ON THE MECHANISM OF LIPOXYGENASE-LIKE ACTION OF BLEOMYCIN-IRON COMPLEXES

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The mechanism of lipid peroxidation catalyzed by bleomycin (BLM)-iron (Fe) complexes has been studied *in vitro* using sodium linoleate as a substrate. BLM-Fe(II)-O_2 and BLM-Fe(III) complexes catalyze lipid peroxidation concomitantly with singlet oxygen evolution. The results from spin trapping methods and gas chromatography-mass spectroscopy (GCMS) analyses suggest that the initial step of lipid peroxidation catalyzed by BLM-Fe complexes is similar to that of soybean lipoxygenase, *viz.*, hydrogen abstration. However, another mechanism might be concerned in the case of BLM-Fe(II)-O_2 complex. BLM-Fe complexes are also capable of enhancing singlet oxygen evolution from the hydrogen peroxide (H_2O_2)-hypochlorite (OCl^-) system.

Bleomycin (BLM) is a useful anticancer drug isolated from *Streptomyces verticillus* by UMEZAWA *et al.*[1] Its major side effect is pneumonitis followed by pulmonary fibrosis.[2] In studies of this complication, we have found that BLM-iron complex catalyzes the lipoxygenase-like reaction concomitantly with singlet oxygen evolution.[3~5]

With ferrous ion and molecular oxygen, BLM forms a so-called perferryl ion-like complex.[6] Perferryl ion is thought to be a promotor of lipid peroxidation.[7~9] However, the mechanism of initiation is still uncertain. BLM is known to make complex not only with ferrous ion but also with ferric ion. The latter complex also catalyzes lipid peroxidation.[10]

In the present study, we have used $^{18}O_2$, electron spin resonance (ESR) spectroscopy and spin trapping methods to investigate the mechanism of the lipoxygenase-like action of BLM-Fe complexes and the evolution of singlet oxygen during the reaction.

Materials and Methods

Chemicals

Bleomycin (BLM) was a gift from Nippon Kayaku Co., Ltd., Japan. Soybean lipoxygenase [E.C.1.13.11.12] and sodium linoleate were purchased from Sigma (St. Louis, MO). The spin trapping agent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was obtaind from Sigma and purified as described in the literature.[11] 2,2,6,6-Tetramethyl-4-piperidone (TMP) was a product of Aldrich (Milwaukee, WI). All other chemicals were reagent grade. BLM-Fe(III) was prepared by mixing equal volumes of BLM (10 mM) and ferric chloride (10 mM). BLM-Fe(II)-$^{18}O_2$ was prepared by mixing equal volumes of BLM (10 mM) and ferrous sulfate (10 mM) under anaerobic conditions, then the gas phase was filled with $^{18}O_2$ (97.5%).

Determination of Lipid Peroxidation Activity

Sodium linoleate of various concentrations was incubated at 37°C in 100 mM Tris-HCl (pH 7.5). Lipid peroxidation was followed by monitoring the oxygen concentration in the incubation medium with a Clark-type oxygen electrode (Yellow Spring Instrument Co.). Reactions were initiated by the rapid addition of BLM-Fe complexes. The initial rate of O_2 consumption (V), is expressed as nmol of O_2 consumed/ml per minute.

Analysis of the Action of $^{18}O_2$ Incorporated Into BLM-Fe(II)

Using a Thunberg-type cell with a side-arm,[12] BLM-Fe(II) was formed under O_2 depleted condition (weak pinkish complex). Then $^{18}O_2$ was introduced in the apparatus and again $^{18}O_2$ was removed after the color-change of BLM-Fe (yellow complex). Under such anaerobic conditions, sodium linoleate was added to BLM-Fe(II)-$^{18}O_2$. The incubation mixture used in this study contained 32 mM sodium linoleate and 5 mM BLM-Fe(II)-$^{18}O_2$ in 200 mM Tris-HCl buffer (pH 7.5). The mixture was kept at 37°C for 5 minutes. The reaction was stopped by adding sodium borohydride and the product was acidified and extracted with dichloromethane and analyzed by GCMS. GCMS analysis was carried out by M-003 (Hitachi, Japan).

