X-ray Absorption Studies of the Cu-Dependent Phenylalanine Hydroxylase from *Chromobacterium violaceum*. Comparison of the Copper Coordination in Oxidized and Dithionite-Reduced Enzymes†

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ABSTRACT: The coordination chemistry of the Cu sites of phenylalanine hydroxylase (PAH) from *Chromobacterium violaceum* has been studied by X-ray absorption spectroscopy (XAS). The EXAFS of the Cu(II) form of the enzyme resembles that of other non-blue copper proteins such as plasma amine oxidases and dopamine-β-hydroxylase and is characteristic of a mixed N/O coordination shell containing histidine ligation. Detailed simulations of the raw EXAFS data have been carried out using full curved-wave restrained refinement methodologies which allow imidazole ligands to be treated as structural units. The results suggest a Cu(II) coordination of two histidines and two additional O/N-donor groups. A reasonable fit to both data sets can be obtained by assuming that the non-imidazole first-shell donor atoms are derived from solvent (H_2O or OH^-). The EXAFS of the reduced enzyme shows major differences. The amplitude of the first shell in the Fourier transform is only 50% of that of the oxidized enzyme, indicative of a substantial reduction in coordination number. In addition, the first shell of the transform is split into two components. Simulations of the reduced data can be obtained by either two histidines at a long distance of 2.08 Å and an O ligand at a short distance of 1.88 Å or two histidines at a short distance of 1.90 Å and one second-row scatterer such as S or Cl at 2.20 Å. Comparison of absorption edge data on the reduced enzyme with data from Cu(I) bis- and tris(1,2-dimethylimidazole) complexes suggests a pseudo-three-coordinate structure.

Phenylalanine hydroxylases (PAH) catalyze the hydroxylation of phenylalanine to tyrosine by O_2. The reaction is a monooxygenation in which one atom of O_2 is incorporated into tyrosine and the other ends up as water. Four electrons are transferred to O_2 in the process, two being derived from breaking the C–H bond of the substrate, and the other two from a reducing cofactor. In the case of PAH, the cofactor is a tetrahydropterin, which is oxidized by two electrons to the dihydropterin during phenylalanine hydroxylation.

In addition to the pterin cofactor, both the mammalian and bacterial PAHs contain a metal ion at their active site. The mammalian enzymes require 1 mol of non-heme iron per subunit for activity (Gottschall et al., 1982), while the bacterial enzyme from *Chromobacterium violaceum* (CV-PAH) contains a single copper atom per mole of enzyme (Pember et al., 1986). The role of the metals is not known with certainty, but reduction to the Fe(II) or Cu(I) is obligatory before catalysis can proceed (Wallick et al., 1984; Marota & Shinman, 1984; Pember et al., 1986). Additionally, there is no evidence that the metal ions undergo redox cycling during catalysis. This suggests that, unlike many other metal-dependent monooxygenases (Stewart & Klinman 1988; Lerch, 1981; Blackburn et al., 1991), metal-bound reduced dioxygen intermediates exist as transitory species, if at all. For both classes of enzyme, a 4a-OH tetrahydropterin is produced as an intermediate (Lazarus et al., 1981, 1982; Pember et al., 1986), and it has been postulated that the active species may be the 4a-peroxy pterin. The metal is believed to serve as the initial binding site for molecular oxygen and may subsequently facilitate transfer of O_2 to the pterin cofactor. Support for this premise has come from the detection of an enzyme-bound O_2 intermediate by kinetics and susceptibility measurements on the Cu PAH (Pember et al., 1989).

Investigation of the coordination chemistry of the Cu centers in the bacterial PAH is important in order to better understand the catalytic mechanism and its relationship to other Cu-dependent monooxygenases. Continuous wave multifrequency EPR has provided evidence for two or three N donors to the Cu(II) centers (McCracken et al., 1988), and pulsed EPR has clearly identified these as histidine residues (Pember et al., 1987a). S-band (1.2 GHz) studies of the interaction of 5-^{14}N- and 5-^{15}N-labeled 6,7-dimethyltetrahydropterin with PAH have established that the pterin is able to coordinate to the Cu(II) center via the 5-N atom without displacement of a histidine ligand (Pember et al., 1987a). This has posed the interesting hypothesis that the metal ion may bind both pterin and O_2 so as to provide spacial proximity for O_2 transfer from Cu to pterin. However, no data are available on the coordination environment of the Cu(I) form of PAH which is the catalytically active species. For this reason, we have embarked on an X-ray absorption spectroscopic study of PAH in both the oxidized and reduced states in order to delineate the coordination chemistry of the enzyme with its substrates and cofactors. In this paper, we present the results of our studies on the Cu(II)–PAH and the first preliminary data on the dithionite-reduced form of the enzyme.

