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Copper(II)/H_2O_2-Mediated DNA Cleavage: Involvement of a Copper(III) Species in H-Atom Abstraction of Deoxyribose Units

Amina Amine,[a,c] Zidane Atmani,[a] Abdelila El Hallaoui,[b] Michel Giorgi,[c] Marcel Pierrot[c] and Marius Réglier[c,*]

[a]*Université Moulay Ismail, Faculté des Sciences de Meknès, Meknès, Morocco*
[b]*Université Mohamed Benabdellah, Faculté des Sciences et Techniques de Fès Sais, Fès, Morocco*
[c]*Chimie, Biologie et Radicaux Libres, UMR-CNRS 6517, case 432, Universités d'Aix-Marseille 1 et 3, Faculté des Sciences et Techniques de Saint Jérôme, av. Escadrille Normandie-Niemen, F-13397 Marseille Cedex 20, France*

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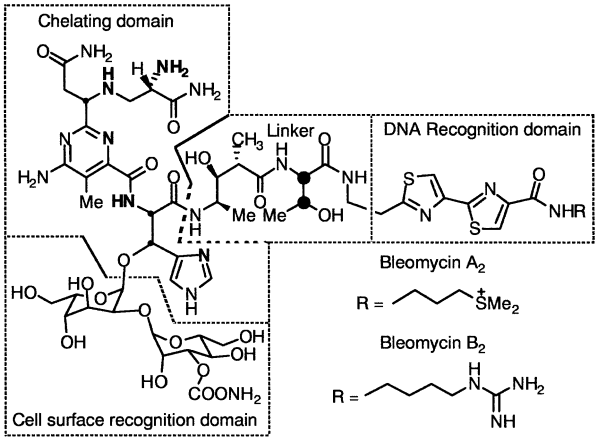
Abstract—A new bis-amido–copper(II) complex **2** has been prepared. In the presence of reducing agents (ascorbate or DTT) under air atmosphere or hydrogen peroxide, complex **2** exhibited interesting nuclease activities in the 1–10 μM concentration range. For explaining the activity observed with hydrogen peroxide, we propose the occurrence of a bis-amido–copper(III) intermediate and an oxidation mechanism involving a H-atom abstraction of deoxyribose moieties of DNA.

Bleomycins (BLM) are well-known glycopeptides clinically employed in the treatment of several neoplastic diseases.[1] The overall structure of BLM can be thought of as containing four distinct regions consisting of (i) a N-terminal domain responsible for the metal binding, oxygen activation and site-selected DNA cleavage, that is the reactive domain, (ii) a C-terminal domain, containing a bithiazole moiety providing the DNA binding affinity and (iii) a disaccharide moiety which is supposed to influence the cell surface recognition and selective accumulation of BLM some cells. These three domains are connected together by a methyl valerate-threonine peptide linker (Scheme 1).

Thanks to the capability to bind metal ions, which activate dioxygen, the clinical efficacy of BLM is due to their faculty to mediate DNA strand scission.[2] In vitro, a lot of transition metal ions such as Fe, Cu, Co, Mn, Ni, Ru, V and Zn are known to bind to BLM and cleave DNA. While the identity of the metal ion responsible for the in vivo DNA degradation remains a subject of debate, some evidence points towards iron as the relevant cofactor.[3] Copper ion also plays an important role in the BLM activity. Early observations seemed to indicate that copper acts as an inhibitor of the BLM activity,[4] but its association to iron increases the action of BLM beyond that exhibited by iron alone.[5] It was also demonstrated that: (i) under O_2 atmosphere copper(I)/BLM could be oxidized to generate oxygen radicals,[6] and (ii) in the presence of dithiothreitol (DTT), copper(II)/BLM was capable of cleaving DNA.[7] In this context, the chemistry of copper associated to BLM and its models have been widely studied and since the first



Scheme 1. Structure of BLM showing its four functional domains (N-atoms involved in metal ion binding are in bold).

*Corresponding author. Tel.: +33-491-288-823; fax: +33-491-983-208; e-mail: marius.reglier@lbs.u-3mrs.fr

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report of DNA cleavage by (1,10-phenanthroline)Cu(I) complex mediated by H_2O_2,[8] a lot of copper complexes having nuclease activity have been reported.[9]

Recently, we focused our attention on the reactivity of copper/oxygen species as possible active species in hydroxylation mediated by copper-containing monooxygenases.[10] One conclusion of our work was that these enzymatic processes could involve copper/oxygen species where copper ion is in high oxidation state such as $Cu(III)_2(\mu\text{-oxo})_2$ or $Cu^{II}O^{\bullet}$ ($\leftrightarrow Cu(III)O^{-}$).[11] In order to find copper complexes capable of cleaving DNA, we developed the synthesis of N-containing tetradentate ligands having two amido groups known to stabilize high copper(III) oxidation state.[12] Here, we report on the synthesis of a new bis-amido–copper(II) complex and the evaluation of its capacity to cleave DNA sequence.

