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EXAFS Studies of Binuclear Iron Complexes as Models for Hemerythrin and Related Proteins

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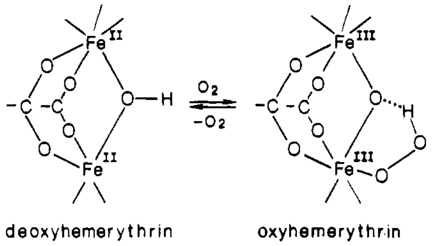
There is increasing interest in oxo-bridged polyiron centers in biology.[2] Binuclear units of this kind occur in hemerythrin,[3] the marine invertebrate oxygen carrier, in ribonucleotide reductase,[4] and in purple acid phosphatases.[5] The reversible binding of dioxygen in hemerythrin has been postulated to occur as shown by eq 1,[6] where the bridging carboxylate groups are contributed from glutamate and aspartate residues of the protein. Following the synthesis of realistic model compounds for both oxidized[7] and reduced[8] forms of the hemerythrin iron core, as well as analogues of the former in which either the oxo bridge was protonated[9] or the bridging carboxylate ligands were replaced by diphenylphosphate groups,[10] we have been working to establish their relevance to the chemical and physical properties of the proteins. Previously, we showed close similarities between the molecular geometry, magnetic exchange interactions, and optical spectroscopic and vibrational properties of azidomethemerythrin and the model compounds $[Fe_2O(O_2CCH_3)_2(HB(pz)_3)_2]$ (**1**) and $[Fe_2O(O_2CCH_3)_2(TACN)_2]^{2+}$ (**2**), where $HB(pz)_3^-$ = hydrotris(1-pyrazolyl)borate and TACN = 1,4,7-triazacyclononane.[11] We now report the results of extended X-ray absorption fine structure (EXAFS) studies of these two complexes as well as the derivatives $[Fe_2O\{O_2P(OC_6H_5)_2\}_2(HB(pz)_3)_2]$ (**3**) and $[Fe_2(OH)(O_2CCH_3)_2(HB(pz)_3)_2]^+$ (**4**). This work addresses in well-defined model systems how sensitive EXAFS spectroscopy is to the important question of modulations in the iron-to-bridging oxygen and nonbonded, e.g. Fe···Fe or Fe···P, distances. Such geometric information is necessary to understand the oxygen binding reaction (eq 1) of hemerythrin as well as the structural biology of ribonucleotide reductase and purple acid phosphatase.



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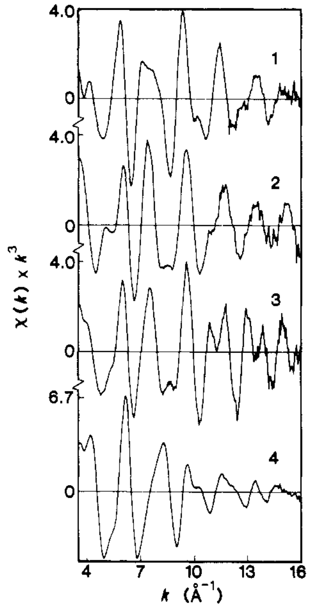

Figure 1. EXAFS spectra of compounds **1**–**4**. The spectra are multiplied by k^3 to enhance the visibility of oscillations at high k. Compounds **1** and **2** each have one oxo bridge and two acetato bridges, compound **3** has one oxo bridge and two diphenyl phosphato bridges, and compound **4** has one hydroxo bridge and two acetato bridges.

Experimental Section

Sample Preparation. Complexes $\mathbf{1}\cdot 4CH_3CN$,[11a] $\mathbf{2}I_2\cdot 0.5CH_3CN$,[11b] $\mathbf{3}\cdot CHCl_3$,[10] and $\mathbf{4}\cdot CH_2Cl_2$[9] were synthesized as described in the literature. The samples were ground to fine powders and mixed well with BN. The mixtures were compressed into uniform pellets for recording of the X-ray absorption data.

