Communications to the editor

CHEMISTRY OF BLEOMYCIN. XXIV. DEAMIDO BLEOMYCIN FROM VIEWPOINT OF METAL COORDINATION AND OXYGEN ACTIVATION

Sir:

Deamido bleomycin (Fig. 1) is a product of bleomycin (BLM) inactivated by BLM hydrolase,[1] which is distributed in a variety of organs and is a kind of aminopeptidases. This enzyme activity in tumor cells is related to their sensitivity to BLM, that is, the higher the enzyme activity, the lower the BLM sensitivity[2]. Recently, the three dimensional structure of metal-complexes of BLM was proposed, and the mechanism of action of BLM was elucidated on a molecular level[3~5], which comprises reductively activated oxygen at the sixth coordination site of BLM-Fe(II) complex.

Fig. 1. Structures of metal-free bleomycin, deamido bleomycin and depyruvamide bleomycin.

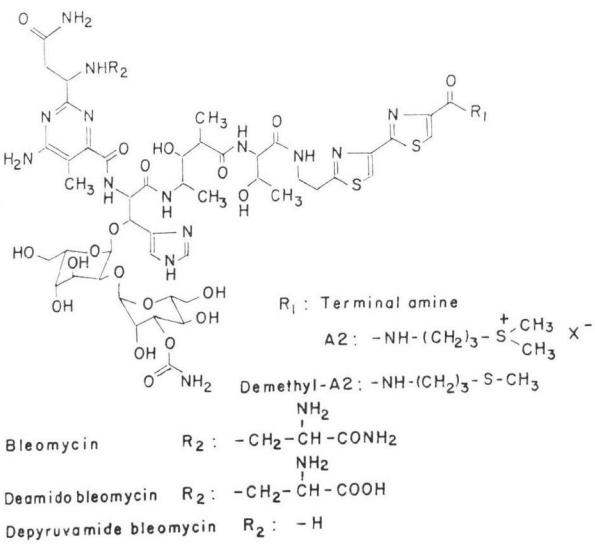
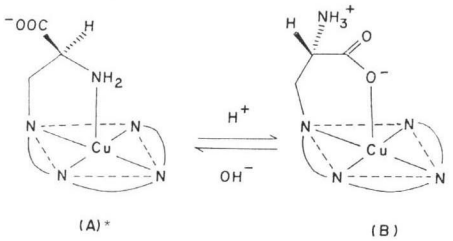


Fig. 2. Coordination structure of deamido bleomycin-Cu(II) complex.



* The same coordination structure as bleamycin-Cu(II) complex[3] taking no account of the 6th coordination site.

The inactivation of BLM by BLM hydrolase occurs only in its metal-free form but not in the copper-complex. This should be due to the masking of the primary amino group of BLM, the recognition site of the enzyme, by coordination. The primary amino group is the axial donor in the metal-complex of BLM. The importance of the fifth axial ligand for the activation of molecular oxygen at the sixth coordination site is inferred from a vast number of works on porphyrin. We have investigated deamido BLM from the viewpoint of the metal coordination and oxygen activation.

In CM-Sephadex column chromatography eluted by a linear gradient between 0.05 M sodium phosphate at pH 6.8 and 1 M sodium chloride, the retention time of deamido BLM A2-Cu(II) complex is about in the middle of those of copper-complexes of BLM demethyl-A2, of which the terminal amine has no charge, and BLM A2, of which the terminal amine has one plus charge (see Fig. 1). As an example, the retention times of copper complexes of BLM demethyl-A2, deamido BLM A2 and BLM A2 obtained by a CM-Sephadex chromatography to separate their mixture were 4.9, 6.8 and 8.9 hours, respectively. If deamido BLM A2-Cu(II) complex has the same coordination structure as that of BLM A2[3], Fig. 2A, the retention time of the deamido BLM A2 complex is expected to be shorter and nearly the same as that of BLM demethyl-A2[6]. The delay of the retention time appears to be due to the partial contribution of structure Fig. 2B, which has one more plus charge than 2A.

The ESR spectrum of deamido BLM-Cu(II) complex at pH 6.8 was slightly different from that of BLM-Cu(II) complex at pH 6.9, but the

Table 1. ESR Parameters for Cu(II) complexes of deamido bleomycin and bleomycin

Complex	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$,G
Deamido bleomycin-Cu(II) (pH 6.8)	2.207	2.055	179.4
Deamido bleomycin-Cu(II) (pH 9.6)	2.211	2.054	171.0
Bleomycin-Cu(II) (pH 6.9)	2.211	2.055	172.4

spectrum of the former at pH 9.6 was close to that of the latter at pH 6.9 (Table 1).

These chromatographic behavior and ESR studies suggest a pH-dependent ligant change shown in Fig. 2 in deamido BLM-Cu(II) complex.

