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RESTRUCTURING OF THE BLEOMYCIN METAL CORE. NOVEL OXYGEN-ACTIVATING LIGANDS WITH SYMMETRIZED STRUCTURE

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Abstract. Novel ligands having symmetrized coordination environment consisting of two histidine units and a pyridine are prepared. Oxygen activating efficiency of the iron complexes of the synthetic ligands increases by introducing electron donating substituent into the pyridine ring.

It has been well established that antitumor antibiotic bleomycin (BLM) exerts its activity by cleaving DNA with active oxygen species generated by its iron core.[1] Many physicochemical data indicated that the β-aminoalaninamide-pyrimidine-β-hydroxyhistidine region of BLM forms an iron complex to activate molecular oxygen (Figure 1 (A)). Previously we have reported efficient oxygen activation by iron complexes of synthetic models of BLM, namely PYML, designed by the direct analogy to the BLM metal core.[2] The structure of the metal complex of PYML appeared to be similar to that of BLM. It was considered that pyridine and imidazole of PYML are coplanar mainly because of the planarity of the peptide bond intervening between the two heterocycles while the primary amino group occupies the axial position due to the tetrahedral nature of the secondary amino nitrogen which allows the aminoalanine side chain to stand straight (Figure 1 (B)).[2] We also found that introduction of an electron-donating group into the pyridine ring enhances the oxygen-activating efficiency of PYML.[3,4] Methoxypyridine and dimethylaminopyridine derivatives of PYML showed oxygen-activating efficiency 1.4 times and 1.8 times strong, respectively, as that of unsubstituted PYML.[5,6]

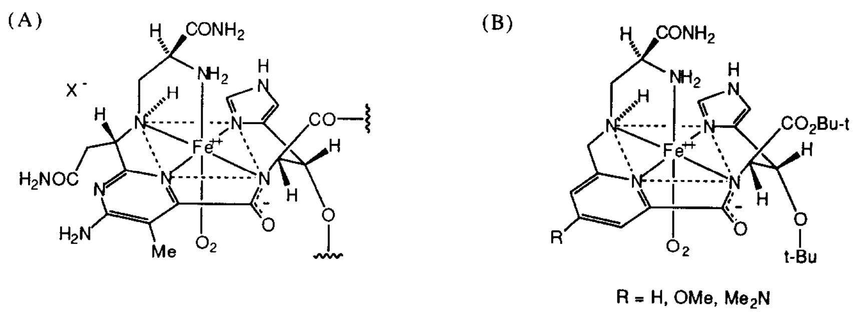


Figure 1. Proposed structure for the iron-oxygen complexes of bleomycin (A) and PYML (B).

Herein we describe our attempt to construct a novel oxygen-activating system by re-assembling the coordination elements contained in the BLM metal core. We designed a new ligand system with symmetrized coordination environment consisting of two histidine units and a pyridine as shown in Figure 2 (A) and named it HPH based on the structure (Histidine-Pyridine-Histidine). Our objective is to obtain a non-BLM type ligand by making the pyridine occupy the axial coordination site as shown in Figure 2 (B). The flexibility of bis(secondary amino) structure was thought to be crucial for HPH to keep up with the conformational change of the ligand skeleton during the dynamic process of oxygen activation.[7,8] We also designed methoxyl derivative MeO-HPH and dimethylamino derivative Me_2N-HPH in order to examine the electronic effects of substituent of the *axial* pyridine of HPH *vs* that of the *equatorial* pyridine of PYML.[9] The electronic effect of the substituent on the pyridine must be effectively transmitted to the oxygen at the other end of the d_{z^2} orbital of iron.

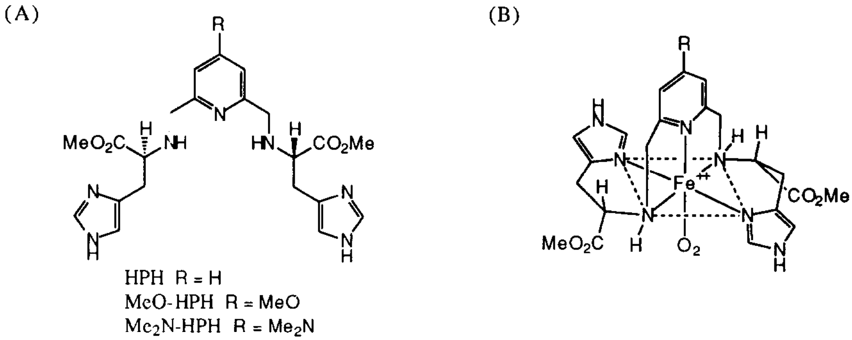
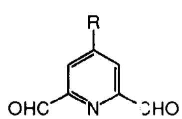
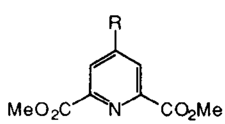


Figure 2. Structure of newly designed ligands HPH, MeO-HPH, and Me_2N-HPH (A) and the anticipated coordination mode (B).

