Molybdenum EXAFS of the *Desulfovibrio Gigas* Mo(2Fe–2S) Protein—Structural Similarity to "Desulfo" Xanthine Dehydrogenase

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ABSTRACT

The molybdenum EXAFS of the Mo(2Fe–2S) protein from *Desulfovibrio gigas* has been examined using fluorescence detection and synchrotron radiation. In the oxidized form the molybdenum environment is found to contain two terminal oxo groups and two long (2.47 Å) Mo–S bonds. Evidence was also found for an oxygen or nitrogen donor ligand at 1.90 Å. Addition of dithionite to the oxidized enzyme results in loss of a terminal oxo group, perhaps due to protonation. In addition, a 0.1 Å contraction in the Mo–S bond lengths is observed. The behavior of both oxidized and dithionite-treated forms is similar to that observed previously with "desulfo" xanthine oxidase.

INTRODUCTION

The Mo(2Fe–2S) protein from *Desulfovibrio gigas* has a molecular weight of 120,000 and contains one molybdenum and six (2Fe–2S) clusters [1, 2]. In its epr and optical

spectroscopic properties, this protein is similar to cyanolyzed deflavo xanthine oxidase [1]. No enzymatic activity is yet known for the *D. gigas* protein, and the possibility exists that the isolation procedure [1] yields a degraded form of an originally active species. To probe more deeply the possible similarity with desulfo xanthine oxidase, x-ray absorption spectra for the *D. gigas* protein have been collected and analyzed.

X-ray absorption is a powerful complement to epr for the study of molybdenum in proteins [3]. Structural details such as bond lengths, coordination numbers, and types of ligands can often be extracted from the extended x-ray absorption fine structure (EXAFS). To date, molybdenum enzyme EXAFS studies have been conducted on nitrogenase [4] and its iron–molybdenum cofactor [5], sulfite oxidase [6, 7], xanthine dehydrogenase [7], xanthine oxidase [8, 9], and nitrate reductase [10].

EXPERIMENTAL

The *D. gigas* Mo(2Fe–2S) protein was isolated according to published procedures [1] and concentrated in 0.1 M pH 7.6 Tris buffer to 90 mg/ml. The spectra were recorded on Beam Line I-5 at the Stanford Synchrotron Radiation Laboratory with a channel-cut Si[2,2,0] monochromator under dedicated (3 GeV,50 mA) conditions using fluorescence detection [11]. The oxidized form of the protein was examined under aerobic conditions. The "reduced" form was generated by the addition of a few grains of sodium dithionite followed by sealing the lucite curvette with a rubber septum and waiting 90 min. This dithionite-treated sample probably contains a mixture of Mo(V) and Mo(IV) species. The spectra were calibrated and the EXAFS was extracted using standard procedures [12]. For the curve-fitting analysis, recently described experimental phase shift and amplitude functions were utilized [7].

RESULTS AND DISCUSSION

The molybdenum K absorption edge region for oxidized and dithionite-reduced *D. gigas* protein is shown in Figure 1. A shoulder is observed in the edge of both forms, and the reduced edge is shifted to lower energy. Similar shoulders and shifts have been observed for sulfite oxidase [6], xanthine dehydrogenase [7], and certain forms of nitrate reductase [10]. The shoulders result from a strong bound state transition [13] that is characteristic of terminal oxygen or sulfur [4].

The Fourier-transformed EXAFS data for oxidized and reduced *D. gigas* protein are presented in Figure 2. Two significant peaks are observed, in regions typical for Mo=O and Mo–SR interactions. A decrease in the Mo=O amplitude is observed upon reduction of the protein. The EXAFS spectra and the best fits are shown in Figure 3, and the results are summarized in Table 1.

The EXAFS of the oxidized protein can be interpreted as consisting primarily of two Mo=O and one or two Mo–SR interactions. As illustrated in Figure 3, the EXAFS for the molybdenum site of the oxidized *D. gigas* protein shows a distinct beat pattern. Comparison with previous results shows that the EXAFS is nearly identical to that of cyanolyzed xanthine dehydrogenase. In the oxidized form, the bond lengths calculated for the *D. gigas* protein are within 0.02 Å of those reported for xanthine dehydrogenase. Futhermore, a significant improvement in the fit is gained by including an oxygen or nitrogen ligand at 1.92 Å, with a reasonable σ of 0.06 Å.

