Vol. 90, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 12, 1979 Pages 375-383

THE PRODUCTION OF HYDROXYL RADICAL FROM COPPER(I) COMPLEX SYSTEMS OF BLEOMYCIN AND TALLYSOMYCIN : COMPARISON WITH COPPER(II) AND IRON(II) SYSTEMS

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Received July 17,1979

Summary : In contrast with BLM(or TALM)-Cu(II) complex system, Cu(I)-O_2 system of BLM(or TALM) as well as the corresponding Fe(II) system evidently produces reactive oxygen radicals as detected by ESR spin trapping. The sulfhydryl compound strongly prevented the generation of hydroxyl radical in BLM(or TALM) -Cu(I)-O_2 system. TALM forms metal complexes similar to BLM. The action mechanism of BLM and TALM has been proposed to be substantially same.

Introduction

Cleavage of cellular DNA by BLM is responsible for the antitumor activity of this antibiotic. In this DNA degradation reaction, ferrous ion and molecular oxygen have been proposed to serve as a specific cofactor.[1,2] We have found that BLM-Fe(II)-O_2 complex system efficiently produces reactive oxygen radicals as detected by BPN(or DMPO) spin trapping and oxidizes phenol and tocopherol.[3] Oberley and Buettner also observed the production of hydroxyl radical from BLM-Fe(II) complex system by DMPO spin trapping.[4] It has been described by Lown and Sim that DNA breakage by BLM is prevented by free radical scavengers such as superoxide dismutase, catalase, and isopropanol.[5] BLM has unique metal-binding sites.[6] In particular, square-pyramidal configuration of BLM-Co(II) and BLM-Fe(II) complexes is well suited to bind O_2 and NO molecules.[7,8] TALM is a glycopeptide antibiotic structurally related to BLM, and was originally isolated as a Cu(II)-chelated complex as well as BLM.[9] Therefore, metal-binding sites and antitumor mechanism of TALM appear to be similar to those of BLM.

Abbreviations used : BLM, bleomycin; TALM, tallysomycin; ESR, electron spin resonance; BPN, N-tert-butyl-α-phenylnitrone; DMPO, 5,5-dimethyl-1-pyroline-N-oxide; DNA, deoxyribonucleic acid; DTT, dithiothreitol

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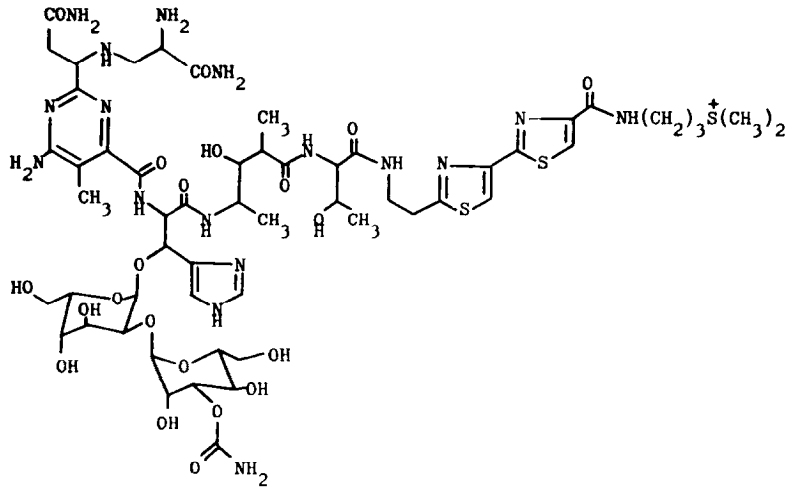
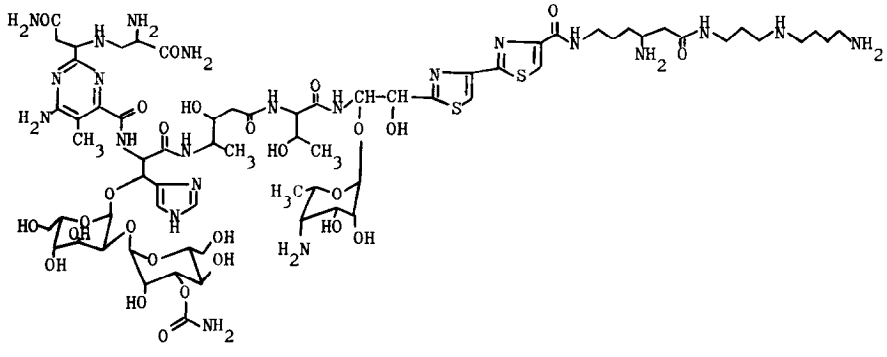


Bleomycin A_2 (BLM)



Tallysomycin A (TALM)