Materials and Methods

Preparation of Enzyme Samples. The enzyme samples used in the present study were obtained by overexpression of re-

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combinant *C. violaceum* PAH in *Escherichia coli* as described in detail in Onishi et al. (1991). Enzyme purity was in all cases judged to be greater than 98% by SDS–PAGE. The enzyme was assayed by the procedure of Pember et al. (1987b), and all enzyme samples had a specific activity greater than 20 units/mg of enzyme. Oxidized enzyme samples were concentrated in an Amicon Centricon concentrator to approximately 3 mM, diluted 25% with glycerol, and transferred to the EXAFS cell [as described previously (Blackburn et al., 1991)]. Reduction of each of these samples was achieved by addition of a 2-fold molar excess of sodium dithionite (four reducing equivalents), by a gas-tight syringe through a septum, to a solution of enzyme made anaerobic by repeated flushing and purging with argon. The reduced enzyme was then transferred anaerobically to an EXAFS cell contained in an argon-purged septum vial and immediately frozen in liquid nitrogen.

Measurement of X-ray Absorption Spectra. Samples were measured on beamline X9A at NSLS, Brookhaven National Laboratory, with an electron beam energy of 2.5 GeV and a maximum stored current of 220 mA. The data were collected with a Si(111) double crystal monochromator and a grazing incidence mirror to reject harmonics. Samples were measured as frozen glasses at 100 K in fluorescence mode using a 13-element Ge detector. A 3-μm Ni foil was placed immediately in front of the detector. Energy calibration was achieved by setting the first inflection point of a copper foil spectrum to 8980.3 eV. For EXAFS measurements, the exit slit was set to 1.0 mm, and the resolution was estimated to be about 3 eV by inspection. For edge studies, a 0.2-mm slit-width was employed, giving an estimated resolution of 1 eV. EXAFS data reduction and analysis were performed as previously described (Blackburn et al., 1991), using curved wave multiple scattering calculations and restrained refinement methods which allow the imidazole ligands to be simulated as geometrically invariant groups. The complete parameter sets, including the parameters which define the internal geometry of the imidazole rings, are given for each simulation in Tables 1–7 of the Supplementary Material. The quality of the fits was determined using a least-squares fitting parameter or fit index, F, defined as

$$F^2 = (1/N)\sum k^6(\chi_i^{theor} - \chi_i^{exp})^2$$

Results

EXAFS of the Cu(II) Enzyme. XAS spectra of two different samples of native [Cu(II)] PAH have been collected. The background subtracted EXAFS of both samples are reproducible within experimental error. Minor differences exist in the region k = 10–12 Å^{-1}, but these are probably not significant with respect to data interpretation and represent differences in signal-to-noise, detector set-up, and/or background subtraction. The overall shape and amplitude of the oscillations resembles the EXAFS of the Cu(II) forms of other non-blue copper proteins (Scott & Dooley, 1985; Knowles et al., 1989; Blackburn et al., 1987; Blackburn, 1989), particularly that of dopamine-β-hydroxylase (Blackburn et al., 1991; Scott et al., 1988; Blumberg et al., 1989), and is typical of mixed imidazole/O donor ligation (Co & Hodgson 1981; Co et al., 1981; Blackburn et al., 1991; Blackburn et al., 1987). The Fourier transforms of the EXAFS data show an intense reproducible first-shell peak and weak outer-shell peaks from the C2/C5 and C3/N4 atoms of coordinated imidazole groups. These latter peaks are less intense than found previously for other members of the non-blue class of copper proteins and indicate a low histidine coordination number.

