Structural Features That Control Oxygen Activation at the Non-Heme Iron Site in Fe(II)–Bleomycin: An Analogue Study

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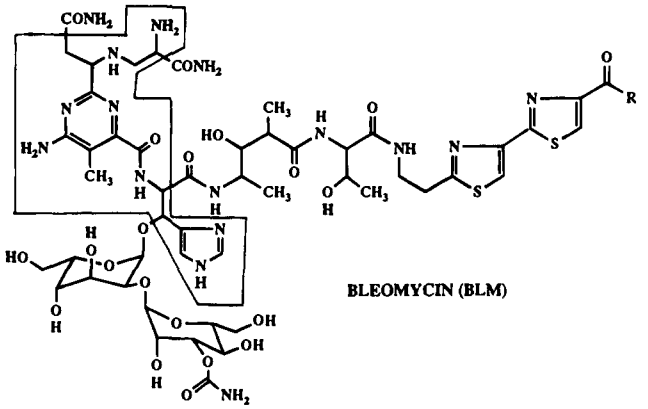
During the past few years, both oxidative damage of DNA and stereoselective oxo transfer to olefinic substrates by the iron chelates of the antitumor antibiotic bleomycin (BLM)[1,2] have drawn special attention since the Fe–BLMs comprise the first examples of mononuclear non-heme iron complexes with significant capacity of O_2 activation.[3] Information regarding



BLEOMYCIN (BLM)

the roles of the different donor groups of the metal binding domain of BLM in the processes of O_2 binding and subsequent reactions at the iron centers in Fe–BLMs is therefore crucial for understanding the phenomenon of O_2 activation at non-heme iron centers. The key intermediate that has been identified spectroscopically in the process of O_2 activation by the Fe–BLMs is the so-called "activated bleomycin". Oxygenation followed by one-electron reduction of the Fe(II) chelate of BLM results in the formation of this low-spin {hydroperoxo}Fe(III) species[4] with a characteristic EPR spectrum (g = 2.26, 2.17, 1.94).[5] In the course of our research with the iron complexes of a designed ligand PMAH that resembles the β-amino-alaninamide–pyrimidine–β-hydroxyhistidine (β-AA–Pm–β-HH) portion of BLM (boxed area), we have recently discovered that reaction of $[Fe^{II}(PMA)]^+$ with dioxygen affords the low-spin intermediate $[(PMA)Fe^{III}–O–OH]^+$, which exhibits an EPR spectrum (Figure 1) identical to that of activated bleomycin and promotes facile DNA cleavage as well as stereoselective oxo transfer to olefinic substrates.[4a] In this Communication, we report the roles of the different donor groups of the β-AA–Pm–β-HH portion of BLM in O_2 activation at the non-heme iron site in Fe(II)–BLM.

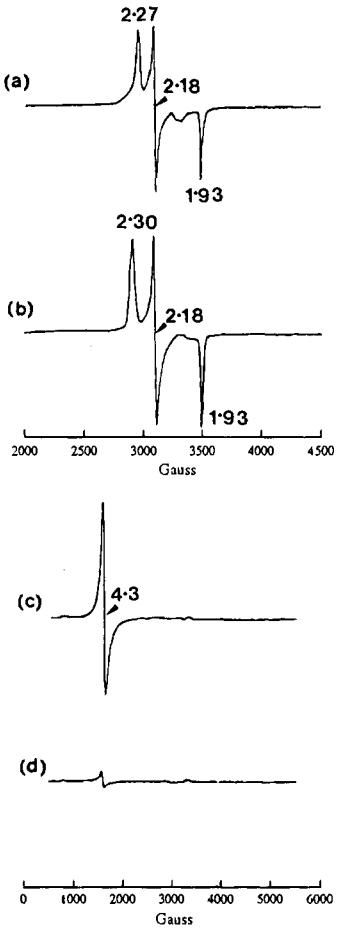
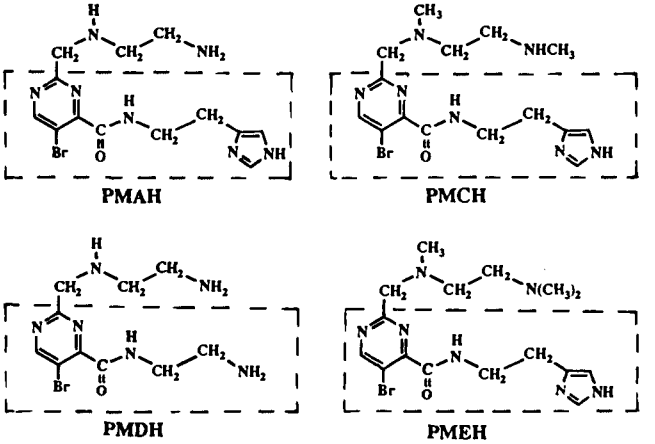


