Copper Monooxygenase Models: Hydroxylation Reactions resulting from Dioxygen Activation by Copper(I) Complexes

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We describe the synthesis and reactivity of new mononuclear copper(I) complexes **4a–c**, which can be considered to be copper monooxygenase models and can be used to achieve hydroxylation of aliphatic C–H bonds by dioxygen activation.

There is presently a great deal of interest in the chemical modelling of the active site of binuclear copper proteins[1,2] that interact with dioxygen such as haemocyanin (Hc, dioxygen carrier),[3] tyrosinase (Tyr, *ortho* hydroxylation of phenols),[4] or dopamine β hydroxylase (DBH, benzylic hydroxylation of dopamine into noradrenaline).[5] For the type III copper containing proteins Hc and Tyr, these investigations aim towards the synthesis of model complexes that exhibit structural and spectroscopic characteristics similar or identical to the original protein[6] and which mimic the chemical reactivity.[7] The latter feature is related to the environment of the metallic centre which proceeds from the structure and complexation sites of the synthetic ligand. We report here some of our results concerning mononuclear copper complexes that mimic some aspects of the chemical reactivity of DBH. Recent structural results for the latter,[8] suggest the presence of two distinct copper centres per subunit with separate functions in the metal catalysed oxidation. The Cu_B centre is involved in dioxygen fixation and is responsible, *via* a copper(II) hydroperoxo species,[9] for the hydroxylation of dopamine. The Cu_A site, at a distance greater than 4 Å, is at the core of a reductant site where ascorbate (reductant *in vivo*) binds and delivers one electron at a time. In binuclear copper(I) Tyr models such as those described by us[10] and Casella *et al.*,[11] the initial association step between the enzyme model and the phenol substrate can easily be achieved between the hydroxy group of the substrate and one of the copper atoms. For the DBH model the initial association step is less obvious unless the phenethylamine (used as a simplified model of dopamine) is already covalently bound to the ligand by an amide group. This is the reason why we used ligands **3a–c** featuring a tripodal nitrogen bearing arms with one or two pyridine rings and two or one phenethylamide groups.

Ligands **3a–c**† were synthesized in two steps, by Michael addition of amines **1a**, **b** to methyl acrylate, followed by amidation of β-amino esters **2a**, **b** with phenethylamine or *N*-methylphenethylamine. Treatment under argon of ligands **3a–c** with 1 equiv. of $Cu(MeCN)_4PF_6$[12] in degassed acetonitrile affords the very dioxygen sensitive copper(I) complexes **4a–c**,† which were isolated by diethyl ether precipitation. Copper(II) complexes **5a**, **b** were obtained in good yields by treatment of 1 equiv. of $Cu(ClO_4)_2 \cdot 6H_2O$ with amides **3a**, **b** in acetonitrile. Crystallization of a saturated acetonitrile solution of complexes **5a**, **b** using the diethyl ether vapour diffusion technique gives blue crystals. Only the crystals of complex **5a** were suitable for an X-ray diffraction analysis.‡ Amide groups are well known to offer two potential binding sites for metal

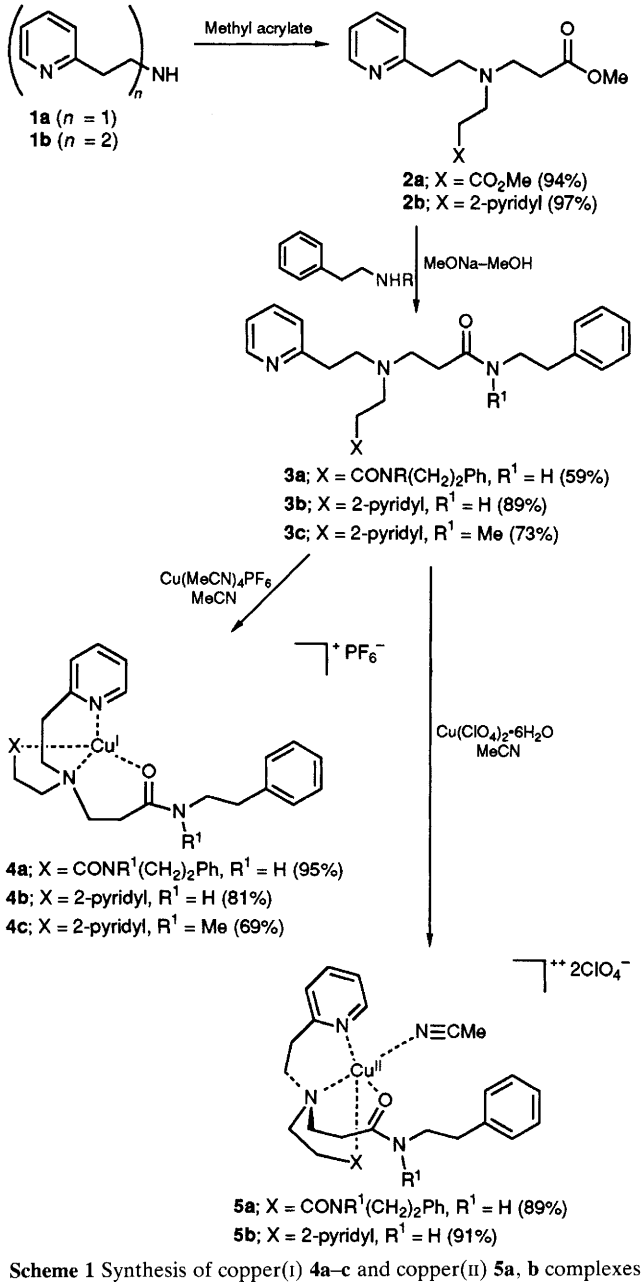
† All new compounds gave spectral and analytical data in agreement with their proposed structure.

