Synthesis and structural characterization of cross-linked histidine–phenol Cu(II) complexes as cytochrome *c* oxidase active site models†

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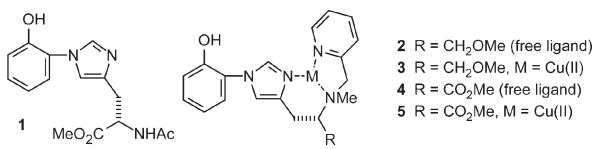
Tridentate cross-linked histidine–phenol Cu(II) ether and ester complexes, chemical analogs of the active site of several heme–copper oxidases, have been synthesized and crystallized.

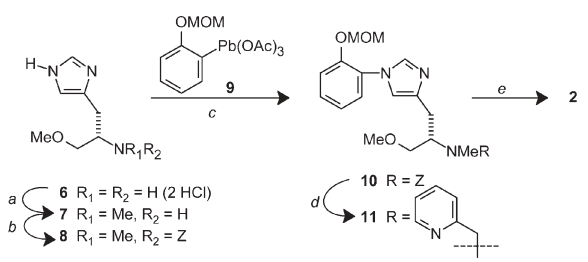
The role of the post-translationally modified tyrosine in the heme-copper oxidases continues to be a subject of great interest. The tyrosine (Tyr244, bovine heart numbering) is cross-linked through C6 to the ε-nitrogen of histidine 240, which serves as a ligand to Cu_B.[1–3] The tyrosine has been postulated to play a structural role[4,5] and/or to function as an electron and a proton donor for the cleavage of the dioxygen bond.[3,6] However, the presence of a tyrosyl radical in the resulting **P** form of the enzyme has not been explicitly demonstrated, and its absence has been attributed to a possible spin coupling between the tyrosyl radical and the paramagnetic Cu_B^{2+}.[6] Several chemical analogs of the tyrosine-histidine cross-link have been synthesized.[7–16] Spectroscopic studies on compound **1** synthesized in our laboratories demonstrated the presence of a UV-generated radical of **1**, with the data supporting the radical residing primarily on the phenol with small delocalization of spin density onto the imidazole.[8,17] Recently, a limited number of Cu-containing complexes, with and without a heme group have been synthesized[18–24] and explored using theoretical calculations.[22,24,25] In general, these models have focused on the effect of the copper on the physicochemical properties of the cross-linked phenol or the dioxygen reactivity of the complexes, and not on the nature of the UV generated cross-linked tyrosyl (phenoxyl) radical (however, see ref. 22).



Herein we present the synthesis of two tridentate ligands for copper (**2** and **4**) that build on our earlier work involving compound **1**.[8] The corresponding copper complexes, **3** and **5**, were crystallized and solid state structures were obtained. Spectrophotometric titrations and time-resolved optical absorption spectroscopy were carried out to provide insight into the nature of the interaction between Cu_B and the proposed tyrosyl radical of the enzyme.

The synthesis of C*c*O ligand mimic **2** benefits from our past studies on copper-catalyzed N-arylation with aryllead reagents (Scheme 1).[26] The synthesis begins with L-histidine methyl ether **6**,[27] which is doubly protected with ethyl chloroformate (amine and imidazole N–H) and reduced with $LiAlH_4$ to give *N*-methyl compound **7** in essentially quantitative yield. Double protection (this time with Z-succinimide), followed by treatment with propylamine (imidazole carbamate removal), affords **8**. Coupling with aryllead(IV) reagent **9** yields **10** in 41% overall yield from **7**. The Z-protection group is removed under standard conditions (95%) and the resulting *N*-methyl compound is subjected to reductive amination with picolyl carbaldehyde to afford **11** (99%). Finally, treatment with HCl gas and neutralization with NaOH yields **2** (89%). Addition of $Cu(ClO_4)_2$ and chloride ion, followed by crystallization, provided **3**. Ester **4** arose from our recent facile methodology for N-methylation of amino acids,[28] which provided *N*-methyl-*N*-benzyl-L-histidine methyl ester in 99% yield from H–His–OMe. Lead coupling with **9** (85%), reductive debenzylation (98%), reductive amination (81%) and removal of the –MOM protection (99%) afforded **4**, with **5** formed in parallel to **3**.