Figure 1. X-band EPR spectra of the oxygenated species derived from (a) $[Fe^{II}(PMA)]^+$, (b) $[Fe^{II}(PMC)]^+$, (c) $[Fe^{II}(PMD)]^+$, and (d) $[Fe^{II}(PME)]^+$ in methanol glass (100 K). The x-axis of the top panel is different from the x-axis of the bottom panel. Selected g values are shown. Spectrometer settings: microwave frequency, 9.43 GHz; microwave power, 13 mW; modulation frequency, 100 kHz; modulation amplitude, 2 G.

The ligand set in the present study consists of PMAH,[4a] PMCH,[6] PMDH,[7] and PMEH[7] (H is the dissociable amide H in all cases. All the donor groups of the metal-binding locus



of BLM have been assembled in these pentadentate ligands in

† Alfred P. Sloan Fellow, 1993–1995.

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(7) ^{13}C NMR spectrum ($(CD_3)_2SO$, 298 K, 300 MHz; δ from TMS): PMDH, 41.09, 42.69, 48.53, 51.17, 54.47, 115.11, 157.72, 161.88, 164.49, 167.69; PMEH, 26.70, 38.97, 41.96, 44.15, 45.63, 52.89, 54.45, 62.16, 114.56, 116.61, 134.26, 134.69, 159.47, 160.26, 163.75, 165.66.

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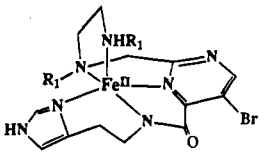
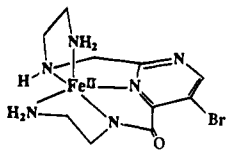
different orders. Ligation of the boxed portion of these ligands (in deprotonated forms) to Fe(II) is characterized by (a) a strong blue band (LMCT, $\epsilon \approx 1800\ M^{-1}\ cm^{-1}$) around 670 nm (Figure S1) and (b) a shift of the ν_{CO} from 1670 (in free ligands) to 1590 cm^{-1} (in complexes).[4a,6,8] Coordination of the amine groups to iron is indicated by the N−H stretching frequencies, the appearance of the EPR spectrum typical of low-spin Fe(III) species,[9] and the red shift of the absorption maximum from ~600 to ~670 nm (Figure S2). These spectral features allowed unambiguous assignment of the structures of the species that we subjected to the O_2 activation test.

In $[Fe^{II}(PMA)]^+$ and $[Fe^{II}(PMC)]^+$, the iron center is coordinated to five nitrogens that belong to the amino groups, pyrimidine and imidazole rings, and the deprotonated amide moiety (structure **a**).[4a,6] Consequently, like Fe(II)-BLM, both



structure **a**

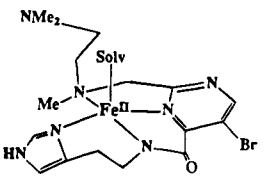


structure **b**

of these complexes (a) bind CO and NO at the sixth site, (b) react with O_2 to give the rhombic activated BLM-like EPR spectrum (g = 2.28, 2.18 and 1.93, signal I hereafter), and (c) induce oxidative DNA damage and stereoselective oxo transfer to olefins. In structure **a**, the trans disposition of the pyrimidine and imidazole groups (aromatic and strong-field N donors) in the basal plane of Fe(II) is essential for effective ligand binding at the sixth site and also for O_2 activation. This is confirmed by $[Fe^{II}(PMD)]^+$. Although the absorption spectrum of this complex is identical to that of $[Fe^{II}(PMA)]^+$ (Fe(II)−N_5 chromophore, structure **b**, Figure S2), it does not bind CO and NO. Furthermore, the reaction between $[Fe^{II}(PMD)]^+$ and O_2 does not afford signal I (Figure 1).[10] Also, $[Fe^{II}(PMD)]^+$ does not exhibit DNA cleavage or oxo transfer capability.