Electron Spin Resonance (ESR) Measurement

All ESR spectra were recorded with JES-FE1X spectrometer (Joel Co., Japan) using 100 kHz field modulation at room temperature. ESR spectra were recorded by using a quartz capillary. The g values of spin adducts were measured using the signal of Mn^{2+} in MnO as a standard.

In the ESR experiments with DMPO, a Thunberg-type cell with two side-arms was used. The cell, which contained the reaction agents in separate side-arms was evacuated and then argon gas was introduced. This procedure was repeated five times. After the last procedure, BLM and iron were mixed in the cell and the BLM-iron complex was mixed with sodium linoleate in the presence of DMPO. In the case of BLM-Fe(II)-O_2, $^{18}O_2$ was introduced after the 5th evacuation. The cell was evacuated again and filled with argon gas. This procedure was repeated twice. Reaction mixtures were immediately transferred into the evacuated quartz capillary by the pressure of argon gas.

In the ESR experiments with TMP, 15 mM sodium linoleate, 50 μM BLM-Fe(II)-O_2 and 0.1 M TMP in 20 mM phosphate buffer (pH 7.5) were incubated under aerobic conditions before the reaction mixture was transferred into the quartz capillary and analyzed by ESR spectrometry.

Chemiluminescence

Chemiluminescence was determined by a Luminescence Reader (Aloka Co., Japan). It was assayed in a cuvette maintained at 37°C with constant stirring. Usually, total counts for 4 minutes incubation after the addition of BLM-Fe complexes to the cuvette, were measured.

In sodium linoleate-BLM-Fe complex systems, sodium linoleate in varying concentrations were preincubated for 4 minutes at 37°C in 20 mM phosphate buffer (pH 7.5). In H_2O_2-OCl^- system, 5 mM H_2O_2 and 5 mM OCl^- were preincubated for 4 minutes at 25°C in 20 mM phosphate buffer of varying pH. Reactions were started by the rapid additions of BLM-Fe complexes.

Results

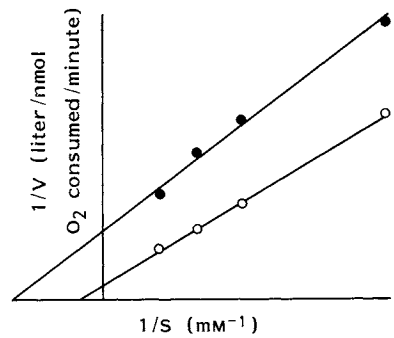
Lipid Peroxidation by BLM-Fe Complexes

The addition of BLM-Fe complex to the sodium linoleate caused oxygen consumption after short lag phase. The Lineweaver-Burk plot revealed the difference of *Km* and Vmax between BLM-Fe(II)-O_2 and BLM-Fe(III) experiments (Fig. 1).

In the case of soybean lipoxygenase system, the reaction product after the incubation at 0°C was mostly 13-hydroperoxy-9-*cis*-11-*trans*-linoleic acid while the peaks of 9-*trans*-11-*trans* and 9-hydroper-

Fig. 1. Relationship between sodium linoleate concentration and O_2 consumption by BLM-Fe complexes (A, B).

○ A, Fe^{2+}, Km=15.4 mM, Vmax=2,500 nmol/minute; ● B, Fe^{3+}, Km=3.7 mM, Vmax=465 nmol/minute.



In experiment A, medium contained 5 μM BLM-Fe(II)-O_2, sodium linoleate of varying concentraion and 0.1 M Tris-HCl buffer at pH 7.5, in a total volume of 2.5 ml. The reaction was initiated by the addition of BLM-Fe(II)-O_2 and maintained at 37°C. Initial rate of O_2 consumption, V is expressed as nmol of O_2 consumed/ml per minute. In experiment B, the incubation medium was the same as in experiment A, except that 5 μM BLM-Fe(III) was used as an initiator.

oxy-10-*trans*-12-*cis*- and -10-*trans*-12-*trans*-linoleic acids were seen in the case of BLM-Fe even after the incubation at 0°C (data not shown).

