# ACC-Oxidase like activity of a copper (II)–ACC complex in the presence of hydrogen peroxide. Detection of a reaction intermediate at low temperature

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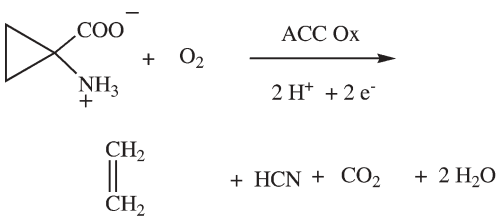
**A Cu(II)–ACC complex [(Bpy)Cu(ACC)($H_2O$)]$ClO_4$ (1) was prepared and its treatment with hydrogen peroxide gave rise to ethylene production in an ACC-Oxidase like activity. A brown species that could be a key intermediate in the reaction was detected at low temperature.**

Ethylene is a hormone involved in many steps of plant growth and development.[1] The final step of its biosynthesis is catalysed by a non-heme iron(II) dependent enzyme, ACC-Oxidase (ACCO), which converts 1-aminocyclopropane carboxylic acid (ACC) into ethylene in the presence of dioxygen and a reductant as summarised in Scheme 1.

The structure of ACCO from *Petunia hybrida* has recently been reported.[2] It confirms that the active site contains a single Fe(II) ion coordinated to three residues (two histidines and one aspartate), constituting a classical coordination sphere within the family of non-heme iron oxidases/oxygenases.[3] While the ACCO mechanism remains unclear at this time, it is reasonably assumed that the first step of the reaction is the coordination of the substrate ACC to the metal ion in a bidentate mode.[4–6] Regarding ACC coordination to metal ions, few data are available in the literature. Spectroscopic and structural data on metal–ACC complexes would thus be of great interest as they may give clues to understanding the catalytic mechanism of the enzymatic system. As a first approach, we focused on the interaction between a copper(II) ion and ACC since many copper–amino acid complexes were reported and crystallised.[7] In the following work we report on the synthesis and the study of a bipyridine(bpy)–copper(II)-ACC complex that reacts with hydrogen peroxide to produce ethylene.

The complex [(bpy)Cu(ACC)($H_2O$)]·$ClO_4$ (**1**) was prepared by adding a methanolic solution of bipyridine to one equivalent of $Cu(ClO_4)_2·6H_2O$, ACC and NaOH in methanol. A blue powder was collected by filtration[8] and single crystals suitable for X-ray structure determination were obtained by slow diffusion of diethyl ether to a methanolic solution of **1**. The cation [(bpy)Cu(ACC)($H_2O$)]$^+$ has a square-base pyramid geometry with the ACC and the bpy moieties forming the basal plane and a water molecule coordinated to the Cu(II) on the axial position (Fig. 1). However, a disordered perchlorate anion was found close to the copper and refined on three different sites. One oxygen atom of each of the three species is lying at a distance ranging from 2.85 to 2.99 Å from the copper ion thus conferring on the latter a pseudo-octahedral geometry (especially in the case of a minor site perchlorate oxygen that is located *ca.* 2.85 Å from Cu). Analysis of the Cambridge Structural Database reveals that the Cu–N(ACC) and Cu–O(ACC) distances are within the range of copper(II)–amino acid distances for complexes of similar geometry (Cu–N = 1.96–2.03 Å, Cu–O = 1.91–1.97 Å).[7] However, it is notable that in the ACC ligand, the C3–C4 distance is slightly shortened at 1.483(6) Å as compared to the same distance measured in free ACC (ranging from 1.490 to 1.497 Å).[9,10] This is accompanied by a closure of the C3–C2–C4 angle. This tendency is confirmed in two other structures of Cu(II)–ACC complexes[11] and has already been




