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Original Scientific Article

Dioxygen Activation by Copper-Bleomycin: Theoretical Considerations

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Abstract. Density functional theory (DFT) calculations are employed to calculate probable reaction intermediates in dioxygen activation by bleomycin-ligated copper – Cu(I)-dioxygen, Cu(I)-superoxo and Cu(II)-hydroperoxo. The electronic structures of these intermediates are discussed with emphasis on their electromerism. Importantly, unlike in dioxygen activation by iron-bleomycin, formation of these reactive intermediates requires that some of the copper-bleomycin bonds be broken. (doi: 10.5562/cca1793)

Keywords: bleomycin, DFT, copper-peroxo, copper-hydroperoxo, dioxygen activation

INTRODUCTION

Bleomycin is a drug whose action involves chelating a metal center and then damaging DNA within living cells. A key intermediate in bleomycin's anti-DNA action is a species known as activated bleomycin, ABLM, which, on the basis of spectroscopic and theoretical studies, appears well described as a bleomycin-ferric-hydroperoxo adduct (*cf.* Figure 1). ABLM's instability has to some extent precluded detailed structural characterization,[1–3] but its formation is generally accepted to entail reaction of ferrous bleomycin with molecular oxygen, followed by a one-electron reduction to yield a formally ferrous-superoxo adduct, whose protonation would lead to ABLM. A somewhat more stable cognate, the Co(III)-hydroperoxo bleomycin adduct, has been characterized spectroscopically, and its inferred structural features appear very similar to those of ABLM.[4–12]

Copper is also known to activate bleomycin and attack DNA in a similar way to (but less much efficiently than) iron-bleomycin.[13] Structural data on Cu-bleomycin is available showing that copper binds in a very similar way to iron,[14,15] with some indication for partial deligation of the amide nitrogen from Cu(I) under certain conditions, supported also by molecular dynamics calculations.[17] Nevertheless, essentially nothing is known on possible reactive intermediates during dioxygen activation by Cu-bleomycin. Here, density functional theory (DFT) calculations are reported on such reactive intermediates, providing a comparison with the iron system.

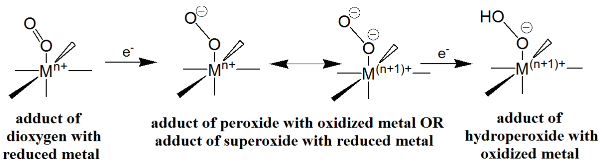

Figure 1. Dioxygen activation by protonation and reduction with transition metals in bleomycin-type coordination environments. The metal M may be iron (II, III) although other metals may perform similar chemistries (*e.g.*, copper). The hydroperoxo species is known as ABLM in iron-bleomycin.

METHODS

Figure 2 shows the models employed in the present work. The bleomycin ligand is modelled as one single chelating unit, comprising all of the potential metal-binding atoms from bleomycin. Bleomycin additionally contains sugar and sulphur-based aromatic systems, which are not included in our models especially as they were also not included in computational models of iron systems by others.[3] Three states at the copper were examined: copper(I) bound to neutral O_2 ($S = 0$ multiplicity for the adduct), its one-electron reduced version (formally Cu(I) bound to superoxide, $S = 1/2$), and Cu(II) bound to hydroperoxide ($S = 1/2$).

Geometry optimization was performed with the B3LYP density functional[17–20] with the 6-31G** basis set as implemented in Spartan.[21] For the SCF calculations, a fine grid was used, and the convergence criteria were set to 10^{-6} (for the root mean square of electron density) and 10^{-8} (energy), respectively. For geometry

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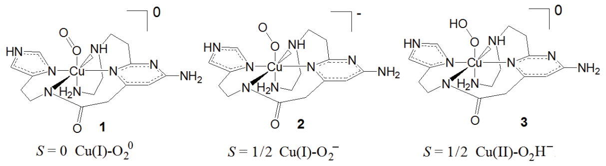

Figure 2. Models employed in the present study.

optimization, convergence criteria were set to 0.001 a.u. (maximum gradient criterion) and 0.0003 (maximum displacement criterion).