‡ *Crystal data* for **5a**: $[C_{29}H_{36}N_4O_2Cu(MeCN)](ClO_4)_2$, M = 776.14. Monoclinic, space group $P2_1/c$, a = 12.833(6), b = 12.141(6), c = 22.95(1) Å, β = 95.49(9)°; V = 3558 Å^3; Z = 4; D_c 1.448 g cm^{-3}. Crystal size 0.15 × 0.15 × 0.4 mm. The final R and R_w values are 0.036 and 0.045, respectively. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

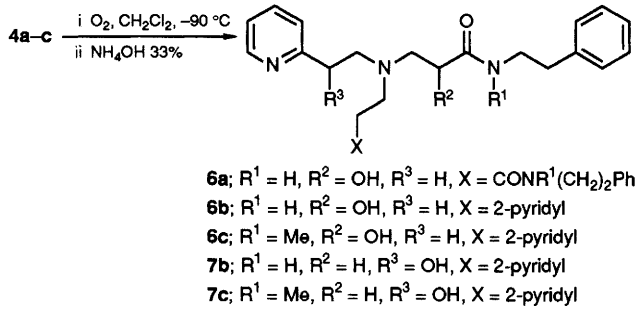
ions complexation (*O- vs. N*-metallation).[13] The crystal structure of complex **5a** reveals that the two amide groups are bound to the copper atom by their oxygen atom which is the most basic site (Fig. 1).

Dissolution of the Cu^{I} complex **4a** in dry CH_2Cl_2 (or EtCN) gives a pale-yellow solution which upon exposure to O_2 at −90 °C rapidly affords a green solution. Following demetallation using aqueous ammonia, analysis of the organic products indicated that 80% of unchanged ligand **3a** was obtained in addition with 20% of the new compound **6a**. A thorough analysis of the spectral data§ clearly indicates that compound **6a** comes from the insertion of one oxygen atom in the position α to one of the amide groups. The copper(I) complex **4b**, when oxidized under identical experimental conditions, affords after decomplexation with aqueous ammonia a mixture of recovered ligand **3b** (74%) and two monohydroxylated compounds **6b** (11%) and **7b** (15%). As shown by the spectral analysis,§ compounds **6b** and **7b** are respectively the hydroxylation product in the position α to the carbonyl and α to the pyridine ring. No reaction occurred when 10^{-3} mol dm^{-3} acetonitrile solutions of complexes **5a**, **b** were treated with 3 equiv. of aqueous hydrogen peroxide solution.

The results that we report here are related to the reactivity of a saturated aliphatic side chain.[14] At this time, it is difficult to provide any evidence for the oxygenated copper species responsible for the hydroxylated products **6a–c** and **7b**, **c**. The copper(I) complexes **4a** and **4b** are so reactive towards dioxygen that until now, our attempts to isolate and characterize the reactive $Cu–O_2$ species were unsuccessful. Furthermore, the possibility involving the formation of a $Cu^{II}OOH$ species by proton abstraction of the amido group by a basic



