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A MODEL STUDY ON THE MECHANISM OF THE AUTOXIDATION OF BLEOMYCIN[1]

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Summary: In order to clarify the mechanism of the self-inactivation of bleomycin, a synthetic model for the metal binding site of bleomycin is treated with Fe(III)-H_2O_2 or Fe(II)-O_2, and the degradation products are isolated and characterized. A breakdown at the amino acid side chain of the metal binding site has been demonstrated.

It has been well documented that antitumor antibiotic bleomycin (BLM) activates dioxygen by the formation of a unique iron complex of the amine-pyrimidine-imidazole region to generate active species closely related to BLM-Fe(II)-O_2^{2-}, and binds to DNA by the bithiazole-terminal amine region.[2] BLM thus cleaves DNA specifically at GC and GT sequences to exert the antitumor activity. However, Umezawa *et al.* isolated the decomposition product by treatment of the metal free BLM with Fe(II) and oxygen, and proposed that the pseudodipeptide side chain of the pyrimidine moiety seemed to undergo decomposition from the NMR study.[3] On the contrary, very recently, Peisach *et al.* observed that in the absence of DNA the metal binding site of the self-

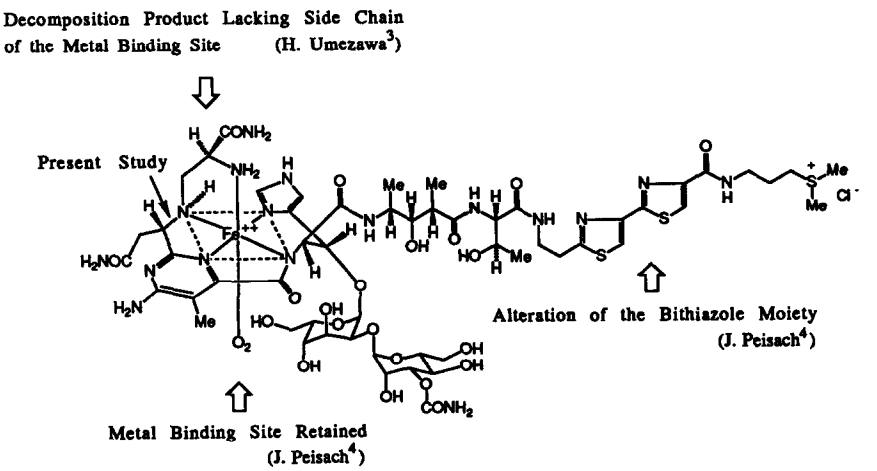
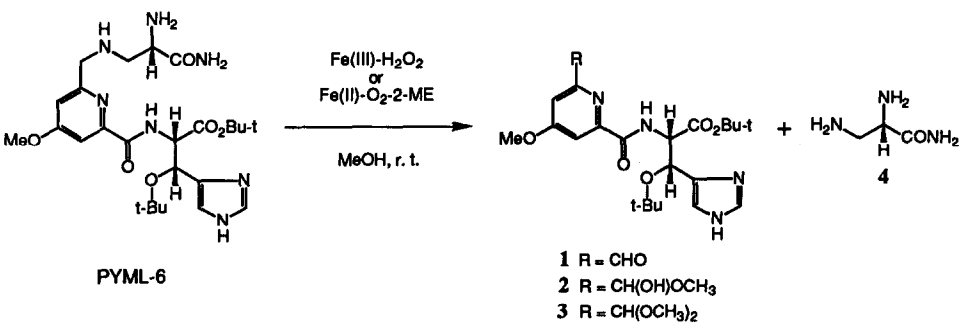


Figure 1 Proposed Positions of Self-Inactivation of Bleomycin.[3,4]

inactivated BLM seemed to be intact whereas the bithiazole moiety seems to be somehow altered, on the basis of ESR, optical, and fluorescence spectra.[4] Furthermore, it should be mentioned here that the catalytic activity for O_2-activation by BLM is limited to turnover number 5 to 10 because of the self-decomposition.[5,6] However, no structural study about the decomposition mode has been investigated in detail. In our continuing model study on BLM, we also experienced partial decomposition of synthetic models in the presence of iron and oxygen. It was thought that the structural assignment of the degradation products may provide a clue for the design of chemically robust man-made BLMs in which the undesired self-inactivation process is suppressed. Herein we report the isolation and the structure determination of the self-degradation products of a BLM model.