RESULTS AND DISCUSSION

Table 1 lists key calculated geometrical parameters for the models of Figure 2, as well as results on reference models of Cu(I) and Cu(II) bleomycin without a dioxygenic (O_2 or OO^- or OOH^-) ligand. One important feature is the fact that dioxygen binding and reduction at the bleomycin-bound copper appears to entail cleavage of at least one copper-nitrogen bonds, as expected since these intermediates would imply coordination numbers distinctly higher than those normally preferred by copper – even though prior to dioxygen binding according to experimental data Cu(II) binds to bleomycin in a manner essentially identical to iron or cobalt, in pentacoordinated manner.[14,15] Thus, two copper-nitrogen bonds are broken in the Cu(I) dioxygen (**1**) (bond lengths longer than 3.5 Å towards the imidazole ring and towards the amine nitrogen trans to the initial empty coordination position). Only the latter bond is clearly broken in models **2** and **3**. Additionally, the bond between the copper and the amine nitrogen cis to the dioxygenic ligand is ~ 2.5 Å in all three models, which is at the limits of what can be described as coordinative copper-nitrogen bonding. This cleavage of copper-nitrogen bonds in models **1–3** is an important difference to the behaviour of iron- and cobalt-bleomycin, where formation of hydroperoxo adducts did not require cleavage of metal-bleomycin bonds.[1,3,5,9]

The O−O bond in model **1** is 1.38 Å for S = 0 and 1.33 for S = 1, consistent with what is measured at this level of theory for a superoxide molecule (1.35 Å for free O_2^- and 1.33 Å for OOH^0), and 0.16 Å longer than the bond length within a free dioxygen molecule (1.22 Å for S = 1 O_2). As such, one may conclude that binding of dioxygen to Cu(I) bleomycin has resulted in an electron transfer, so that the adduct is better described as Cu(II)-superoxo – much like the adduct of ferrous hemoglobin with dioxygen is better described as

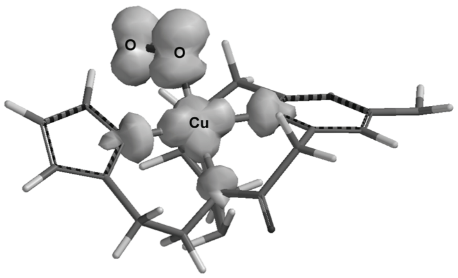

Figure 3. Spin density in model **2**.

Table 1. Key DFT-derived distances and angles (Å and º, respectively) for models employed in the present study

1, S = 0 Cu(I)−OO^0			
Cu−O	1.82/1.86[(d)]	Cu−N(amide)[(b)]	1.90
O−O	1.38	Cu−N(imid)[(c)]	3.78
Cu−O−O	69º	Cu−N(py)[(d)]	1.94
Cu−N(*cis*)[(a)]	2.46	Cu−N(*trans*)[(a)]	3.83
1, S = 1 Cu(I)−OO^0			
Cu−O	2.08/2.90[(d)]	Cu−N(amide)[(b)]	1.95
O−O	1.33	Cu−N(imid)[(c)]	3.52
Cu−O−O	115º	Cu−N(py)[(d)]	2.07
Cu−N(*cis*)[(a)]	2.07	Cu−N(*trans*)[(a)]	2.15
2[(e)], S = 1/2 Cu(I)−OO^-			
Cu−O	1.93/1.93[(d)]	Cu−N(amide)[(b)]	2.00
O−O	1.46	Cu−N(imid)[(c)]	3.91
Cu−O−O	68º	Cu−N(py)[(d)]	2.06
Cu−NH_2(*cis*)[(a)]	2.62	Cu−N(*trans*)[(a)]	4.24
3, S = 1/2 Cu(II)−OOH			
Cu−O	1.93/2.65[(d)]	Cu−N(amide)[(b)]	1.96
O−OH	1.47	Cu−N(imid)[(c)]	2.03
Cu−O−OH	102º	Cu−N(py)[(d)]	2.06
Cu−N(*cis*)[(a)]	2.59	Cu−N(*trans*)[(a)]	3.69
4, S = 0 Cu(I)			
Cu−O	−	Cu−N(amide)[(b)]	1.98
O−O	−	Cu−N(imid)[(c)]	2.01
Cu−O−O	−	Cu−N(py)[(d)]	1.96
Cu−N(*cis*)[(a)]	3.00	Cu−N(*trans*)[(a)]	2.17
5, S = 1/2 Cu(II)			
Cu−O	−	Cu−N(amide)[(b)]	1.92
O−O	−	Cu−N(imid)[(c)]	2.07
Cu−O−O	−	Cu−N(py)[(d)]	2.02
Cu−N(*cis*)[(a)]	2.16	Cu−N(*trans*)[(a)]	2.26