The Action of O_2 Attached to BLM-Fe(II)

Fig. 2(A) shows the GCMS analysis of the products of sodium linoleate catalyzed by BLM-Fe(III) under $^{18}O_2$ condition. Peaks with *m/e* 312 and 101 are ^{18}O-containing products. The GCMS spectrum of the products catalyzed by BLM-Fe(II)-$^{18}O_2$ under anaerobic condition is shown in Fig. 2(B). If the $^{18}O_2$ molecule of this complex is to be transferred to linoleate, we would expect the presence of ^{18}O-containing products, *m/e* 312 or 101. However, our data indicated that no such products were formed.

Experiments with DMPO

Incubation of sodium linoleate and BLM-Fe(II) with DMPO in the absence of oxygen yields only a baseline signal (data not shown). When sodium linoleate and BLM-Fe(III) with DMPO were incubated under argon condition and analyzed by ESR, a DMPO adduct was observed (Fig. 3A). Analysis of the hyperfine constants suggests that the radical species trapped is carbon centered radical ($a_N = 16.5$ G, $a_H\beta = 23.8$ G).[13,14] Indeed, this signal is very similar to that obtained when sodium linoleate was incubated with soybean lipoxygenase in the presence of DMPO under argon condition. (Fig. 3B)

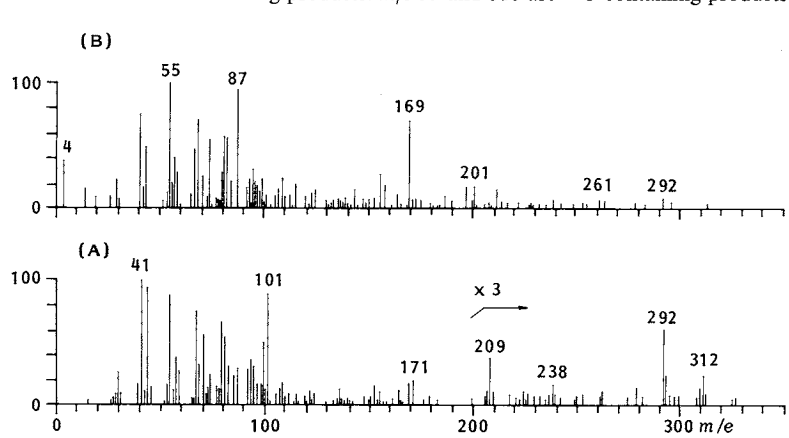
As can be seen in Fig. 3(C), by using sodium linoleate and BLM-Fe(II)-O_2 with DMPO under argon condition, two different DMPO adducts were obtained. One adduct, which we label signal a, is identified as a carbon centered radical adduct ($a_N = 16.5$ G, $a_H\beta = 23.8$ G). This signal diminishes with time. Hyperfine splitting constants ($a_N = a_H\beta = 14.9$ G) are obtained for the second DMPO adduct. The values are consistent with hydroxyl radical adduct of DMPO.[13,14]

Experiments with 2,2,6,6-Tetramethyl-4-piperidone

As shown in Fig. 4(A), incubation of sodium linoleate and BLM-Fe(II)-O_2 with TMP at 37°C yields a ESR signal. This signal coincides with the signal of H_2O_2-OCl^- system (Fig. 4B).

