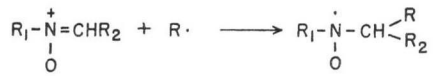
Communications to the editor

FORMATION OF SUPEROXIDE AND HYDROXY RADICALS IN IRON(II)-BLEOMYCIN-OXYGEN SYSTEM: ELECTRON SPIN RESONANCE DETECTION BY SPIN TRAPPING

Sir:

The technique of spin trapping of short-lived radical intermediate (R·) by nitrones or nitroso-compounds,[1] has recently been applied to studies of free-radicals in biological systems. Much of this increased interest results from studies on the role of the oxygen-centered radicals, $O_2^{\cdot-}$ and ·OH, in biological systems.[2,3] The basic reaction can be described as follows:



The resultant spin adduct is usually a relatively stable nitroxide radical which can be characterized by electron spin resonance (ESR) method, and then it is possible to identify the radical R· from the ESR parameters in favorable cases.

It has been suggested that the degradation of DNA in Fe(II)-bleomycin system is dependent on the oxidation of a Fe(II)-bleomycin-DNA complex, and that the formation of an oxygen-labile Fe(II)-bleomycin complex is related to DNA cleavage.[4,5] Oxidation of the bleomycin-Fe(II) complex by oxygen is expected to produce a variety of potentially reactive free-radical species which participate in the degradation of DNA.

In this communication, the generation of $O_2^{\cdot-}$ and ·OH radicals from Fe(II)-bleomycin system in the presence of oxygen has been demonstrated by the production of the spin adducts of N-*tert*-butyl-α-phenylnitrone(BPN). BPN has the advantage of forming very stable spin adducts.

Bleomycin-A_2 purified was a gift from Nippon Kayaku Co. Ltd., and BPN was obtained from Aldrich Chemical Company. The reaction mixture for spin trapping consisted of 1: 1 bleomycin-A_2-Fe(II) complex(1.0~0.02 mM) and BPN (0.08 M; ethanol solution) in buffered solution (pH 6.9). Oxygen was bubbled through the mixture for approximately 5 seconds, and then an aliquot of the sample solution was rapidly transferred to a quartz flat cell for ESR examination at 25°C. The time course of ESR spectral changes of the 1: 1 bleomycin-A_2-Fe(II) complex (1.0 mM) by oxygen bubbling was investigated at pH 6.9 and measured at 77 K. X-Band ESR measurements were made using a JES-FE-3X spectrometer equipped with 100 KHz field modulation. The g-values and hyperfine splittings were determined relative to Li-TCNQ(g=2.0026), FREMY's salt (a_{10}^N=13.09 G), and Mn(II) in MgO(ΔH_{3-4} =86.9 G).

The oxygen bubbling to the 1: 1 bleomycin-Fe(II) complex(1.0 mM) in the presence of BPN resulted in the generation of the ESR spectrum shown in Fig. 1A. This spectrum can be analyzed in terms of the parameters: g=2.0057 and a^N=15.3 G. These values are essentially identical to those found for the ·OH spin adduct

Fig. 1. ESR spectra obtained by oxygen bubbling of Fe(II)-bleomycin complex in the presence of BPN

(A) 1.0 mM Fe(II)-bleomycin complex and 0.08 M BPN;

(B) 0.02 mM Fe(II)-bleomycin complex and 0.08 M BPN.

Conditions of ESR spectroscopy: microwave power, 10 mW; modulation amplitude, 0.5 G; time constant, 0.01(A) and 0.1(B) second scan time, 4 minutes.

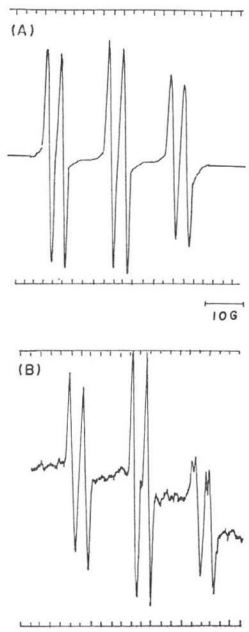
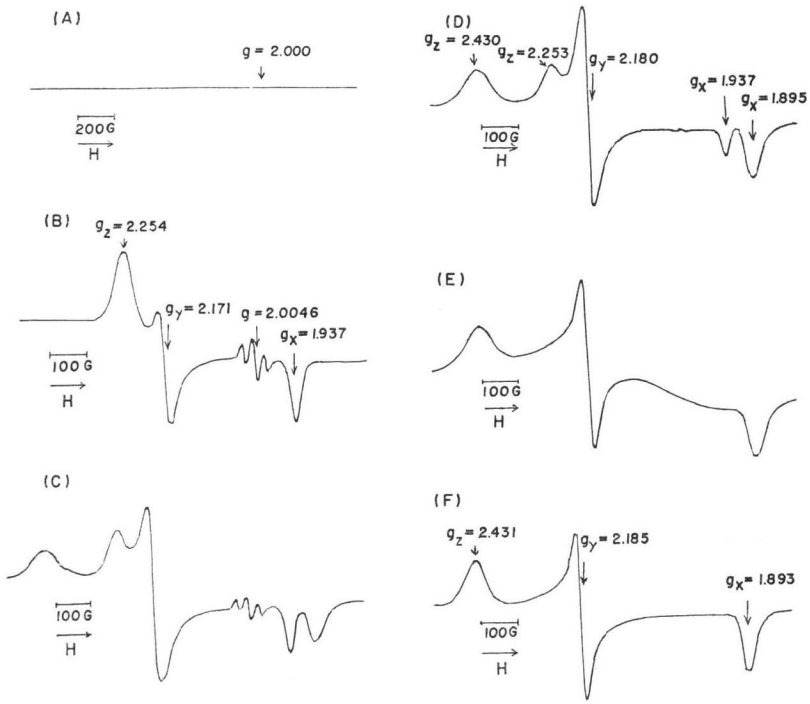


