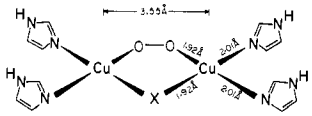

Figure 2. Model proposed for oxyhemocyanin active site. Two copper atoms separated by 3.55 Å are each bound to two imidazoles and two oxygen atoms in an approximately square-planar geometry, bridged by a bound peroxide and a protein ligand X. It should be emphasized that EXAFS analysis alone cannot give orientation information. The model is derived from a synthesis of EXAFS and edge results and other published chemical and spectroscopic data.

were not constrained to have integer values, indicated 2.0 imidazole groups at 2.01 Å, 2.0 oxygen atoms at 1.92 Å, and 1.1 copper atoms at 3.55 Å around each copper atom. This analysis identifies the presence of two imidazole ligands in contrast to the single-filtered-shell method used in the earlier studies[2,3] where only the number of atoms in the first shell was determined and imidazoles were suggested to be present on the basis of absorption edge and other spectroscopic information.

Oxyhemocyanin data from another mollusc, the α and β components of *Helix pomatia*, were collected and gave similar results. All the curve-fitting results are summarized in Table I. These results suggest that the binding sites for the intact *Megathura crenulata* and the α and β components of *Helix pomatia* hemocyanin are identical, within the experimental error of the technique. However, the small difference could also be the result of slight strain on the binuclear copper site due to different protein conformations, which might explain the different oxygenation curves observed for the α and β components of *Helix pomatia*.[23]

From this structural information and earlier spectroscopic results, it is possible to propose a model for the oxyhemocyanin binding site. The model emerging from our EXAFS analysis suggests two copper atoms separated by 3.55 Å. Each copper is in an approximately square-planar geometry and is bound to two histidines with an average Cu–N distance of 2.01 Å and two oxygen atoms at an average distance of 1.92 Å. The Cu–Cu distance of 3.55 Å precludes the possibility of direct Cu–Cu bonding but is reasonable for bridging by an oxygen atom. The bridging concept has also been suggested as a superexchange path for an antiferromagnetic coupling between two Cu(II) ions, explaining the lack of an EPR signal for oxyhemocyanin.[14] The single atom bridging model is consistent with the high coupling constant.[15] Tyrosine has been previously suggested as a possible bridging ligand, on the basis of an observation that tyrosine becomes accessible upon the removal of copper.[10,11] However, the lack of an observed charge transfer between tyrosine and Cu(II) and the high pK_a value for tyrosine make this suggestion questionable. The fourth ligand bound to copper can then naturally be assigned as the bound dioxygen (peroxide). The other oxygen atom from the peroxide cannot be located in the curve-fitting analysis. This can be explained by the minimal contribution to the scattered wave by a single low Z atom at a long distance, in the presence of many other atoms. On the basis of EXAFS and other studies, two models are most feasible, either bridging of the peroxide end to end or bridging through a single oxygen. Resonance Raman studies indicate that the peroxide oxygen atoms are equivalent.[6] These studies and a transition dipole–vector coupling model[14] both favor the end-to-end alternative (Figure 2). Preliminary results from application of the imidazole group fitting technique to EXAFS data on deoxyhemocyanin also indicate the presence of two imidazoles bound to each copper atom,[24] consistent with the proposed oxyhemocyanin model. Studies of deoxy, half-apo, and dimer forms of hemocyanin will be reported in a subsequent communication.[24]

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Metalloprotein EXAFS. A Group Fitting Procedure for Imidazole Ligands

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The EXAFS (extended X-ray absorption fine structure) technique has been demonstrated to be useful in extracting detailed structural information about active sites in metalloproteins and metalloenzymes.[1] The curve-fitting techniques now used in our laboratory make use of a parameterized function to model the effect of each single shell of atomic scatterers on the absorption coefficient of the central metal atom.[2] Until recently, for ligands consisting of more than one shell of atoms, it was necessary to include optimizable parameters for each shell. Second-shell atoms required a different set of parameters than first-shell atoms due to differences in scattering characteristics.[3] We have now developed a method for fitting the EXAFS of a *rigid* multiatom ligand as a whole ("group fitting"[4]) and tested its utility for fitting imidazole ligands to copper atoms in several copper proteins. A major advantage of the group fitting technique is that it allows a reduction in the number of variables used in the fit. Another important advantage lies in the technique's ability to account for different phase and amplitude behavior in the second and third shell of scatterers, thereby enhancing the technique's sensitivity toward other outer shell atoms.

