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Formation and spectroscopic characterization of the dioxygen adduct of a heme–Cu complex possessing a cross-linked tyrosine–histidine mimic: modeling the active site of cytochrome *c* oxidase†

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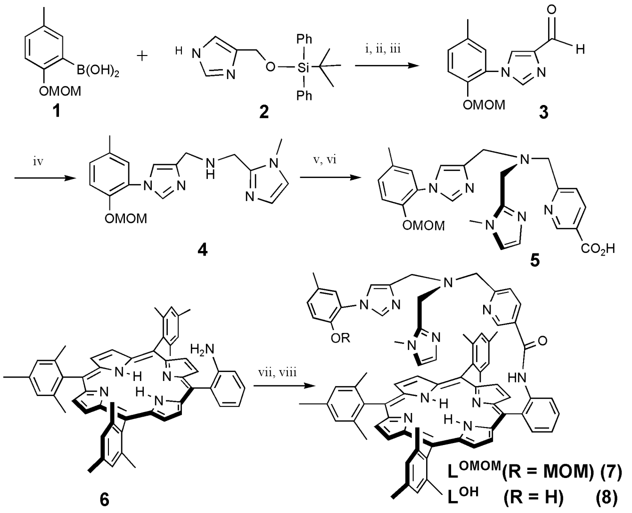
A binucleating porphyrin with covalently appended copper chelates having a cross-linked imidazole–phenol group as the novel active site model of cytochrome *c* oxidase has been prepared, and the dioxygen adduct of its iron(II)–copper(I) complex was spectroscopically characterized.

Cytochrome *c* oxidase (C*c*O), the terminal enzyme of the respiratory chain, catalyzes the 4e/4H$^+$ reduction of dioxygen to water without generating toxic reactive intermediates, conserving the released energy for the synthesis of ATP.[1] The active site of O_2 reduction is comprised of heme a$_3$/Cu$_B$ binuclear moiety in which one of the copper-bound histidines is covalently cross-linked to a tyrosine residue between the C6 of Tyr244 and the ε-nitrogen of His 240 (in the bovine enzyme sequence).[2] This unprecedented Tyr-His cross-link is proposed either to function as an electron and a proton donor to the dioxygen bound to heme a$_3$ or to fix Cu$_B$ in a certain configuration and distance from heme a$_3$ during the catalytic O_2 reduction.[3] A number of heme-based dinuclear Fe–Cu complexes have been reported as model compounds in the hope of unraveling the mechanism of O_2 reduction in the active site of C*c*O.[4] However, the reported heme-based models are devoid of the Tyr-His cross-linkage. There are a few recent reports[5] about the syntheses and physicochemical investigations of cross-linked phenol–imidazoles. Very recently, Karlin and co-workers[6] reported copper complexes with imidazole–phenol cross-links as an initial synthetic model for the Cu$_B$ site in C*c*O. Herein we report the first example of constructing a heme-containing model with covalently appended copper chelates having a cross-linked imidazole–phenol group as a novel C*c*O model compound.

The synthetic routes to the desired compounds are shown in Scheme 1.‡ The aldehyde **3** is prepared firstly from the coupling of methoxymethyl (MOM)-protected 2-hydroxyphenylboronic acid (**1**) with 4(5)-(*tert*-butyldiphenylsilanyloxymethyl)-1*H*-imidazole (**2**),[7] followed by removing the silyl protecting group and then oxidized by activated MnO_2. It is noteworthy that none of the desired product is obtained when 1*H*-imidazole-4-carbaldehyde is employed for the coupling. The MOM-protected phenylboronic acid (**1**) can be obtained in 65% overall yield by sequential reactions involving metalation (*n*-BuLi/ether/−70 °C) of MOM-protected 2-bromo-4-methylphenol, and treatment with $B(OMe)_3$, followed by acidic work-up. Treatment of **3** with 2-aminomethylimidazole in methanol generates the corresponding Schiff base intermediate, which is consequently reduced by $NaBH_4$ *in situ* to give **4** in a yield of 65%. The tripodal ligand **5** is isolated by reacting the amine **4** with methyl 6-chloromethylnicotinate in the presence of K_2CO_3 in CH_3CN, and then hydrolyzing in a KOH solution. The prepared tripodal ligand is an important building block in assembling C*c*O active site models. The condensation reaction between **5** and the porphyrin **6** (2-[10,15,20-tris-(2,4,6-trimethylphenyl)-porphyrin-5-yl]-phenylamine) is performed in the presence of Et_3N/2-chloromethylpyridinium iodide in CH_2Cl_2 to give the covalent conjugate L^{OMOM} (**7**) in 63% yield. Finally, the MOM group is removed with bromotrimethylsilane in CH_2Cl_2 at − 30 °C to regenerate the phenolic hydroxyl group and the hydroxyl free ligand L^{OH} (**8**)‡ is obtained in a moderate yield (60%).