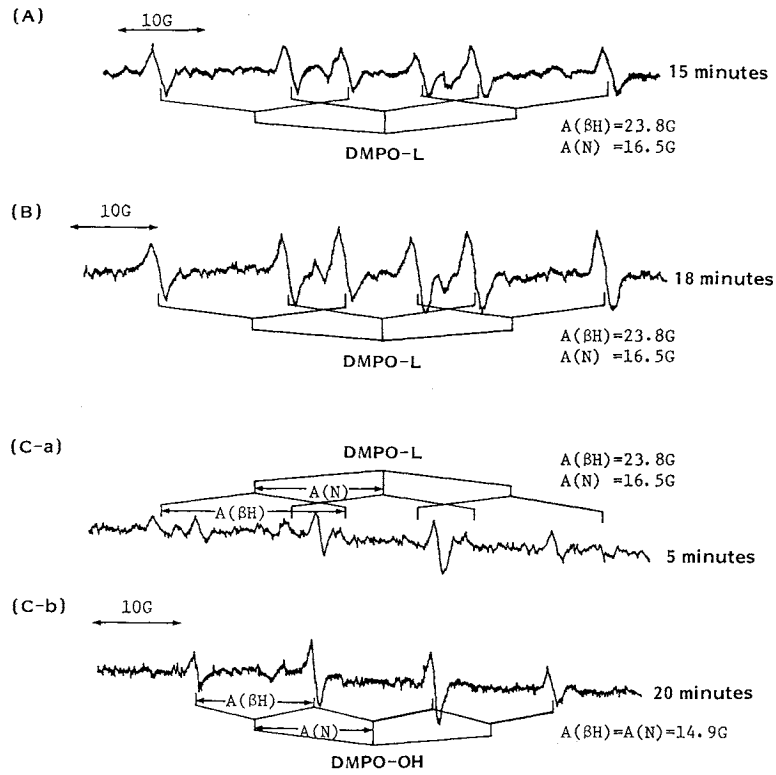
Fig. 2. GC-MS analyses of the products of sodium linoleate catalyzed by BLM-Fe complexes.

(A) Lipoxgenase-like reaction of BLM-Fe(III) in $^{18}O_2$, (B) $^{18}O_2$ transfer from BLM-Fe(II)-$^{18}O_2$? *m/e* 101 and 312 are ^{18}O containing products. *m/e* 99 and 310 are ^{16}O containing products.



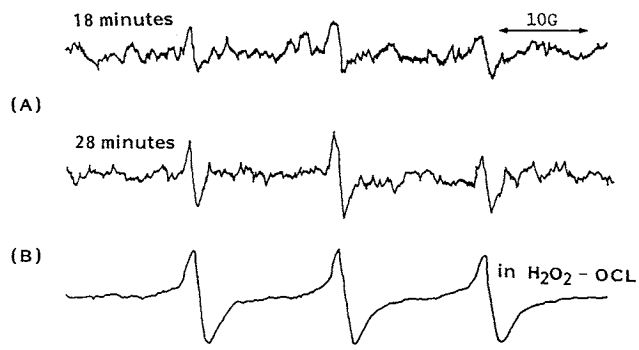
(A) Under $^{18}O_2$ condition 32 mM sodium linoleate was catalyzed by 5 mM BLM-Fe(III) in 0.2 M Tris-HCl buffer (pH 7.5). (B) Under anaerobic condition, 32 mM sodium linoleate was catalyzed by BLM-Fe(II)-$^{18}O_2$ in 0.2 M Tris-HCl at pH 7.5. The reaction mixtures were kept at 37°C for 5 minutes.

Fig. 3. Production of DMPO adduct.



The incubation mixture for the detection of DMPO-radical adducts contained: (A) 30 mM sodium linoleate, 2 mM BLM-Fe(III) and 0.1 M DMPO in 0.1 M Tris-HCl buffer at pH 7.5 in argon. (B) 30 mM sodium linoleate, soybean lipoxygenase (0.5 mg), and 0.1 M DMPO in Tris-HCl buffer in argon. (C) 30 mM sodium linoleate, 2 mM BLM-Fe(II)-O_2 and 0.1 M DMPO in Tris-HCl buffer in argon.