The essence of our standard curve-fitting analysis is the adjustment of structure-dependent parameters in the function chosen to model the EXAFS until the fit with the experimentally observed data is optimized by least squares. In general, any atom in the vicinity of the absorber contributes to the EXAFS and the fitting procedure optimizes a distance and number for each atom. The group-fitting technique makes use of the fact that, within a given ligand of n atoms, the distance and number for $n-1$ of the atoms are highly correlated with these parameters for the other atom. Thus, only one distance and one number need be optimized, with the other parameters being constrained to obey certain relationships with the optimized parameters. These relationships are then defined by fits of model compounds containing only the multiatom ligand.

For example, in order to determine group-fitting parameters for imidazole ligands around a copper atom, $Cu(Im)_4^{2+}$ was used as a model. Three tetrakis(imidazolato)copper(II) compounds (the nitrate, perchlorate, and sulfate salts) were studied. They all give, within experimental error, identical spectra, a typical one being shown in Figure 1. The Fourier transform of the data in

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(3) When there are other atoms lying between the absorber and the scatterer of interest, these intervening atoms cause distortions of the primary outgoing wave, resulting in a different absorber–scatterer phase shift and backscattering amplitude function. This distortion cannot be accounted for using only first-shell fitting parameters. (See: Cramer, S. P.; Dawson, J. H.; Hodgson, K. O.; Hager, L. P. *J. Am. Chem. Soc.* **1978**, *100*, 7282.)

(4) A similar technique is also used in refinements of X-ray crystallographic data where a rigid ligand such as a phenyl ring is described as a group having only one x,y,z coordinate and a group temperature factor.

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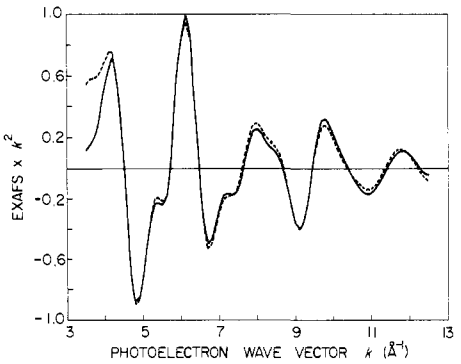

Figure 1. Imidazole group fitting on $Cu(Im)_4(ClO_4)_2$. The Fourier filtered data (solid line) are fit with imidazoles and oxygen atoms of the anion (dashed line).

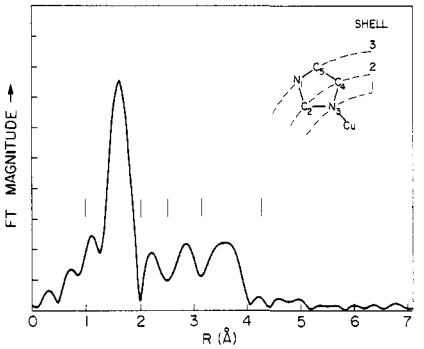

Figure 2. Fourier transform for $Cu(Im)_4(ClO_4)_2$ over the k range of 3.5 to 12.5 Å^{-1} (with k^3 weighting). The major peak is attributed to the scattering from the coordinated nitrogen atom (N_3) of the imidazole (shell 1). The first minor peak is the scattering from the oxygen atom of the anion. The second and third minor peaks are the backscattering peaks due to shell 2 (C_2, C_4) and shell 3 (N_1, C_5) of the imidazole. The bars indicate the windows used for filtering out each peak.