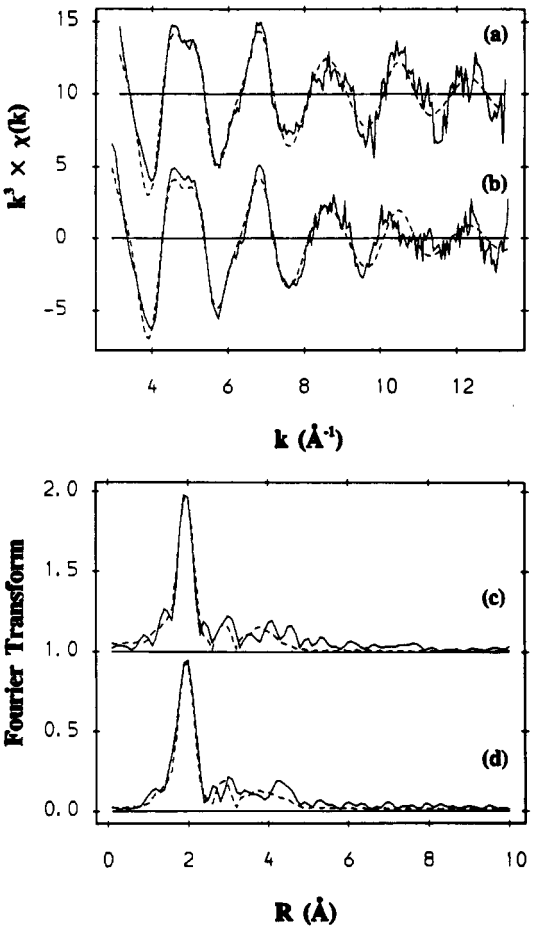

FIGURE 1: Experimental (solid) versus simulated (dashed) EXAFS and Fourier transforms for the Cu(II) center in oxidized phenylalanine hydroxylase from *C. violaceum*. (a) May 1991 data simulated using two imidazole groups and two additional O/N atoms at 1.98 Å, with a Debye–Waller term of 0.009 Å^2 (F = 1.88). (b) Nov 1990 data simulated with the identical parameter set (F = 1.79). (c) Fourier transform of trace a. (d) Fourier transform of trace b.

Table I: Parameters Used To Simulate the EXAFS of Oxidized and Reduced PAH

shell	distance (Å)	Debye–Waller (Å^2)
(A) Oxidized PAH, May 1991 Data, F = 1.88		
2 N (imidazole)	1.98	0.008
2 O/N	1.98	0.009
(B) Reduced PAH, Fit 1, F = 1.76		
2 N (imidazole)	1.89	0.012
1 S	2.20	0.015
(C) Reduced PAH, Fit 2, F = 2.98		
2 N (imidazole)	1.90	0.010
1 N (imidazole)	2.03	0.011
(D) Reduced PAH, Fit 3, F = 1.75		
1 O	1.88	0.002
2 N (imidazole)	2.08	0.008

Initial simulations were performed by searching for least-squares minima corresponding to different combinations of imidazole groups and additional low-Z (O or N) atoms in the first coordination sphere. In these simulations, the Cu–imidazole and other Cu–N/O first-shell interactions were refined as a single shell, using the same phase shift [Cu–N refined on $[Cu(imid)_4](NO_3)_2$, (Strange et al., 1987)]. The results are shown in Figures 1 and 2 and Table IA. Figure 1 shows the best fit obtained using two imidazole groups and two additional low-Z atoms (O or N) at 1.98 Å. This fit has been superimposed on both of the two experimental data sets

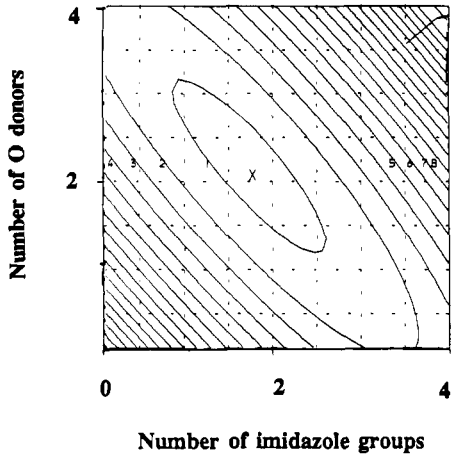

FIGURE 2: Contour map of the three-dimensional surface which describes the variation of least-squares fit index (F) as a function of the number of coordinated histidine and O/N-donor ligands (May 91 data). The number of imidazole groups and O/N atoms were varied between 0 and 4, and the distances and Debye–Waller terms were fixed at the values given in the legend to Figure 1. F_{min} = 1.85 at 1.77 histidines and 2.05 O/N donors. The lower contour in the diagram corresponds to F = 2.04 and the upper contour to F = 16.32. The increment between contours is 0.75.

