



## BLEOMYCIN MODEL COMPLEX BEARING A CARBAMOYL DERIVED SUBSTITUENT CAPABLE OF COORDINATION TO THE CHELATED METAL ION AS A SIXTH LIGAND

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**Abstract:** A new, simple bleomycin (BLM) model compound bearing a carbamoyl group mimicking BLM's sugar-tagged carbamoyl moiety was synthesized and the physicochemical properties of its divalent metal complexes were studied. The Fe complex of the model compound exhibited enhanced oxygen activating ability. Copyright © 1996 Elsevier Science Ltd

Bleomycin (BLM) is a family of glycopeptide antibiotics isolated as a Cu(II) complex from a culture of *Streptomyces verticillus*.<sup>1</sup> The therapeutic effect of BLM is believed to be yielded by cellular DNA cleavage which occurs in the presence of a metal ion such as Fe(II) and dioxygen.<sup>2</sup> The Fe(II) chelate of BLM activates dioxygen by oxygenation followed by reduction of the chelated ferrous ion. BLM's sugar moiety is believed to play an important role as an environmental factor for the efficient dioxygen activation.<sup>3</sup> Furthermore, the carbamoyl group in BLM's sugar moiety has been suspected to coordinate to the ferrous ion as a sixth ligand.<sup>4</sup>

Several model compounds of BLM's metal coordination site have been synthesized and the effect of the BLM's sugar moiety on the oxygen activating ability as well as a DNA cleaving ability have been investigated.<sup>5</sup> Recently, Boger et al. synthesized several BLM model compounds complete with or without disaccharide moiety and studied the DNA cleaving activity of the models concluding that the carbamoyl group may have little effects on the metal complexation, oxygen activation and DNA cleavage.<sup>5c-d</sup>

On the other hand, in the course of the study on the simplified BLM model compounds, the introduction of a bulky alkyl group, such as *t*-butyl or lauryl group, as a steric factor in place of the sugar moiety was proved to enhance the stability of the Fe-complex and oxygen activating ability.<sup>6</sup> However, the role of the carbamoyl group in BLM's sugar moiety remained unclarified in such studies. These facts prompted us to undertake synthesis and studies of a new, simple BLM model compound **1** (Fig. 1)

bearing an alkyl tagged carbamoyl group in place of BLM's sugar moiety. During the study, we have observed the coordination of the carbamoyl group to the chelated metal ion in **1**-Fe(II) and Co(III) complexes. Contrary to the preceding results,<sup>5c-d</sup> we have also observed the enhancement of oxygen activating ability in **1**-Fe complex. Here, we wish to make a preliminary report of these results including the synthesis of compound **1** (Fig. 1).

The synthetic procedure of **1** is summarized in Scheme 1. Pyridine carboxylic acid derivative **1** which was prepared from the known aldehyde,<sup>7</sup> was hydrolyzed to free acid and coupled with L-histidine methyl ester by the action of diphenylphosphoryl azide (DPPA) to form the derivative **2** (quantitative). Saponification of the

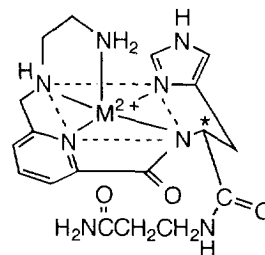
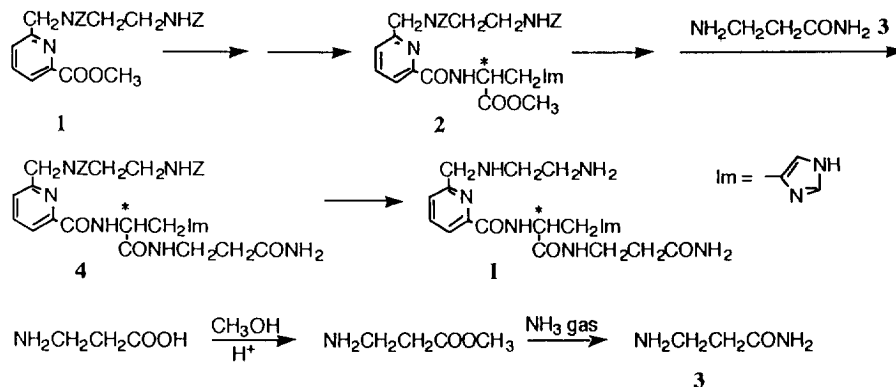


Figure 1. Structure of BLM model compound **1**

ester moiety of **2** followed by the coupling with  $\beta$ -alanylamine **3**, prepared from  $\beta$ -alanine, in the presence of DPPA gave the protected model compound **4** (79%). The final removal of Z-groups on compound **4** was performed by hydrogenation in the presence of a Pd catalyst to yield the model compound **I** (quantitative).<sup>8, 9</sup>



