From natural bleomycins to man-designed bleomycins

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Abstract – Bleomycin (BLM) has a number of important biochemical functions and each partial structure of BLM seems to have a separate role in the biochemical functions. In order to clarify such biomechanisms in terms of organic reactions, pyridine model compounds (PYML) are designed, synthesized, and characterized. These results have provided the most reliable evidence for the proposed metal-binding sites of bleomycin and a man-made oligopeptide, PYML(6) showed a remarkable oxygen-activation virtually equivalent to natural BLM. The first man-designed bleomycin, PYML(6)-the bithiazole-terminal amine moiety has shown a remarkably efficient oxygen activation comparable to bleomycin and an efficient DNA cleaving activity in vitro. Novel man-made bleomycins consisted of the PYML and other DNA-binding sites are also designed and synthesized.

INTRODUCTION

Bleomycins (1, BLM A2; 2 BLM B2) are a family of glycopeptide antitumor antibiotics discovered by H. Umezawa et al. from cultures of Streptomyces verticillus as copper chelates in 1966 and differing only in the amino side-chain at the C-terminus of the common linear structural unit, bleomycinic acid (3, R=OH). BLM are now clinically used in the treatment of Hodgkin's lymphoma, carcinomas of the skin, head, and neck, and tumors of the testis mostly in combination with radiation or other chemotherapeutic agents (ref. 1). The structure was finally determined by Takita et al. (ref. 2, 3) in 1978 after extensive degradation study with the help of X-ray analysis of the Cu(II) complex of P-3A, a biosynthetic intermediate of BLM (ref. 4) and supporting evidence for the pyrimidine moiety with synthetic model compounds (ref. 5). A BLM consists of a linear hexapeptide and a disaccharide and a terminal amine as shown in Fig. 1. In addition to its medicinal importance, a BLM is one of the most ingeniously elaborated compounds by nature, because the sequence selective cleavage of cellular DNA with BLM or the biological effect is now considered to be due to three chemical characteristics of the
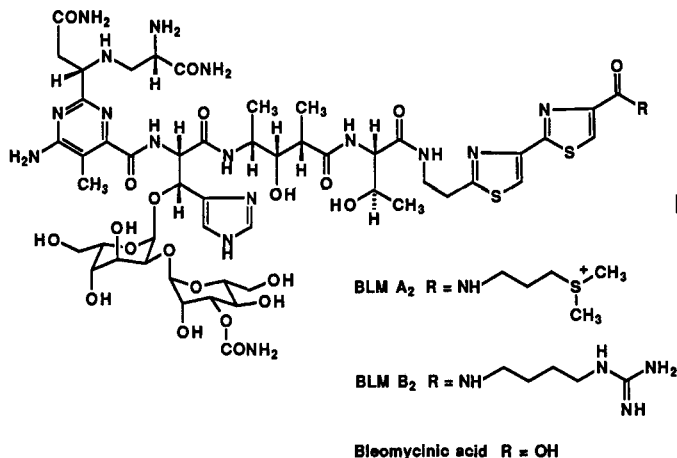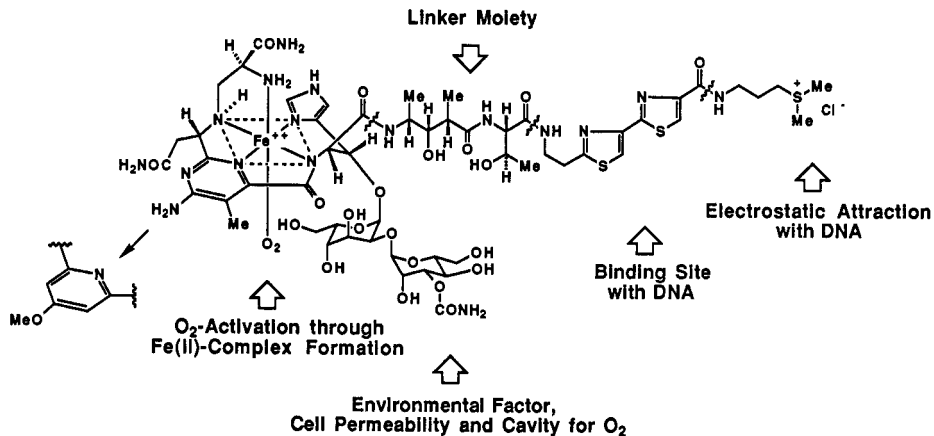

