atom in rubredoxin which has tetrahedral FeS_4 coordination.[8] However, UV/visible, EPR, Mössbauer, and Raman spectra of Dx are distinctly different than those measured for rubredoxin,[8−14] leading to two different proposed models for metal coordination for the Dx site: According to the first one, Fe is coordinated by four cysteine residues as FeS_4 in a distorted tetrahedral geometry.[5] The second model, based on a comparison of the spectroscopic properties of Dfx with a ferric diethylenetriaminepentaacetic acid model complex, favors a seven-coordinated geometry with oxygen and nitrogen ligands.[15]

The fact that apo-Dx can be prepared and readily reconstituted with Fe^{3+} [5] as well as other metal ions such as Ni^{2+} and Co^{2+} [16] led us to prepare the Cd^{2+}−substituted Dx for ^{113}Cd NMR measurements. The ^{113}Cd chemical shifts of metalloproteins are extremely sensitive to the nature and the number of the ligands as well as their geometrical arrangement in the metal coordination sphere.[17−20] In particular, resonances from ^{113}Cd bound exclusively by cysteinyl sulfur ligands are the most deshielded and appear between ca. 600 and 750 ppm, whereas resonances from sites consisting of a combination of oxygen and nitrogen ligands occur in the 0 to 300 ppm range.

Using recombinantly expressed Dx,[21] apo-Dx was prepared as described[5,16] and dissolved in 0.6 mL of 0.5 M Tris base without β-mercaptoethanol after the last trichloroacetic acid

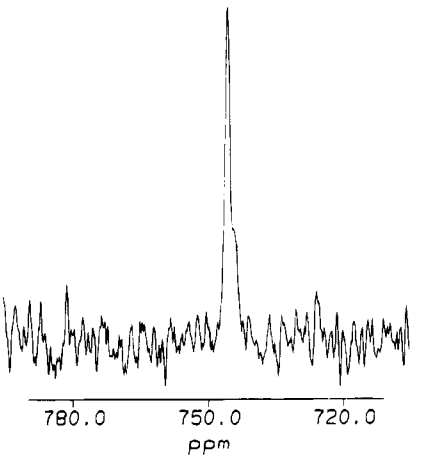

Figure 1. Proton-undecoupled ^{113}Cd NMR spectrum (110.9 MHz) of 4.4 mM recombinant apo-Dx in 10 mM TrisCl in 90% H_2O/10% D_2O, pH 7.4 at 25 °C after the addition of 0.5 equiv of $^{113}CdCl_2$. It is the result of 53 650 scans, acquired with a 60° tip angle and a 0.891 s repetition time, after the application of a 10 Hz exponential line broadening. It has been referenced vs external 0.1 M $Cd(ClO_4)_2$ in 90% H_2O/ 10% D_2O.

Table 1. Metal−Sulfur Bond Lengths and Angles of MS_4 Centers in Rubredoxin and Alcohol Dehydrogenase

center	bond dists (Å)	center	bond angles (deg)
		Rubredoxin[a]	
Fe−S6	2.32	S6−Fe−S9	114.5
Fe−S9	2.29	S6−Fe−S39	111.3
Fe−S39	2.28	S6−Fe−S42	106.1
Fe−S42	2.27	S9−Fe−S39	103.4
		S9−Fe−S42	109.8
av	2.29	S39−Fe−S42	111.9
std dev	0.022		
		av	109.5
		std dev	4.07
		Alcohol Dehydrogenase[b]	
Zn−S97	2.377	S97−Zn−S100	107.3
Zn−S100	2.331	S97−Zn−S103	113.2
Zn−S103	2.257	S97−Zn−S111	101.5
Zn−S111	2.344	S100−Zn−S103	107.4
		S100−Zn−S111	118.9
av	2.327	S103−Zn−S111	108.6
std dev	0.051		
		av	109.5
		std dev	5.94

[a] Data for *D gigas* rubredoxin taken from ref 8 with residue numbers of cysteinyl ligands indicated. [b] Bond lengths and bond angles determined from 1.8 Å resolution crystal structure of equine LADH obtained from Brookhaven National Laboratory Protein Data Bank (accession number 2ohx).

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precipitation. A half equivalent of $CdCl_2$ (96.3 atom % ^{113}Cd) was added to apo-Dx from a stock solution[22] and the sample was buffer-exchanged against 10 mL of 10 mM TrisCl in 90% H_2O/10% D_2O, pH 7.4 (uncorrected pH meter reading).

The ^{113}Cd NMR spectrum of Cd^{2+}-substituted desulforedoxin (Cd-Dx) exhibited a single resonance at 746 ppm (Figure 1). This chemical shift is substantially deshielded and indicative of exclusive sulfur ligation.[17] Tetrahedral cysteinyl ligation can be assigned by analogy to two other metal sites, the FeS_4 site of *Desulfovibrio gigas* rubredoxin,[8,20] and the stuctural ZnS_4 site in horse liver alcohol dehydrogenase (LADH).[23,24] Cd^{2+}-substituted rubredoxin yields a single ^{113}Cd-NMR resonance at 723.6 ppm[20] while the structural zinc site of LADH similarly substituted exhibits a ^{113}Cd-NMR resonance with a chemical shift of 751 ppm.[25]

Metal coordination in the structural zinc binding site in LADH exists in a distorted tetrahedral arrangement[23,24] with standard deviations in metal−sulfur bond length of 0.051 Å and S−Zn−S bond angles of 5.94 degrees (Table 1). In contrast, the metal site in *D. gigas* rubredoxin is closer to tetrahedral geometry with smaller deviations in both S−Fe−S bond angles and S−Fe bond distances (Table 1).[8] The ^{113}Cd chemical shift of 746 ppm for Cd−Dx compared to 751 ppm for Cd−LADH and 724 ppm for Cd−rubredoxin indicates that a strained tetrahedral geometry for the Dx metal site is likely. Furthermore, the observed downfield chemical shift precludes any participation by oxygen or nitrogen ligands in coordinating the metal in the Dx site.

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(22) A stock solution of [$^{113}CdCl_2$] was prepared by dissolving ^{113}CdO (Oakridge National Laboratory) in concentrated HCl, evaporating the solvent, and taking the salt up in water.

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