Scheme 1. Synthetic procedure of model compound **I**

The coordination chemistry of the model compound with several divalent metal ions was investigated by spectroscopic methods in Tris-HCl buffer (pH 7.2). The electronic and EPR features of the **I**-Cu(II) complex closely resembled those of the BLM-Cu(II) complex.<sup>10</sup> The results indicate that **I**-Cu(II) forms a BLM-Cu(II)-like pyramidal type complex. The model compound **I** also forms a complex with Fe(II) under anaerobic condition which was confirmed by electronic spectroscopy (Fig. 2-1). The Fe(II) complex was easily oxidized to form stable Fe(III) complex in the presence of atmospheric air as shown in Fig. 2-2. As it was mentioned earlier, the introduction of a bulky group as a steric factor in place of the BLM's sugar moiety has been effective to obtain stable Fe(III) complex in a simple BLM model.<sup>6</sup> However, the current alkyl tagged carbamoyl group in **I** is far smaller compared to such preceding steric factors. This suggests that the role of the  $\beta$ -alanylamine portion in compound **I** is more than a simple steric factor preventing a possible attack of  $\text{OH}^-$  ion to the chelated Fe(III) by a steric repulsion or creating hydrophobic cavity around the metal.

From the electronic absorption study, it is also observed that the Fe(III) complexes of **I** was reduced by a common reducing agent such as dithiothreitol (DTT) through the formation of the Fe-DTT adduct and reoxidized by oxygen to show the reversible redox reaction like the BLM-Fe complex (Fig. 2-3, 4). Formation of the

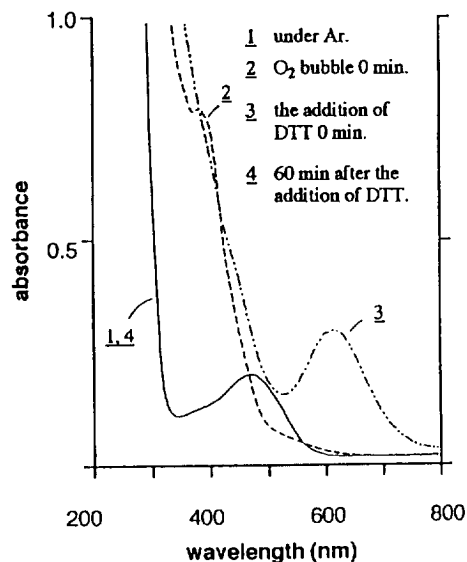


Figure 2. Reversible redox reaction of Fe(II) complex. The concentration of Fe<sup>I</sup> complex was 0.5 mM in 50 mM Tris-HCl (pH 7.2), and the spectra were taken under the following condition; **1**, under Ar ( $\lambda_{\text{max}} = 471 \text{ nm}$ ); **2**,  $\text{O}_2$  bubbling to the sample **1** ( $\lambda_{\text{max}} = 386 \text{ nm}$ ); **3**, the addition of DTT (1.0 mM) to the sample **2**; **4**, 60 min after the addition of DTT to the sample **2**.

Fe(III) complexes and their reversible redox reactions were further confirmed by the EPR method. Thus, the I-Fe(III) complex exhibited a characteristic signal ( $g_x = 2.355$ ,  $g_y = 2.183$ ,  $g_z = 1.882$ ) due to a low-spin Fe(III) complex (Fig. 3-a). These values are almost identical to those of BLM-Fe(III) complex ( $g_x = 2.432$ ,  $g_y = 2.185$ ,  $g_z = 1.898$ ).<sup>11</sup> Figure 3 shows the EPR spectral change in the I-Fe(III) complex after the addition of DTT for 10s and 300s. At the initial reaction stage of the I-Fe(III) complex with DTT, the signal due to the I-Fe(III)-DTT complex was detected ( $g_x = 2.253$ ,  $g_y = 2.171$ ,  $g_z = 1.940$ , Fig. 3-b). About 300s after the addition of DTT, the signal due to a low-spin Fe(III) complex appeared again (Fig. 3-c).

Generation of hydroxyl radicals in the redox reaction of the Fe(II) complex of **I** with oxygen was investigated by EPR spin trapping experiment using *N*-*t*-butyl- $\alpha$ -phenylnitron (BPN) as a spin trapping agent. The EPR parameters, triplet of doublet with a  $g$  factor of  $g^H = 2.01$  and  $a^N = 15.0G$  were identical with those of the hydroxyl radical-PBN spin adduct.<sup>12</sup> The oxygen activating ability of I-Fe complex estimated from the intensity of the EPR signal of the spin adduct was 75 % of that of the BLM standard. The number is higher than that of the related model compound having the bulkier *t*-butyl group as the steric factor.<sup>6d</sup>

Presumably, the coordination of the carbamoyl moiety to the chelated Fe ion prior and after the coordination and the activation of oxygen molecule is responsible for the observed high oxygen activating ability of the I-Fe complex.