The spectral correspondence between reduced *D. gigas* protein and reduced cyano-
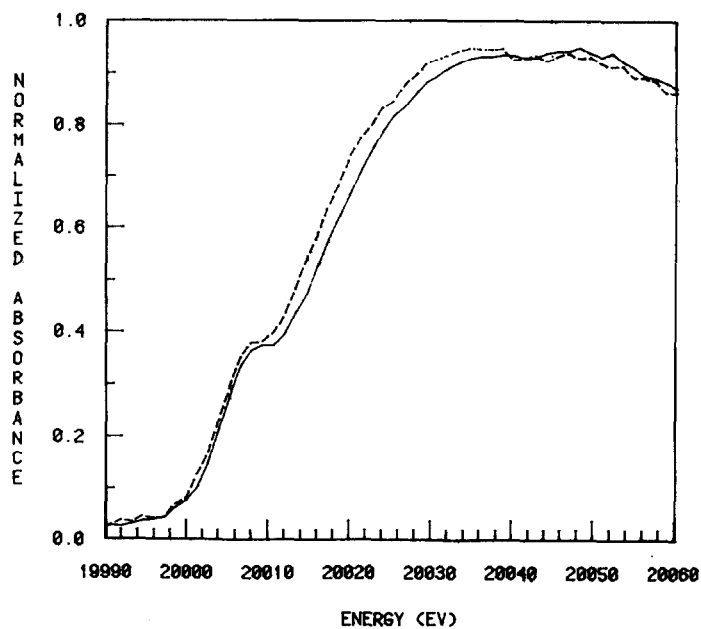
FIGURE 1. The molybdenum K absorption edge region for oxidized (—) and dithionite-reduced (---) *D. gigas* Mo protein.

FIGURE 2. Fourier transformed EXAFS for oxidized (—) and dithionite-reduced (---) *D. gigas* Mo protein. Transform range: k = 4–12 Å^{-1}, k^3 weighting.
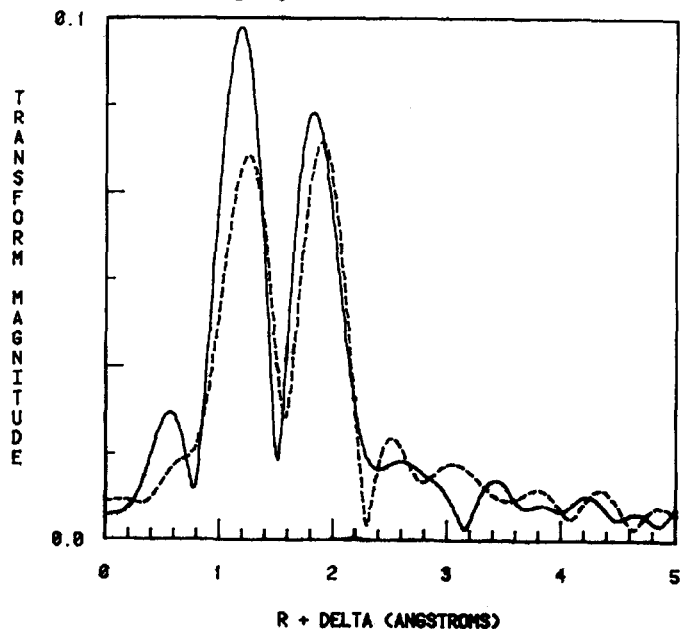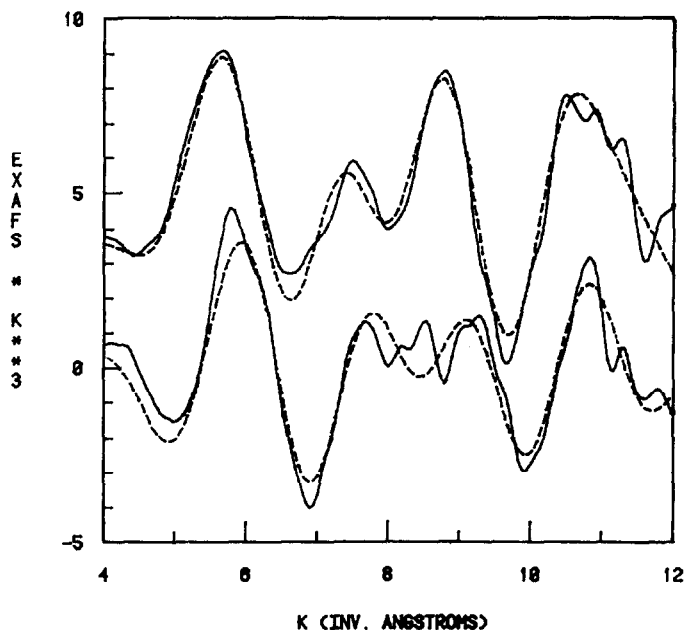
FIGURE 3. EXAFS spectra (—) and best fits (---) for oxidized (top) and dithionite-reduced (bottom) *D. gigas* Mo protein.

lyzed xanthine dehydrogenase is less striking. A single Mo=O at 1.68 Å as well as one or two sulfurs at 2.38 Å were sufficient to reproduce the general spectral features. No longer does an oxygen at 1.9 Å improve the fit, but a Mo–O,N interaction at 2.11 Å can be included.