Data Collection and Analysis. X-ray absorption data were collected at the Stanford Synchrotron Radiation Laboratory on the focused beam line II-2 under dedicated conditions (3 GeV, 30–50 mA). The radiation was monochromatized by a Si(220) double-crystal monochromator. The spectra were collected as transmission data and are an average of 3, 8, 5, and 11 scans for compounds **1**–**4**, respectively. Energy calibration was performed by means of an Fe-foil internal standard,[12] assigning the first inflection point of the Fe absorption edge as 7111.2 eV. The data reduction was performed as described previously in detail.[13,14] In brief, the averaged data were reduced by subtracting a smooth polynomial pre-edge, extrapolated from the measured pre-edge. A three- or four-region polynomial spline was fit above the edge and subtracted from the observed data, after which the data were normalized to the atomic falloff. The spline points were chosen to minimize the residual low-frequency background while not reducing the observed amplitude of the EXAFS as judged by monitoring the Fourier transforms. The normalized, background-subtracted data were converted to k space by assuming a threshold energy (E_0) of 7130 eV. The photoelectron wave vector k is defined by $k = (2m_e(E - E_0)/\hbar^2)^{1/2}$, where m_e is the electron mass.

Data analysis of the EXAFS was performed with curve-fitting techniques using empirical parameters, as described in ref 15. The following models were used to obtain phase and amplitude parameters for different Fe–X scattering pairs: Fe–O and Fe–C, $Fe(acetylacetonate)_3$;[16] Fe–N, $[Fe(TPP)(imidazole)_2]Cl$;[17] Fe–Fe, $[(8\text{-hydroxy-2-methylquinolinato})_2Fe]_2O$.[18] All curve fitting was based on least-squares minimization[14] using k^3-weighted data. Fourier filtering was used to isolate the first (O, N) and second (Fe, C, N, O, P, B) shells. Fitting was done for each of these two individual filtered shells as well as for the combined shells in unfiltered data (about 0.75–5.5 Å) and gave the same

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Table I. Results of Single and Multiwave Fits to EXAFS for Compounds **1–4**[a]

	Fe–O		Fe–N		Fe–O′		Fe···Fe		Fe···X		
compd/bridge	R, Å	n^c	R, Å	n^c	R, Å	n^c	R, Å	n^c	R, Å	n^c	F^b
1	2.10	2.7									0.97
	2.09	3.1	2.26	1.4							0.94
μ-oxo, acetato	2.07	3.5	2.23	2.5	1.80	1.2					0.77
	2.07	3.5	2.23	2.6	1.80	1.2	3.16	1.7			0.59
structure	2.043	2	2.164	3	1.784	1	3.146	1			
2	2.10	2.3									1.06
	2.09	2.6	2.25	1.8							1.01
μ-oxo, acetato	2.06	2.8	2.23	2.4	1.78	1.3					0.75
	2.06	2.8	2.23	2.3	1.78	1.3	3.07	1.3			0.64
structure	2.042	2	2.179	3	1.781	1	3.063	1			
3	2.10	2.7									1.01
	2.08	2.9			1.80	0.9					0.91
μ-oxo, diphenyl phosphato	2.07	3.5	2.25	1.4	1.81	1.1					0.88
	2.07	3.5	2.25	1.4	1.81	1.1	3.33	1.8			0.74
	2.07	3.7	2.24	1.7	1.82	1.2	3.30	2.5	3.23	1.8[d]	0.39
structure	2.045	2	2.157	3	1.808	1	3.337	1	3.210	5[e]	
4	2.02	3.9									1.02
	1.98	3.2	2.10	3.6							0.92
μ-hydroxo, acetato	1.98	3.2	2.10	3.7			3.44	1.0			0.89
	1.98	3.1	2.10	3.8			3.44	1.0	3.09	4.8[f]	0.75
structure	1.985	3	2.102	3			3.439	1	3.10	9[g]	

[a] EXAFS fitting range $k = 4\text{–}16$ Å^{-1}. Errors are estimated to be about ±0.03 Å for distances and about 25% for coordination numbers.[15a] Each fit has two variable parameters per wave. [b] F, the goodness of fit, is defined as $\{[\sum k^6(\text{data} - \text{fit})^2]/(\text{no. of points})\}^{1/2}$. [c] The number ($n$) of ligands at the distance (R) indicated. [d] Parameters for the P wave modeled with an S wave; see text. [e] The average includes two P and three C atoms. [f] Parameters for a C wave. [g] The average includes three C atoms, five N atoms, and one B atom.