(Figure 1a,b), and it can be seen that agreement is satisfactory. The imidazole groups have been simulated as complete structural groups, and complete details of the imidazole ring geometries employed in these and subsequent fits are given in Tables 1–7 of the Supplementary Material. Figure 2 shows the three-dimensional surface which is mapped out by the least-squares fitting parameter, F, as the number of coordinated imidazole and O/N groups are each varied between 0 and 4. It can be seen that the surface exhibits a long valley within which a local minimum is found. This minimum lies between 1.0 imidazole/3.0 O/N donors and 2.5 imidazoles/1 O/N donor. Thus it is clear that the Cu(II) center is almost certainly coordinated by four ligands, and it is unlikely that the number of bound histidines exceeds two. The value of the first-shell distance (1.98 Å) is normal for EXAFS-derived equatorial Cu(II)–imidazole bond lengths in non-blue copper proteins (Blackburn et al., 1987, 1991; Scott et al., 1988; Strange et al., 1987) and model compounds (Orpen et al., 1990; McFadden et al., 1975) and is also consistent with equatorial Cu(II)–OH_2 coordination.

Further simulations were carried out to test whether any improvement in the least-squares fitting parameter, F, accrued as the result of splitting the first shell into Cu–N(imidazole) and Cu–O interactions, using a Cu–O phase shift refined on a Cu(II)–phenolate model compound (Blackburn et al., 1988). This procedure resulted in a decrease in the Cu–O bond length to 1.93 Å. Cu–O bond lengths of 1.90–1.95 Å are often exhibited in monodentate complexes with phenolate or carboxylate ligands (Orpen et al., 1989), and a value of 1.93 Å would thus be consistent with the presence of Cu–O (Tyr, Asp, Glu). However, splitting the first shell did not lead to a decrease in the value of F, which remained unchanged for the November 1990 data and increased from 1.88 to 2.08 for the May 1991 data. In addition, the Debye–Waller term for the Cu–O term increased from 0.009 to 0.014 Å^2, opposite to what would be expected for a stronly bound Cu–O-type interaction. We conclude that whereas EXAFS cannot unambiguously exclude the presence of O-donor ligands other than water, confirmation by other spectroscopic techniques [e.g., UV/vis for phenolate–Cu(II) charge transfer] is necessary before any firm assignment can be made. In fact, additional evidence is more consistent with solvent as the source of the O–donor

