Unfortunately, in the O_2-bound series, only two examples are known (Table I): with thiolate,[8b] the distance is 2.37 Å; with thioether,[18] it is 2.49 Å. Recently, a series of sulfur-donor-ligated ferrous–CO complexes has been examined with EXAFS spectroscopy[21] and the Fe–S bond length has been found to increase by about 0.1 Å upon going from thiolate (2.32 Å) to any other type (thiol, thioether, or disulfide) of sulfur donor (2.40–2.41 Å). Taken together, the available data suggest that there is an appreciable lengthening of the Fe(II)–S(axial) bond trans to O_2 or CO for nonthiolate relative to thiolate sulfur donors and strongly support the assignment of the sulfur in oxy-P-450 and oxy-chloroperoxidase as a thiolate type.

In summary, compelling and direct evidence for a sulfur atom, most likely thiolate, as the trans axial ligand in oxy-P-450-CAM and oxychloroperoxidase has been obtained. Iron–sulfur bond distances of 2.37 Å have been determined in both cases. A sulfur donor ligand has now been detected by EXAFS analysis for all of the isolable intermediates in the P-450 reaction cycle and for the ferrous–CO state;[19,21] with chloroperoxidase, this represents the second state where a sulfur donor ligand has been detected by EXAFS.[22] Possible mechanistic implications of having a thiolate ligand in the P-450 system have been discussed.[2,23]

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Differential Anomalous X-ray Scattering Evidence for the Existence of μ-$H_3O_2^-$ Bridging Ligands in Solution

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In aqueous solution, transition-metal aquo ions $M(OH_2)^{n+}$ frequently undergo hydrolysis to μ-hydroxy or μ-oxo bridged dimers and polymers.[2] This reaction is generally presumed[3] to occur via condensation of the primary hydrolysis product $M(OH)^{(n-1)+}$; however, an alternative mechanism[4] involves initial formation of $H_3O_2^-$ bridged species. The $H_3O_2^-$ bridge may also be relevant to electron-transfer reactions, where one mechanism[5] involves H-atom transfer via an $H_3O_2^-$ bridged intermediate. Although recent crystallographic characterization of μ-$H_3O_2^-$ bridged Cr, Mo, and W clusters[4,6,7] offer support for these mechanisms, their significance depends on the extent to which $H_3O_2^-$ bridged species exist in solution. The $H_3O_2^-$ anion has been found in both bridging[4,6,7] and unligated[8] environments, but to date it has been structurally characterized only in crystalline materials. Vapor tensiometry[9] and Mössbauer spectroscopy[10] have provided indirect evidence for μ-$H_3O_2^-$ bridge formation in solutions of Cr and Fe complexes; however, these experiments are neither structurally definitive nor, in the case of the Mössbauer work, necessarily representative of aqueous solutions. We have obtained differential anomalous X-ray scattering (DAS) evidence for the existence of $H_3O_2^-$ bridged species in aqueous solution.

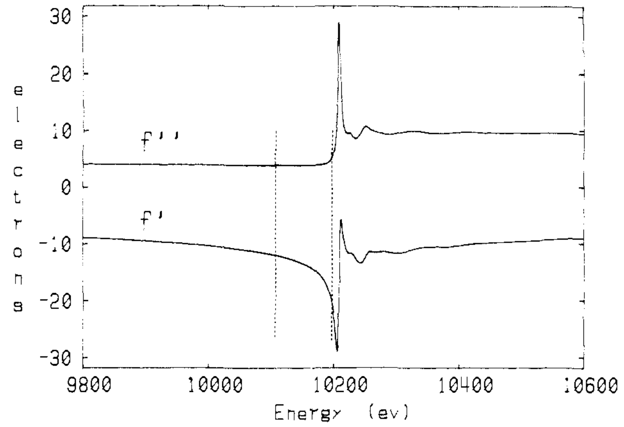

Figure 1. Experimental f_w' and f_w'' values for solution II in the vicinity of the W L_{III} absorption edge. Identical values were observed for solution I. f_w'' values were obtained from absorption measurements by using the optical theorem. f_w' values were calculated from f_w'' data by using the Kramers-Kronig relation. Vertical lines indicate energies for data collection (corresponding to $f_w' = -12.0$ and -20.3 electrons).