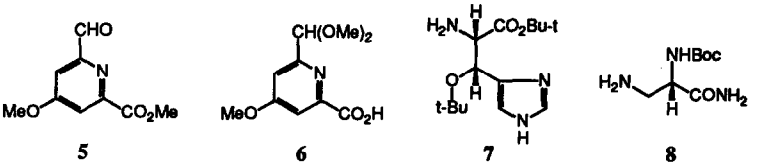
Previously, we have demonstrated that a synthetic model with a 4-methoxypyridine nucleus and a *tert*-butyl group, namely PYML-6, is comparable to BLM in dioxygen activation.[7,8] We now examined the degradation of PYML-6 with Fe(III)-H_2O_2 or Fe(II)-O_2 in detail (Scheme 1). Thus, to an orange-colored solution of PYML-6 (1 eq) and $Fe(ClO_4)_3 \cdot 6H_2O$ (1 eq) in methanol was added aqueous hydrogen peroxide (30 eq) under argon atmosphere to give a yellow solution. After being stirred for 15 hours at room temperature, the reaction mixture was concentrated and subjected to silicagel preparative TLC (developed with CH_2Cl_2 : MeOH = 10 : 1) to give an intractable mixture of aldehyde **1**, hemiacetal **2**, and acetal **3** (Rf 0.45~0.50 on the above TLC), which was further treated with saturated tartaric acid at room temperature for 12 hours to afford aldehyde **1** as a single product (13% yield based on PYML-6). The reaction mixture also afforded (S)-2,3-diaminopropionamide **4** dihydrochloride in 9% yield based on PYML-6 after chromatography on microcrystalline cellulose (eluted with *n*-PrOH : Pyridine : AcOH : H_2O = 15 : 10 : 3 : 4). On the other hand, self-inactivation of PYML-6 with Fe(II)-O_2 system also gave the same products in almost the same yields. A solution of PYML-6 (1 eq), $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (1 eq), and 2-mercaptoethanol (10 eq) in methanol was stirred for 20 hours in the presence of oxygen at room temperature. Work-up of the reaction gave a mixture of **1**, **2**, and **3**, which was transformed into aldehyde **1** by acid treatment (10% yield based on PYML-6). The degradation product **1** was shown to be catalytically inactive; Fe(II) complex of aldehyde **1** did not mediate epoxidation of β-methylstyrene, whereas

Scheme 1

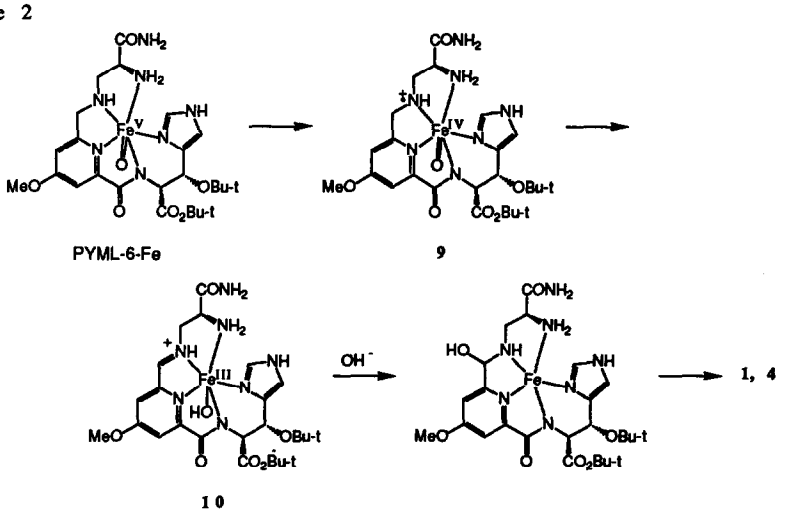


PYML-6-Fe(II) complex catalyzed epoxidation of olefinic substrates.[6,7] The degradation of PYML-6 was not observed in the control experiments using (i) Fe(II)-O_2 (without reducing agents), (ii) 2-mercaptoethanol-O_2 (without iron), (iii) Fe(III) (without H_2O_2), or iv) H_2O_2 (without iron).