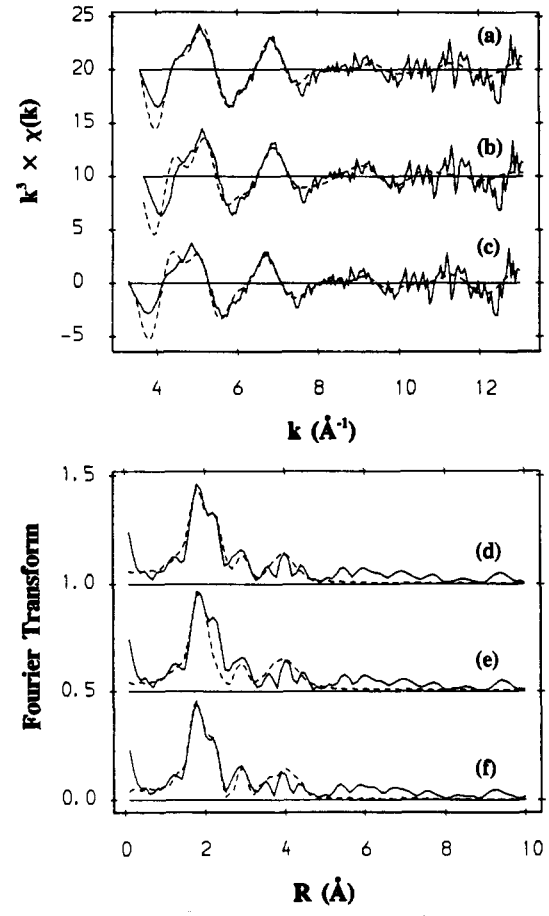

FIGURE 3: Experimental (solid) versus simulated (dashed) EXAFS and Fourier transforms for the Cu(I) center in dithionite-reduced phenylalanine hydroxylase from *C. violaceum*. (a) Data simulated with two imidazoles at 1.89 Å and one S/Cl at 2.20 Å, (F = 1.76). (b) Alternative fit with the S/Cl ligand replaced with a complete imidazole group at 2.03 Å (F = 2.98). (c) Alternative fit with one O at 1.88 Å and two imidazoles at 2.08 Å (F = 1.74). Traces d, e, and f represent the Fourier transforms of traces a, b, and c, respectively.

interaction, since coordination positions are available for binding of exogenous ligands. In particular, kinetic and EPR studies support preequilibrium complex formation between 6,7-dimethyltetrahydropterin and Cu(II) as an intermediate in the reductive activation of the enzyme (Pember et al., 1986, 1987a).

EXAFS of the Cu(I) Enzyme. EXAFS spectra and associated Fourier transforms of dithionite-reduced PAH are shown in Figure 3. These spectra correspond to dithionite reduction of the May 1991 oxidized sample shown in Figure 1a. Comparison of the amplitude and shape of the first shell of the FT indicates a substantial change in coordination at the Cu(I) center. The amplitude is reduced by at least 50%, and the peak is split into two well-resolved components. Curve-fitting analysis indicates that the data can be simulated by two imidazoles with Cu–N(imid) = 1.89 Å (F = 2.82), which is shorter by 0.1 Å than the Cu–N(imid) distance found in the oxidized enzyme. The two-imidazole fit on its own does not reproduce the split peak in the FT of the reduced enzyme. The latter can be well fit by including a second row scatterer (S/Cl) at 2.20 Å which decreases the value of the fit index by 62% (Table IB, F = 1.76). The significance of this extra shell in the FT is not clear at the present time since the data are of worse signal-to-noise than for the oxidized enzyme and care must be taken not to overinterpret the split shell at this time. The value of the Cu–N(imid) bond length is toward the short end of the range found for pseudo-three-coordinate complexes

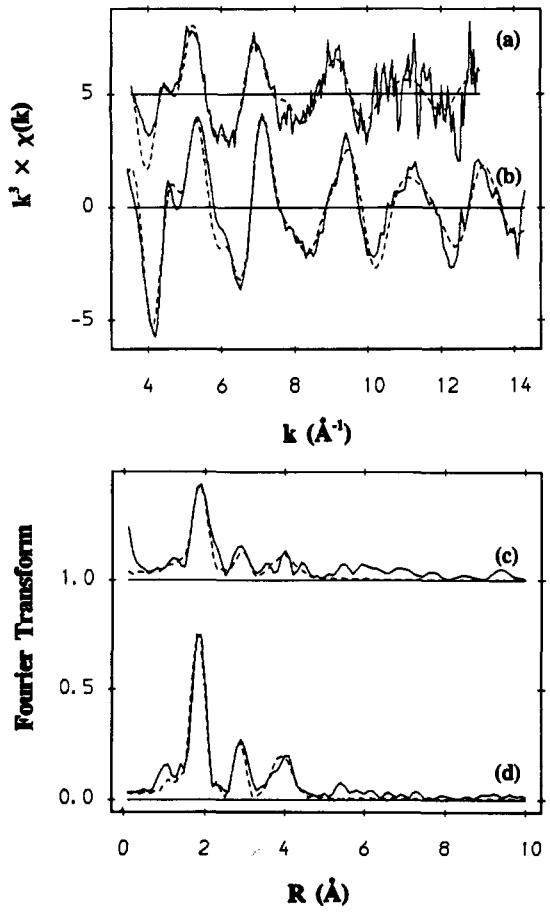

FIGURE 4: Difference analysis of the EXAFS of the Cu(I) center in *C. violaceum* phenylalanine hydroxylase. (a) Difference spectrum generated via subtraction of the S/Cl wave at 2.20 Å from the experimental data of the dithionite-reduced enzyme (solid line). This spectrum has been simulated by 1.6 imidazoles at 1.89 Å (dashed line) ($F = 1.50$). (b) Experimental (solid) versus simulated (dashed) EXAFS of the two-coordinate model complex $[Cu(1,2\text{-dimethylimidazole})_2]^+$. The simulation utilized two imidazole groups at the crystallographic distance of 1.86 Å ($F = 0.73$).