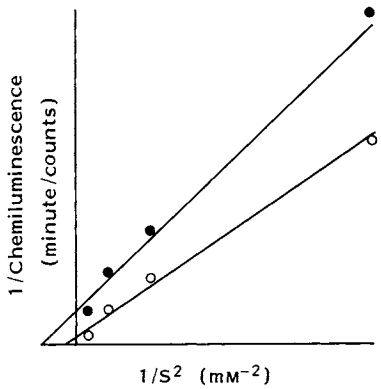
Fig. 4. Production of TMP adduct.



The incubation mixture for the detection of TMP adduct contained: (A) 15 mM sodium linoleate, 50 μM BLM-Fe(II)-O_2 and 0.1 M TMP in 20 mM phosphate buffer at pH 7.5 (B) 100 mM hydrogen peroxide, 100 mM hypochlorite and 0.1 M TMP in 20 mM phosphate buffer at pH 7.5. The instrumental parameters were: Scan range, 5.0 mT; modulation amplitude, 0.1 mT; scan time, 8 minutes; time constant, 1.0 second; microwave power, 10 mW; amplitude, 5.0×10^3.

Fig. 5. Comparison of chemiluminescence caused by BLM-Fe complexes with sodium linoleate.

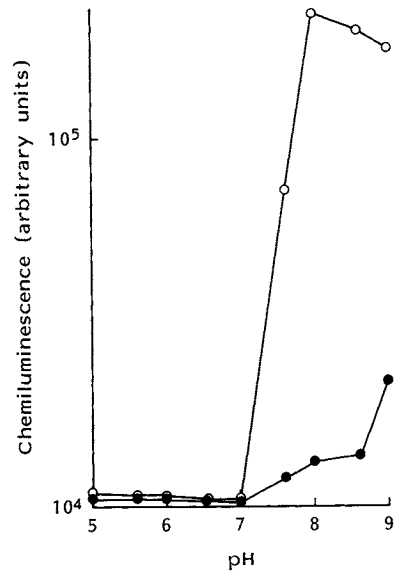
○ A, Fe^{2+}, $Km=13.4$ mM; ● B, Fe^{3+}, $Km=8.4$ mM.



The incubation media contained (A) 5 μM BLM-Fe(II)-O_2 or (B) 5 μM BLM-Fe(III), sodium linoleate of varying concentrations in 20 mM phosphate buffer at pH 7.5. The reaction was initiated by the addition of BLM-Fe complexes and maintained at 37°C. Total counts from 0 to 4 minutes, incubation interval after addition of BLM-Fe complexes to the cuvette, were measured.

Fig. 6. Effect of pH on photoemission in H_2O_2-OCl^--BLM-Fe systems.

○ Fe^{2+}, ● Fe^{3+}.



5 mM H_2O_2 and 5 mM OCl^- in 20 mM phosphate buffer at varying pH were preincubated for 4 minutes at 25°C under the aerobic condition. Reactions were initiated by addition of 50 μM BLM-Fe(II)-O_2 or BLM-Fe(III). Chemiluminescence was then measured for 4 minutes.

Chemiluminescence Studies of the BLM-Fe Complexes

Addition of BLM-Fe complexes to buffered solution of sodium linoleate caused photoemission without any sensitizers. Fig. 5 shows that chemiluminescence intensity is much stronger when BLM-Fe(II)-O_2 is added to the substrate than the case of BLM-Fe(III). Monomol($^1\Delta_g \rightarrow {}^3\Sigma_g^-$) and dimol($^1\Delta_g + {}^1\Delta_g \rightarrow 2{}^3\Sigma_g^-$) emission from the singlet oxygen are observed from H_2O_2-OCl^- reaction.[15] We found that BLM-Fe complexes enhanced the light emission of this system at neutral pH. Activity of BLM-Fe(II)-O_2 was also higher in this system (Fig. 6). Because the Luminescence Reader (Aloka) can only record the visible light emission, Fig. 6 shows that the chemiluminescence would be resulted from the dimol reaction of the singlet oxygen, and the singlet oxygen might evolve from one molecule of the substrate.