Figure 1 over the k range of 3.5–12.5 Å^{-1} is shown in Figure 2. The major peak at 1.6 Å[5] is attributed to the nearest-neighbor scattering [i.e., scattering from the coordinated nitrogen atoms (N_3) of the imidazoles]. The minor peak at 2.2 Å is contributed to partly from the side lobe of the main peak (resulting from truncation of the data over a finite range) and partly from the loosely coordinated oxygen atoms of the anions. This transform peak is dependent on the physical state of the sample, being smaller for data collected on $Cu(Im)_4^{2+}$ in solution. This suggests that the axial anions are more loosely coordinated in solution. The backscattering peak at 2.8 Å is due to the second shell of carbon atoms (C_2, C_4) from the imidazoles (see inset of Figure 2). The broadened peak at 3.6 Å is the backscattering peak due to N_1 and C_5 of the imidazoles, the third shell of atoms in the imidazole group. Each shell of atoms in the imidazole group can be independently fit to an expression of the form:[2,6]

$$\chi(k) \simeq [c_1 \exp(c_2k^2)/k^{c_3}] \sin(a_1 + a_2k + a_3/k) \quad (1)$$

The linear phase parameter a_2 contains the distance information, whereas the amplitude parameter c_1 contains information as to the number of scatterers in the shell. In the standard curve fitting analysis, c_1 and a_2 are adjustable for each shell. However, in the case of the three imidazole shells, the c_1's and a_2's are correlated among themselves in such a way that the amplitude-dependent c_1's for the three shells can be constrained to remain in a constant ratio and the distance-dependent a_2's for the three shells can be constrained according to the geometry of the imidazole ring. Therefore, for the entire imidazole group, only two parameters are adjustable, one to predict the coordination number (c_1) and one to obtain the $Cu-N_3$ distance (a_2). Two assumptions are implicit in applying the above constraints: (1) the copper–imidazole pair is treated as a symmetric planar structure, and (2) the $Cu-N_3$ distances in the models are similar to the corresponding distances in the unknowns (proteins). These assumptions appear to be at least approximately satisfied on considering X-ray crystallographic studies of a variety of copper–imidazole compounds.[7-13] The determination of the other parameters in eq 1 (c_2, c_3, a_1, a_3) proceeded as follows. The Fourier peaks due to the three shells of the imidazole group were independently filtered (as indicated by the bars in Figure 2) and retransformed into k space. Each filter was then curve fit to the expression in eq 1 to give an initial set of optimized values for the parameters in each shell. These parameters were then used as starting values in a fit to the full data set using all shells and varying the parameters one shell at a time. Cycling through the shells to obtain one set of completely optimized parameters for all shells gave rise to the definition of the constraints on c_1 and a_2 for the second and third imidazole shells and also yielded fixed values for c_2, c_3, a_1, and a_3 for each shell.

For a test of effectiveness of the imidazole group fitting procedure, EXAFS spectra were recorded on several copper–imidazole complexes as well as two copper complexes of histidine-containing peptides. The parameters used in the group-fitting procedure were determined from fits on data from $Cu(Im)_4(ClO_4)_2$ as described above. The results of imidazole group fits on all of the copper–imidazole models studied are summarized in Table I. The relationship between c_1 in eq 1 and the number of imidazoles calculated (Table I) was defined by the fit on the reference compound, $Cu(Im)_4(ClO_4)_2$. For each compound, the curve fitting included contributions from the imidazoles and other appropriate atoms which were indicated to be in the copper coordination sphere (e.g., the oxygen atoms of the anions in the $Cu(Im)_4^{2+}$ complexes).

