Nature of carbon monoxide binding of iron(II)-bleomycin

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The CO vibrations of the Fe(II)-CO adduct complexes of bleomycin (BLM) and its synthetic analog(PYML) have been determined at 1973 and 1980 cm^{-1}, respectively. The model PYML-Fe(II) complex ($P_{1/2}^{CO} = 3.8 \times 10^{-2}$ torr) binds CO with much greater affinity than the native BLM-Fe(II) complex ($P_{1/2}^{CO} = 2.2 \times 10^{-1}$ torr). Of special interest is the fact that the rate of CO rebinding to the ferrous BLM complex ($k = 7.7 \times 10^{2}$ $M^{-1} \cdot s^{-1}$) is clearly slower than that to the corresponding PYML complex ($k = 7.4 \times 10^{3}$ $M^{-1} \cdot s^{-1}$). The steric effect of the sugar portion of BLM molecule appears to have an important influence on the present low affinity of CO binding and slow rate of CO rebinding.

Iron-bleomycin *CO affinity* *Photolysis* *Steric effect*

1. INTRODUCTION

Iron-BLM causes DNA cleavage in conjunction with molecular oxygen and reducing agent [1,2] and bears analogy with the properties of Fe(II)-porphyrins such as in hemoglobin and cytochrome P-450 towards O_2 [3]. The BLM-Fe(II) complex readily forms a stable, diamagnetic complex with CO [4,5] and its CO adduct does not mediate strand scission of DNA. Indeed, carbon monoxide is a biological ligand for iron-BLM, just as for hemoproteins. The present report on CO stretching frequency, CO affinity constant, and rate constant of CO rebinding for the BLM-Fe(II) complex evidently reveals that the BLM structure results in significant change for the nature of CO binding, compared with a simple model ligand of BLM, namely PYML.

2. EXPERIMENTAL

Purified BLM-A_2 was the gift of Nippon Kayaku Co. Ltd, and the model ligand PYML was synthesized according to [6]. The Fe(II) complexes of BLM and PYML were exposed to an atmosphere of CO at pH 8.3 (0.05 M borate buffer) in a Thunberg cuvette. Infrared spectral data were measured on a Nicolet 7199 FT-IR spectrometer, and a cell fitted with CaF_2 windows and set to 0.1 mm was used. The CO affinity was determined in terms of $P_{1/2}^{CO}$ (pressure units), the pressure of gaseous ligand at which half of the Fe(II) complexes (3.0×10^{-4} M) is ligated. Photolysis was achieved by pulsing Rhodamine 590 in a phase-R flash lamp (DL-10), rated duration of 300 ns. In aqueous solution (pH 8.3), the photodissociation of the samples (3.0×10^{-4} M) was measured by monitoring ΔA at a fixed wavelength.

Abbreviations: BLM, bleomycin; PYML, *N*-[6-{[(*S*)-2-amino-2-carbamoylethyl]amino]methyl}pyridine-2-carbonyl]-L-histidine

3. RESULTS AND DISCUSSION

As clearly shown in hemes and hemoproteins, the infrared stretching frequencies of bound CO would provide a useful information for the nature of CO binding [7]. Fig.1 represents FT-IR spectra of the BLM-Fe(II) complex and its CO adduct in D_2O. The BLM-Fe(II)–CO complex gave ν_{CO} at 1973 cm^{-1} and the PYML-Fe(II)–CO complex at 1980 cm^{-1}. The positions of these bands are closer to those of the CO adducts of certain iron(II)-porphyrins, Fe(diethylprotoporphyrinato)(*N*-methylimidazole) ($\nu_{CO} = 1970$ cm^{-1}) and Fe(meso-tetra-

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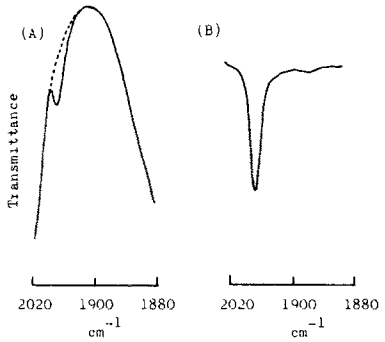

Fig.1. FT-IR spectra of the BLM-Fe(II) complex (---) and its CO adduct (——) in D_2O(A) and difference spectrum of the BLM-Fe(II)–CO complex minus the BLM-Fe(II) complex (B).

