Synthesis of heme–tris(imidazolyl)methane copper complex, the first binuclear complex bearing three imidazole ligands for copper, as an active site model of cytochrome *c* oxidase

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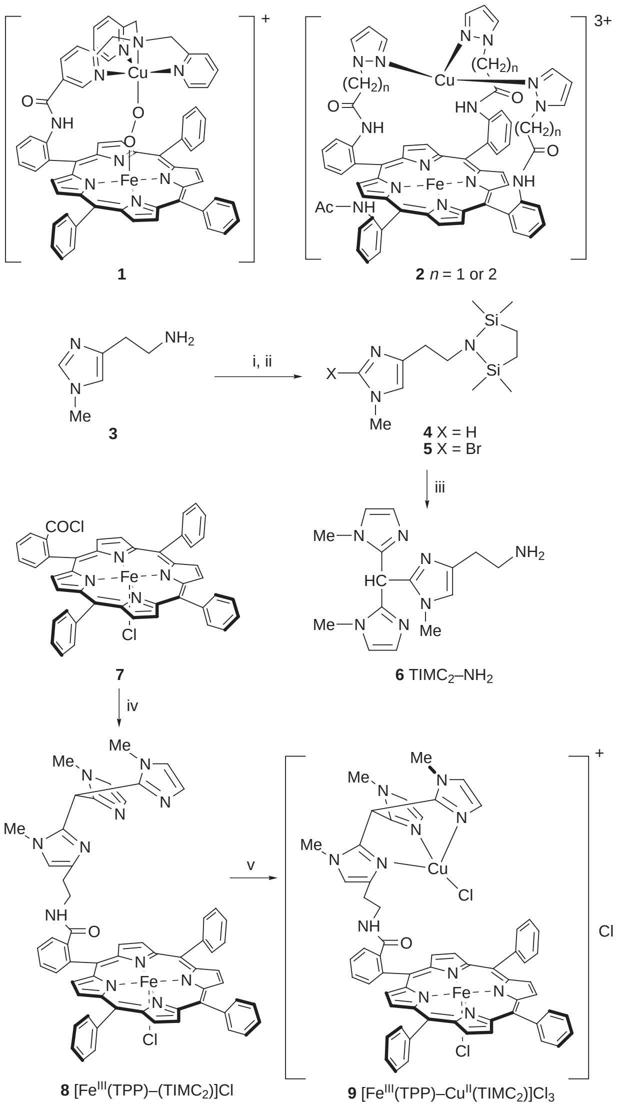
As an active site model of cytochrome *c* oxidase, a new covalently-linked binuclear complex with three imidazole ligands for copper, heme–tris(imidazolyl)methane copper, was prepared and spectroscopically characterized.

Cytochrome *c* oxidase (CcO) is a terminal respiratory protein complex which catalyzes the four-electron reduction of O_2 to water in aerobic organisms.[1] The structures of bovine heart[2] and *Paracoccus denitrificans*[3] CcOs have been independently revealed by X-ray crystallography. Heme a_3 and Cu_B, which form a unique binuclear center, are directly responsible for dioxgen reduction. The histidinyl imidazole axially ligates to the iron in heme a_3, while Cu_B has the three histidinyl imidazole ligands for the copper. The separations between the iron and the copper atoms in the active site were determined to be 4.5 Å for the bovine heart and 5.2 Å for the bacterial CcO.

