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PRODUCTION OF FREE RADICALS FROM PHENOL AND TOCOPHEROL BY BLEOMYCIN-IRON(II) COMPLEX

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Summary : Oxygen-bubbling of 1:1 bleomycin(BLM)-Fe(II) complex efficiently generates hydroxy radical which is inhibited by catalase. Hydroxy radical produced from BLM-Fe(II)-O_2 system oxidizes 2,6-di-tert-butyl-p-cresol and α-tocopherol to form the corresponding phenoxy and tocopheroxyl radicals, respectively. These free radicals were identified by the ESR hyperfine structures.

Introduction

BLM binds to and cleavages DNA in a reaction that depends on the presence of ferrous ion and molecular oxygen.[1] In the DNA degradation by BLM, ferric ion and anaerobiosis cannot replace ferrous ion and aerobiosis, respectively.[2] Divalent metal ions, Cu(II), Zn(II), and Co(II), inhibit BLM induced damage to DNA.[3] The breakage of DNA is also inhibited by chelating agents such as deferoxamine and EDTA.[3] In addition, it has been that reducing agents[4] and superoxide radical[5] stimulate DNA degradation reaction by BLM. These observations strongly suggest that reactive free radicals such as $O_2^{\cdot-}$ and ·OH may be responsible for oxidative cleavage of DNA by BLM. Recently, the production of oxygen radicals in BLM-Fe(II) complex system was detected by ESR spin trapping technique.[6,7] However, oxidative reaction of organic substances by free radicals generated from BLM-Fe(II) complex has never been investigated. This paper has described that hydroxy radical produced from BLM-Fe(II)-O_2 system attacks phenol and tocopherol to form the corresponding phenoxy and tocopheroxyl radicals, respectively.

Experimental

BLM-A_2 purified was a gift from Nippon Kayaku Co. Ltd. DL-α-Tocopherol and catalase (bovine liver; 3000 units/mg) were obtained from Sigma Company. DMPO and 2,6-di-tert-

Abbreviations used : BLM, bleomycin; ESR, electron spin resonance; DMPO, 5,5-dimethyl-1-pyroline-N-oxide; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid

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butyl-p-cresol were purchased from Aldrich Chemical Company, and DMPO was purified by filtration with charcoal.

The reaction mixture for spin trapping consisted of 1:1 BLM-Fe(II) complex(0.5 mM; aqueous solution of pH 6.9) and DMPO(0.05 M; ethanol solution). Oxygen was bubbled through the reaction mixture for 5 seconds, and then an aliquot of the sample solution was rapidly transferred to a quartz flat cell for ESR examination. Free radicals produced from 2,6-di-tert-butyl-p-cresol or tocopherol by BLM-Fe(II) complex were also detected by the same procedure, except for the replacement of DMPO by the phenol or tocopherol. X-Band ESR measurements were made using a JES-FE-3X spectrometer equipped with 100 KH_z field modulation at 20°.

Results and Discussion

The spectrum of Figure 1A, which has 1:2:2:1 quartet pattern, $a^N = a_\beta^H = 15.2$ G, and g=2.0058, is characteristic of hydroxy radical adduct of DMPO.[8] In addition, this reaction was strongly inhibited by catalase which is scavenger of H_2O_2(see Figure 1B). The experimental result of DMPO spin trapping shows efficient production of ·OH radical by oxygen-bubbling of 1:1 BLM-Fe(II) complex, consistent with the previous reports.[6,7] On the basis of the present result, the previous ESR studies of BLM-Fe complex, and spin trapping using N-tert-butyl-α-phenylnitrone,[6] it is proposed that superoxide and hydroxy radicals are produced during the reversible redox reaction of the 1:1 BLM-Fe complex as follows :

$$\text{BLM-Fe(II)} + O_2 \longrightarrow \text{BLM-Fe(III)} + O_2^{\cdot-}$$

$$\text{BLM-Fe(III)} \xrightarrow{\text{reducing agent}} \text{BLM-Fe(II)}$$

$$O_2^{\cdot-} + H^+ \underset{}{\overset{pK_a=4.4}{\rightleftharpoons}} HO_2\cdot \quad [9]$$

$$O_2^{\cdot-} + HO_2\cdot \xrightarrow{H_2O} O_2 + H_2O_2 + OH^-$$

$$O_2^{\cdot-} + H_2O_2 \longrightarrow O_2 + OH^- + \cdot OH \text{ (Haber-Weiss reaction)}$$

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2$$

Buettner and Oberley have reported that the $O_2^{\cdot-}$ spin adduct of DMPO is considerably unstable ($T_{1/2}$=35 sec at pH 8).[10] In xanthine-xanthine oxidase system as a superoxide generating system, Lai and Piette also detected a weak ESR signal of the ·OH radical adduct of DMPO, suggesting that $O_2^{\cdot-}$ is converted into ·OH.[11]