Analysis of four tetrakis(imidazolato)copper(II) compounds all predict $Cu-N_3$ distances to within ±0.02 Å of the crystallographic distances. The group-fitting technique also predicts the correct coordination number to within 10%, except for $Cu(Im)_2(Im^-)Cl$ which is 20% lower than the expected coordination number. The low value for this complex can be explained by the static spread of $Cu-N_3$ distances observed in the crystal structure. The same phenomenon is observed when curve fitting with single atoms is performed. Analysis of copper complexes with histidine-containing peptides predicts the correct $Cu-N_3$ distances to within ±0.02 Å. Analysis of the copper–carnosine complex

Table I. Imidazole Group Fits for Copper Imidazole Models

	EXAFS		crystallographic		
compd	$Cu-N_3$ distance, Å	no.	$Cu-N_3$ distance, Å	no.	ref
$Cu(Im)_4(ClO_4)_2$[a]	2.00	4.0	2.00	4	7
$Cu(Im)_4(NO_3)_2$	2.01	3.9	2.01	4	8
$Cu(Im)_4SO_4$	2.02	3.7	2.01	4	9
$Cu(Im)_2(Im^-)Cl$	2.00	3.2	2.02	4	10
Cu-carnosine[b]	2.00	1.2	2.01	1	11
Cu-GGH[c]	1.93	2.0	1.96	1	12
Cu-His[d]	1.95	1.5	1.95[e]		13

[a] All of the $Cu-N_3$ distances and coordination numbers are calibrated with respect to this compound. [b] (β-Alanyl-L-histidinato)copper(II) dihydrate. [c] (Glycylglycyl-*O*-methyl-L-histidinato)copper(II). [d] Copper(II) L-histidine in 1:2 molar ratio solution [0.1 M Cu(II)]. [e] This distance was obtained from the crystal structure of the (L-histidinyl-L-threoninato)copper(II) hydrate complex.[13a]

(5) The distances quoted are in R (frequency) space. A phase shift of 0.3–0.6 Å should be added to obtain the real distances.
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Table II. Imidazole Group Fits for Metalloproteins

protein	$Cu-N_3$ distance, Å	no.	ref
plastocyanin (*Phaseolus vulgaris*)			
oxidized	1.98	2.0	14
reduced	2.05	1.8	14
hemocyanin (*Megathura crenulata*)			
oxygenated	2.01	2.0	15
deoxygenated	1.95	2.0	16

predicts 1.7 imidazoles at 2.00 Å. The predicted distance is consistent with the crystallographic distance, but the predicted coordination number is much higher than actually occurs. However, including the contribution of backscattering by the peptide carbons at ca. 3.0 Å causes the number of imidazoles predicted to drop to 1.2, within the 20% experimental uncertainty. Application of this technique to the copper complex of the tripeptide glycylglycyl-*O*-methyl-L-histidine [Cu(GGH)] predicts 2.0 imidazoles at 1.93 Å. Again, the predicted $Cu-N_3$ distance is correct, but two imidazoles instead of one are suggested, even when the peptide carbons are included in the fit. The crystal structure of this complex[12] indicates that the peptide carbons, peptide nitrogens, and carboxy oxygen are arranged in such a way as to simulate an imidazole group, causing the fitting procedure to predict an additional imidazole. This type of problem may be unique to small copper–peptide complexes, since there are no known protein examples of copper coordination to peptide amides adjacent to a ligating histidine. EXAFS was also recorded on a solution of Cu(II) and histidine in a 1:2 molar ratio. Application of the group fitting technique to these data predicts the presence of 1.5 imidazoles at 1.95 Å. One explanation may be that the solution exists as a mixture of two species, with one and two imidazoles coordinated per copper atom. An alternate explanation is that two imidazoles are coordinated at slightly different distances, resulting in prediction of a lower coordination number due to static disorder. The $Cu-N_3$ distance obtained is consistent with the crystallographic distance in copper–histidine complexes.[13] For further investigation of the utility of this technique in fitting imidazole ligands, fits were performed on two copper compounds where no imidazoles are present [$Cu(acac)_2$ and $Cu(NH_3)_4^{2+}$]. In both cases, when fitting with imidazoles and other appropriate atoms, numbers of imidazoles significantly less than one were obtained. If imidazoles only are assumed to be present, entirely unsatisfactory fits result.