These degradation products were identical to the authentic samples synthesized independently. Methyl 6-formyl-4-methoxypyridine-2-carboxylate **5**[8] was converted into acetal-acid **6** (96% yield) by treatment with methanol and *p*-toluenesulfonic acid, followed by hydrolysis. Coupling of the acid **6** with *erythro*-β-*tert*-butoxy-L-histidine *tert*-butyl ester **7**[9] was effected by DPPA[10] to give dipeptide **3** (94% yield; FABMS, m/z 494, MH^+), which was transformed into the aldehyde **1** by treatment with tartaric acid (82% yield).[11] HPLC, 400 MHz ^1H NMR and FABMS of the degradation product **1** was identical with the synthetic **1** in all respects except a slight epimerization at α-carbon of the hydroxyhistidine moiety [(2*S*,3*R*)/(2*R*,3*R*) > 10/1]. On the other hand, acid treatment of (*S*)-3-amino-2-[(*tert*-butoxycarbonyl)amino]propionamide **8**[12] gave **4** (quantitative), which was identical with the degradation product **4** by 400 MHz ^1H NMR and FABMS. It was evident that these degradation products are not contaminants because PYML-6 was prepared *via* a route which does not involve **1**, **2**, **3**, or **4** as synthetic intermediates.[7,8]



Scheme 2



Thus, we demonstrated the decomposition of the amino acid appendage of the metal binding site in the self-degradation of a synthetic model of BLM. The present results are consistent with

the aforementioned study on the self-inactivated BLM by Umezawa.[3] The degradation can be explained by a mechanism analogous to that for the N-dealkylation reaction caused by cytochrome P-450 enzyme.[13] A single electron transfer from the secondary amino nitrogen of PYML-6 gives a cation radical **9** (Scheme 2). Subsequent abstraction of a labile hydrogen atom would generate a cation **10**, which produces amine **4** and aldehyde **1** *via* a hydroxylated intermediate. Efforts are currently under way to design new BLM models resistant against such degradation.

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REFERENCES AND NOTES

1. Synthetic Studies on Antitumor Antibiotic, Bleomycin. XXX. For Part XXIX, see Ohno, M; Otsuka, M. In "Recent Progress in the Chemical Synthesis of Antibiotics"; Lukacs, G., Ohno, M. Eds.; Springer-Verlag: Heidelberg; *in press.*

2. (a) Sugiura, Y.; Takita, T.; Umezawa, H. In "Metal Ions in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1985: pp81-108. (b) Hecht, S. M. *Acc. Chem. Res.*, **1986**, *19*, 383. (c) Stubbe, J.; Kozarich, J. W. *Chem. Rev.*, **1987**, *87*, 1107.

3. (a) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Iitaka, Y.; Umezawa, H. *J. Antibiot.*, **1978**, *31*, 1073. (b) Umezawa, H.; Takita, T. *Structure and Bonding*, **1980**, *40*, 73.

4. Nakamura, M.; Peisach, J. *J. Antibiot.*, **1988**, *41* 638.

5. Sugiyama, H; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.*, **1986**, *108*, 3852.

6. Kaku, Y.; Otsuka, M.; Ohno, M. *Chem. Lett.*, **1989**, 611.

7. Sugano, Y.; Kittaka, A.; Otsuka, M; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.*, **1986**, *27*, 3635.

8. Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron*, **1988**, *44*, 2821.

9. Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron*, **1988**, *44*, 2811.

10. Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.*, **1972**, *94*, 6203.

11. Spectral data of **1**. IR (KBr) 2960, 1735, 1720, 1670, 1600, 1510, 1460, 1430, 1295, 1250, 1185 cm^{-1}; ^1H NMR ($CDCl_3$, TMS) δ 1.20 (9H, s), 1.44 (9H, s), 3.97 (3H, s), 4.99 (1H, dd, J = 3.7, 8.3 Hz), 5.15 (1H, d, J = 3.7 Hz), 7.01 (1H, s), 7.55 (1H, d, J = 2.6 Hz), 7.63 (1H, d, J = 1.2 Hz), 7.90 (1H, d, J = 2.6 Hz), 8.95 (1H, br d, J = 8.3 Hz), 10.04 (1H, s); FABMS m/z 447 (MH^+).

12. Otsuka, M.; Kittaka, A.; Iimori, T.; Yamashita, H.; Kobayashi, S.; Ohno, M. *Chem. Pharm. Bull.*, **1985**, *33*, 509.

13. Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.*, **1984**, *17*, 9.

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