Fig. 2. Time course of ESR spectral changes of Fe(II)-bleomycin complex by oxygen bubbling at pH 6.9: (A), 0 second; (B), 3 seconds; (C), 6 seconds; (D), 10 seconds; (E), 30 seconds; and (F), 90 seconds
The concentration of Fe(II)-bleomycin complex was 1.0 mM.



of BPN[1] and thus we propose that •OH radical is produced in this condition. At 0.02 mM concentration of the 1: 1 bleomycin-Fe(II) complex, on the other hand, the $O_2^{\bar{\bullet}}$ or $\bullet O_2H$ radical adduct of BPN was detected with g=2.0057, a^N= 14.9 G, and a_β^H=2.8 G[1] (see Fig. 1B). Therefore, the formation of superoxide ion ($O_2^{\bar{\bullet}}$) or its protonated form ($\bullet O_2H$) is postulated in the condition of low Fe(II)-bleomycin concentration. The pKa for the acid-base equilibrium of the $\bullet O_2H$ radical species, $\bullet O_2H \rightleftharpoons O_2^{\bar{\bullet}} + H^+$, is 4.4± 0.4.[6] It is still uncertain as to whether BPN traps $O_2^{\bar{\bullet}}$ directly or $\bullet O_2H$ (followed by protonation) which is in equilibrium with $O_2^{\bar{\bullet}}$. The present result is consistent with the observation that the DNA degradation by Fe(II)-bleomycin system is inhibited by superoxide dismutase at low concentration of bleomycin, but not at high concentration.[7] At low concentration of Fe(II)-bleomycin, it seems that diffusible $O_2^{\bar{\bullet}}$ species is presumably an important participant in the DNA degradation reaction. The previous ESR study indicated that the 1: 1 bleomycin-Co(II) complex has square-pyramidal geometry and oxygen molecule is incorporated to the vacant sixth coordination site of the cobalt.[8] In the case of the 1: 1 bleomycin-Co(II) complex (1.0 mM), the ESR signal of the •OH spin adduct with BPN was not as strong as in the case of the corresponding Fe (II) complex. On the other hand, the oxygen bubbling to the 1: 1 Cu(II) and Zn(II) complexes of bleomycin(1.0 mM) generated no ESR signals of the spin adducts.

Fig. 2 shows the time course of ESR spectral changes of the 1: 1 bleomycin-Fe(II) complex by the oxygen bubbling. Although the bleomycin-Fe(II) complex is ESR inactive, the exposure of this complex to oxygen yielded ESR active species. At initial reaction stage, the low-spin Fe(III) complex (g_z=2.254, g_y=2.171, and g_x= 1.937) together with a minor radical species near g=2.005 was clearly observed (see Fig. 2B). However, the unstable Fe(III) complex is transient species. At final reaction stage, another stable Fe(III) complex was formed and its ESR feature is typical of the rhombic low-spin type with g_z=2.431, g_y=2.185, and g_x=1.893 (see Fig. 2F). The 1: 1 bleomycin-Fe(III) complex generated by the oxidation of the corresponding Fe(II) complex easily underwent the reduction by

a reducing agent, in contrast with the 1:1 bleomycin-Co(III) complex. During the reversible redox reaction of the bleomycin-iron complex, reactive free-radical species such as $O_2^{-\cdot}$ and $\cdot OH$ are produced as demonstrated by the present ESR spin trapping. Despite the ability of Cu (II), Co(II), Zn(II), and Fe(II) ions to form complexes with bleomycin, in this regard, it is reasonable that only Fe(II) has significant activity on the DNA degradation by bleomycin.[4]

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