containing two short and one longer Cu–X interactions (Blackburn et al., 1984, 1989; Sorell & Borovick, 1987; Karlin et al., 1988), and hence the presence of a ligand at a longer distance is quite consistent with the observed imidazole ligation. A Cu–S/Cl interaction is unexpected, although the presence of a Cu(I)–S interaction has recently been confirmed in the EXAFS of the reduced form of dopamine-β-hydroxylase (DBH) (Scott et al., 1988; Blackburn et al., 1991), which is also a mononuclear Cu-dependent monooxygenase. However, PAH is mechanistically distinct from DBH in that the latter contains two nonequivalent mononuclear Cu centers, and no pterin cofactor, and there is thus no basis to propose structural similarity between the two enzymes.

For these reasons, we have attempted to simulate the reduced data using structural models which involve only O or N (imidazole) coordination. Figure 3b shows the best fit which could be obtained when the long Cu–S interaction was replaced by a complete imidazole group. The distances refined to 1.90 Å for the two short imidazoles and 2.03 Å for the long imidazole group (Table IC, $F = 2.98$). This fit is no improvement over two imidazoles on their own and fails to reproduce the shape of the k = 4–6-Å^{-1} region. Figure 3c shows the best fit obtained with one Cu–O interaction at 1.88 Å and two imidazoles at 2.08 Å (Table ID). The value of the fit index ($F = 1.75$) is now equivalent to that with the Cu–S/Cl wave, but the fit is visually worse in the k = 4–6-Å^{-1} region.

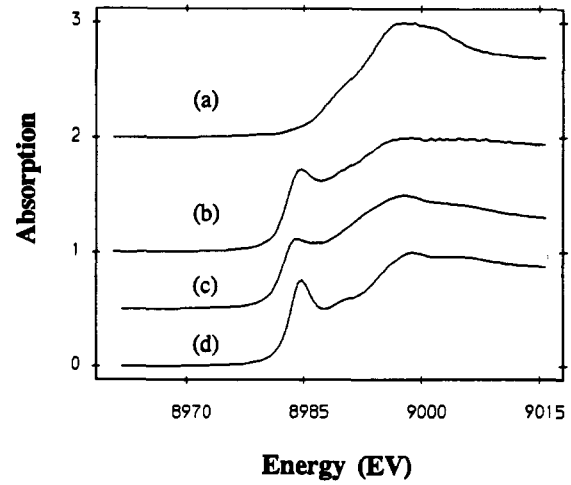

FIGURE 5: Absorption edge data for the Cu centers in *C. violaceum* phenylalanine hydroxylase and relevant model complexes. Si(111) crystals were used for measurements of the edges of both protein and model systems, with a slit width of 0.2 mm. The resolution was estimated to be 1 eV by inspection. (a) Oxidized PAH; (b) dithionite-reduced PAH; (c) three-coordinate $[Cu(1,2\text{-dimethylimidazole})_3]^+$; (d) two-coordinate $[Cu(1,2\text{-dimethylimidazole})_2]^+$.

As a further check on the validity of the models suggested by the simulations, we have performed an analysis using difference techniques. Figure 4 shows the data for the reduced enzyme after subtraction of a theoretical Cu–S wave equivalent to that used in the simulation above (Figure 3a). Figure 4b shows the experimental EXAFS of $[Cu(1,2\text{-dimethylimidazole})_2]^+$, which contains a linear two-coordinate Cu(I) center with Cu–N(imid) = 1.86 Å (Sanyal et al., manuscript in preparation) and a simulation of the data with metrical parameters close to the crystallographic values. Strong similarities are evident between the difference spectrum in Figure 4a and the model complex in Figure 4b. Furthermore, simulation of the difference spectrum with two imidazoles at 1.89 Å gives a good fit to the data ($F = 1.80$). Refinement of the first-shell Debye–Waller term to a value closer to that found in the model complex results in a Cu(I)–imidazole coordination number of 1.6 ($F = 1.50$) for the difference spectrum. This fit is also shown in Figure 4a. It should be noted that the k = 4–6-Å^{-1} region of the spectrum is now well fit. Indeed, simulations have shown that the relative amplitudes of the beat pattern in this region of the spectrum are very sensitive to the Cu–N(imid) first-shell distance. In the present case, the observed pattern (low k peak < high k peak) is characteristic of a Cu–N(imid) <1.90 Å, and as Cu–N(imid) approaches 2.0 Å the amplitudes of the two peaks tend to equalize (Figure 3c). This analysis indicates that the imidazole contribution is entirely consistent with an essentially two-coordinate site with Cu–N(imid) = 1.89 ± 0.02 Å.