Discussion

The role of iron in the initiation of lipid peroxidation has been examined in a variety of systems.[9,16,17] BLM is known to form complexes with various transition metals. When mixed with ferrous ion under the aerobic condition, BLM forms so-called perferryl ion-like complex.[6] We have proposed that this complex catalyzes the lipoxygenase-like reaction concomitantly with singlet oxygen evolution.[5,12] BLM can form a complex with ferric ion, too, even under anaerobic conditions.[18] This complex, BLM-Fe(III) also catalyzes lipid peroxidation.[10] Although these two complexes have shown quite similar EPR spectra (moderate tetragonal, moderate rhombic[19]), their activities are considerably different, as exemplified in Fig. 1. Differences between the respective *Km* and Vmax values could be ascribed to the structural difference between BLM-Fe(III) and BLM-Fe(II)-O_2, suggesting that the latter two species are not

interconverted during the lipid peroxidation reaction.

Perferryl ion is believed to be a potent initiator of lipid peroxidation. But the precise mechanism is not yet clarified. As for the mechanism of cyclooxygenase, PETERSON presented a model in which the dioxygen molecule bound to the heme-iron of the active center of the enzyme was transferred to the polyunsaturated fatty acid.[20] However, our data indicate that the mechanism of lipid peroxidation catalyzed by BLM-Fe(II)-O_2 is not dioxygen activation.

Soybean lipoxygenase is a non-heme iron dioxygenase. Many investigators have supported the idea that hydrogen abstraction from the substrate with oxygen addition thereafter is the catalytic mechanism.[21] In this model, the Fe(III) in yellow lipoxygenase can be reduced by the substrate linoleic acid, and the iron shuttles between the Fe(II) and Fe(III) states during the aerobic reaction.

Using a spin trapping agent, DMPO, we were able to demonstrate the presence of a carbon centered radical adduct, especially when BLM-Fe(III) was used as the catalyst under the anaerobic condition (Fig. 3). This signal resembled that obtained in the case of sodium linoleate-soybean lipoxygenase system. We assigned, therefore, the radical species generated in the BLM-Fe(III)-linoleate system to the carbon centered linoleate radical.

Our results suggest that the initial step of lipid peroxidation by BLM-Fe(III) is hydrogen abstraction similar to the model proposed for soybean lipoxygenase.

In the case of BLM-Fe(II)-O_2, a carbon centered linoleate radical was detectable, but a hydroxyl radical adduct was predominant. Based upon the results presented here, we suppose that the initial step of lipid peroxidation by BLM-Fe(II)-O_2 may also be the hydrogen abstraction. However, another mechanism might be concerned during the reaction. Hydroxyl radical production by BLM and Fe(II) has been reported previously by the use of ESR with DMPO,[22,23] although a previous report has shown that the detection of DMPO-OH does not unequivocally prove the existence of the hydroxyl radical.[24] It is possible that superoxide anion radical was trapped during the reaction of BLM-Fe(II)-O_2 and sodium linoleate under aerobic conditions.

The detection of 1,268-nm chemiluminescence emitted when the singlet oxygen ($^1\Delta_g$) returns to its ground state is claimed to be the best assay for the singlet oxygen.[15,25] Using this method, KANOFSKY presented evidence that the BLM-Fe(III) complex catalyzed the singlet oxygen evolution from linoleate hydroperoxide, and the maximal evolution was attained in the coexistence of an equal amount of linoleate.[26]

Chemical methods for detecting the singlet oxygen have been said to be not so specific. Another specific assay for the singlet oxygen has been developed by MOAN and WOLD.[27] This method consists of detecting signals of *N*-oxyl derivative in the ESR spectroscopy with TMP as the trapping agent (Fig. 4).

