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ENANTIOMERIC BLEOMYCIN MODEL COMPOUNDS BEARING LONG ALKYL-CHAIN

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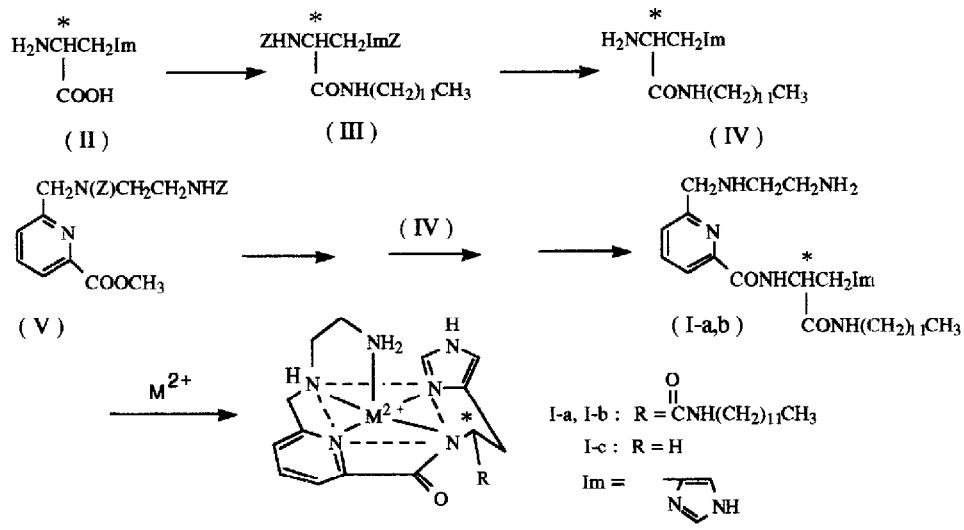
Abstract. Enantiomeric bleomycin model compounds bearing long alkyl-chain were synthesized and the physicochemical properties of their metal complexes were studied. The Fe complexes of these compounds exhibited a strong DNA cleaving activity in a chiral discriminative manner.

Bleomycin (BLM) is a glycopeptide-derived antitumor antibiotic agent[1]. Therapeutic action of BLM takes place when it forms metal complex with ferrous ion and activates molecular oxygen, thereby inducing degradation of DNA[2]. BLM metal complex is also known to oxidize simple organic molecules like olefins[3]. Several model compounds of BLM's metal coordination site have been synthesized and the effects of substituents on the O_2 activating ability on their metal complexes have been investigated[4]. Among these, model compounds having tert-butyl[4b,c] or hexadecyl group[4e] are intriguing because their Fe complexes exhibited enhanced O_2 activating ability. However, the effects of the introduction and the configuration of such hydrophobic moiety on the physicochemical properties as well as DNA cleaving activity of the model compounds are still ambiguous. These prompted us to undertake the synthesis and the study of new enantiomeric BLM model compounds, I-a and -b, having lauryl group as a hydrophobic moiety in a chiral manner. These compounds would be useful for the better understanding about the role of hydrophobic moiety and provide a chiral selective oxidizing catalyst.

Synthetic procedure of I-a and -b is summarized in scheme 1. D-Histidine (II) was treated with benzyloxycarbonyl chloride (Z-Cl) followed by condensation with laurylamine in the presence of diphenylphosphoroazidate (DPPA)[5] to form the derivative III (78.1%). Removal of Z-group by hydrogenation in the presence of Pd catalyst yielded the free amine IV (98.7%). Meanwhile, pyridinedicarboxylic acid derivative V, which was prepared from the known ester[4e], was saponified to the free acid and condensed with free amine IV by the action of DPPA to form the protected model compound (87.1%). Final deblocking of the foregoing material was performed in a similar manner as above to yield the model compound I-a (77.3%)[6-a]. Using L-histidine instead of D-histidine by the same synthetic route gave I-b, as expected[6-b]. The model compounds thus obtained have a lauryl group in a chiral manner.

Model compounds I-a and -b form micellar type aggregate in an aqueous solution. The critical micelle concentration (c.m.c) of the compounds, measured by surface tension method using a Wilhelmy tensiometer at 25°C, was 2×10^{-5}M in 0.1M Tris-HCl buffer (pH = 7.2). On the other hand, Cu(II) complex of I-a did not show a clear c.m.c., though, strongly aggregated at concentrations higher than 6×10^{-5}M at the same pH. Model compound I-c, which has the same formula as I-a or b except for not having lauryl group, is soluble in water and does not form aggregate under any condition.



Scheme1. Synthetic procedure and the formation of metal complex of I-a and -b.

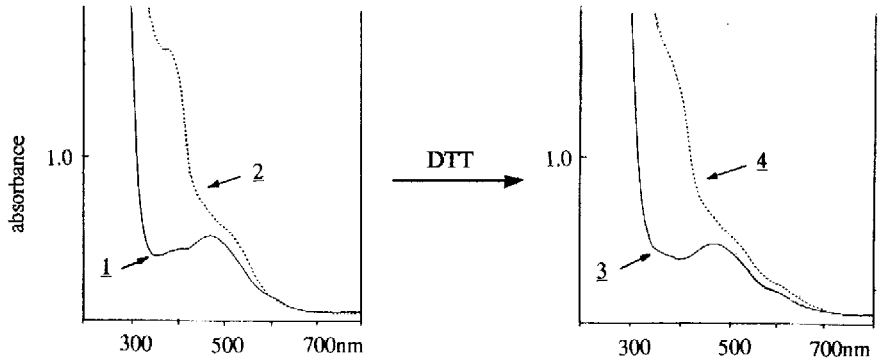
The coordination chemistry of the model compounds with several divalent metal ions was investigated spectrophotometrically. All compounds formed metal complexes with their UV λmax. values[7] close to that of the corresponding BLM metal complexes[8,9] and are supposed to have BLM like structures. In the case of Fe complex of I-c, however, precipitation of $Fe(OH)_3$ was observed while the sample was exposed to air in a short period of time. This is due to the oxidation of Fe(II) to Fe(III) after the coordination of oxygen as the 6th ligand of the complex and subsequent nucleophilic attack of OH^- ion to the metal. On the other hand, no such precipitation was observed for the Fe complexes of I-a or -b even after the prolonged exposure to air. Thus, the stability of I-a and -b Fe complexes is enhanced by the introduction of lauryl group which is supposed to create the hydrophobic cavity around the Fe(III) and prevent the attack of OH^-.