Coordination of the primary NH_2 group of the β-AA portion is also essential for providing a strong crystal field and for formation of the low-spin Fe(III)−hydroperoxo intermediate.[11] Thus, $[Fe^{II}(PME)]^+$, in which the NMe_2 group is not coordinated (structure **c**, λ_{max} 600 nm),[12] does not (a) bind CO, (b) give rise to signal I, or (c) promote DNA cleavage. Also, addition of $Et_3N{\cdot}HCl$ to methanolic solution of $[Fe^{II}(PMA)]^+$ or $[Fe^{II}(PMC)]^+$ causes protonation of the axial amine group (λ_{max} shifts from 670 and 600 nm, Figure S2). Under such a condition, both $[Fe^{II}(PMA)]^+$ and $[Fe^{II}(PMC)]^+$ fail to produce signal I upon oxygenation, and no DNA cleavage is observed.

The spectral and reactivity parameters of the iron complexes of the four designed ligands in this work clearly demonstrate that ligation of the five nitrogens located in the primary and



structure **c**

secondary amines, pyrimidine and imidazole rings, and the deprotonated amide moiety is required for the Fe(II) center to bind O_2 and give rise to the low-spin $[(ligand)Fe(III)-O-OH]^+$ intermediate, the species responsible for oxidative DNA damage and oxo transfer capabilities. The β-AA−Pm−β-HH portion of BLM provides this combination around iron in Fe(II)−BLM.[13] Thus, much like the iron−porphyrin complexes,[18] the Fe(II) centers of these non-heme systems also require a set of strong-field ligands with extended π systems in the basal plane and one axial donor in order to bind and activate O_2. It also appears that the e-accepting capacity of the π system in the basal plane dictates the integrity of the O−O bond. In case of the porphyrin complexes, greater extent of e-transfer from the Fe−O−O unit to the π system of porphyrin results in the scission of the O−O bond and formation of the ferryl (or perferryl) species.[19] In the case of the present analogues (and Fe(II)−BLM), the extent of π-bonding appears to be just enough for the Fe(III)−hydroperoxo intermediate to form, but not enough to cause any O−O bond scission. More experiments, designed to support this hypothesis, are in progress in this laboratory.

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Supplementary Material Available: Absorption spectra of $[Fe^{II}(PMA)]^+$, $[Fe^{II}(PMC)]^+$, and $[Fe^{II}(PMD)]^+$ in methanol (Figure S1); absorption spectra of $[Fe^{II}(PMA)]^+$, $[Fe^{II}(PME)]^+$, and $[Fe^{II}(PMA)]^+$ + 1 equiv of $Et_3N{\cdot}HCl$ in methanol (Figure S2) (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) The product is high-spin $[Fe^{III}(PMD)]^{2+}$ (g = 4.3).

(11) This is supported by the facts that (i) depyruvamide−BLM (BLM without the β-AA portion) affords only high-spin Fe(III) complex and (ii) at low pH (when the NH_2 group is protonated), Fe(III)−BLM is high-spin.[10]

(12) Alkylation of the NH_2 group of PMAH makes axial coordination difficult. With PMCH, structure **a** is obtained in methanol only in presence of excess base. In the case of PMEH, axial ligation is not achieved even under strongly basic conditions.

(13) Loss of the imidazole and/or the primary amine group from this locus therefore inhibits the formation of the low-spin Fe(III)−hydroperoxo intermediate and causes inactivation of BLM, as observed with the modified BLMs.[14-17]

(14) Depyruvamide−BLM forms a high-spin iron complex with no O_2 activation capability. See: (a) Umezawa, H.; Hori, S.; Sawa, T.; Yoshioka, T.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 419. (b) Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 913.

(15) Methylation of the α-amino group of the β-AA moiety modulates BLM activity. Only the Nα monomethyl derivative (one Me group on the primary NH_2 group) shows activities comparable to those of BLM. See: Fukuoka, T.; Muraoka, Y.; Fujii, A.; Naganawa, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 114.

(16) The Fe(II) complex of *N*-acetyl-BLM neither induces oxidative DNA damage and nor binds CO. See: Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Biochemistry* **1980**, *19*, 4096.

(17) Desimidazolyldeglycobleomycin A_2, the agent without the imidazole in the β-HH portion of the metal-chelating locus, exhibits diminished DNA cleavage and no sequence specificity. See: Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 5631.

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