The trinuclear cluster $[M_3O_2(O_2CR)_6(H_2O)_3]^{2+}$ (M = W, Mo; R = CH_3, C_2H_5) can be crystallized as a monomer (isolated M_3 clusters) at low pH or as a dimer or polymer at higher pH.[6,7] In the dimer and polymer, partial deprotonation of the water ligands leads to the formation of $H_3O_2^-$ bridges between M_3 clusters. We have used DAS to determine the local atomic environment of the tungstens in two different solutions of W_3 clusters. Solution I was 0.3 M in HCl, thus giving fully protonated (monomeric) W_3 clusters. Solution II contained LiCl in place of HCl, thus favoring dimer and polymer formation.

X-ray scattering is a useful technique for determining the radial distribution function (RDF) of electron density about the average atom in a sample. However, since *all* atomic correlations contribute to the RDF, solute structure determination is extremely difficult for dilute multicomponent solutions. The recent availability of synchrotron radiation, providing a high-flux source of tunable X-rays, has made feasible the development[11] of DAS, in which scattering is measured at two wavelengths.

The X-ray scattering factor $f(k,E)$ for an atom is given by $f(k,E) = f_0(k) + f'(k,E) + if''(k,E)$ where $k = (4\pi \sin \theta)/\lambda$ is the wavevector and E is the incident photon energy. The anom-

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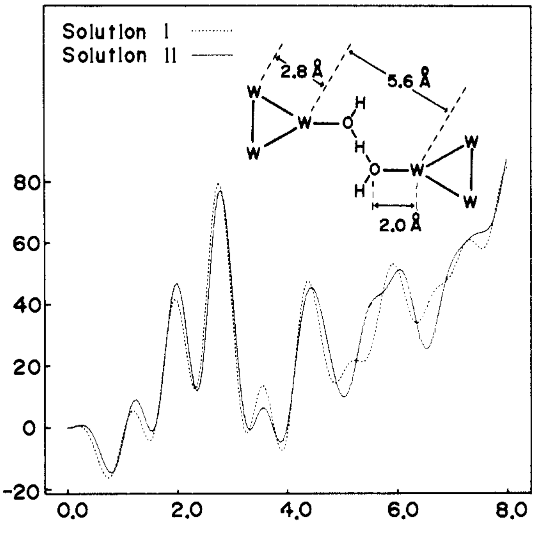

Figure 2. Tungsten differential distribution functions for solutions I and II. The first three large peaks due to intracluster correlations. The shoulder at 5.6 Å in solution II is due to W-W correlations which are clearly absent in solution I. Inset: Schematic W structure for solution II consitent with EXAFS and DAS results. Two W_3 clusters are bridged by a single $H_3O_2^-$ in a trans geometry. For clarity, the other atoms in the cluster have been omitted.

alous scattering factors, f' and f'' show significant energy dependence only in the vicinity of an atom's absorption edge. If scattering intensities are measured at two energies near a W absorption edge, their normalized difference contains only terms involving W scattering, since only f_w' and f_w'' have changed. The Fourier transform of this difference gives a differential distribution function (DDF), consisting of a weighted sum of those correlation functions that involve tungsten. Since the DDF represents the average electron density about *only* the tungsten atoms, it is much more useful than the RDF for solute structure determination, especially in complex systems.

DAS and extended X-ray absorption fine structure (EXAFS) data were collected at the Stanford Synchrotron Radiation Laboratory. Solutions I and II were each 0.077 M in the W_3 cluster (0.13 atom % W, 3.9 wt % W), which was prepared as previously described.[12] The dramatic energy dependence of the W anomalous scattering factors in the vicinity of the W edge is shown in Figure 1. Scattering data were measured by using a coupled θ-2θ scanning mode in reflection geometry at 10 196.8 and 10 106.8 eV. As indicated (Figure 1), there is a large change in f_w' and a negligible change in f_w'' between these energies.