**Scheme 1** Reaction catalysed by ACC Oxidase.

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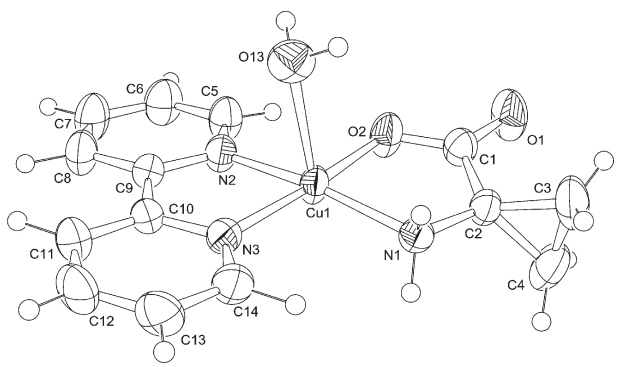


**Fig. 1** ORTEP drawing (ADP shown at the 50% probability level) of [(bpy)Cu(ACC)($H_2O$)]$^+$ cation. The perchlorate anion has been omitted for clarity. Selected bond lengths (Å): Cu1–N1 = 2.005(2), Cu1–O2 = 1.916(2), Cu1–N2 = 1.985(3) , Cu1–N3 = 2.011(3), Cu1–O13 = 2.421(3).

observed in M(II)–ACC pyridoxal Schiff base complexes (M = $Cu^{II}$ and $Ni^{II}$).[12]

The X-band EPR spectra of **1** were recorded at 130 K in MeOH or in $H_2O$–glycerol (9 : 1) frozen solutions. They are consistent with a copper(II) ion in a square-planar derived geometry in accordance with the solid-state structure. The simulations with automatic parameter fitting were performed for rhombic symmetry[13] and allowed us in both cases to distinguish two distinct hyperfine couplings with the nitrogen atoms of the ligands (for instance in MeOH, coupling constants expressed in $10^{-4}$ T, the contribution of naturally abundant $^{63}Cu$ and $^{65}Cu$ is considered, but here the values refer to $^{63}Cu$: $g_x = 2.0503$, $A_{x\mathrm{Cu}} = 19.9$, $A_{x\mathrm{N}(1)} = 5.5$, $A_{x\mathrm{N}(2)} = 13.5 - g_y = 2.0508$, $A_{y\mathrm{Cu}} = 18.5$, $A_{y\mathrm{N}(1)} = 5.5$, $A_{y\mathrm{N}(2)} = 13.5 - g_z = 2.2325$, $A_{z\mathrm{Cu}} = 184$, $A_{z\mathrm{N}(1)} = 14.6$, $A_{z\mathrm{N}(2)} = 6.5$). One set of coupling constants [labelled N(1)] is probably accounting for the nitrogen of the ACC and the second [labelled N(2)] for the two nitrogens of the bipyridine. This indicates that the structure of the complex, and more precisely the bidentate coordination of ACC, is conserved in solution.

We then studied the reactivity of complex **1** with $H_2O_2$ in water and in methanol. Reactivity assays were performed as follows: 1 mL of a millimolar solution of **1** (either in $H_2O$ or in MeOH) were introduced in sealable tubes of 16 mL; hydrogen peroxide was then added through the septum with a syringe and the evolved ethylene was measured by removing 0.2 to 1 mL of the headspace with a gastight syringe and injecting the sample into a gas chromatograph.[14] When complex **1** was treated with 10 equiv. of hydrogen peroxide in water or in methanol, a small amount of ethylene was detected (0.5 and 0.7% yield *vs.* initial complex respectively). Alkalinisation of the media was followed by a spectacular increase in the activity (Fig. 2A). The evolved ethylene reached a maximum with 6 equiv. of sodium hydroxide in $H_2O$ and with 3 equiv. in MeOH (31% and 67% of conversion respectively). In the same conditions (1 mM in $H_2O$ or MeOH, presence or absence of base, 10 mM of hydrogen peroxide), unbound ACC was hardly oxidised to produce ethylene, indicating that the observed activity is not due to free ACC in solution that could have been released from the complex. Furthermore, a 1 mM solution of [$Cu(ClO_4)_2 \cdot 6H_2O$ + ACC] was prepared and ethylene production was investigated in the presence of 10 equiv. of $H_2O_2$ as a function of added NaOH (Fig. 2A). In these conditions, ethylene production was found to be 2 to 10 times lower than that observed with complex **1** depending on the conditions. Indeed, in $H_2O$, with 6 equiv. of base, ethylene is produced with a yield of 9% (*vs.* 31% with **1**) and in MeOH with 3 equiv. of NaOH, with a yield of 14% (*vs.* 67% with **1**). These results underline the importance of controlling the coordination of the amino acid to the metal ion.