The possible coordination of the carbamoyl group of **I** to the metal ion was investigated by IR spectroscopy.<sup>13</sup> The free ligand (**I**) exhibited three  $\nu_{CO}$ 's at  $1644\text{cm}^{-1}$  (terminal carbamoyl),  $1651\text{cm}^{-1}$  (secondary amide, incapable of coordination to the metal ion) and  $1673\text{cm}^{-1}$  (secondary amide, capable of coordination to the metal ion). Ligation of the ligand **I** to Fe(II) is characterized by a shift of the  $\nu_{CO}$  from  $1673\text{cm}^{-1}$  (in free ligand) to  $1557\text{cm}^{-1}$  (in complex).<sup>13a</sup> Coordination of the carbamoyl group to the Fe(II) ion is indicated by the shift of carbamoyl  $\nu_{CO}$  from  $1644\text{cm}^{-1}$  in the free state to  $1521\text{cm}^{-1}$  in the complex. We have also observed a similar shift of  $\nu_{CO}$  by the coordination of the carbamoyl group to Co(III) in the cobalt complex of **I**.<sup>14</sup> Although these spectral features suggest the coordination of the carbamoyl group to the chelated Fe ion, the present data are not sufficient to illustrate the precise structure of the complex and X-ray crystal analysis of the I-Co(III) complex will be needed.

The results of the current study, however, clearly demonstrate that the introduction of a simple alkyl tagged carbamoyl moiety in place of the BLM's sugar moiety into a simplified BLM's metal coordination site is effective

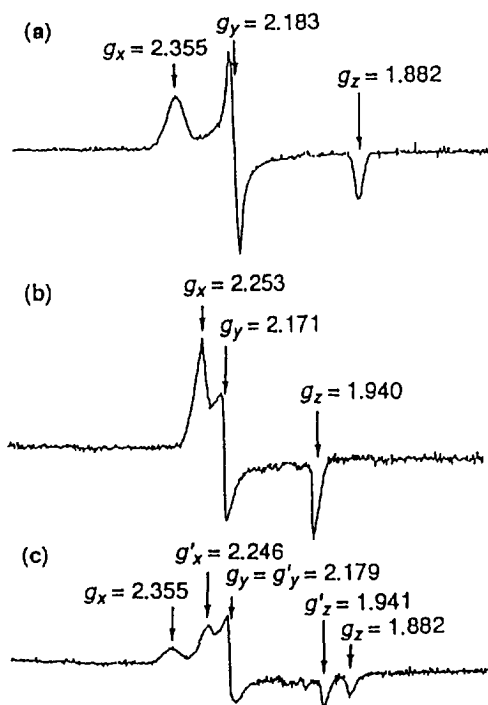


Figure 3. Time course of EPR spectral change of the I-Fe(II) complex (2.0mM) pH 7.2 : (a) 10s after the oxygen bubbling to I-Fe(II) complex : (b) 10s after the addition of DTT (4.0 mM) to the sample **a** : (c) 300s after the addition of DTT (1.0 mM) to the sample **a**

for the enhancement of the stability of the model compound-Fe(III) complex as well as the oxygen activating ability. These effects are possibly brought by the coordination of the carbamoyl group to the chelated metal ion and the observations presented here will contribute to elucidate more precise role of the carbamoyl group in BLM's sugar moiety. The study of the biological effects such as the strand scission of DNA mediated by I-Fe complex is now under investigation and will be reported elsewhere.

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9. White powder.  $[\alpha]_D^{20} = +5.6^\circ$  (C = 0.25, MeOH : H<sub>2</sub>O = 4 : 1), Electrospray Mass for C<sub>18</sub>H<sub>26</sub>N<sub>8</sub>O<sub>5</sub> m/z 403(M+H). Satisfactory NMR spectrum was obtained for the sample.
10.  $\lambda_{\max}$  values were observed at 606nm for the I-Cu(II) complex and 595nm for the BLM-Cu(II) complex. EPR g values were observed at  $g_{\parallel} = 2.200$ ,  $g_{\perp} = 2.040$ ,  $A_{\parallel}(G) = 190$  for the I-Cu(II) complex;  $g_{\parallel} = 2.211$ ,  $g_{\perp} = 2.055$ ,  $A_{\parallel}(G) = 183$  for the BLM-Cu(II) complex.
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14. IR bands (nujol, cm<sup>-1</sup>);  $\nu_{\text{CO}}$  1557, 1521(carbamoyl), 1651 for I-Fe(II) complex.  $\nu_{\text{CO}}$  1599, 1624(carbamoyl), 1659 for I-Co(III) complex.

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