Absorption Edges. Figure 5a,b compares absorption edges for oxidized and reduced PAH. As expected for a non-blue Cu(II) site, the oxidized enzyme exhibits a featureless edge with a half-maximal position around 8990 eV (Kau et al., 1987). The reduced enzyme on the other hand exhibits a well-resolved peak at 8984.5 eV. Such peaks have been studied in detail and shown to be characteristic of two-coordinate or pseudo-three-coordinate Cu(I) sites in trigonal or T-shaped geometries (Kau et al., 1987; Blackburn et al., 1989). Illustration of the relationship between coordination number and 8984 eV peak intensity is provided in Figure 5c,d. Figure 5d shows the absorption edge of the linear two-coordinate $[Cu(1,2\text{-dimethylimidazole})_2]^+$, which clearly shows an intense edge peak at 8984.8 eV. When 1 mol of 1,2-dimethylimidazole

is added to a solution of the bis complex in CH_2Cl_2, a three-coordinate adduct is formed (Sanyal et al., 1991), the structure of which is believed (from EXAFS) to be T-shaped with two short and one long imidazole groups. The edge of this adduct (Figure 5c) shows a strongly attenuated 8984 eV peak with respect to the two-coordinate precursor. Comparison with reduced PAH suggests that the coordination number of the Cu(I) centers in the reduced enzyme is probably greater than two-coordinate, since the intensity of the 8984 eV peak is also attenuated with respect to that of the bis model complex. Thus a pseudo-three-coordinate structure involving two short imidazoles and one longer Cu–X interaction (as suggested from the EXAFS) is also suggested from absorption edge analysis.

Discussion

The results of the present study confirm previous spectroscopic conclusions on the coordination environment of the Cu centers in oxidized CV-PAH and provide the first structural insights into the coordination chemistry of the active reduced form. Taking into account the uncertainties of EXAFS analysis of coordination numbers (±25%), we estimated that the Cu(II) centers in the oxidized protein are coordinated to two histidine ligands, with an additional two O/N-donor groups completing the equatorial ligation. In general, EXAFS does not detect axial coordination, and our data therefore give no indication of the presence or absence of axially coordinated ligands. Dithionite reduction leads to a site of lower coordination number, most consistent with a pseudo-three-coordinate structure involving two short histidines and a third ligand at a longer distance. Although the third ligand is best fit by a S/Cl interaction, further studies are required to confirm this assignment.

The reductive activation of CV-PAH is known to be a complex process. Mild reducing agents such as ascorbate and ferrocyanide which reduce the copper center in many other copper proteins do not reduce the copper. The pterin cofactor is only able to reductively activate CV-PAH by ca 10% and only in the presence of substrate (Pember et al., 1986). The oxygen substrate readily oxidizes the activated enzyme back to the inactive Cu^{2+} form. Thus full activity is measured only by including strong reducing agents such as DTT or cysteine in the assay mixture. Other strong reducing agents such as dithionite quantitatively reduce CV-PAH but are not suitable for activity measurements because they rapidly consume the oxygen substrate. The role of the thiol reagents is unclear at the present time, but there is evidence for thiol complex formation with the enzyme, possibly at the Cu(I) centers (Pember et al., 1986). The observation of a Cu–S/Cl interaction in the dithionite-reduced enzyme may be relevant to the mechanism of thiol activation but, alternatively, could be the result of coordination of exogenous S ligands, either during enzyme isolation or from reduced S-containing species which may be produced as impurities in the dithionite by disproportionation.