Several hypotheses can be considered for the role of the base. One can first think of a modification of **1** in an alkaline solution. However, very few spectral changes (UV–visible or EPR) were detected upon addition of sodium hydroxide suggesting that the complex is poorly affected. A second hypothesis is that the addition of base helps to deprotonate the hydrogen peroxide and increases its nucleophilicity favouring then the formation of a Cu–peroxide adduct. To investigate this possibility, we measured the activity as a function of pH (Fig. 2B). The ACC conversion reached a maximum at pH ~11.8 (corresponding to the addition of 6 equiv. of NaOH) which is the p$K_a$ of the $H_2O_2/HOO^-$ couple. After 6 equiv. of added base, the pH increased very slowly and the

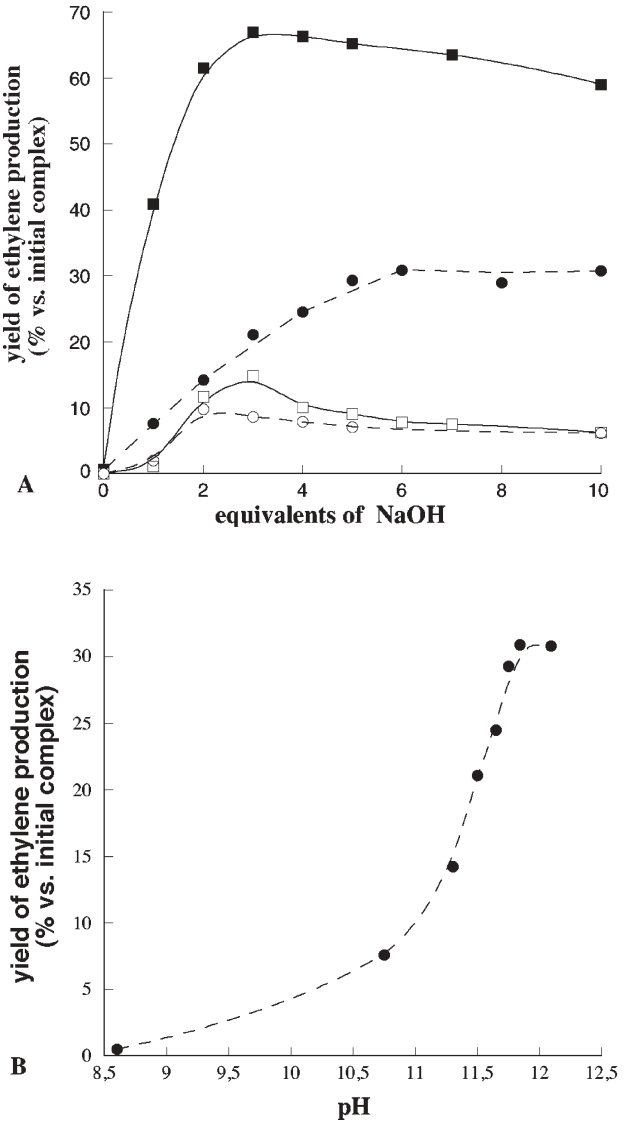


**Fig. 2** Ethylene production by complex **1** in the presence of $H_2O_2$. (A) ethylene produced in the presence of 10 equiv. of $H_2O_2$ (100 μL of a 0.1 M solution) as a function of NaOH added by: 1 mL of a 1 mM solution of complex **1** either in water (---●---) or in methanol (—■—) and by 1 mL of a 1 mM mixture of ($CuClO_4 \cdot 6H_2O$ + ACC) either in water (---○---) or in methanol (—□—). (B) ethylene produced by complex **1** in water as a function of pH. The pH was adjusted by addition of NaOH.

activity was found to be rather stable. This could indicate that the deprotonation of the hydrogen peroxide is a favourable factor for the reaction and that a copper–peroxide intermediate could be involved in the oxidation of ACC.