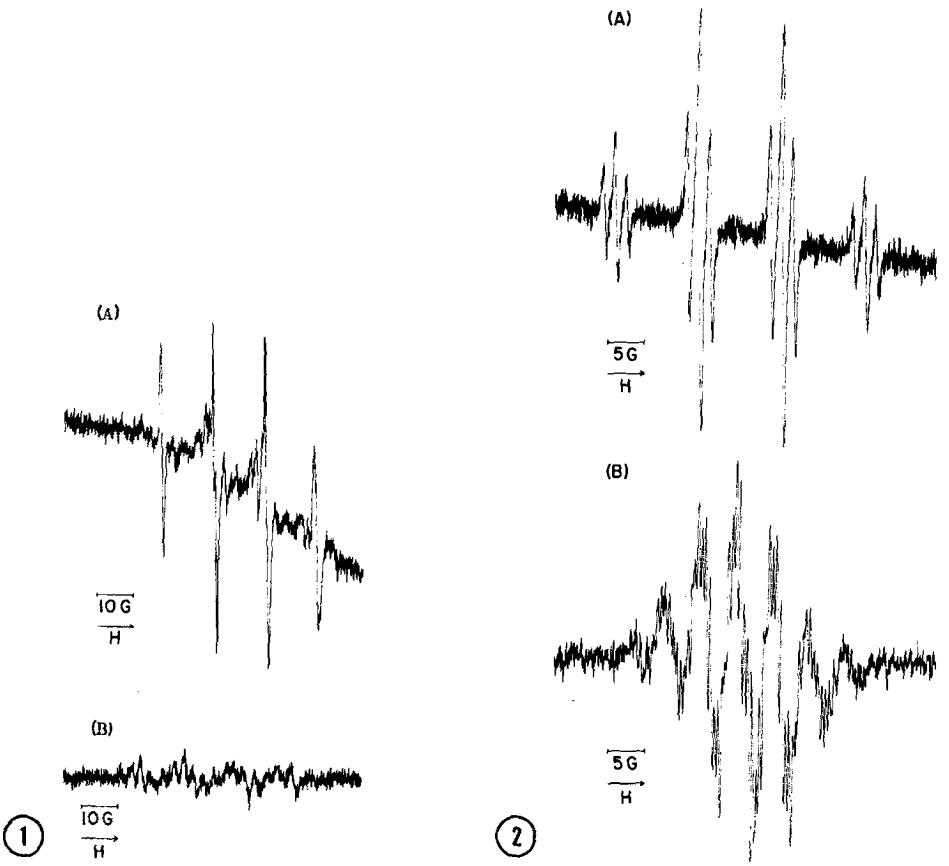


Figure 1 ESR spectra of DMPO spin adduct produced by BLM-Fe(II) complex

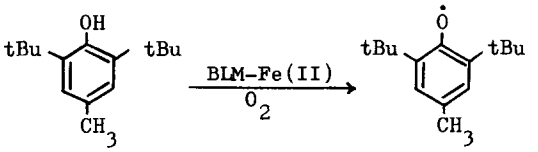
(A) 0.5 mM BLM-Fe(II) complex and 0.05 M DMPO; and
(B) 0.5 mM BLM-Fe(II) complex, 5 mg catalase, and 0.05 M DMPO
Conditions of ESR spectroscopy : microwave power, 10 mW; modulation amplitude, 0.5 G; and time constant, 0.03 sec.

Figure 2 ESR spectra of free radicals from 2,6-di-tert-butyl-p-cresol and α-tocopherol produced by BLM-Fe(II) complex

(A) 0.5 mM BLM-Fe(II) complex and 0.05 M 2,6-di-tert-butyl-p-cresol; and (B) 0.5 mM BLM-Fe(II) complex and 0.05 M α-tocopherol
Conditions of ESR spectroscopy : microwave power, 10 mW; modulation amplitude, 0.5 G(A) and 0.2 G(B); and time constant, 0.03 sec.

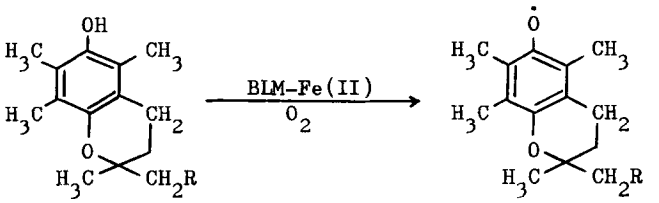
Figure 2 shows the ESR spectra for radicals formed by oxidation with BLM-Fe(II) complex system of 2,6-di-tert-butyl-p-cresol and DL-α-tocopherol. The free radical from 2,6-di-tert-butyl-p-cresol is identified to the corresponding phenoxy radical by means of the hyperfine structure of quartet of triplets and g-value(g=2.0053)(see Figure 2A). The quartet splitting

(1:3:3:1) is clearly associated with the three hydrogen atoms of the para-methyl group, and the triplet splitting(1:2:1) with meta-ring hydrogen atoms.



The hydrogen interaction constants were 10.8 G for para-methyl protons and 1.7 G for meta-ring protons.

On the other hand, the radical from α-tocopherol is typical of α-tocopheroxyl free radical. The seven splittings observed in Figure 2B are from the six approximately equivalent C-2 and C-6 methyl protons, and each of these lines is split by the C-3 methyl and C-5 methylene protons.



The hydrogen interaction constants and g-value were estimated to be 5.5 G(C-methyl protons), 4.6 G(C-6 methyl), 0.8 G(C-3 methyl and C-5 methylene), and g=2.0052. In fact, these values correspond well to those of the phenoxy radical from 2,4-di-tert-butyl-5,6-di-methyl-phenol (ortho-methyl protons=5.4 G and meta-methyl protons=1.3 G).[12]

Ferrous ion and molecular oxygen serve as a specific cofactor in the cleavage of DNA by BLM. Therefore, evidence presented in this report suggests that the ·OH radical produced by BLM-Fe(II)-O_2 complex system is also responsible for the degradation of DNA by BLM. The formation of malondialdehyde as a product of BLM-DNA reaction,[13] which requires a C-C bond cleavage in deoxyribose, may be produced by the attack of reactive ·OH radical at the 4' position of a deoxyribose in DNA.

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