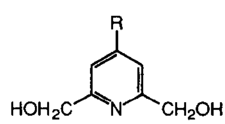
HPH, MeO-HPH, and Me_2N-HPH were synthesized from pyridine-2,6-dicarbaldehyde derivatives (**1**, **2**, and **3**) and histidine methyl ester. While unsubstituted pyridine derivative **1** is commercially available, methoxy derivative **2** and dimethylamino derivative **3** were prepared from previously synthesized dimethyl ester, **4** and **5**,[4,6] respectively. Treatment of diester **4** and **5** with $NaBH_4$ in CH_2Cl_2 - MeOH gave diol **6** and **7** in 73% and 54% yields, respectively. Subsequent oxidation with MnO_2 in CH_2Cl_2 afforded dialdehyde **2** and **3** in 61% and 54% yields, respectively. Schiff base formation of dialdehyde **1**, **2**, and **3** with histidine methyl ester in the presence of molecular sieves 3A followed by hydrogenation (H_2, Pd-C, MeOH) gave HPH, MeO-HPH, and Me_2N-HPH in 38%, 34%, and 49% yields, respectively.[10]



1 R = H
2 R = MeO
3 R = Me_2N



4 R = MeO
5 R = Me_2N



6 R = MeO
7 R = Me_2N

Table 1 shows the ESR parameters for the copper complexes of the synthetic ligands. Although the Cu(II) complex of Me_2N-HPH exhibited somewhat deviated parameters, all of the copper complexes can be regarded to have basically the same coordination geometry. This indicates that all ligands formed copper complex with axial symmetry consistent with the coordination mode shown in Figure 2 (B).

Table 1. ESR parameters for the Cu(II) complexes of synthetic ligands and bleomycin.

Complex	$g_\perp$	$g_{//}$	$A_{//}$
HPH-Cu(II)	2.057	2.234	156.1
MeO-HPH-Cu(II)	2.072	2.240	160.0
Me_2N-HPH-Cu(II)	2.082	2.370	153.3
BLM-Cu(II)	2.055	2.211	168.9

It was gratifying that these synthetic ligands, in fact, exhibited oxygen acivating capability as demonstrated by the ESR spin trapping experiments. In the presence of oxygen, HPH-Fe(II) showed a small, but evident signal for the spin adduct as shown in Figure 3 (A). The introduction of methoxyl group greatly enhanced the oxygen activating capability and dimethylamino group further amplified the signal of the spin adduct (Figure 2 (B) and (C)). The increment of the oxygen activating power was rather great compared with the case of PYML. That is, the oxygen-activating efficiencies of MeO-HPH and Me_2N-HPH are 5 times and 8 times strong, respectively, as that of unsubstituted HPH. This profound influence of the electron donating group presumably resulted from the axial coordination of the pyridine ring, inducing a large energy splitting of the *d*-orbital of the iron. Notably,

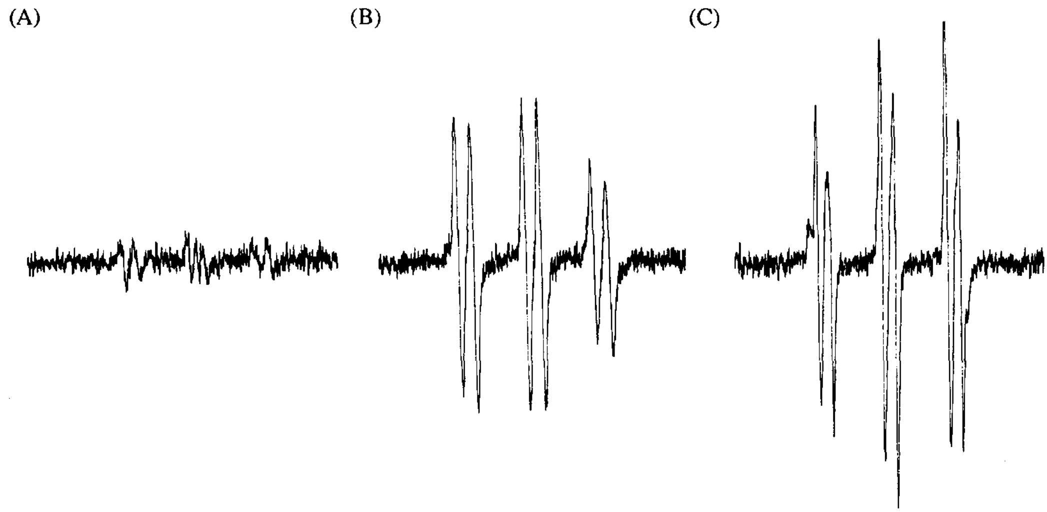


Figure 3. ESR spin trapping of HPH-Fe(II) (A), MeO-HPH-Fe(II) (B), and Me_2N-HPH-Fe(II) (C) complexes in the presence of *N*-tert-butyl-α-phenylnitrone.

Table 2. Correlation between oxygen-activating capability of the synthetic ligand and the Hammet constants of the substituent on the pyridine ring.

Ligand	Substituent on pyridine	σ_p	Oxygen activation
HPH	H	0.00	1
MeO-HPH	MeO	-0.27	5
Me_2N-HPH	Me_2N	-0.83	8

the oxygen-activating power of these ligands is well in accordance with the inductive effect, i. e. σ_p value,[11] of the substituents on the pyridine ring (Table 2). Moreover, the formation of the spin adduct was amplified by the addition of reducing agent such as sodium dithionite to recycle the ferric complex, indicating the oxygen activation by the iron complexes of HPH, MeO-HPH, and Me_2N-HPH to be a catalytic process.

Thus, we are successful to design a new ligands with efficient oxygen-activating power by symmetrizing the structure of BLM metal core. The present results indicates that these can be used as warhead for sequence specific DNA-cleaving molecules by introducing appropriate DNA-affinity site.

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