The results of application of the imidazole group fitting technique to some histidine-containing copper proteins being studied in this laboratory are summarized in Table II. Other appropriate first-shell atoms (in addition to the imidazoles) were included in the fit. Results for a blue copper protein (plastocyanin) and an oxygen-carrying protein (hemocyanin) are presented. The oxidized and reduced plastocyanin show coordination of two imidazoles (consistent with the low-resolution X-ray crystallographic results[17]) at 1.98 and 2.05 Å, respectively. Similar studies on hemocyanin indicate that two imidazoles are coordinated to each copper atom for both oxygenated and deoxygenated forms. The details of these metalloprotein studies will be reported in other communications.[14-16]

These results indicate that the imidazole group-fitting technique can be successfully used to reveal the structural details of histidine ligands in copper metalloproteins. The extension of the group fitting technique to other commonly occurring biological ligand structures, such as porphyrins or carbon monoxide, is currently being pursued.

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New, Systematic, Good Yield Syntheses of Boron Hydrides: Preparation of B_4H_{10} and B_5H_{11}. A Practical Conversion of B_5H_9 to $B_{10}H_{14}$

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One of the principal handicaps in the investigation of the chemistry of the intermediate boron hydrides B_4H_{10} and B_5H_{11} has been the absence of simple preparative procedures which would provide these materials in relatively large quantities in good yield.[1,2] We report here a new systematic approach to boron hydride syntheses which not only meets these requirements for the preparation of B_4H_{10} and B_5H_{11} but has also been extended to the preparation of $2\text{-}BrB_4H_9$ and produced a simple conversion of B_5H_9 to $B_{10}H_{14}$.

The systematic nature of these syntheses relates to our observation that hydride ion can be abstracted from certain boron hydride anions to give as one of the final products a neutral boron hydride which contains one more boron atom than the anionic starting material. The simplest reaction observed (*1*) involves

$$BX_3 + [N(n\text{-}C_4H_9)_4][BH_4] \xrightarrow[CH_2Cl_2]{\text{room temperature}} [N(n\text{-}C_4H_9)_4][HBX_3] + {}^1/_2B_2H_6 \quad (1)$$
$$X = Cl, Br$$

$[N(n\text{-}C_4H_9)_4][BH_4]$[3] and appears to be quantitative. The tetra-*n*-butylammonium salts of the previously unreported anions $HBBr_3^-$ and $HBCl_3^-$ are stable, free-flowing solids under a dry atmosphere at room temperature [NMR data:[4] $HBBr_3^-$, $\delta_{^{11}B} = -13.0$ ($J_{^{11}B-^1H} = 176$ Hz); $HBCl_3^-$, $\delta_{^{11}B} = 3.1$ ($J_{^{11}B-^1H} = 158$ Hz)].

Tetraborane(10) and pentaborane(11) are prepared by reactions 2 and 3 in which 1:1 molar ratios of reactants are stirred vigorously in the absence of a solvent. Tetraborane(10) and pentaborane(11)

$$[N(n\text{-}C_4H_9)_4][B_3H_8] + BBr_3 \xrightarrow[1\text{ h}]{0\ ^\circ C} B_4H_{10} + [N(n\text{-}C_4H_9)_4][HBBr_3] + \text{solid BH residue} \quad (2)$$

$$K[B_4H_9] + BCl_3 \xrightarrow[2.5\text{ h}]{-35\ ^\circ C} B_5H_{11} + K[HBCl_3] + \text{solid BH residue} \quad (3)$$

are obtained in 65% and 60% yields, respectively. These yields are based upon the amount of boron in the borane anion. In both reactions the borane anion is completely consumed. The starting materials, $[N(n\text{-}C_4H_9)_4][B_3H_8]$ and KB_4H_9, for these reactions

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