The structure of **3** as determined by single-crystal X-ray analysis is given in Fig. 1;‡ the corresponding Cu-complex **5** is virtually identical.[29] The geometry about the copper atom is a tetragonal distorted square pyramid with three nitrogens (pyridine, imidazole and amine) and a chloride in a square plane and a weakly coordinated chloride ion in an axial position (Cu–Cl: 2.75 Å). The axial chloride forms the equatorial chloride–copper bond in the adjacent monomeric unit. Adjacent ligands are rotated by 180°. The dihedral angle between the phenol and the histidine in the solid state was determined to be −25.7° for both complexes. The



Scheme 1 *Reagents and conditions*: (a) (1) CH_3CH_2OCOCl, 99%, (2) $LiAlH_4$, reflux, 99%; (b) Z-succinate, then propylamine; (c) **9**, $Cu(OAc)_2$, 41% from **7**; (d) (1) cyclohexadiene, Pd/C, 95%, (2) picolyl carbaldehyde, $Na(OAc)_3BH$, 99%; (e) $HCl_{(g)}$, then NaOH, 89%.

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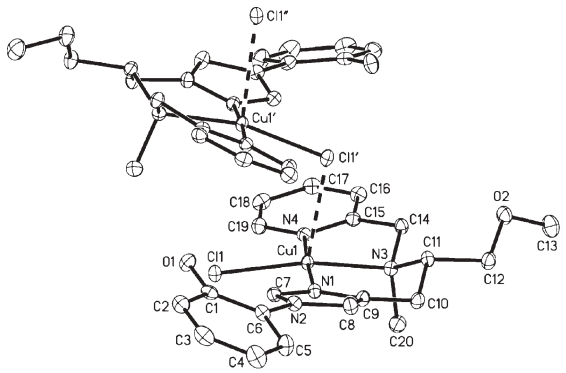

Fig. 1 Crystal structure of **3** showing two formula units and the weakly coordinated axial chlorine atom, Cu1···Cl′ 2.7527(8) Å. Thermal ellipsoids are drawn at the 50% probability level and hydrogen atoms are omitted for clarity. Symmetry code: ′ = $x - 1/2$, $3/2 - y$, $1 - z$; ″ = $x - 1$, y, z.

angle is closer to planar than that observed in the bovine enzyme (44°)[3] or in compound **1** (−36.3°).[8]

Spectrophotometric titrations were performed on both ligands and Cu-complexes to determine the pK_a of the phenolic proton. Fig. 2(A) and (C) show the UV-visible spectra recorded over the pH range 3.1–9.3 for the tridentate ether ligand **2** and pH 4.9–9.4 for the Cu-complex, **3**, respectively. Singular value decomposition (SVD) and global pK_a fitting[8] provided the pK_a values and the intermediate spectra of the different protonation states (Fig. 2(B) and (D)). Three pK_a values were observed for the ligand, 4.4, 7.3 and 8.9, and are attributed to the pyridine, imidazole and the phenol, respectively. The phenolic pK_a is similar to that observed for the His-phenol cross-linked compound **1** (8.34) but significantly lower than that of unperturbed tyrosine (10.1).[8] For the Cu-complex, a single pK_a value of 7.8 was observed for the phenolic proton. A lower pK_a would be expected for the ligands through resonance delocalization of the phenolate anion by the *N*-linked imidazole or through an inductive electron withdrawing effect;[11,15,24] further reduction is expected upon coordination of the imidazole to copper.[11] A similar phenolic pK_a (8.0) was recently reported for a Zn(II) complex containing an imidazole–phenol cross-link.[24] We observed pK_a values of 4.4, 5.9 and 8.7 for ligand **4** and 7.8 for Cu-complex **5**.