We found, in addition, that the BLM-Fe complexes stimulated the light emission of the H_2O_2-OCl^- system at neural pH (Fig. 6). KHAN and KASHA established that the singlet oxygen was generated in the hydrogen peroxide-hypochlorite system and the emission spectra of the chemiluminescence thereby were at the infrared region in the monomol reaction ($^1\Delta_g \rightarrow {}^3\Sigma_g^-$), and at the visible region in the dimol reaction ($2^1\Delta_g \rightarrow 2^3\Sigma_g^-$, *etc.*).[15]

The mechanism of this singlet oxygen formation has been represented as follows (1):

$$ClO^- + H_2O_2 \rightarrow Cl-O-O^- + H_2O \rightarrow Cl^- + {}^1O_2 + H_2O \quad (1)$$

$$L-O-O^- \rightarrow L^- + {}^1O_2 \quad (2)$$

We now assume that BLM-Fe might stimulate the second step of this reaction. KANOFSKY proposed that the real substrate for the singlet oxygen formation was linoleate hydroperoxide and the RUSSEL's mechanism was a plausible explanation for the evolution of the singlet oxygen in the BLM-Fe(III) systems.[26,28] The participation of the RUSSEL's mechanism is undeniable, however, with regard to the tentative observation of the stimulation of photoemission by BLM-Fe in the reaction (1), we propose that BLM-Fe catalyzes the reaction (2) in the same manner.

One further question that remains to be elucidated is why BLM-Fe(II)-O_2 catalyzes stronger photoemission than BLM-Fe(III).

The clarification of this question will probably require the further investigation of the action of the O_2 molecule attached to the iron during the reaction.

In conclusion, the results obtained indicate that the reaction mechanism of lipid peroxidation catalyzed

by BLM-Fe complexes is similar to that of soybean lipoxygenase, *viz.*, hydrogen abstraction. We propose a tentative mechanism of the singlet oxygen evolution catalyzed by BLM-Fe complexes. However, more work will be required to make clear the relationships between lipid peroxidation concomitantly with the singlet oxygen evolution and the side effect of BLM.