Synthesis

Ligand **1** was synthesized in 57% yield from diphenic acid chloride and 2 equivalents of L-histidine methyl ester in dichloromethane. The corresponding copper(II) complex **2** was quantitatively obtained in methanol by complexation of NaH–bis-deprotonated ligand **1** with copper triflate ($CuTf_2$). Recrystallization of copper(II) complex **2** from dichloromethane using the diethyl ether vapor diffusion technique afforded blue crystals, which were suitable for X-ray diffraction analysis.[13] The structure of the molecule is shown in Figure 1 where some relevant bond distances and angles are indicated (Scheme 2).

DNA Cleavage

The nuclease activity of the copper(II) complex **2** was studied using a reductant (ascorbate or DTT) in the presence of air, or hydrogen peroxide. DNA cleavages were carried out under aerobic conditions with a reaction mixture (20 μL total volume) containing 200 ng of supercoiled pUC19 DNA in 10 mM Tris–HCl buffer (pH 7.6), an appropriate amount of the copper(II) complex **2** (1–10 μM) and reducing agents (ascorbate, 100 μM or DTT, 100 μM) or hydrogen peroxide (100 μM). The reaction mixtures were incubated at 30 °C for 2 h. At the end of the reaction, 6 μL of bromophenol blue were added and the samples were immediately deposited and run on a 0.9% agarose gel containing 1 μl of ethidium bromide. The gels were run at room temperature at a constant current (90 V) for 1 h in TAE buffer. Bands of DNA were visualized by UV light and photographed (Fig. 2).

As shown in Figure 2, the copper(II) complex **2** exhibited significant DNA cleavage reactivity in the 1–10 μM concentration range. The amount of DNA breaks increase as a function of the concentration of the copper(II) complex **2** as evidenced by the progressive transformation of plasmid form I to form II and then to form III (Fig. 2). When DNA was incubated with **2** alone, less than 20% of form II were observed (Fig. 2, lanes 11–13). This is due to the presence of reducing agent in the reaction mixture.

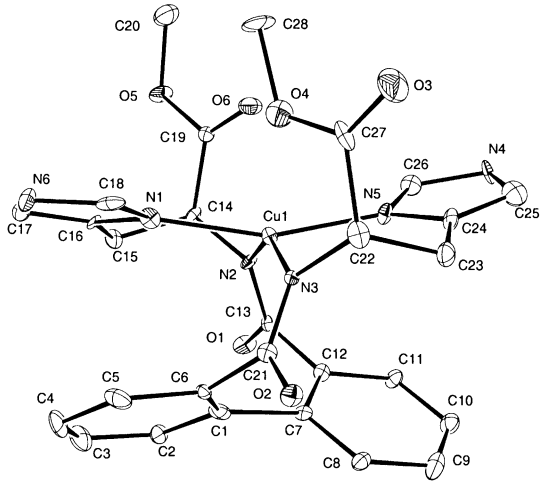
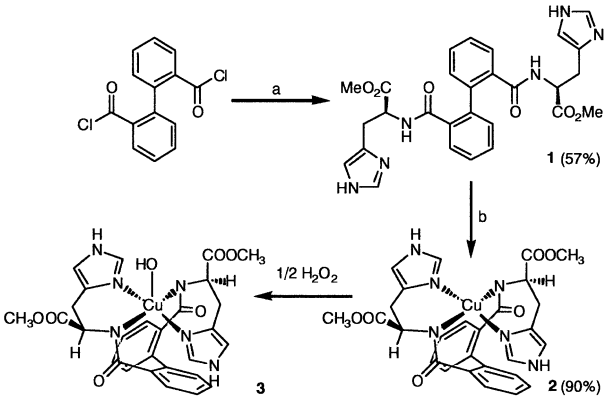


Figure 1. Molecular structure and atom numbering for the copper(II) complex **2**. Selected bond distances in Å: N1–Cu1 2.029 (1), N2–Cu1 1.9825 (9), N3–Cu1 1.992 (1), N5–Cu1 1.9977 (9). Selected bond angles in °: N1–Cu1–N2 88.83 (4), N1–Cu1–N3 96.20 (4), N1–Cu1–N5 160.41 (4), N2–Cu1–N3 148.92 (4), N2–Cu1–N5 96.00 (4), N3–Cu1–N5 89.44 (4).



