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A NOVEL BLEOMYCIN MODEL COMPOUND BEARING HYDROPHILIC STERIC FACTOR EXHIBITED HIGH OXYGEN ACTIVATING CAPACITY

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Abstract: The bleomycin (BLM) model compound bearing hydrophilic steric factor was synthesized and the physicochemical properties of the metal complexes were studied. Iron complex of the model compound exhibited BLM like reversible redox cycle and higher oxygen activating activity compared to the corresponding model compound bearing hydrophobic steric factor.

Bleomycin (BLM) is a family of glycopeptide antitumor antibiotics and is clinically used against several types of cancer.¹ Therapeutic action of BLM takes place when it forms metal complex with ferrous ion and activates molecular oxygen, thereby inducing the degradation of DNA.² BLM metal complex is also known to oxidize simple organic molecules like olefins.³ Many studies have been reported on the synthesis of model compounds which simplified the metal coordination site of BLM. Among these, model compounds which have hydrophobic steric factor, *tert*-butyl group, in place of the disaccharide moiety existing around the 6th coordination sphere of BLM are intriguing because their iron complexes exhibited enhanced O₂ activating ability.⁴ We have previously reported the synthesis of a BLM model compound II bearing a long alkyl chain (lauryl group) as a hydrophobic steric factor around the 6th coordination sphere (Fig.1).⁵ The Fe-complex of compound II was found to exhibit enhanced stability and high O₂ activating ability compared to the compound I which lacks the steric factor. Thus the introduction of steric factor was found to be effective for the creating a highly active BLM model compound. The compounds mentioned above bears hydrophobic steric factor around 6th coordination sphere, despite the fact that natural BLM has hydrophilic disaccharide moiety as the steric

factor. These prompted us to undertake a study of a new BLM model compound III having tris(hydroxymethyl) aminomethane derivative as a hydrophilic steric factor in a chiral manner. The model compound IV bearing a *tert*-butyl group as the hydrophobic steric factor was also synthesized to compare the effect of the nature of the steric factor on the physicochemical properties and oxygen activating ability of the model compounds.



Figure 1. Structure of Model Metal Complexes

The model compound III was prepared as follows. Tris(hydroxymethyl) aminomethane 1 was treated with tert-butyl dimethylsilyl chloride (TBDMS-Cl) followed by coupling with N^{α} -(Fmoc)-Nim-(Trt)-L-histidine 3 in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxy benzotriazole (HOBt) in DMF⁶ to give the derivative 4 (94%). Removal of Fmoc group with piperidine⁷ yielded the free amine 5 (97%). The free amine 5 was coupled with pyridinecarboxylic acid derivative 9 in the same manner as above to afford the fully protected model compound (90%). Deprotection of the foregoing material with a mixture of trifluoroacetic acid (TFA), *m*-cresol and thioanisole⁸ yielded model compound III (71 %) possessing tris(hydroxymethyl) aminomethane in a chiral manner.⁹ Synthesis of the model compounds IV bearing *tert*-butyl group was accomplished by the modified method of the reported procedure.⁵ Coupling of (Z)-protected L-histidine 6 with *tert*-butyl amine in the presence of diphenylazidophosphate (DPPA) afforded the amide 7 in 90 %. Deprotection of 7 by hydrogenation in the presence of Pd catalyst followed by coupling with 9 by the action of DPPA provided the protected model compound (87 %). Final removal of Z-group of the foregoing material was performed in a similar manner as above to yield model compound IV-L (92 %).^{9b}



Scheme 1. Synthetic Procedure of Model Compounds

Cu^{II} complexes of the model compounds III and IV in Tris-HCl buffer (pH 7.2) showed characteristic UV-Vis absorption with the absorption maximum at 604 nm, which is very similar to the natural BLM-Cu^{II} complex (absorption maximum at 595 nm).¹⁰ EPR spectra of the Cu^{II} complexes exhibited similar parameters¹¹ to these of the BLM-Cu^{II} complex. These results suggest that both compound III and IV form pyramidal type complexes with Cu^{II} ion like BLM-Cu^{II} complex.

The formation of Fe^{II} complexes of the model compounds III and IV in pH 7.2 under an anaerobic condition were confirmed by UV-Vis spectroscopic study. The Fe^{II} complexes of both III and IV were easily oxidized to form stable Fe^{III} complexes in the presence of oxygen or air (Fig. 2, 1, 2). On the other hand, the Fe^{II} complex of model compound I which does not have the steric factor was very unstable under the same condition and the precipitation of Fe(OH)₃ was observed. The Fe^{III} complexes of III and IV were easily reduced by the action of dithiothreitol (DTT) and regenerate the corresponding Fe^{II} complexes (Fig. 2, 4), presumably through the formation of DTT adduct of the Fe^{III} complex which showed characteristic absorption spectrum around 630 nm (Fig. 2, 3). Thus, the introduction of the steric factor, either hydrophobic or hydrophilic, was proved to be effective to enhance the stability of Fe^{III} complex as well as to promote the reversible redox reaction.