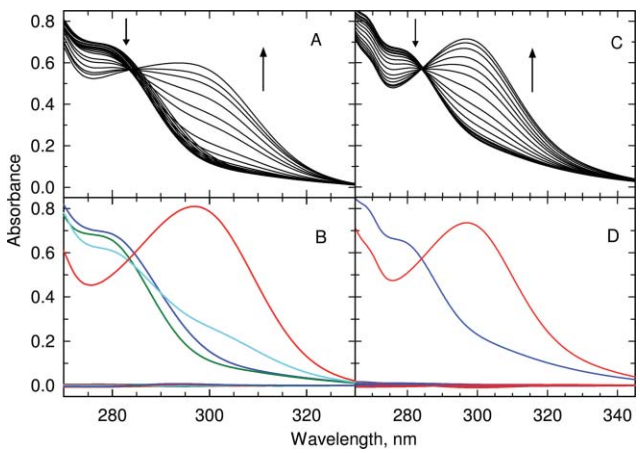

Fig. 2 Spectrophotometric titrations of an aqueous solution of ligand **2** (0.62 mM, pH 3.1–9.3) (panel A) and Cu-complex **3** (0.27 mM, pH 4.9 to 9.4) (panel C). (B) The spectral forms of the different protonation states of **2** (blue, fully protonated; green, pyridine unprotonated; cyan, imidazole deprotonated; red, fully deprotonated). (D) The spectra of the different protonation states of the Cu–ether complex **3** (blue, protonated; red, deprotonated).

Kitagawa and co-workers recently reported an increase in the pK_a of a cross-linked imidazole–phenol ligand when coordinated to Cu(II) (pK_a ~10), which they supported by theoretical calculations.[22] However as pointed out by van der Donk and co-workers,[24] these calculations were carried out on a conformation in which the O–H of the phenol group was intermolecularly H-bonded to the imidazole due to a large angle (66°) between the planes of the phenol and the imidazole rings. This is not the case for our Cu-complex or the enzyme active site, in which the angles between the phenol and imidazole rings were −25.7 and 44°, respectively, precluding a H-bonded structure.[3,8]

We investigated the presence of a phenoxyl radical in the tridentate ligands and Cu-complexes at room temperature by time-resolved UV-Vis spectroscopy following excitation at 266 nm. Time-resolved optical absorption difference spectra (post- minus pre-photolysis) of the tridentate ether ligand **2**, recorded between 100 ns and 500 μs after UV photolysis, show a positive peak at ~330 nm in the UV region and a weak broad band at ~480 nm (Fig. 3(A)); these are similar absorbance maxima we reported previously for compound **1**.[8] SVD and global exponential fitting revealed three lifetimes of 0.14, 1.1 and 43 μs. The corresponding intermediate spectra, extracted using a sequential mechanism, are shown in Fig. 3(B). The 0.14 μs lifetime is attributed to the decay of the triplet state,[30] and the 1.1 μs lifetime to the decay of the phenoxyl radical. Similar lifetimes were obtained for the ester

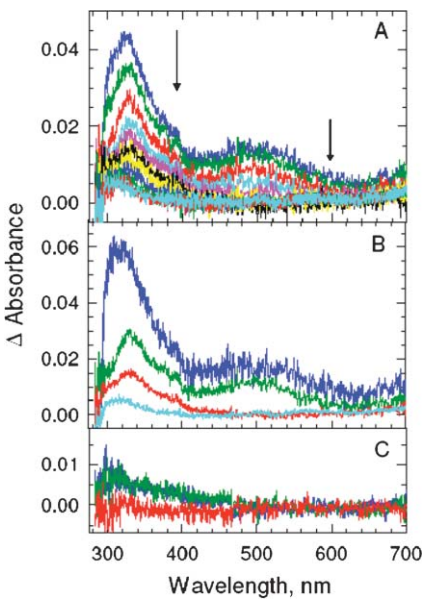

Fig. 3 (A) Time-resolved difference spectra (post- minus pre-photolysis) of an aqueous solution of the tridentate ether ligand **2** following excitation at 266 nm (Nd:YAG, 7 ns pulse). The spectra were recorded from 100 ns to 500 μs. (B) Intermediate spectra of the tridentate ether ligand were extracted on the basis of a unidirectional sequential mechanism following SVD/global exponential fitting analysis. The blue, green, red and cyan traces represent intermediates 1, 2, 3 and 4, respectively. (C). Time-resolved difference spectra of an aqueous solution of the Cu–tridentate ether complex **3** following excitation at 266 nm (Nd:YAG, 7 ns pulse).