Scheme 2. Reagents: (a) L-histine methyl ester, CH_2Cl_2; (b) NaH/MeOH then $Cu(CF_3SO_3)_2$ in MeOH.

	1	2	3	4	5	6	7	8	9	10	11	12	13
pUC19 DNA		Ascorbate			H_2O_2			DTT			-		
II	6	59	88	47	94	46	34	55	24	17	9	5	17
III	0	41	0	0	6	0	0	0	0	0	0	0	0
I	94	0	12	53	0	54	66	45	76	83	91	95	83

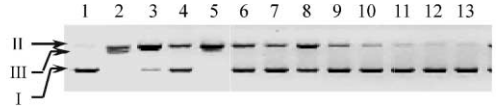


Figure 2. Distributions (%) of pUC19 DNA forms before (lane 1) and after (lanes 2–13) cleavage by copper(II) complex **2**. The copper(II) complex **2** was activated by 100 μM ascorbate (lanes 2–4) or 100 μM H_2O_2 (lanes 5–7) or 100 μM DTT (lanes 8–10) or incubated without cofactors (lanes 11–13). The concentration of **2** varied from 10 μM (lanes 2, 5 and 8) to 5 μM (lanes 3, 6 and 9) and 1 μM (lanes 3, 7 and 10).

Discussion

In order to control if the observed DNA cleavages involve diffusible hydroxyl radicals (HO•), radical scavengers (ethanol, DMSO or glycerol) were used as potential inhibitors of the strand scission activity. When DNA cleavage was performed with **2**/ascorbate or DTT/O_2 in the presence of 10% ethanol, 0.4 M DMSO or glycerol, 50–80% inhibition was observed, while DNA breaks were not affected when **2**/H_2O_2 was used as the oxidizing system. Considering that HO• scavengers did not affect the reactivity of the complex **2** activated by H_2O_2, we assume that a copper/oxygen species might be responsible for DNA cleavage. In order to obtain information regarding the mechanism and the active species resulting of DNA oxidative cleavage, we studied the reaction of the copper(II) complex **2** with H_2O_2. Addition of H_2O_2 (5 equiv) to a 1 mM acetonitrile solution of complex **2** caused a rapid decoloration from blue-green to yellow. Since the new copper complex exhibited an EPR silent spectrum and that the reaction did not produce any formation of O_2 as evidenced by measurement with a Clark electrode, we propose that upon reaction with H_2O_2 the copper(II) complex **2** is transformed into a copper(III) species **3**.

Very recently, Mayer described the oxidation of dihydroantracene (DHA) and 2,4-di-*tert*-butylphenol (DTBP) by an isolated copper(III) complex $[Cu(Pre)]^+$.[14] As for metal-oxo complexes which oxidize hydrocarbons by a H-atom abstraction mechanism, Mayer attributes the reactivity of $[Cu(Pre)]^+$ by: "its thermodynamic affinity for a H-atom ($\equiv H^+ + e^-$) rather than any radical character within the oxidant."

In order to check the reactivity of our putative copper(III) complex **3** produced by interaction of **2** with an excess of H_2O_2, we decided to study oxidation of substrates having C–H with various bond strengths such as DHA and indane. We found that **2**, in the presence of H_2O_2, catalyzes the oxidation of DHA into anthraquinone accompanied by small quantities of anthracene and anthrone (Table 1, entry 1). On the contrary, with indane substrate, which has stronger benzylic C–H bonds, the same conditions produced only a small amount of 1-indanone (Table 1, entry 6). Control experiments show that in absence of either **2** (Table 1, entry 5) or hydrogen peroxide (Table 1, entry 4) the oxidation of DHA did not occur. In order to check the the ligand effect, we studied the reaction with copper triflate. In this case, we observed that DHA and indane were transformed into anthraquinone and 1-indanone, respectively (Table 1, entries 3 and 7). These conditions produce HO• radical by Fenton like mechanism,[15] and thus the results confirm that **2**/H_2O_2 system does not produce diffusible oxygen radical species. According to these observations, we propose a mechanism involving a benzylic H-atom abstraction from DHA by the copper(III) species **3**. This process leads to a water molecule, the benzylic radical DHA• and regenerates the copper(II) complex **2** which can participate in a catalytic cycle again after having reacted with H_2O_2. Then the DHA• radical can react with dioxygen to give the hydroperoxide AntOOH. Since the formation of anthraquinone is also observed under anaerobic conditions (Table 1, entry 2) another way could be the oxidation of DHA• into DHA^+ which could be the precursor of either anthracene after deprotonation or AntOOH after reaction with H_2O_2. The decomposition of AntOOH would lead to anthrone which can give anthraquinone after further oxidation. We confirmed that when anthrone was submitted to the reaction conditions it was transformed quantitatively into anthraquinone (Scheme 3).