The cycle of the reversible redox reaction in the presence of the reducing agent DTT, which is characteristic to the BLM-Fe complex, was also confirmed by EPR (Fig.3, c, d).

The spectrum c is assigned to that of the DTT adduct of the III-Fe^{III} complex which was shown in the UV-Vis study. The III-Fe^{III}-DTT adduct should have small rhombic splitting of the g values than the III-Fe^{III} complex. The EPR parameters ($g_x = 2.234$, $g_y = 2.175$, $g_z = 1.943$) are similar to those of BLM-Fe^{III}•H₂S adduct ($g_x = 2.223$, $g_y = 2.148$, $g_z =$ 1.999).12 Thus, we detected the formation of the intermediate DTT adduct of the Fe^{III} complex of the BLM model compounds during the cycle of the redox reaction by UV-Vis and EPR studies for the first time.

The formation of the iron complexes and their reaction with oxygen were further examined by EPR study. Figure 3 shows the time course of the spectral change of the III-Fe^{II} complex after the exposure to oxygen. At the initial reaction stage of the III-Fe^{III} complex with oxygen, signals due to the two low-spin Fe^{III} complexes, and a high-spin Fe^{III} complex were detected (Fig.3, **a**). Although the high-spin species (g = 4.238) is stable, the low-spin Fe^{III} complex exhibiting small rhombic splitting of the g values ($g_x = 2.313$, $g_y = 2.182$, $g_z = 1.932$), which is considered as the activated Fe^{III}-O₂H complex¹², was transient and turned to the stable low-spin Fe^{III}-OH complex.



Figure 2. Reversible redox reaction of III-Fe^{II} complex. The concentration of III-Fe^{II} complex was 0.5 mM in 50 mM Tris-HCl (pH 7.2), and the spectra were taken under the following condition; <u>1</u>, under N₂ (λ max = 475 nm); <u>2</u>, O₂ bubbling to the sample 1 (λ max = 475 nm); <u>3</u>, the addition of DTT(0.5 mM) to the sample <u>2</u>; <u>4</u>, 30 min after the addition of DTT to the sample <u>2</u>. Essentially similar results were obtained for IV-Fe^{II} complex.



Figure 3. Time course of EPR spectral change of the III-Fe^{II} complex after oxygen bubbling at pH 7.2 (left) : (a) 5s : (b) 600s, and after DTT addition (right) : (c) 5s : (d) 300s. The concentration of the complex and DTT were 1.0 and 1.2 nM, respectively.

Neither transient species nor high-spin species was observed in the reaction of the IV-FeII complex with oxygen. At the final reaction stage, EPR signals due to a low-spin FeIII -OH complex with a large rhombic splitting $(g_x = 2.354, g_y = 2.182, g_z = 1.893)$ was detected (Fig.3, b). The IV-FeII complex also showed EPR signals of low-spin species, $g_x = 2.363$, $g_y = 2.175$, $g_z = 1.888$. These values are typical of low-spin FeIII complex like BLM-FeIII ($g_x = 2.432$, $g_y = 2.185$, $g_z = 1.898$).¹² These EPR experiments suggest that introduction of the hydrophilic steric factor stabilized the transient FeIII -O2H complex

During the redox reaction of the Fe^{II} complex of III or IV with O_2 , O_2 was activated to generate hydroxy radical which was demonstrated by EPR spin-trapping experiment. The EPR spectra obtained by the reaction of the III- or IV-Fe^{II} complex with oxygen in the presence of N-tert-butyl- α -phenyl nitrone (BPN) showed characteristic parameters, g = 2.01, $\alpha^{N} = 15.2$ G. These parameters are identical with those of the hydroxy radical-BPN spin adduct.¹² The O₂ activating capacity of the complexes, III and IV, to that of the BLM standard estimated by the spin trap experiment were 74 % and 52 %, respectively. The EPR studies clearly showed that the structural feature and the reactivity of the III iron complex are close to those of the BLM iron complex. Also, the introduction of the hydrophilic steric factor to the BLM model compound was shown to be more effective for the generation of hydroxy radical compared to the introduction of hydrophobic steric factor.

In conclusion, we are successful to design the model compound III resembling BLM like EPR behavior and reactivity. The high water solubility and oxygen activating ability will also make the III-Fe complex as a feasible model as the BLM-Fe complex. However, the present data are not sufficient to illustrate precise role of steric factor in natural BLM and further investigations are in progress.

References and Notes

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- a) white powder. [a]D20 +2.7° (C=0.5, MeOH), MS(FAB) for C19H29N7O5•3HClO4 m/z 736(M+) 9. b) white powder. [α]D20 -7.0° (C=0.5, MeOH), MS(FAB) for C19H29N7O2 m/z 388(M+H) Satisfactory NMR spectra were obtained for the each sample.
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