Table II. Interatomic Distances in the Second Shell of Scatterers around Fe[a]

compd/bridge	scatterer	no. of atoms/Fe	dist range, Å	mean dist, Å
1	Fe–C	2	3.004–3.014	3.010
	N	3	3.081–3.129	3.098
μ-oxo, acetato	Fe	1	3.146	3.146
	C	3	3.195–3.256	3.214
	B	1	3.280–3.293	3.287
	O	2	3.341–3.357	3.348
2	Fe–C	1	2.933	2.933
	C	6	2.974–3.008	2.995
μ-oxo, acetato	Fe	1	3.063	3.063
	C	1	3.079	3.079
	O	2	3.310	3.310
3	Fe–N	3	3.059–3.132	3.086
	C	3	3.174–3.259	3.209
μ-oxo, diphenyl phosphato	P	2	3.205–3.217	3.211
	B	1	3.293–3.302	3.298
	Fe	1	3.337	3.337
	O	2	3.556–3.583	3.569
4	Fe–N/O	5	3.009–3.035	3.021
	C	3	3.150–3.196	3.178
μ-hydroxo, acetato	B	1	3.218	3.218
	O	2	3.386–3.454	3.420
	Fe	1	3.439	3.439

[a] Data are taken from the crystal structure determinations of **1**–**4**.[11a,b,10,9]

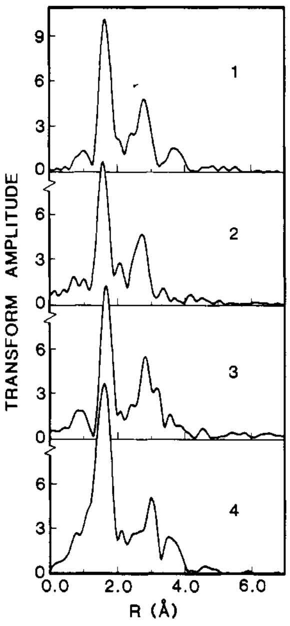

Figure 2. Fourier transforms of the EXAFS data for compounds **1**–**4**. The second shell is shifted toward higher R values as the Fe···Fe distance is increased (Table I). The peak positions are offset to lower R by an atom-dependent phase shift of about 0.4 Å.

results. As a control, fitting of raw EXAFS data was carried out for compound **3**. The results were the same as when the wide filter window was used. The fits reported were all done with the wide window. In the fits, only the structure-dependent parameters c_0 and a_1, i.e. the distance and the number of atoms in each given shell, were allowed to vary.[15b] EXAFS is insensitive to small changes in the atomic number of the scattering atom, making N and O atoms indistinguishable. Assignments of the scatterers as N or O were made according to the known structures.

Results and Discussion

Given the excellent quality of the data to high resolution (k = 16.5 or 17.0 Å^{-1}), it was possible to compare the results for the four compounds to determine how certain changes in structure are reflected in the amplitude and/or phase of the EXAFS oscillations. Furthermore, it was interesting to compare EXAFS data for the various model compounds with data from hemerythrin. Raw data are provided as supplementary material.

In Figure 1, EXAFS (multiplied by k^3) spectra are compared for the four compounds. There is a distinct similarity in the frequency of the EXAFS for the two oxo-bridged complexes, **1** and **2**, although the amplitudes of individual oscillations differ somewhat. For the phosphate-bridged compound **3** the first few oscillations are also quite similar to those in acetate-bridged compounds **1** and **2**, while the higher k oscillations have a different structure, presumably due to the increased Fe···Fe distance and the presence of the heavier P scatterer in the shell. The EXAFS spectrum of the hydroxo-bridged compound **4** is markedly different, however, from that of the other compounds in its frequency, amplitudes, and amplitude envelope, revealing the strong influence on the EXAFS of the absence of a short Fe–O distance and the increase of the Fe–Fe distance to 3.44 Å (Tables I and II). This difference is also reflected in the shape and amplitude of the derived Fourier transforms (Figure 2).