A number of heme-based binuclear Fe–Cu complexes have been prepared[4] to elucidate the mechanism of O_2 reduction and/or to reproduce an EPR-silent, antiferromagnetic coupled Fe^{III}–X–Cu^{II} moiety, which are the characteristics of the resting state of CcO. We recently reported that the reduced form (Fe^{II}–Cu^{I}) of the heme–tris(pyridylmethyl)amine copper complex reacted with dioxygen to give a stable oxygenated complex **1** in a μ-1,2-peroxo fashion at ambient temperature.[5] To the best of our knowledge, this is the first well-characterized dioxygen adduct of a CcO modeling complex. Although the coordination to the copper ions is considered to affect the binding and reduction of dioxygen significantly, there have been no examples of a binuclear model complex having three imidazole ligands for the copper ion similar to the native ones. As a close model with a Cu–L_3 moiety, we reported the oxidized form of heme–trispyrazolyl copper complex **2**,[6] which, however, did not exhibit a large enough formation constant with copper ion to allow further study. Therefore, we designed and synthesized a new model complex, heme–tris(imidazolyl)methane copper [**Fe^{III}(TPP)–Cu^{II}(TIMC$_2$)Cl$_3$**]. Tris(imidazolyl)methane is a tridendate chelating ligand which can give stable complexes with copper ion.[7] The two components, porphyrin and tris(imidazolyl)methane, are connected through an amide linkage to maintain a suitable geometry and separation of the two metal ions.

The synthetic routes to the desired complex are shown in Scheme 1.‡ (N^{τ}-Methylhistamin-2-yl)-bis(N-methylimidazol-2-yl)methane **6** (**TIMC$_2$-NH$_2$**) could be obtained *via* the coupling between silyl-protected N^{τ}-methy-2-bromohistamine **5** and the lithiated anion of bis(N-methylimidazol-2-yl)methane[8] under Pd(PPh$_3$)$_4$-catalyzed conditions.[9] N^{τ}-Methylhistamine **3** was prepared from commercially available histamine dihydrochloride in three steps according to the reported methods[10] in 71% overall yield. There were some prerequisites for the protection of the NH$_2$ group: first, the protecting group should be robust enough under the following strong basic conditions involving BuLi, and second, the group should be removable under mild conditions and should be easily separable from the reaction mixture. Thus, a cyclic disilyl group[11] was chosen as the protecting group. A solution of **3** and DBU§ was treated with 1,1,4,4-tetramethyl-1,4-dichlorosilylethylene to give the cyclic disilazole **4** in 90% yield. Bromination at the C-2 position of **4** was accomplished with BuLi and 1,2-dibromo-1,1,2,2-tetrafluoroethane in 94% yield with conservation of the disilyl protecting group. Litiation of bis(N-methylimidazol-2-yl)methane at CH$_2$ was performed with BuLi in THF, followed by the addition of Pd(PPh$_3$)$_4$ and **5** . The mixture was



Scheme 1 *Reagents and conditions*: i, DBU, 1,1,4,4-tetramethyl-1,4-dichlorosilylethylene, CH$_2$Cl$_2$, 2 h, room temp., 90%; ii, BuLi, Et$_2$O , 1h, −78 to −35 °C, 1,2-dibromo-1,1,2,2-tetrafluoroethane, −78 to −25 °C; iii, BuLi, bis(N-methylimidazol-2-yl)methane, THF, 1 h, −78 to −20 °C, Pd(PPh$_3$)$_4$, overnight, 50 °C; iv, **6**, Pr$^i_2$NEt, CH$_2$Cl$_2$, 2 h, 0 to 20 °C; v, CuCl$_2$·6H$_2$O, MeOH–CH$_2$Cl$_2$, 1 h, room temp.

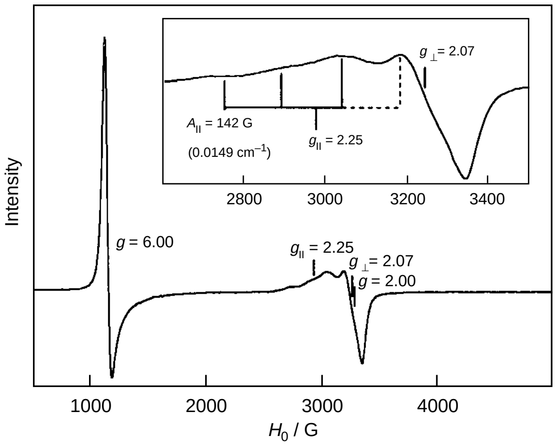


Fig. 1 EPR spectrum of **9** {**[Fe^{III}(TPP)–Cu^{II}(TIMC$_2$)]Cl$_3$**} in CH_2Cl_2 at 6 K, microwave frequncy = 9.3423 GHz, power = 1 mW

heated overnight at 50 °C to enhance the coupling reaction. The removal of the starting compounds by sublimation under high vaccum conditions afforded **TIMC$_2$-NH$_2$ 6** in 25% yield.