EXAFS data for both solutions show shells only at 2.0 (W-O) and 2.8 Å (W-W), confirming that the clusters remain intact. These spectra are essentially identical, reflecting the fact that EXAFS is insensitive to longer distance interactions[13] and thus probes only the intracluster structure.

Examination of the crystal structure for the W_3 cluster[7] reveals that the intracluster structure gives rise to four shells of atoms around each W. These are located at 2.0 (W-O), 2.8 (W-W, W-C, and W-O), 4.5 (W-O and W-C), and 6.0 Å (W-C). Peaks corresponding to each of these shells are found in Figure 2, confirming that the experimental DDFs accurately reflect the W intracluster correlations. Since the intracluster structure is the same in both solutions, the close similarity of these peaks for both DDFs indicates the small relative error of the data.

Systematic errors in the scattering data can give rise to low-amplitude structure in the DDF. These errors have relatively low frequencies in *k* space and are thus most significant at low *R* (0-1.5 Å). Since the experimental DDFs have essentially the same structure even at low *R*, systematic errors cannot reasonably account for differences between the DDFs at higher *R*.

The only significant difference between the DDFs occurs at 5.6 Å. This difference, a shoulder for solution II which is absent for solution I, is clearly larger than the relative error in the data. As this feature is observed only for solution II, it cannot be due to *intra*cluster correlations. Although in principle attributable to solvent structure, the appearance of a solvent peak of this intensity *only* in solution II is implausible, given the small change in pH between solutions I and II. The light cations (H^+ for I, Li^+ for II) make a negligible contribution to the W DDF; hence this difference at 5.6 Å must be due to pH-dependent changes in the *inter*cluster structure. A structural model consistent with these observations is shown in Figure 2 (inset).

The pH of solution II was 1.3-1.4, indicating the release of 0.5-0.65 H^+/W_3 cluster. If every deprotonation leads to formation of an $H_3O_2^-$ bridge, this would indicate formation of 1.0-1.3 bridges/W_3 cluster, or an average of 0.4 W neighors/W. Although quantitation of a poorly resolved shoulder is difficult, the size of the 5.6-Å feature (approximately 0.5 W neighbors/W) is consistent with the observed pH. The observed 5.6-Å distance matches the intercluster W-W distance expected for trans $H_3O_2^-$ bridged W clusters[6,7] and is inconsistent with the 3-4-Å lengths expected for μ-O, μ-OH, μ-OH_2 bridges or the 5.0-Å length of a gauche $H_3O_2^-$ bridge.[4] The present work thus demonstrates that a significant fraction of the W_3 clusters in solution II are bridged by $H_3O_2^-$ in a trans geometry.

The chemical significance of the $H_3O_2^-$ bridge has been discussed previously.[4,6] By demonstrating directly that such species exist in significant concentration in aqueous solution, the present work provides support for the importance of μ-$H_3O_2^-$ bridges in aqueous transition-metal chemistry. Of possibly greater significance is the demonstration that DAS can be used to determine solute structure in dilute complex solutions. Previously, DAS has been used primarily to study disordered samples, i.e., liquids[14] and a variety of amorphous solids.[11,15,16] The present work represents one of the first applications of DAS to discrete molecular complexes. To our knowledge, this also represents the lowest concentration for which DDFs have been successfully determined. Notably, our data were acquired in less than 6 h/sample.

Our success is due to the scattering strength of W and the large $\Delta f'$ near the W L_{III} edge, as well as to the high X-ray flux available at SSRL. Many transition metals have $\Delta f'$ values within a factor of 3 of $\Delta f_w'$. Hard X-ray sources with fluxes 2-3 orders of magnitude higher than the source we used should be available in the future. Given these facts, and longer measurement times, structural characterization of solutes as dilute as 1 mM may be possible via DAS.

NMR and EXAFS are often used in structural studies of metalloproteins. In comparison with these techniques, DAS has the advantages of being applicable to most elements and sensitive to the long-distance (5-10 Å) interactions, which cannot be detected by EXAFS. Our work suggests that DAS is applicable to many more systems, and at lower concentrations, than has been appreciated previously.

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Supplementary Material Available: Plots of normalized X-ray scattering intensity in *k* space (3 pages). Ordering information is given on any current masthead page.

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