The results of the curve fitting analyses are summarized in Table I and are based on fittings over the range k = 4–16 Å^{-1}. Crystal structure determinations of **1–3** have shown that, in the first coordination shell of Fe, the average long Fe–O (acetate) distance is 0.11–0.15 Å shorter than the average Fe–N distance.[7,10,11] In the curve fitting, the O/N atoms could have been included in the model by using one O or N wave while floating the Debye–Waller term[15b] to accommodate the range of distances. With data ranging to k_{max} = 16 Å^{-1}, however, the resolution is approximately that which should allow separate waves for O and N atoms. This latter approach significantly improved the fits, by an average decrease of more than 0.1 in F, the goodness of fit (Table I), for first shell, and therefore these results are given in Table I. It should be noted that the Fe–N distances from the EXAFS fits are in general 0.05 Å longer than those from the crystal structure determination. Also, the coordination number determination for O and N suffers from high correlation, whereas the *sum* of coordination numbers obtained is consistent with the actual structures. It is likely that the slightly larger Fe–N distances derive from correlation with the Fe–O wave, possibly coupled with a small error in the transferability of Fe–N parameters.

Curve fits for compounds **1–3** showed a remarkable sensitivity to the presence or absence of a short Fe–O (μ-oxo) distance. F decreased by an average of 0.3 for the filtered first shell fit, and as expected, the fit of the frequency of the EXAFS was greatly improved when the short Fe–O distance was added to fits (filtered and unfiltered data) of complexes **1–3**. Attempts to include a short Fe–O shell for hydroxo-bridged compound **4** at different stages of the refinements consistently led to an increase of the initial Fe–O distance to ~1.95 Å and, owing to high correlation, unrealistic values of both the distance to and number of other first shell atoms.

For all four compounds the second shell of atoms contains not only Fe but also one or more of the atoms P, O, N, C, and B with the distance distribution given in Table II. These atoms have a scattering power very different from that of Fe,[19] and their inclusion in the refinements is necessary to obtain a good fit. It is not always possible, however, to resolve atoms as close in distance as occurs for the second shells of these complexes,[20] and thus inclusion of a second type of scatterer can result in unreasonable refinement results even though the quality of the fit is improved. For the two (μ-oxo)bis(μ-acetato) complexes, **1** and **2**, inclusion of C both altered the Fe···Fe distance and gave a very low or negative number of Fe atoms, while the number of C atoms increased. In both compounds the Fe atoms are surrounded by 6–8 C/N atoms at distances very close to the Fe···Fe distance (3.06 and 3.15 Å, Table II), and it is characteristic that fitting C only (no Fe) for the second shell gives a better fit for compounds **1** and **2** than fitting Fe (with no C).

Second shell fits for compound **3** require Fe–P phase and amplitude parameters. Unfortunately, no acceptable Fe–P parameters could be derived from model compounds thus far investigated, and parameters for S from $[Fe(S_2C_8H_8)_2]^-$[21] were used instead. This approximation should be reasonable since S and P are expected to have quite similar scattering behavior. The inclusion of an Fe–S wave improved the fit (F decreased dramatically from 0.74 to 0.39), whereas inclusion of C resulted in a negative number of C atoms while decreasing F from 0.74 to only 0.57. Figure 3 illustrates the marked improvement of the fits upon addition of the short oxygen and, especially, the sulfur atoms in the refinements for compound **3**.

For compound **4**, where the Fe···Fe distance is more isolated from other Fe···X distances (3.44 Å, Table II), a resonable fit was obtained including both Fe *and* C, as reported in Table I.