Stepwise metalation of the porphyrins, **7** and **8**, begins with addition of excess $FeBr_2$ in THF at reflux, followed by extraction with an aqueous Na_2EDTA solution, yielding the corresponding mononuclear Fe^{II} porphyrins, L^{OMOM}Fe^{II} and L^{OH}Fe^{II}, respectively. Addition of copper salt $[Cu(CH_3CN)_4]^+$ $CF_3SO_3^-$ gives the desired Fe^{II}/Cu^I complexes with similar UV–vis spectra to those of their mononuclear Fe$^{\mathbf{II}}$ complexes, [L^{OMOM}Fe^{II}Cu^I]$^+$ (**9**), ESI–MS *m*/*z* = 1330.5 (*M*$^+$); [L^{OH}Fe^{II}Cu^I]$^+$ (**10**), ESI–MS *m*/*z* = 1286.5 (*M*$^+$).

Both **9** and **10** react with O_2 at − 30 °C in CH_3CN to give the dioxygen adducts [L^{OMOM}Fe^{III}–O$_2$–Cu^{II}]$^+$ (**11**) and [L^{OH}Fe^{III}–O$_2$–Cu^{II}]$^+$ (**12**), respectively. The formation of the corresponding peroxo species was evidenced by the following observations: (1) upon exposure of the reduced form **9** or **10** to O_2, its UV–vis spectra show distinctive changes with clear isosbestic points. The Soret band shifts from 429 nm to 421 nm, and the Q-band at 533 nm disappears (Fig. 1), which indicates the formation of a dioxygen adduct as described for those of our previously isolated peroxo-bridged Fe–O$_2$–Cu species.[8] (2) ESI mass spectra of the dioxygen adducts show a distribution of peaks centered at 1362.5 (*M*$^+$) for **11**, and 1318.5 (*M*$^+$) for **12**. The observed isotope distribution of peaks agrees very well with the simulated pattern based on the ratio of Fe^{II}–Cu^I : O$_2$ = 1 : 1. The expected increase in mass of 4 is observed when **11** (*m/z*, *M*$^+$, 1366.5) or **12** (*m/z*, *M*$^+$, 1322.5) forms from $^{18}O_2$. (3) The resonance Raman spectra of **11** shows an isotope dependent peak at 801 cm^{-1} which shifts to 755 cm^{-1} with ^{18}O-labeled dioxygen, and **12** displays a similar isotope sensitive band at 799 ($^{16}O_2$) and 752 cm^{-1} ($^{18}O_2$), respectively (Fig. 2). The observed isotopic shifts are in good agreement with the value



Scheme 1 *Reagents and conditions:* (i) cat. [Cu(OH)TMEDA]$_2$Cl$_2$, CH$_2$Cl$_2$, O$_2$, rt, 75%; (ii) *n*-BuN$^+$F$^-$, THF, rt, 93%; (iii) MnO$_2$, CHCl$_3$, reflux, 85%; (iv) *a*, 2-aminomethylimidazole, Et$_3$N, MeOH, *b*, NaBH$_4$, 65%; (v) methyl 6-chloromethylnicotinate, K$_2$CO$_3$, CH$_3$CN, rt, 61%; (vi) KOH, THF, rt, 86%; (vii) 2-chloromethylpyridinium iodide, **5**, Et$_3$N, CH$_2$Cl$_2$, rt, 63%; (viii) Me$_3$SiBr, CH$_2$Cl$_2$, − 30 °C, 60%.

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† Electronic supplementary information (ESI) available: experiment procedures for preparing metal porphyrins and oxygenation reaction. See http://www.rsc.org/suppdata/cc/b3/b311538k/

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calculated from the harmonic oscillator approximation of the O–O stretching vibration [$\Delta_{calcd}(^{16}O_2/^{18}O_2)$ = 46 cm^{-1}]. These observed ν(O–O) values are similar to those of previous reported dioxygen adducts in the peroxy state.[4,8] (4) Both **11** and **12** are EPR silent in a frozen solution (CH_3CN, 77K), which indicates the presence of the strong antiferromagnetic coupling between the two metals.