Fe(III) Complexes of I-a and -b were reduced by common reducing agent such as dithiothreitol (DTT) and re-oxidized by air or oxygen as shown in Fig. 1. This kind of reversible redox reaction is known to be characteristic to BLM-Fe complex. The result indicates that the Fe complexes of I-a and -b activate molecular oxygen and generate hydroxyl radical. The generation of hydroxyl radical was confirmed by an spin-trapping experiment using BPN in the presence of DTT and oxygen. From the ESR spectrum of BPN spin-adduct (triplet of doublets, g 2.01, a^N 15.0 G), O_2 activating ability of the complexes was estimated to be 58% to that of the BLM standard.

The Fe complex of I-a or -b was incubated aerobically with closed circular plasmid DNA(pBR322). Conversion of the closed circular DNA to nicked and linear DNA was monitored by agarose gel electrophoresis, followed by gel staining. The complexes exhibited strong DNA cleaving activity at the concentration of 1.5×10^{-4} M. The results are summarized in Table 1. Although their O_2 activation ability was about half that of BLM as mentioned above, their DNA cleaving activity was only 1/20-30 compared with that of BLM. It is considered that Fe-BLM or Fe-BLM model compounds capable of binding to DNA and producing diffusible oxygen radicals bring about DNA scission. Therefore, the low reactivity of the Fe complexes of I-a and -b is likely

resulted from the lack of an apparent DNA binding site, such as bithiazole moiety of BLM, in the model compounds. Rather interesting observation is that the complex of I-b, derived from L-histidine, exhibited greater activity compared with the antidope complex I-a (approximately 1.5 times) as indicated in Table 1.

Fig.1 Reversible redox reaction of I-a Fe(II) complex.



The concentration of I-a Fe(II) complex was 0.5mM in 50mM Tris-HCl(pH7.2), and the UV spectra were obtained under following conditions ; 1, under N_2 (λmax = 478nm) ; 2, 1min after O_2 bubbling to sample 1 (λmax = 392nm) ; 3, 10min after the addition of DTT(1mM) to sample 2 ; 4, 20min after the addition of DTT to sample 2.

Table 1. Strand scission of pBR322 DNA by the Fe complex of I-a, I-b, and BLM.

sample / ratio(%) of DNA	A DNA	B DNA+I-a	C DNA+I-b	D DNA+BLM
nicked DNA	5.9	43.9	67.0	68.6
linear DNA	none	none	1.6	4.5
closed circular DNA	93.0	56.3	31.2	26.7

The samples contained 0.5mg of DNA, 2mM tris-HCl (pH7.5), and the following additions : A, none ; B, 15μM I-a Fe(II) complex plus 1mM DTT ; C, 15μM I-b Fe(II) complex plus 1mM DTT ; D, 0.8μM BLM-B4 Fe(II) complex plus 65μM DTT. The reaction samples were incubated at 23°C for 20min under an aerobic condition. After agarose gel electrophoresis, the bands were stained with ethidium bromide and the ratio(%) of each DNA were determined by densitometric readings.

Since I-a and -b lack the apparent DNA binding site, the association of the complex with DNA, if possible, must be governed by weak surface interaction. The minor groove of DNA helices is known to be in hydrophobic circumstances[10], therefore, a surface interaction between the minor groove of DNA and lauryl group of the complexes is possible to occur. Barton et. al. reported[11] that enantiomeric metal complexes of phenanthrolines bind to DNA helices stereoselectively, inducing stereoselective photoactivated cleavage of DNA. It was suggested[12] that chiral complexes form noncovalent bond with DNA helices in two modes with chirally

preferential manner : intercalatively bound mode and surface-bound mode. Thus, we propose that the observed chiral discriminative scission of DNA was resulted from the difference of the chirality of the hydrophobic lauryl group which could cause varied degrees of association with DNA. However, the present data are not sufficient to illustrate precise mechanism and further investigation are in progress.

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6. (a) White powder. $[\alpha]_D^{20}$ -1.5° (c = 0.5%, MeOH), M^++2 501 : (b) White powder. $[\alpha]_D^{20}$ +1.5° (c = 0.5%, MeOH), M^++2 501 : Satisfactory NMR spectra were obtained for the both samples.
7. Following λmax values were observed ; 607nm for I-a Cu(II) and I-b Cu(II) ; 606nm for I-c Cu(II) ; 596nm for BLM Cu(II)[8] ; 475nm for I-a Fe(II) and I-b Fe(II) ; 476nm for I-c Fe(II) ; 476nm for BLM Fe(II)[8] ; 392nm for I-a Fe(II) + O_2 ; 385nm for BLM Fe(II) + O_2[9].
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