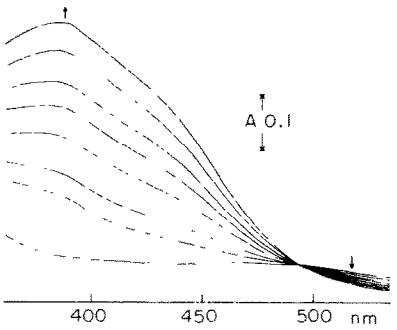

Fig.2. Spectral changes occurring upon titration of the BLM-Fe(II) complex with the following pressures of carbon monoxide at 20°C; 0, 20, 30, 49, 79, 102, 136, and 745 mmHg.

($\alpha,\alpha,\alpha,\alpha$ - *o* - pivalamidophenyl)porphyrinato)(*N*-methylimidazole) (1969 cm^{-1}), rather than those of hemoglobin-CO (1951 cm^{-1}) and cytochrome P-450–CO (1940 cm^{-1}) [7].

Fig.2 shows the CO titration plot of the BLM-FE(II) complex which gives optical spectral changes with isosbestic point. The carbon monoxide affinities as $P_{1/2}^{CO}$, the pressure for half-saturation of the BLM-FE(II) and PYML-Fe(II) complexes are listed in table 1 along with those of some hemes and hemoproteins. The half-saturation values were estimated on the basis of the equilibrium constants determined by the usual visible spectral procedures of CO titrations. In terms of $P_{1/2}^{CO}$, the PYML-Fe(II) complex binds CO approximately 6 times better than does the BLM-Fe(II) complex. The CO affinity of the BLM-Fe(II) complex is nearly equivalent to those of hemoglobin (T) and monopyridinepropanolmesoheme, whereas the PYML-Fe(II) complex has the CO affinity corresponding to myoglobin. Although the electronic effect on (ν_{CO} is important, the steric interaction is the common denominator in CO affinity. The model PYML-Fe(II) complex binds CO with significantly higher affinity than does the BLM-Fe (II) complex. Probably, steric bulk near the binding site in BLM distorts the Fe-CO geometry and lowers the CO affinity of the BLM-Fe(II) complex relative to the unconstrained model. In hemes and hemoproteins, it has been observed that the infrared (ν_{CO} value correlates with the CO binding affinity ($P_{1/2}^{CO}$), namely the lower the ν_{CO}, the lower the affinity for CO, and

Table 1

CO affinity of iron-bleomycin and iron-porphyrins

Compound	$P_{1/2}^{CO}$(20–25°C), torr	Refs
Fe(BLM)	2.2×10^{-1}	This work
Fe(PYML)	3.8×10^{-2}	This work
Myoglobin	1.2×10^{-2}–2.8×10^{-2}	9
Hemoglobin(T)	1×10^{-1}–2.8×10^{-1}	10,11
Hemoglobin(R)	1×10^{-3}–4×10^{-3}	10,11
(Mesoheme methyl ester) (pyridine)	12.5	12
(Monopyridinepropanolmesoheme)	2.0×10^{-1}	12
Fe(meso-tetra($\alpha,\alpha,\alpha,\alpha$-*o*-pivalamidophenyl)-porphyrinate)(1,2-dimethylimidazole)	8.9×10^{-3}	13
Fe(meso-tri(α,α,α-*o*-pivalamidophenyl)-β-*o*-5-(1-imidazoyl)varleramidophenylporphyrinate	2.2×10^{-5}–3.0×10^{-6}	13

that the hemoprotein structure functions to reduce importantly the affinity for CO [14]. Interestingly, a similar phenomenon is recognized in the present Fe(II) complexes of BLM and PYML.