The pH-dependent axial donor change was more clearly demonstrated in the ESR spectrum of the Co(II) complex (Fig. 3). The 1:1 Co(II) complex of deamido BLM was obtained by mixing the metal-free ligand (1.0 mM) and cobalt nitrate (1.0 mM) in aqueous solution (pH 6.8 and 9.6) under fully deaerated condition, and then the X-band ESR spectra were measured at 77 K using a JES-FE-3X spectrometer. At pH 9.6, the 1:1 deamido BLM-Co(II) complex showed an ESR spectrum ($g_{\perp}=2.276$, $g_{\parallel}=2.023$, $A_{\parallel}^{Co}=91.1$ G) similar to the 1:1 BLM-Co(II) complex ($g_{\perp}=2.272$, $g_{\parallel}=2.025$, $A_{\parallel}^{Co}=92.5$G)[4] at pH 6.8. The ESR feature is characteristic to a square-pyramidal configuration with axial primary amino nitrogen coordination. At pH 6.8, on the other hand, the ESR spectrum of the deamido BLM-Co(II) complex ($g_{\perp}=2.301$, $g_{\parallel}=2.020$, $A_{\parallel}^{Co}=112.1$ G) is distinctly different and is close to that of the 1:1 depyruvamide BLM-Co(II) complex ($g_{\perp}=2.368$, $g_{\parallel}=2.025$, $A_{\parallel}^{Co}=123.3$ G) at pH 6.8 which has an aquo molecule as the fifth axial ligand (SUGIURA, unpublished). In depyruvamide BLM metal complex (see Fig. 1 for the metal-free structure[7]*), a square-pyramidal configuration like BLM is impossible due to the lack of the C_3-fragment needed to form the axial bridge. These ESR results strongly suggest that at physiological pH a weaker axial donor, probably carboxyl group, occupies the fifth axial position in the deamido BLM-Co(II) complex, as shown in Fig. 2B.

The previous result of the spin trapping ex-

Fig. 3. ESR spectra of deamido bleomycin-Co(II) complex at pH 9.6 (A) and pH 6.8 (B).

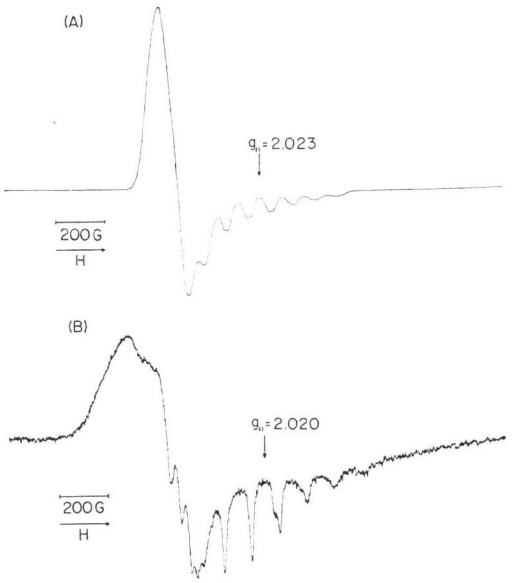
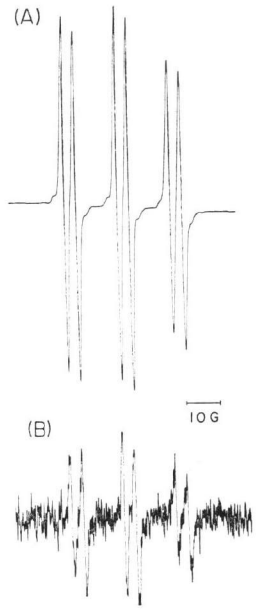


Fig. 4. ESR spectra obtained by oxygen bubbling of bleomycin-Fe(II)(A) and deamido bleomycin-Fe(II) (B) complexes in the presence of N-tert-butyl-α-phenylnitrone.

Condition of ESR spectroscopy: microwave power, 10 mW; modulation amplitude, 0.5 G; time constant, 0.01 (A) and 0.03 (B) sec.; scan time, 4 min.; gain, 2.0×10^2 (A) and 7.9×10^3 (B).



* Direct formation of depyruvamide BLM, not *via* ring-closure[8], from BLM was also suggested (MURAOKA *et al.*, unpublished).

periment demonstrated the efficient production of hydroxy radical at a high concentration (1.0 mM) of the 1:1 BLM-Fe(II) complex[5]. However, the formation of hydroxy radical by the 1:1 deamido BLM-Fe(II) complex under the same conditions (pH 6.9) was remarkably low, and its spin concentration of free radical was approximately estimated to be 1/90 of that of the BLM system (Fig. 4). It is presumed that the large difference in oxygen activation is attributable to the difference of the fifth axial coordination donor between deamido BLM and BLM as presented in the Cu(II) and Co(II) complexes. The activity of deamido BLM for DNA cleavage reaction has been estimated at approximately 1% to that of BLM[9]. Thus, the lower biological activity of deamido BLM should be due to the less effective oxygen-activation of its Fe(II)-complex, of which the axial coordination site is predominantly occupied by the carboxyl group at physiological pH.

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