In any event, the picture which emerges is that of a highly coordinative unsaturated Cu(I) site. The value of the coordination number and bond length for the Cu(I)–imidazole shell both suggest a maximum of two coordinated imidazole groups. A large number of model studies have indicated that such a site would be expected to pick up one additional weakly bound ligand so as to form a distorted three-coordinate Cu(I) complex. Furthermore, most Cu(I) complexes which bind dioxygen are known to have distorted three-coordinate structures (Karlin & Gultneh, 1987; Tyeklar & Karlin, 1989; Vigato et al., 1990), whereas two-coordinate Cu(I) complexes generally do not bind O_2 or CO (Sorrell & Borovick, 1986, 1987; Pasquali & Floriani, 1984). For example, [Cu(1,2-dimethylimidazole)$_2$]$^+$ does not bind O_2 or CO, but, upon addition of 1 molar equiv of (1,2-dimethylimidazole) to the bis complex in CH_2Cl_2, dioxygen and carbonyl adducts can be formed (Sanyal et al., 1991). The structure of the Cu(I) site in PAH is thus well set up for O_2 binding, although the identity of the third ligand and its relationship to the mechanism of thiol activation are unclear at this time. An attractive hypothesis is that, under catalytic conditions, the pterin cofactor takes the place of this third ligand, providing a three-coordinate site for O_2 coordination, such that the oxygen substrate is correctly oriented for reacting with the cofactor to form the 4a-peroxy pterin. Further studies are underway to investigate these and other aspects of the coordination chemistry of reduced CV-PAH.

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A Ligand-Induced Conformational Change in the Estrogen Receptor Is Localized in the Steroid Binding Domain†

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ABSTRACT: Upon binding estrogen, the estrogen receptor (ER) is proposed to undergo some form of conformational transition leading to increased transcription from estrogen-responsive genes. In vitro methods used to study the transition often do not separate heat-induced effects on the ER from estrogen-induced effects. The technique of affinity partitioning with PEG-palmitate was used to study the change in the hydrophobic surface properties of the ER upon binding ligand with and without in vitro heating. Upon binding estradiol (E_2), the full-length rat uterine cytosolic ER undergoes a dramatic decrease in surface hydrophobicity. The binding of the anti-estrogen 4-hydroxytamoxifen (4-OHT) results in a similar decrease in surface hydrophobicity. These effects are independent of any conformational changes induced by heating the ER to 30 °C for 45 min. The use of the human ER steroid binding domain overproduced in *Escherichia coli* (ER-C) and the trypsin-generated steroid binding domain from rat uterine cytosolic ER demonstrates that the decrease in surface hydrophobicity upon binding E_2 or 4-OHT is localized to the steroid binding domain. Gel filtration analysis indicates that the change in surface hydrophobicity upon binding ligand is an inherent property of the steroid binding domain and not due to a ligand-induced change in the oligomeric state of the receptor. The decrease in surface hydrophobicity of the steroid binding domain of the ER upon binding E_2 or 4-OHT represents an early and possibly a necessary event in estrogen action and may be important for "tight" binding of the ER in the nucleus.

The steroid receptor superfamily of DNA binding, nuclear transcription factors includes the estrogen receptor (ER)[1] (Evans, 1988). Early studies using controlled proteolysis as well as more recent cloning of the cDNA for the ER have revealed distinct functional domains (Green et al., 1986;

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[1] Abbreviations: ER, estrogen receptor; hsp, heat shock protein; ERE, estrogen response element; E_2, estradiol; 4-OHT, 4-hydroxytamoxifen; PEG, poly(ethylene glycol); ATPP, aqueous two-phase partitioning; PMSF, phenylmethanesulfonyl fluoride; HAP, hydroxylapatite; EDTA, ethylenediaminetetraacetic acid; BCIP, 5-bromo-4-chloro-3-indolyl 1-phosphate; NBT, nitro blue tetrazolium; U, unoccupied ER; EO, E_2-occupied ER; AO, 4-OHT-occupied ER; UH, unoccupied heated ER; EOH, E_2-occupied heated ER; AOH, 4-OHT-occupied heated ER; DES, diethylstilbestrol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; TAMZ, tamoxifen aziridine; V_0, void volume; SEM, standard error of the mean; kDa, kilodalton(s).

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