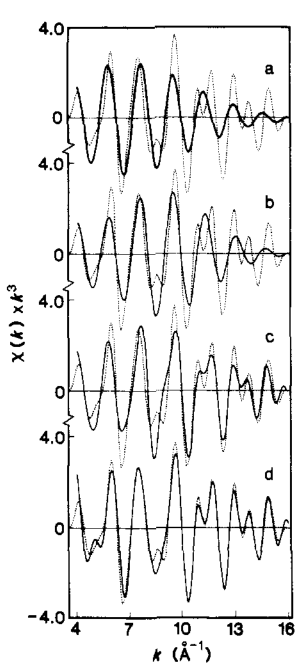

Figure 3. Comparison of fits for compound **3** upon inclusion of (a) only long oxygen distances, (b) O(long) + O(short), (c) O + O + N + Fe, and (d) O + O + N + Fe + S in the refinements: data, dotted line; fit, solid line. A Fourier filter of 1–5.5 Å was applied to the back transform of the observed data.

The Fe–Fe distances as determined from EXAFS deviate by less than 0.014 Å from those obtained in the crystal structure analyses, except for the diphenyl phosphate bridged compound, **3**. There the Fe···Fe distance is shorter by 0.037 Å while the Fe···P distance is longer by 0.020 Å. It may be noted that the Fe···Fe and Fe···P distances in **3** are both significantly shorter than the respective values of 3.36 and 3.27 Å reported from a recent EXAFS study of a polynuclear Fe(III)–ATP (4:1) complex.[22]

When the EXAFS of the compounds **1–4** in Figure 1 are compared with the EXAFS of azidomethemerythrin from *Phascolopsis gouldii* as shown in Figure 3 of ref 23, there is a remarkable similarity between the protein and compound **1**, $[Fe_2O(O_2CCH_3)_2(HB(pz)_3)_2]$. The Fe–O–Fe angle of 123.6° in **1** is outside the range where multiple scattering[24] should have any significant effect on the Fe···Fe distance obtained from the EXAFS. It is therefore likely that the somewhat high number of Fe atoms obtained for the azidomethemerythrin fit[23] is due to the severe interference of C and other light atoms at about the same Fe···X distance as Fe and not from multiple scattering effects. The Fe···Fe distance obtained in the fits of azidomethemerythrin would then be 3.20 Å, comparable to the 3.15-Å distance in compound **1** and substantially shorter than the values of 3.38[23] and 3.54 Å[25] obtained from the earlier EXAFS analyses.

The similarity between azidomethemerythrin and compound **1** is also seen by comparing their edges, which are superimposable. The energy resolution in the recorded spectra is degraded, however, by the presence of a focusing mirror before the monochromator, making a detailed study of the edge structure difficult. At the present resolution level the edges from compounds **1** and **2** are superimposable, the edge from compound **3** is shifted slightly to higher energy, and that for compound **4** is shifted to higher energy by less than 2 eV.

Fits were also performed over the k range 4–12 Å^{-1}, the typical working range for proteins where low iron concentrations usually

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give a high noise level in data at higher *k*. Over this more limited range, three-wave fits of the first shell became impossible and correlation effects between Fe and other atoms in the second shell increased significantly, increasing the calculated number of Fe atoms.

Conclusions

This investigation has shown that EXAFS spectra of oxo- and hydroxo-bridged iron complexes **1**–**4** are highly sensitive to the presence or absence of a short μ-oxo Fe–O distance and that this geometric feature can be unambiguously determined. This result provides further evidence in confirmation of the conclusions reached about the presence of such a short iron–oxo bridging distance in azidomethemerythrin.[24,26] The ability of EXAFS spectroscopy to differentiate Fe–oxygen bridge bond lengths in the 1.78 vs. 1.95 Å distance ranges should prove to be valuable in assessing the stereochemistry of semimet forms of hemerythrin, of deoxyhemerythrin (cf. ref 25), and of the binuclear iron centers in ribonucleotide reductase and purple acid phosphatase. It has also been demonstrated that great care must be exercised in determining the second-shell contributions to the EXAFS, since the Fe$\cdots$Fe scattering can be severely influenced by the presence of C or other low *Z* atoms at the same distance range from Fe as Fe itself. This perturbation can affect especially the determination of the number of neighboring Fe atoms, a problem that is further exacerbated if the *k* range of data is more limited, as is often the case with EXAFS data for dilute metalloproteins. On the positive side, however, the dramatic improvement of the fit for the phosphate ester bridged complex **3** upon addition of the P(S) atom to the second shell augurs well for the potential utility of EXAFS spectroscopy to delineate structural details of iron phosphate centers in biology.