The simplest model that can explain the current results is presented in Scheme 1. The
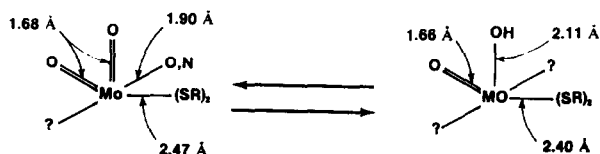
SCHEME 1. Partial structures of *D. gigas* protein Mo site.

TABLE 1. Summary of Curve-Fitting Results

Sample	Mo–O$_t$			Mo–S						Function
	Number[a]	R, Å[b]	σ, Å	Number[a]	R, Å[b]	σ, Å	Number[a]	R, Å[b]	σ, Å	Value[c]
Oxidized	2	1.67	0.045	2	2.47	0.072	—	—	—	0.84
D. gigas protein	2	1.68	0.047	2	2.47	0.075	1	1.90	0.055	0.53
	2	1.68	0.041	1	2.47	0.036	1	1.89	0.044	0.52
	1.9	1.68	0.041	1.7	2.47	0.070	1.0	1.90	0.050	0.52
Dithionite-treated	1	1.66	0.046	2	2.39	0.080	—	—	—	0.61
D. gigas protein	0.9	1.66	0.041	1.4	2.40	0.06	0.7	2.11	0.07	0.61

[a]Values reported as integers were held fixed during optimization.
[b]Estimated error is ±0.03 Å.
[c]Defined as $[\Sigma k^6 (X_{obs} - X_{calc})^2/N]^{1/2}$.

loss or protonation of one of the terminal ligands upon molybdenum reduction has been proposed for several other molybdenum enzymes, and the shortening of the thiolatelike bond lengths has also been observed. This is the first time that an Mo–O bond length at around 1.9 Å has been clearly demonstrated in the EXAFS of an oxidized protein, although in the other spectra such an interaction could not be ruled out.

Scheme 1 is ambiguous for several reasons. First, lacking a biological assay or alternate spectroscopic probe, the homogeneity of the molybdenum sites in this protein as isolated cannot be guaranteed. In fact, about 15% of the resting enzyme appears as Mo(V) by epr. The dithionite-reduced sample most likely contained a mixture of Mo(V) and Mo(IV) states under the experimental conditions employed. If different species have bond length differences less than about 0.1 Å, EXAFS analysis will simply give a weighted average structure.

A second source of ambiguity involves EXAFS amplitudes. The possibility of overlapping components, and the correlation of predicted coordination number with the Debye-Waller factor make it difficult to discern the number of sulfur ligands. This is less of a problem for determination of terminal oxo groups, because no other bond lengths are in that region and reasonable values for σ have been established. The final factor makes it difficult to discern the number of sulfur ligands. This is less a problem for terminal oxo groups because no other bond lengths are in this region, and nearly invariant values for σ have been established. A final limitation on the EXAFS structure determination is the insensitivity to weak interactions. There might well be water molecules or nitrogen ligands coordinated to the molybdenum site whose contribution to the EXAFS is too small to observe.

Despite all these limitations, the structures proposed in Scheme 1 do clarify some of the questions that have arisen concerning the *D. gigas* protein. First of all, no Mo–Fe interaction was observed under the aerobic conditions employed. This occurred despite the fact that a large amount of iron is present in this protein and that an increase in the relaxation of the Mo(V) signal is seen when the Fe/S centers are reduced and paramagnetic [5]. The Mo site, therefore, appears to be mononuclear and akin to the Mo cofactor enzymes such as sulfite oxidase, xanthine oxidase, nitrate reductase, and formate dehydrogenase, rather than the cluster type of site found in the Fe–Mo cofactor of nitrogenase. The *D. gigas* protein also resembles sulfite oxidase, desulfo xanthine oxidase, and *Chlorella* nitrate reductase because the molybdenum possesses two terminal oxygens in the oxidized form, one of which is lost or protonated upon reduction. Finally, the Mo–S bond lengths in the oxidized form fall close to those of desulfo xanthine oxidase (2.47 Å). The cause of this slight elongation over more normal Mo–thiolate bond lengths (~2.42 Å) is unknown. Previously, the slow reduction behavior of the molybdenum signal has hinted at similarity with desulfo xanthine oxidase [1]. Thus, the similarity between the Mo sites of these two proteins, hinted at by similar spectra and redox properties, is seen to have an underlying structural basis.

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