Fig. 1 Bleomycins

BLM A₂ R = NH~

BLM B₂ R = NH~

Bleomycinic acid R = OH

Fig. 2 Assumed Mechanism of Biological Functions

glycopeptides. The bithiazole and terminal amine residues are believed to contribute to the binding to DNA (ref. 6) and the β-aminoalanine -pyrimidine- β-hydroxy-histidine moiety appears to be capable of dioxygen activation by chelation with ferrous ion (ref. 4, 7), and the sugar moiety might be essential for the permeability to the cell membrane as shown in Fig. 2.

Then, the structure of BLM was confirmed by the total synthesis of the deglyco-bleomycin in 1981 and also BLM-A2 in 1982 from our laboratories (ref. 8) and other laboratories (ref. 9) in 1982.

TRANSITION-METAL BINDING SITE OF BLEOMYCIN

As BLM contains many heteroatoms which can bind metals, various structures for the transition-metal binding sites have been proposed. Among these, we believe that the most reasonable one is Umezawa's structure (Fig. 2), because it was presented based on the X-ray structural analysis of the Cu(II) complex of P-3A, a biosynthetic precursor of BLM isolated from a culture broth. We have designed our synthetic models for the metal binding site, namely pyridine-model ligands (PYMLs), according to the Umezawa's structure.

The key features of our first series of PYML compounds include the following; 1) replacement of the pyrimidine nucleus by a pyridine, 2) use of a simplified side chain (((s)-2-amino-2-carbamoylethyl)amino) methyl group, and 3) use of erythro- -hydroxy-L-histidine to L-histidine (ref. 11). The 1:1 divalent metal complexes of PYML-1 and the dioxygen analogue adduct complex were shown to be remarkably close to those of the corresponding BLM-mental complexes. The spin-trapping experiments showed that hydroxyl radicals are generated in the PYML-1-Fe(II)-O$_2$ complex system and that the radical spin concentration is approximately 18% of that of the BLM-Fe(II) complex system (Table 1). Thus, PYML-1 was considered to be the most simplified structural unit required for the metal binding and dioxygen activation.

An attempt to yield more oxygen-sensitive complex was carried out. We have studied the various steric and electronic factors of the PYML skeleton to clarify the relationships between the designed structures and the capability to activate dioxygen. In particular, the disaccharide moiety of BLM appeared to be very important to construct a hydrophobic molecular cavity to accommodate dioxygen. A model having a tert-butyl group, PYML-4, was considered to be a good candidate to realize the effective formation of a hydrophobic cavity. In fact, the dioxygen activating ability of PYML-4 increased up to 71% of that of BLM, demonstrating the profound effect of the tert-butyl group as a steric factor (Table 1) (ref. 12)

Table 1 ESR Spin Trapping of PYML-Fe(II)-O$_2$ Complex Systems

Fe(II) complex	Relative spin concentration
BLM	100
PYML-6	97
PYML-4	71
PYML-7	58
PYML-1	18

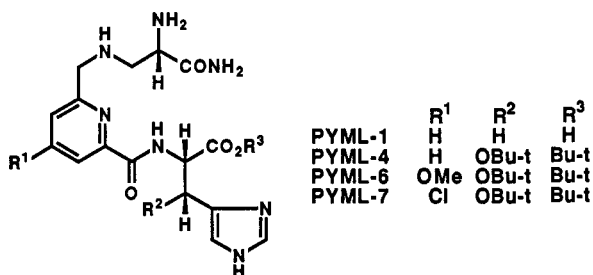|	R^1	R^2	R^3
PYML-1	H	H	H
PYML-4	H	OBu-t	Bu-t
PYML-6	OMe	OBu-t	Bu-t
PYML-7	Cl	OBu-t	Bu-t