5-(2-Carboxyphenyl)-10,15,20-triphenylporphyrin was used as the porphyrin moiety. In order to avoid complications during the insertion of the two different metal ions to two different ligand sites, iron(III) was introduced into the porphyrin ligand prior to coupling with the **TIMC$_2$** moiety. The acid chloride **7**, obtained quantitatively from the free acid, was coupled with **6** in the presence of $Pr^i{}_2NEt$ in CH_2Cl_2 to give the mononuclear complex **8** {**[Fe^{III}(TPP)–(TIMC$_2$)]Cl**; m/z = 993, λ_{max}/nm (ε/M^{-1} cm^{-1}) = 378 (23 200), 417 (46 700), 511 (5170), 576 (2410), 695 (1380), 775 (520)} in 31% yield. The final desired complex **9** {**[Fe^{III}(TPP)–Cu^{II}(TIMC$_2$)]Cl$_3$**} was prepared quantitatively by the addition of $CuCl_2\cdot6H_2O$ in MeOH–CH_2Cl_2. Its electronic spectrum [λ_{max}/nm (ε/ M^{-1} cm^{-1}) = 378 (26 900), 417 (44 700), 510 (6560), 577 (2330), 654 (2110), 687 (2110)] was similar to that of the mononuclear heme complex. Its electrospray mass spectrum showed a cluster of peaks at m/z 1128 as a base peak, which is assignable to the monocation **[Fe^{III}(TPP)–Cu^{II}(TIMC$_2$)Cl$_2$]$^+$**. The distribution of isotope peaks was in good accord with simulation. The high-resolution mass spectrum (FAB, NBS) also gave an acceptable mass number within 10 mmeu [Calc. for $C_{60}H_{47}N_{11}OFeCuCl_2$ ([M - Cl]$^+$): 1128.4053. Found: 1128.4101]. The EPR spectrum of this complex showed signals at g = 6.00 and 2.00, which are characteristic of typical high spin iron(III) porphyrins, and signals at $g_{||}$ = 2.25 and $g_\perp$ = 2.07 derived from Cu^{II} (Fig. 1). The lack of a meaningful magnetic interaction in the EPR spectrum indicated a large separation of the two paramagnetic centers . The four-split hyperfine structure of Cu^{II} centered at $g_{||}$ was observed with $A_{||}$ = 142 G (0.0149 cm^{-1}). It has been shown that as the coordination geometry of copper in a CuL_4 center is distorted from square planar to teterahedral, $g_{||}$ increases and $A_{||}$ decreases with an antiparallel linear correlation.[12] Comparing the present $g_{||}$ and $A_{||}$ values with those of the reported Cu^{II} complexes, we conclude that the coordination geometry of the copper is distorted tetragonal rather than planar. This spectroscopic evidence substantiated the designed structure of **[Fe^{III}(TPP)–Cu^{II}(TIMC$_2$)]Cl$_3$**. Of all the CcO models, this is the first binuclear complex bearing three imidazole ligands coordinated to copper.

The reaction between the reduced form (Fe^{II}–Cu^{I}) of the present complex and dioxygen is now under investigation, along with a comparison with our previous binuclear oxygenated complex (**1**).

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Notes and References

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‡ Experimental details will be reported elsewhere. All new compounds were fully characterized by spectroscopic methods and elemental analyses. Stated yields refer to isolated compounds. Electronic spectra were measured in CH_2Cl_2.

§ Simple tertiary amines like Et_3N were ineffective for this reaction. Since the removal of generated HCl is critical for the completion of the reaction, application of amines more basic than imidazole, such as DBU, is essential.

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