Fig. 3 depicts the results for rebinding of CO to the BLM-Fe (II) and PYML-Fe(II) complexes from the flash photolysis experiments, and in these cases the reactions obeyed good second order kinetics. The CO recombination rate constants of the BLM-Fe(II) and PYML-Fe(II) complexes are remarkably different from those of hemoglobin ($k = 0.6\text{–}0.9 \times 10^5$ $M^{-1} \cdot s^{-1}$) and myoglobin ($5.4\text{–}6.9 \times 10^5$ $M^{-1} \cdot s^{-1}$) [15]. Of interest is the fact that the rate of CO rebinding to the ferrous BLM complex ($k = 7.7 \times 10^2$ $M^{-1} \cdot s^{-1}$) is slower than that to the corresponding PYML complex (7.4×10^3 $M^{-1} \cdot s^{-1}$) by a factor of about 10. It is known that PYML corresponds to the β-aminoalanine-pyrimidine-β-hydroxyhistidine portion of BLM, and is able to mimic the metal binding and dioxygen activation by BLM ligand. The visible absorption characteristic ($\lambda_{max} = 390$ nm ($\epsilon = 2000$) and Mössbauer spectrum ($\delta_{Fe} = +0.18$ and $\Delta E_Q = 0.51$ mm/s) of the PYML–Fe (II)–CO complex are indeed similar to those ($\lambda_{max} = 380$ nm ($\epsilon = 3000$), $\delta_{Fe} = +0.19$ mm/s and $\Delta E_Q = 0.66$ mm/s) of the BLM-Fe (II)–CO complex [16]. However, the two iron (II) complexes differ distinctly in the binding affinity and rebinding rate of CO. Herein, it is of special interest to note that the CO binding constants of BLM-Fe(II) complex are close to those of Fe(C_3-capped porphyrin)(1,2-dimethyl imidazole) complex ($P_{1/2}^{CO} = 1.4 \times 10^{-1}$ torr and $\nu_{CO} = 1984$ cm^{-1}) and Fe(C_2-capped porphyrin)(1,2-dimethylimidazole) complex ($P_{1/2}^{CO} = 2.0 \times 10^{-1}$ torr and $\nu_{CO} = 1999$ cm^{-1}) [17]. If we can consider hydrogen bonds between the carbamoyl group of sugar and the amide group of β-aminopropionamide in the BLM molecule, a steric environment similar to iron-capped porphyrin is formed in the BLM-iron complex as illustrated in fig.4. Indeed, the CPK model strongly supports the formation of such the hyrdogen bonds. Therefore, the present results indicate that the steric effect of the BLM ligand, in particular the sugar portion, has an important influence on the nature of carbon monoxide binding of the BLM-Fe(II) complex.

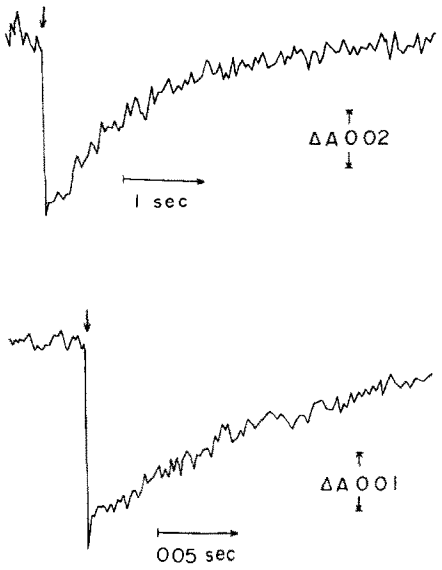

Fig.3. Rebinding of CO to the BLM-Fe(II) complex(upper) and PYML-Fe(II) complex(lower). The arrow indicates the time of the flash, and the measurement was carried out at 390 nm and 20°C.

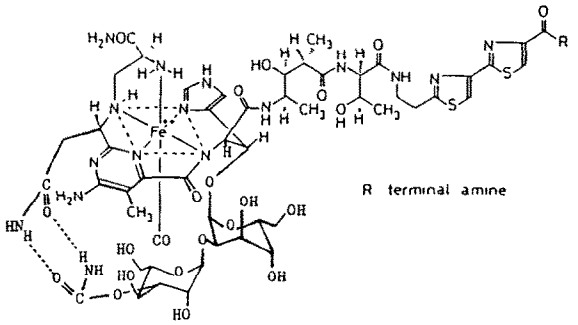

Fig.4. Proposed environment near bound CO of the BLM-Fe(II) complex.

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