The formed peroxo species are stable at − 30 °C in CH_3CN, and on warming of the solution to room temperature (after removal of excess O_2 *in vacuo*), the dioxygen adducts [$L^{OMOM}Fe^{III}$–O_2–Cu^{II}]$^+$ (**11**) and [$L^{OH}Fe^{III}$–O_2–Cu^{II}]$^+$ (**12**) exhibit interesting differences. For **11**, the major decomposed product is the μ-oxo complex formulated as [$L^{OMOM}Fe^{III}$–O–Cu^{II}]$^+$ [*m*/*z*, 1346.6 (M^+)] with UV–vis features [λ_{max} = 440 nm (Soret)] similar to the reported μ-oxo analogues.[8,9] By contrast, no μ-oxo final species is observed for **12**. The final decomposed product demonstrates features like that of the hydroxo ferric porphyrin derivatives.[10] The EPR spectrum (MeCN, 77 K) of the product shows signals at g = 5.56 and 1.99 corresponding to a high spin iron(III) porphyrin, and signals at $g_{\|}$ = 2.23 and $g_{\perp}$ = 2.06, which are assigned to a S = 1/2 Cu(II) ion in a tetragonal field.[11] We tentatively formulate the product as [$L^{OH}Fe^{III}$–OH, Cu^{II}]$^{2+}$. The decomposition mechanism and further product characterization are in progress.

In summary, a novel heme-based binucleating ligand incorporated with *N*-(2′-hydroxyphenyl)imidazole moiety as a C*c*O's Cu_B site mimic has been designed and successfully prepared. The oxygenation reaction with its iron(II)–copper(I) complex has been preliminarily investigated by various spectroscopic methods.

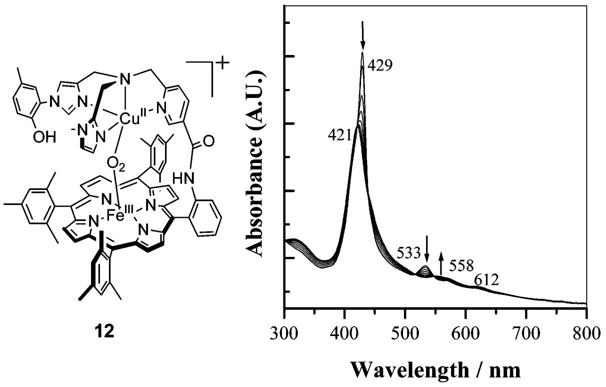

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Fig. 1 UV–visible spectral changes of **10**, [$L^{OH}Fe^{II}Cu^{I}$]$^+$, to **12** upon exposure to dioxygen in CH_3CN at −30 °C.

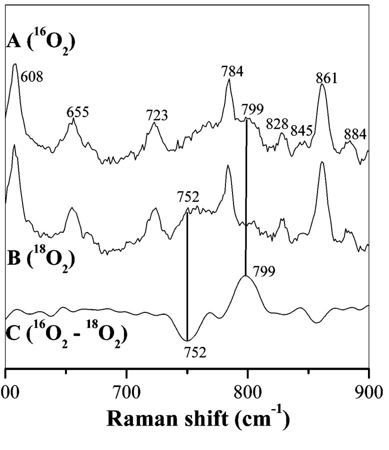

Fig. 2 Resonance Raman spectra of **12** formed from $^{16}O_2$ (A) and $^{18}O_2$ (B). The difference spectra A minus B is shown as trace C (3% toluene in CH_3CN, −30 °C, 413 nm excitation).

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Notes and references

‡ Synthetic details will be reported elsewhere. All new compounds were fully characterized by spectroscopic methods. Stated yields refer to isolated compounds and the purity was guaranteed by chromatography. Data for L^{OH} (**8**), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (d, J = 8.0, 1 H), 8.75 (d, J = 4.8, 2 H), 8.67 (d, J = 4.4, 2 H), 8.63 (d, J = 3.6, 4 H), 8.03 (d, J = 6.0, 1 H), 7.85 (d, J = 7.2, 1 H), 7.83 (s, 1 H), 7.76 (s, 1 H), 7.54 (t, 1 H), 7.24 ~ 7.26 (m, 6 H), 7.20 (s, 2 H), 7.14 (s, 1 H), 6.82 (d, J = 8.0, 1 H), 6.77 (d, J = 6.0, 1 H), 6.70 (d, J = 8.0, 1 H), 6.59 (d, J = 8.0 Hz, 1 H,), 6.28 (s, 1 H), 6.03 (s, 1 H), 3.29 (s, 2 H), 3.25 (s, 2 H), 3.16 (s, 2 H), 2.86 (s, 3 H), 2.60 (s, 3 H), 2.58 (s, 6 H), 2.13 (s, 3 H), 1.85 (s, 3 H), 1.82 (s, 6 H), 1.79 (s, 3 H), 1.75 (s, 6 H), − 2.54 (s, 2 H). IR (KBr) 3411, 3318, 3026, 2916, 2855, 1697, 1683, 1674, 1652, 1599, 1578, 1558, 1520, 1472, 1457, 1446, 1399, 1377, 1344, 1284, 1257, 1217, 1188, 1131, 1070, 968, 804 cm^{-1}. HR-MS (FAB, NBA) Found: 1170.5869. Calcd for $C_{76}H_{72}N_{11}O_2$: [M + H]$^+$, 1170.5870.

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