Fig. 3 PYMLs

As for the electronic factor, we assumed that the increased π-electron density on the N-atom of the pyridine ring would increase the capacity to activate dioxygen for the Fe(II) complex, and *vice versa*. Thus, PYML-6 and -7 were designed and synthesized (ref. 13). It was gratifying that PYML-6 with a methoxyl substituent showed oxygen activation virtually equivalent (97%) to that of BLM (Table 1). On the other hand, an electron withdrawing chloro group induced a reverse effect in PYML-7 less active (55%) than unsubstituted PYML-4 (Table 1), suggesting an important influence of the π-electron density at the N-atom of the pyridine ring on the dioxygen activation. Consequently, it seemed that, in PYML-6, the overall effect of the sterically bulky tert-butyl group and the electron donating nature of the methoxyl substituent reproduced a metal binding and dioxygen activating property comparable to BLM.

DNA CLEAVAGE WITH MAN-DESIGNED BLEOMYCIN

The efficient oxygen activation with PYMLs provided a basis for the exploitation of specific cleaving agents of DNA and new anticancer compounds if the disaccharide moiety is not critical for the permeability of the cell membrane. Thus, the DNA binding site, tripeptide S, was coupled with the PYML-6 to afford PYML(6)-bleomycin (Figure 4) (ref. 14).
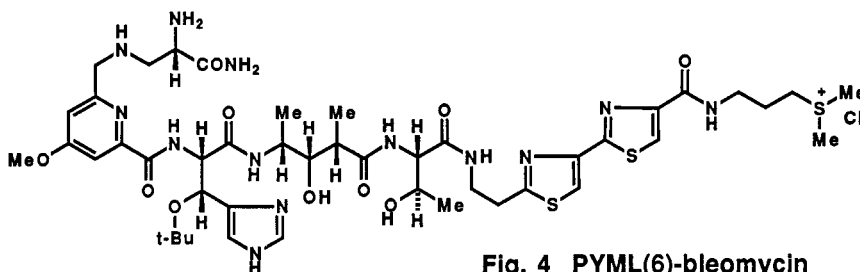
Fig. 4 PYML(6)-bleomycin

The PYML(6)-bleomycin-Fe(II) complex system showed a potent DNA cleaving activity in the presence of molecular oxygen and reducing agent. As the PYML-6-Fe(II) complex was virtually inactive in the DNA cleaving reaction, a DNA affinity site seems to be essentital for the PYML ligands to deliver a metal center to an appropriate site of the DNA helix. The nucleotide sequence cleavage mode by PYML(6)-bleomycin system was considerably similar (but not identical) to that of the corresponding BLM system.

CONCLUSION

From the study of PYML series of compounds, it has been strongly supported that the β-aminoalanine-pyrimidine-β-hydroxyhistidine moiety of bleomycin is essential as the reaction site for the metal binding and dioxygen activation. Therefore, it is worthwhile to develop some useful oxidants based on the biofunction of bleomycin.

The PYML(6)-bleomycin, the first man-designed bleomycin, cleaves DNA efficiently in vitro and the sequence specificity of the DNA cleavage is very similar to that of bleomycin. However, the permeability of the model compound to the cell membrane is not clear yet, but must be very critical for the anticancer activity. Furthermore, the role of the pentanoic acid

and threonine moiety is not yet completely understood. Although the
present study is still at the early stagy of the man-designed bleomycins,
we believe that there is good hope for the design of some useful
bleomycin homologues that will approach to an anticancer agent of
different organ selectivity.

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