Scheme 1 Synthesis of copper(I) **4a–c** and copper(II) **5a**, **b** complexes



Scheme 2 Reaction of complexes **4a–c** with dioxygen

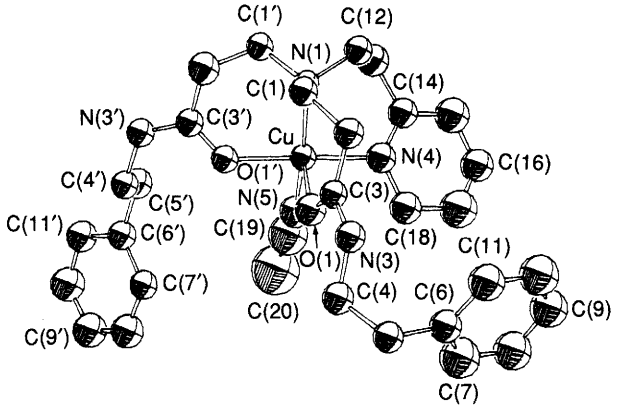


Fig. 1 ORTEP plot of complex **5a** emphasizing the central copper coordination sphere; selected bond lengths (Å) and bond angles (°): Cu–O(1), 2.12 (1); Cu–O(1′), 1.96 (1); Cu–N(1), 2.05 (1); Cu–N(4), 2.00 (1); Cu–N(5), 1.97 (2); O(1′)–Cu–N(5), 82.9 (5); O(1′)–Cu–N(1), 93.9 (4); O(1′)–Cu–N(4), 168.1 (5); N(4)–Cu–O(1), 94.6 (4); O(1′)–Cu–O(1), 93.9 (4); N(5)–Cu–N(4), 87.4 (6); N(5)–Cu–O(1), 101.0 (5); N(1)–Cu–N(4), 93.5 (6); N(5)–Cu–N(1), 163.5 (6); N(1)–Cu–O(1), 95.4 (4)

§ *Selected spectroscopic data*. All signals in the ^1H and ^{13}C spectra of compounds **6a**, **b** and **7b** have been assigned by two dimensional ^1H–^{13}C spectroscopy. Compound **6a**: IR ($CHCl_3$): ν(OH) 3410, ν(NH) 3300 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.43 (d, *J* 4.4 Hz, 1H), 7.67 (t, *J* 7.6 Hz, 1H), 7.07–7.34 (m, 12H), 4.14 (dd, *J* 8.5, 3.7 Hz, 1H), 3.56 (q, *J* 6.7 Hz, 4H), 3.30 (m, 2H), 2.65–3.23 (m, 10H), 2.26 (t, *J* 5.8 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 33.49 (CH_2), 34.59 (CH_2), 35.58 (CH_2), 35.80 (CH_2), 40.08 (CH_2), 40.53 (CH_2), 50.71 (CH_2), 53.37 (CH_2), 57.51 (CH_2), 69.81 (CH), 121.61 (CH), 123.98 (CH), 126.38 (CH), 126.46 (CH), 128.51 (CH), 128.57 (CH), 128.72 (CH), 129.00 (CH), 137.08 (CH), 138.84 (C), 139.03 (C), 148.42 (CH), 159.85 (C), 171.98 (C), 172.66 (C). Compound **6b**: IR ($CHCl_3$): ν(OH) 3410, ν(C=O) 1665, ν(C=N) 1600, ν(C=C) 1530, 1460 and 1440 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.48 (d, *J* 4.1 Hz, 2H), 7.61 (td, *J* 7.7, 1.8 Hz, 2H), 7.07–7.36 (m, 9H), 4.14 (dd, *J* 8.4, 4.5 Hz, 1H), 3.54 (q, *J* 6.5 Hz, 2H), 3.02 (m, 6H), 2.88 (m, 6H). Compound **7b**: IR ($CHCl_3$): ν(OH) 3480 ν(NH) 3320, ν(C=O) 1650 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.57 (d, *J* 4.6 Hz, 2H), 7.70 (td *J* 7.5, 1.3 Hz, 2H), 7.59 (br s, 1H), 7.19–7.34 (m, 9H), 4.89 (dd, *J* 8.2, 3.1 Hz, 1H), 3.44 (q, *J* 6.3 Hz, 1H), 3.33 (m, 2H), 2.71–3.26 (m, 8H), 2.34 (br t, *J* 6 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.99 (C), 161.36 (C), 159.89 (C), 148.85 (CH), 148.53 (CH), 139.27 (C), 136.26 (CH), 136.74 (CH), 128.65 (CH), 128.45 (CH), 126.27 (CH), 123.81 (CH), 123.69 (CH), 122.34 (CH), 121.60 (CH), 71.21 (CH), 61.21 (CH_2), 53.64 (CH_2), 50.98 (CH_2), 40.57 (CH_2), 35.63 (CH_2), 34.50 (CH_2), 33.51 (CH_2).

μ-peroxo copper(II) species[15] can be excluded because when the *N*-methyl derivative **4c** was used the same hydroxylated products **6c** (10%) and **7c** (17%), were obtained. We can also mention that the copper(II) complexes **5a**, **5b** remain unreactive even in the presence of a threefold excess of hydrogen peroxide; this probably indicates that a $Cu^{II}OOH$ complex is not formed, at least not easily, by such a treatment. But, the present results clearly show that it is possible to conceive mononuclear models of DBH. The appropriate environment of the copper atom allows the formation of copper–dioxygen species that are able to hydroxylate the aliphatic part of the ligand in the position β to the tripodal nitrogen. However the latter does not occur on the phenethylamide part of the ligand as expected. Further modelling is required to master fully the structure and topology of the copper ligand in order to achieve hydroxylation on the proper methylene group as in DBH. The approach used here needs to be improved to construct a copper complex with catalytic hydroxylation properties towards an exogenous ligand. For this purpose the problem of the association process of dopamine into the copper coordination sphere still remains to be solved.

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