[(a)] Positions *cis* and *trans* relative to the OOH ligand.
[(b)] Amide nitrogen atom.
[(c)] Imidazole nitrogen atom.
[(d)] Distances to both oxygen atoms are shown.
[(e)] A bidentate version of the model was also identified, which was 16 kcal mol^{-1} less stable and is hence not discussed any further.

Table 2. Key DFT-derived partial charges and spins from NPA (spin given in brackets)

		Partial charge (spin)	
Model / Formal description	Spin state	Cu	O_2 or OOH
1 / Cu(I)−OO^0	$S = 0$	1.42 (0.00)	−0.84 (0.00)
	$S = 1$	1.40 (0.67)	−0.73 (1.1)
2 / Cu(I)−OO^-	$S = 1/2$	1.26 (0.48)	−1.26 (0.46)
3 / Cu(II)−OOH	$S = 1/2$	1.42 (0.67)	−0.75 (0.21)

ferric-superoxo.[22,23] The partial atomic charges shown in Table 2 for the oxygen atoms in model **1** indeed reveal negative charge; whereas in the $S = 0$ state antiferromagnetic coupling is problematic in terms of population analyses, in the $S = 1$ state the 1.1 spin units on the O_2 ligand are clearly in line with a superoxide ligand rather than with a O_2 molecule (be it singlet or triplet). Interestingly, the $S = 0$ was found to feature a bidentate O_2 ligand, whereas the $S = 1$ state featured a monodentate O_2; in both cases a local minimum corresponding to the alternative hapticity isomer could not be located. The $S = 1$ was found to be more stable than $S = 0$ by 13 kcal mol^{-1}.

The O−O bond in model **2** is 1.45 Å, similar to what is predicted at the same level of theory for a peroxo ligand[22,24,25] and longer by ~ 0.1 Å than the superoxide-like bond predicted in model **1**. This suggests that model **2** may be described as Cu(II)-O_2^{2-}, as opposed to Cu(I)-O_2^-. Thus, at this stage the dioxygen molecule has been reduced by two electrons – which is the most one can achieve in terms of activating O_2 with a biological copper center.[26] In fact the O−O bond in this copper(II)-peroxo model is distinctly longer than the 1.33 – 1.36 Å seen in related Fe(III)-peroxo models in heme and non-heme environment.[3,24,25] The geometrical parameters within the Cu−O−O−(H) moiety in **3**, and especially the 1.47 Å O−O distance, are generally in line with previously reported data on related species, showing a clean Cu-hydroperoxo structure,[26] as also supported by the negative charge on the OOH (equal in magnitude to the charge computed for the superoxo ligand in **1**) and by the negligible spin density (0.26 units).

Figure 3 illustrates the spin density in model **2**. The unpaired electron appears distributed in a manner drastically different from what is seen typically in related Fe(II)-superoxo / Fe(III)-peroxo adducts. Thus, in model **2** the spin density at the oxygen atoms is distributed mostly in orbitals located in the metal-oxygen-oxygen plane, while in the iron counterpart it is distributed perpendicular to this plane.[24,27,28] The values of the spin density computed on the two oxygen atoms highlighted in Figure 3 are 0.27 and 0.12, respectively, amounting to less than 0.5 and consistent with the geometrical data of Table 1 in predicting an $S = 1/2$ copper center (hence, Cu(II)) bound to a diamagnetic peroxo ligand – albeit with strong mixing between the orbitals of the metal and peroxide.

CONCLUSION

To conclude, the density functional theory data shown here predicts that dioxygen activation by copper-bleomycin will require deligation of two of the bleomycin ligands away from the copper – which is a notable difference compared to the pathway followed for the same reaction by iron bleomycin. Another important difference is in the fact that the Cu(I)-superoxo/ Cu(II)-peroxo has a distinctly clearer peroxo character at the ligand, compared to related Fe(II)-superoxo / Fe(III)-peroxo moieties previously described at the same level of theory.

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