However, Buettner and Oberley recently reported that the hydroxyl spin adduct signal of DMPO is not detected in TALM-Fe(II) system but in TALM-Cu(II) system, and that Cu(II) may have a role in the action of TALM in contrast to the apparent role of Fe(II) for BLM.[10] On the other hand, it has been known that BLM-Cu(II) complex does not cause scission of DNA in vitro, and that the Cu(II) ion of the BLM complex is reductively transferred to a cellular sulfhydryl-containing protein as follows[11]:

$$\text{Cu(II)-BLM} \xrightarrow{\text{HS-Protein}} \text{Cu(I)-S-Protein} + \text{BLM}$$

In this paper, it has been described that hydroxyl radical can be produced in the Cu(I) complex systems of BLM and TALM in contrast with their Cu(II) systems, the presence of excess sulfhydryl compounds prevents production of the free radical from the Cu(I) systems, and the action mechanism of TALM is substantially same to that of BLM.

Experimental

BLM-A_2, BPN, and copper(I) acetate were obtained from Nippon Kayaku, Aldrich, and Alfa, respectively. TALM-A was kindly supplied by Prof. Hamao Umezawa. The reaction mixture for spin trapping consisted of 1:1 BLM(or TALM)-Cu(I) complex(1.0 mM; aqueous solution of pH 6.9) and BPN(0.08 M; ethanol solution). The spin trapping experiments and the detection of free radicals from L-ascorbic acid(or DL-α-tocopherol) were performed according to the previous procedure.[3] X-Band ESR spectra were recorded with a JES-FE-3X spectrometer equipped with 100 KHz field modulation, and a quartz flat cell was used for ESR measurements at 20°C.

Results and Discussion

Figure 1 shows the experimental results of the spin trapping by BLM-Cu(I)-O_2 system, together with those by the corresponding Cu(II) and Fe(II) systems. In contrast with BLM-Cu(II) complex system(Figure 1B), BLM-Cu(I) complex system resulted in the generation of strong ESR signal(Figure 1C). The ESR parameters, g=2.0057 and a^N=15.3 G, are typical of the •OH spin adduct of BPN.[3,12] It is reasonable that the oxidation of reduced BLM-Cu(I) complex by oxygen generates BLM-Cu(II) complex and oxygen radicals as follows :

$$\text{Cu(I)-BLM} + O_2 \longrightarrow \text{Cu(II)-BLM} + O_2^{\cdot-}$$

$$2\ O_2^{\cdot-} + 2\ H^+ \longrightarrow H_2O_2 + O_2$$

$$O_2^{\cdot-} + H_2O_2 \longrightarrow O_2 + OH^- + \cdot OH$$

Copper(I)-induced generation of superoxide has been observed even in human red cell membrane.[13] Recently, we showed that 1:1 BLM-Cu(II) complex has substantially a square-pyramidal configuration.[14] In contrast with square-planar configuration, square-pyramidal arrangement is known to be favored in the Cu(I) complexes.[15] Dabrowiak et al. have reported that the DC polarogram of 1:1 BLM-Cu(II) complex

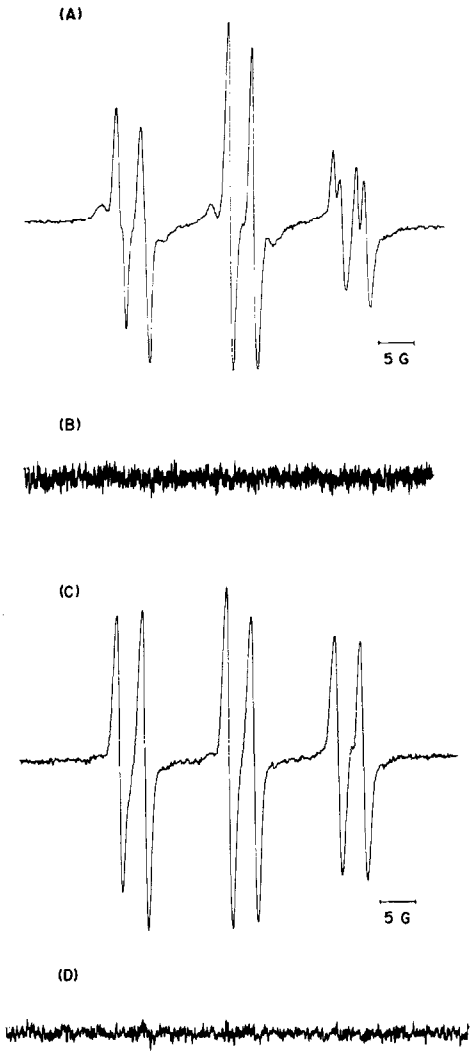


Figure 1 ESR spectra obtained by oxygen bubbling of BLM-metal complexes in the presence of BPN