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Registry No. **1**, 86177-70-0; **2**, 103835-68-3; **3**, 96502-34-0; **4**, 90886-30-9; ribonucleotide reductase, 9040-57-7; purple acid phosphatase, 9013-05-2.

Supplementary Material Available: Listings of raw data for compounds **1**–**4** (20 pages). Ordering information is given on any current masthead page.

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Raman Spectral Study on the Solution Structure of the Chromium(III)-edta Complex

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The complex of chromium(III) with ethylenediaminetetraacetate (edta) was first prepared by Britzinger et al.[1] and was formulated as H[Cr(edta)]. However, Schwarzenbach et al.[2] later proposed the formula [Cr(Hedta)(H_2O)], in which the edta coordinates as a quinquedentate ligand with a protonated carboxylate group. This structure was later supported by an IR study[3] and recently was confirmed by a single-crystal X-ray analysis.[4] The sodium salt of the chromium(III)–edta complex, however, was shown by IR analysis to contain sexidentate coordinated edta.[5] The sexidentate structure was later confirmed by X-ray crystallographic analysis of the ammonium and rubidium salts of $[\mathrm{Cr(edta)}]^-$[6] and, also, of the lithium and potassium salts.[7]

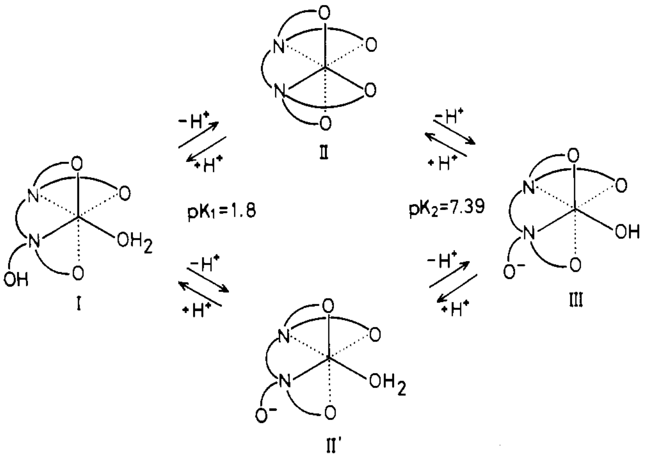

Figure 1. Possible equilibria of Cr(III)–edta (pK_a values from ref 8).

In addition to structural interests, chromium(III)–edta has attracted considerable attention because of its anomalously rapid substitution reactions in solution.[8-11] It is well-known that the edta complex of chromium(III) has two acid dissociation steps, which have been interpreted as the loss of a proton from the uncoordinated carboxylic acid group followed by the loss of a proton from the coordinated water molecule (shown by I $\leftrightarrow$ II' $\leftrightarrow$ III in Figure 1).[2,8,10] Thus, it was believed that the edta remained coordinated to Cr(III) as a quinquedentate ligand over a wide pH range, although a sexidentate complex had been proposed at slightly acidic pH. Very recently, however, Wheeler and Legg[12] were able to show by deuterium NMR spectroscopy that edta forms a sexidentate complex with Cr(III) between pH 3.5 and 6.5 (shown as I $\leftrightarrow$ II $\leftrightarrow$ III in Figure 1).

Raman spectroscopy also has a high potential for characterizing the structure of coordination compounds in aqueous solution.[13-16] Krishnan and Plane[16] reported that the Raman spectra exhibit very intense bands in the 400–500-cm^{-1} region due to skeletal vibrations in complexes where some covalency is expected between metal and ligand. They also noted that the spectra of Cu(II)–edta and Hg(II)–edta complexes changed at both low and high pH, consistent with a single protonation of the ligand in acid solution

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