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References

1) UMEZAWA, H.; K. MAEDA, T. TAKEUCHI & Y. OKAMI: New antibiotics, bleomycin A and B. J. Antibiotics 19: 200~209, 1966
2) RUDDERS, R. A. & G. T. HENSLEY: Bleomycin pulmonary toxicity. Chest 63: 626~628, 1973
3) TETSUKA, T.; S. KAWAI, K. KONNO & H. ENDO: The control mechanism of collagen biosynthesis. (in Japanese) Seikagaku 42: 468, 1970
4) TAKEDA, K.; S. KAWAI, F. KATO, T. TETSUKA & K. KONNO: Stimulation of prolyl hydroxylase activity by bleomycin. J. Antibiotics 31: 884~887, 1978
5) TETSUKA, T.: Activation of oxygen by bleomycin. (in Japanese) Proc. Jpn. Cancer Assoc. 37: 195, 1978
6) SUGIURA, Y.: Bleomycin-iron complexes. Electron spin resonance study, ligand effect, and implication for action mechanism. J. Am. Chem. Soc. 102: 5208~5215, 1980
7) HOCHSTEIN, P.; K. NORDENBRAND & L. ERNSTER: Evidence for the involvement of iron in the ADP-activated peroxidation of lipids in microsomes and mitochondria. Biochem. Biophys. Res. Commun. 14: 323~328, 1964
8) NOGUCHI, T. & M. NAKANO: Effect of ferrous ions of microsomal phospholipid peroxidation and related light emission. Biochim. Biophys. Acta 368: 446~455, 1974
9) AUST, S. D. & B. A. SVINGEN: The role of iron in enzymatic lipid peroxidation. *In* Free Radicals in Biology. Vol. 5, *Ed.*, W. A. PRYOR, pp. 1~28, Academic Press, 1982
10) EKIMOTO, H.; K. TAKAHASHI & A. MATSUDA: Lipid peroxidation by bleomycin-iron complexes *in vitro*. J. Antibiotics 38: 1077~1082, 1985
11) GREEN, M. J. & H. A. O. HILL: Chemistry of oxygen. *In* Method in Enzymology. Vol. 105. *Ed.*, L. PACKER, pp. 3~22, Academic Press, 1984
12) KIKUCHI, H. & T. TETSUKA: Free radicals in the lipoxygenase-like reaction of bleomycin. *In* Medical, Biochemical and Chemical Aspects of Free Radicals. *Ed.*, O. HAYAISHI *et al.*, pp. 1101~1104, Elsevier, 1989
13) FUJITA, Y.: Radicals observed in the rat brain mitochondria by spin trapping method. (in Japanese) *In* Free Radicals in Clinical Medicine. Vol. 2. *Ed.*, M. KONDO, pp. 39~49, Nihon Igakukan, 1987
14) BRITIGAN, B. E.; G. M. ROSEN, Y. CHAI & M. S. COHEN: Do human neutrophils make hydroxyl radical? J. Biol. Chem. 261: 4426~4431, 1986
15) KHAN, A. U. & M. KASHA: Chemiluminescence arising from simultaneous transitions in pairs of singlet oxygen molecules. J. Am. Chem. Soc. 92: 3293~3300, 1970
16) BRAUGHLER, J. M.; L. A. DUNCAN & R. L. CHASE: The involvement of iron in lipid peroxidation. J. Biol. Chem. 261: 10282~10289, 1986
17) MARTON, A.; N. SUKOSD-ROZLOSNIK, A. VERTES & I. HORVATH: The effect of EDTA-Fe(III) complexes with different chemical structure on the lipid peroxidation in brain microsomes. Biochem. Biophys. Res. Commun. 145: 211~217, 1987
18) BURGER, R. M.; J. PEISACH & S. B. HORWITZ: Activated bleomycin. J. Biol. Chem. 256: 11636~11644, 1981
19) LOEW, G. M. H.: An analysis of the electron spin resonance of low spin ferric heme compounds. Biophys. J. 10: 196~212, 1970
20) PETERSON, D. A.; J. M. GERRARD, G. H. R. RAO, E. L. MILLS & J. G. WHITE: Interaction of arachidonic acid and heme iron in the synthesis of prostaglandins. *In* Advance in Prostaglandin and Thromboxane Research. Vol. 6. *Ed.*, SAMUELSSON *et al.*, pp. 157~161, Raven Press, 1980
21) PETERSSON, L.; S. SLAPPENDE, M. C. FEITERS & J. F. G. VLIEGENHALT: Magnetic susceptibility studies on yellow and anaerobically substrate-treated yellow soybean lipoxygenase-1. Biochim. Biophys. Acta 913: 228~237, 1987
22) OBERLEY, L. W. & G. R. BUETTNER: The production of hydroxyl radical by bleomycin and iron (II). FEBS Lett. 97: 47~49, 1979
23) SUGIURA, Y.: Production of free radicals from phenol and tochopherol by bleomycin-iron (II) complex. Biochem.

Biophys. Res. Commun. 87: 649~653, 1979
24) FINKELSTEIN, E.; G. M. ROSEN & E. J. RAUCKMAN: Spin trapping of superoxide and hydroxyl radical: Practical aspects. Arch. Biochem. Biophys. 200: 1~16, 1980
25) KHAN, A. U.; P. GEBAUER & L. P. HAGER. Chloroperoxidase generation of singlet Δ molecular oxygen observed directly by spectroscopy in the 1- to 1.6-μm region. Proc. Natl. Acad. Sci. U.S.A. 80: 5195~5197, 1983
26) KANOFSKY, J. R.: Singlet oxygen production by bleomycin. J. Biol. Chem. 261: 13546~13550, 1986
27) MOAN, J. & E. WOLD: Detection of singlet oxygen production by ESR. Nature 279: 450~451, 1979
28) KELLOGG, R. E.: Mechanism of chemiluminescence from peroxy radicals. J. Am. Chem. Soc. 91: 5433~5436, 1969