(A) 0.1 mM BLM-Fe(II) complex and 0.08 M BPN,
(B) 1.0 mM BLM-Cu(II) complex and 0.08 M BPN,
(C) 1.0 mM BLM-Cu(I) complex and 0.08 M BPN, and
(D) 1.0 mM BLM-Cu(I) complex, 10 mM DTT, and 0.08 M BPN
Conditions of ESR spectroscopy : microwave power, 10 mW; modulation amplitude, 0.5 G; scan time, 8 min.

exhibits a one electron metal centered reduction wave of Cu(II)$\longrightarrow$Cu(I) at -0.53 V.[16] As shown in Figure 1D, on the other hand, the addition of excess sulfhydryl compounds such as dithiothreitol and 2-mercaptoethanol strongly

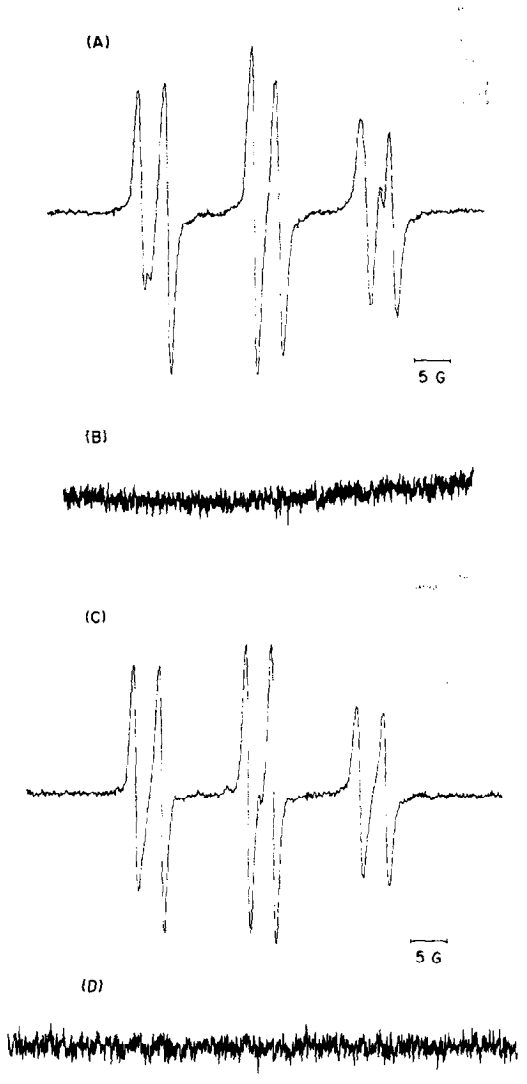


Figure 2 ESR spectra obtained by oxygen bubbling of TALM-metal complexes in the presence of BPN

(A) 0.1 mM TALM-Fe(II) complex and 0.08 M BPN,
(B) 1.0 mM TALM-Cu(II) complex and 0.08 M BPN,
(C) 1.0 mM TALM-Cu(I) complex and 0.08 M BPN, and
(D) 1.0 mM TALM-Cu(I) complex, 10 mM DTT, and 0.08 M BPN
Conditions of ESR spectroscopy : microwave power, 10 mW; modulation amplitude, 0.5 G; scan time, 8 min.

prevented the generation of oxygen radicals in BLM-Cu(I)-O_2 complex system, in contrast with the corresponding BLM-Fe(II)-O_2 complex system.[3] Probably, excess sulfhydryl compound removes the Cu(I) from BLM-Cu(I) complex and then

stabilizes as a Cu(I)-S complex. In fact, sulfhydryl sulfur is strong donor atom for Cu(I). BLM-Cu(II) complex does not cause the degradation of DNA *in vitro*, but *in vivo*.[17] A sulfhydryl-containing protein which reduces Cu(II) to Cu(I) in the BLM complex and binds the resulting Cu(I) ion, has been identified by Umezawa and his colleague.[11] Therefore, the generation of reactive oxygen radicals from the complex system between the copper-liberated BLM and a second metal ion, probably Fe(II) ion, seems to make a contribution to a cellular mechanism for the activity of BLM-Cu(II) complex.