At low temperature (−30 °C in methanol), the addition of 10 equiv. of hydrogen peroxide to complex **1** in the presence of a few equivalents of base resulted in the appearance of a brown coloration, stable for few minutes at −30 °C, and characterised by an absorption band centered at *ca.* 440 nm (Fig. 3). Capdevielle and Maumy observed a similar brown coloration when copper(II) salts were treated by a large amount of hydrogen peroxide. The brown complex was isolated and described as an oxidising agent for several substrates. The raw formula of the intermediate was found to be $CuO_2H$ but its exact structure was never completely

 

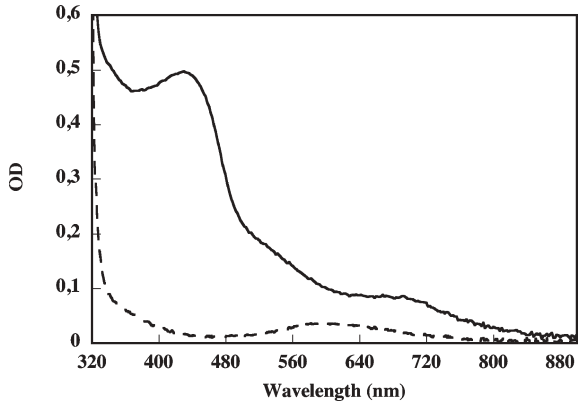


**Fig. 3** UV–visible spectra of 1 mM solution of **1** in MeOH with 5 equiv. of NaOH at −30 °C : initial complex (---); brown intermediate 20 s after addition of 10 equiv. $H_2O_2$ (—).

elucidated.[15] In our case the brown intermediate observed could be of a similar nature and, as we have previously discussed, it could contain a copper–peroxide moiety. The nature and exact structure of this intermediate remains to be elucidated by further experimental data.

In this study we have prepared a copper(II)–ACC complex which is able to convert the ACC amino acid into ethylene in the presence of hydrogen peroxide. To our knowledge, this is the first example of a well-characterised coordination compound containing a metal–ACC motif. Furthermore, this complex constitutes the first bioinspired functional model of ACCO. Investigations to determine the exact nature of the brown intermediate and its implication in the oxidation of ACC into ethylene are in progress.†

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## Notes and references

† *Crystal data* for **1**: $C_{14}H_{16}N_3O_7ClCu$, $M_w$ = 437.29, monoclinic, blue crystal (0.4 × 0.2 × 0.2 mm$^3$), $a$ = 12.0940(1) Å, $b$ = 19.5096(3) Å, $c$ = 7.5502(2) Å, $\beta$ = 102.8066(7)°, $V$ = 1737.14(6) Å$^3$, space group $P2_1/c$, $Z$ = 4, $\rho$ = 1.672 g cm$^{-3}$, $\mu$(MoKα) = 14.54 cm$^{-1}$, 15442 reflections measured in the 2.71–27.48° $\theta$ range, 3586 unique ($R_{int}$ = 0.034), 251 parameters refined on $F^2$ to final indices $R[F^2 > 4\sigma F^2$:3265 reflections] = 0.0478, $wR$[3586 reflections] = 0.1117 [$w = 1/[\sigma^2(F_o^2) + (0.0465P)^2 + 2.5576P]$ where $P = (F_o^2 + 2F_c^2)/3$]. All hydrogen atoms were found experimentally, included into the calculations but not refined. The perchlorate anion was found to be disordered with a major and two minor species: indeed the oxygen atoms were disordered and refined on three sites with occupancies equal to 0.6, 0.2 and 0.2 respectively, the chloride and the O3 oxygen being common to the three species. The final residual Fourier positive and negative peaks were equal to 0.787 and −0.579, respectively. Crystal data were collected on a Nonius KappaCCD diffractometer at 293 K. Solution and refinement calculations were performed using SIR92[16] and SHELX-97[17] respectively. CCDC 288376. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b515374c

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