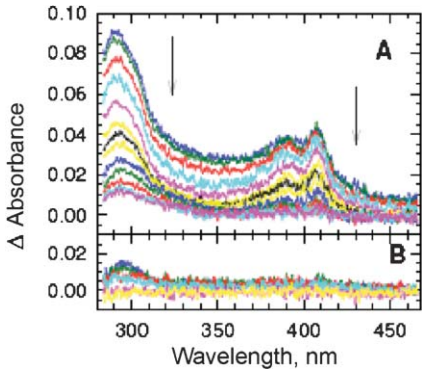

Fig. 4 Time-resolved difference spectra (post- minus pre-photolysis) of an aqueous solution of tyrosine (1.76 mM) in the absence (panel A) and presence of copper (2.12 mM) (panel B). The spectra were recorded from 100 ns to 0.5 ms following excitation at 266 nm (Nd:YAG, 7 ns pulse).

ligand (0.26, 1.3 and 19 μs). Upon incorporation of copper into the tridentate ether and ester complexes, no absorbance bands were detected upon UV photolysis (Fig. 3(C)) suggesting that at room temperature the decay of the phenoxyl radical, presumably through quenching by the copper, is too fast for our time resolution. Kitagawa and co-workers did not observe any absorbance bands in the transient absorption spectra of their Cu(II) complex containing an imidazole–phenol cross-link; however, the authors concluded that the phenoxyl radical was generated based on the decay of the absorbance at 400 nm with a half-life of 44 μs.[22] Our results clearly show the absence or quenching of the transient radical signal in the Cu-complex on ~100 ns time scale (Fig. 3(C)).

In order to investigate the effect of copper on the formation and decay of the phenoxyl radical, we recorded time-resolved absorption spectra of tyrosine in the absence and presence of stoichiometric amounts of copper (Fig. 4(A) and (B), respectively). In absence of copper, two apparent lifetimes were resolved, 1.2 and 58 μs. The latter lifetime is similar to what we reported previously (77 μs);[8] the lifetime of 1.2 μs is attributed to the decay of the triplet state. It is clear that in the presence of copper, the expected spectral changes in both the UV and visible region associated with the formation of the tyrosyl radical are absent, most likely due to quenching of the excited state by the paramagnetic copper.

In conclusion, our results indicate that the pK_a of the cross-linked phenol in the copper complexes is significantly lower than that of unperturbed phenol. This supports the proposal that the cross-linked tyrosine in the enzyme may facilitate proton delivery to the active site. Our time-resolved experiments show the formation of a phenoxyl radical in the tridentate ligands upon UV photolysis, which is quenched in the copper complexes. The presence of a UV-generated radical in both ligands and the copper complexes is currently being investigated by EPR and FT-IR difference spectroscopy.

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Notes and references

‡ Crystals of **3** were grown from a solution containing MeOH and NaCl. *Crystal data*: $C_{20}H_{24}Cl_2CuN_4O_6$, M = 550.87, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 6.6563(11), b = 16.198(3), c = 20.176(3) Å, U = 2175.4(6) Å^3, T = 90(2) K, Z = 4, μ(Mo-Kα) = 1.3 mm^{-1}, 28893 reflections measured, 6365 unique (R_{int} = 0.047) which were used in all calculations. The Flack parameter refined to −0.026(11). The final $wR(F^2)$ was 0.089 (all data). CCDC 640795. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b703835f

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