Figure 2 shows the experimental results of the spin trapping by TALM-metal complex systems, and the obtained results are remarkably similar to those in the corresponding BLM complex systems. The generation of hydroxyl radical was not observed in TALM-Cu(II)-O_2 system, whereas Cu(I) and Fe(II) complex systems clearly generated reactive oxygen radicals. However, TALM-Cu(I)-O_2 system was also prevented the production of hydroxyl radical by the sulfhydryl compound. In respect of the reversibility of redox reaction, TALM(or BLM)-Cu(I) complex system is not so effective as the corresponding Fe(II) complex system.
In strand scission of DNA by TALM as well as BLM, it appears more likely that ferrous ion and molecular oxygen have important effect as a cofactor. These results are quite different from the published result.[9] TALM is an antibiotic related to BLM. In fact, TALM and BLM form similar metal complexes(see Table I),

Table I ESR Parameters for Cu(II) and Co(II) Complexes of Bleomycin A_2 and Tallysomycin A

Complex	$g_\perp$	$g_\parallel$	$A_\parallel$,G	A^N,G
BLM-Cu(II)	2.055	2.211	183	
BLM-Co(II)	2.272	2.025	92.5	13
BLM-Co(II)-O_2	2.007	2.098	20.2	
TALM-Cu(II)	2.057	2.210	178	
TALM-Co(II)	2.270	2.025	92.2	13
TALM-Co(II)-O_2	2.008	2.101	20.0	

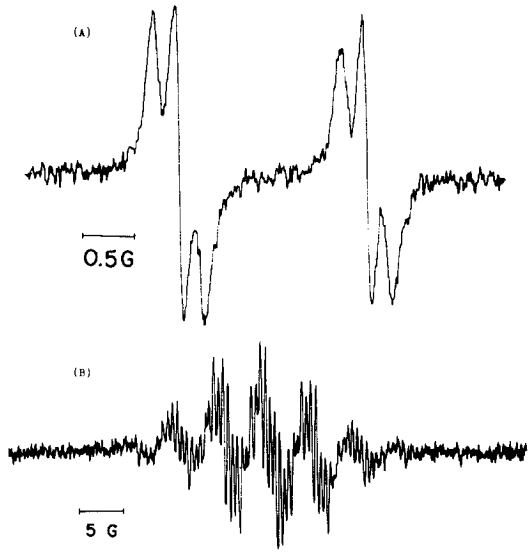
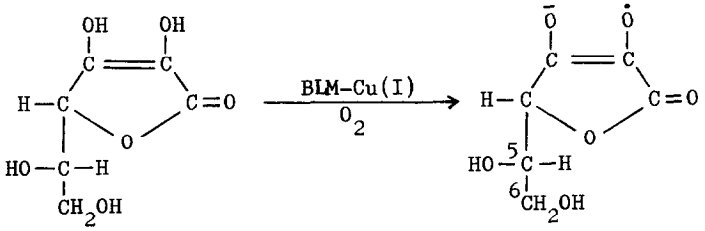


Figure 3 ESR spectra of free radicals from L-ascorbic acid and α-tocopherol by BLM-Cu(I) complex system

(A) 1.0 mM BLM-Cu(I) complex and 0.05 M L-ascorbic acid; and
(B) 1.0 mM BLM-Cu(I) complex and 0.05 M α-tocopherol
Conditions of ESR spectroscopy : microwave power, 10 mW; modulation amplitude, 0.05 G(A) and 0.2 G(B); and scan time, 4 min(A) and 8 min(B).

though TALM-A has a second Cu(II)-binding site, namely L-β-lysylspermidine portion.[18] In addition, it is well-known that low-valence Fe(II) and Cu(I) complexes have higher affinity for O_2 than high-valence Fe(III) and Cu(II) complexes.

Figure 3 shows the ESR spectra of free radicals formed by oxidation of L-ascorbic acid and DL-α-tocopherol with BLM-Cu(I) complex system. The ESR feature of the radical from L-ascorbic acid exhibited a triplet splitting of each of doublet structure with a splitting constant of 1.7 G. The triplet structure has the intensity ratio of approximately 1:2:1 and a splitting constant of 0.18 G. The origins of the doublet and triplet are due to interactions of the unpaired electron with the proton situated on the carbon atom in position 5 and with two equivalent protons attached to the carbon atom in position 6, respectively.



The free radical from α-tocopherol is characteristic of α-tocopheroxyl radical which has been already detected in oxidation of α-tocopherol by BLM-Fe(II)-O_2 system.[3] Similar results were also obtained from the reaction of L-ascorbic acid(or DL-α-tocopherol) and TALM-Cu(I)-O_2 system.

In conclusion, the production of hydroxyl radical by Cu(II) and BLM(or TALM) system has never been observed. The Cu(I) complex system as well as the corresponding Fe(II) system clearly generated reactive oxygen radicals. The sulfhydryl compound strongly prevented the production of the free radical from the Cu(I) complex system. The action mechanism of BLM and TALM should be substantially same.

Acknowledgment

Gratitude is due to Prof. Hamao Umezawa for kind encouragement and Dr. Tomohisa Takita for pertinent advice.

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