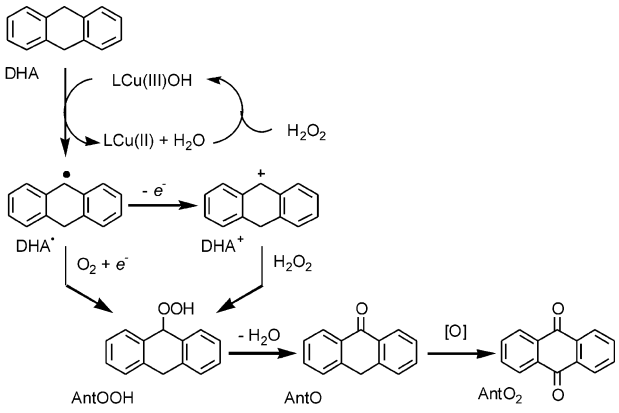
Table 1. Oxidations catalyzed by copper(II) complexes in the presence of H_2O_2 (product distributions were determined by gas chromatography)[a]

	Conditions	Products		
	DHA +	Anthracene (%) +	AntO (%) +	$AntO_2$ (%)
1	**2**/H_2O_2/air	1.5	2.5	38
2	**2**/H_2O_2/Ar	1.9	0.2	27
3	$CuTf_2$/H_2O_2/air	9.2	2.7	21.5
4	**2**/air	2.2	—	0.8
5	H_2O_2/air	6	1.7	1.2
	Indane +	1-Indanol (%)	+	1-Indanone (%)
6	**2**/H_2O_2/air	—		4.3
7	$CuTf_2$/H_2O_2/air	6.4		41.8

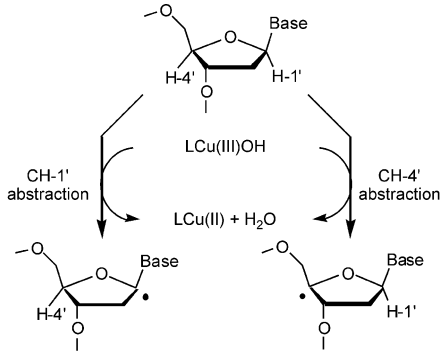
[a]Oxidations were performed in the presence of copper catalyst (10%), H_2O_2 (10 equiv/substrate) in MeOH at 60 °C for 6 h.

Summary

A new bis-amido–copper(II) complex has been prepared and characterized by X-ray diffraction spectroscopy. Its ability to interact and cleave DNA molecules has been demonstrated. We showed that the copper(II) complex **2**/H_2O_2 system, assumed to produce a copper(III) species **3**, is able to oxidize hydrocarbon substrates with weaker C–H bond such as DHA (78 kcal mol^{-1}), but is unreactive towards indane which has a stronger benzylic C–H bond (84.7 kcal mol^{-1} for ethylbenzene). While C–H bond strength data for deoxyribose unit are not available in the literature, we can assume that the CH-1′ and CH-4′ bond strengths of DNA deoxyribose unit are



Scheme 3. Mechanism proposed for oxidation of DHA by the copper(II) complex **2**/H_2O_2 system.



Scheme 4. Mechanism proposed for DNA cleavage.

weaker compared to the benzylic C–H bond of indane. This proposal is all the more reasonable since Walling et al. have studied the kinetics of the abstraction by *tert*-butyloxy radical of acetal H-atoms of several compounds compared to the benzylic H-atom of toluene.[16] In all cases, the ratio $k_{acetal}/k_{toluene}$ which was found to be up to 30 denote a weakness of the acetal C–H bond compared to a benzylic C–H bond. In this context, it is reasonable to propose a similar mechanism for the oxidative degradation of DNA mediated by **2**/H_2O_2, in which the first step is a CH-1′ and/or CH-4′-atom abstraction by the copper(III) species **3** leading radicals precursor of DNA degradation products (Scheme 4).[17]

References and Notes

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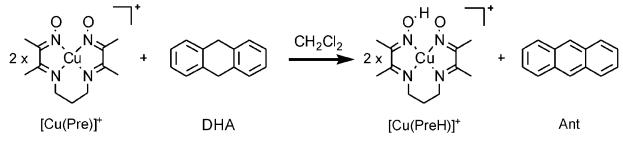

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