# Lipid Peroxidation by Synthetic Analogues of Iron Bleomycin: Possible Role of a Low-Spin {Hydroperoxo}iron(III) Intermediate in Lipid Peroxidation Induced by Bleomycin

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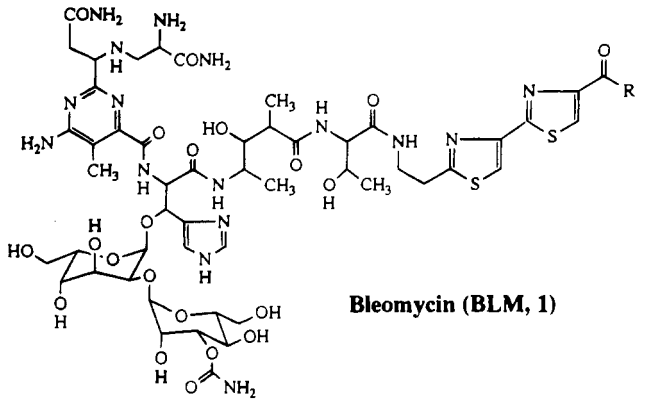
The iron complexes $[Fe(PMA)]^{n+}$ ($n$ = 1, 2) of a designed ligand PMAH (H is a dissociable amide H) that mimics the metal-binding portion of the antitumor drug bleomycin (BLM) promote facile lipid peroxidation in the presence of $O_2$ or $H_2O_2$. These peroxidation reactions are not induced by singlet oxygen or •OH radical. The active intermediate, detected spectroscopically, is a low-spin {hydroperoxo}iron(III) species formulated as $[(PMA)Fe^{III}-O-OH]^+$. This highly oxidizing intermediate causes H atom abstraction from a variety of organic substrates including lipids. With linoleic acid and arachidonic acid as the substrates, the two model complexes mainly afford the 13-OOH and the 15-OOH positional isomers, respectively. The same predominant products, albeit in higher yields, are obtained in enzymatic peroxidation with soybean lipoxygenase. The Fe chelates of BLM also induce lipid peroxidation, a reaction that could be responsible for the lung damage observed during BLM therapy. Similarities in the overall characteristics of the peroxidation reactions suggest that a low-spin {hydroperoxo}iron(III) intermediate could be involved in lipid peroxidations by the Fe-BLMs.

## Introduction

The bleomycin (BLM, **1**) family of glycopeptide antibiotics is in clinical use against several types of cancer.[1] The drug



**Bleomycin (BLM, 1)**

binds $Fe^{2+}$, as well as a number of other transition metal ions, to produce metallobleomycins (M-BLMs) which inflict damage to DNA in the presence of oxygen[2−5] or under UV illumination.[6] Selective uptake of BLM by cancer cells, followed by Fe-BLM-mediated oxidative cleavage of cellular DNA, is believed to be responsible for the antineoplastic action of the drug. One-electron reduction of oxygenated Fe-BLM results in the formation of "activated bleomycin", a low-spin iron−oxo species with a characteristic EPR spectrum[7] which catalyzes scission of the DNA backbone via breaks in the deoxyribose units and free base release.[4] Activated bleomycin is likely to be responsible for both the therapeutic efficacy of BLM and its major and serious side effect, pulmonary fibrosis.[8−10] The lung injury observed following bleomycin therapy is characterized by interstitial oedema, accumulation of inflammatory cells, and intraalveolar haemorrhage. These changes eventually lead to pulmonary fibrosis, the result of enhanced production and deposition of matrix components in the lung interstitium. Current therapy for BLM-induced lung damage has been inadequate. Corticosoids are most often used. In line with the proposed mechanism of nature and action of activated bleomycin,[4] antioxidants and iron chelators are also found to be beneficial.

The mechanism of BLM-induced fibrosis, the syndrome that limits the clinical use of the drug, probably involves the action of highly active oxidants on the components of the cell membranes. Since activated bleomycin is highly oxidizing in nature[7,11] and promotes facile oxo transfer to olefinic substrates,[12] the interaction between lipids and Fe-BLMs could be relevant to BLM-mediated lung damage. Indeed, several *in vitro* studies[13−17] have indicated that the iron chelates of BLM and peplomycin (another member of the BLM family) catalyze lipid peroxidation, with product distributions much like that produced by 15-lipoxygenase.[18,19] Yet, at this time, factors like (a) complications in cell biology during inflammatory stages,[9] (b)

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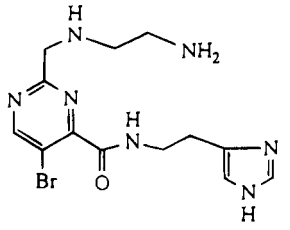
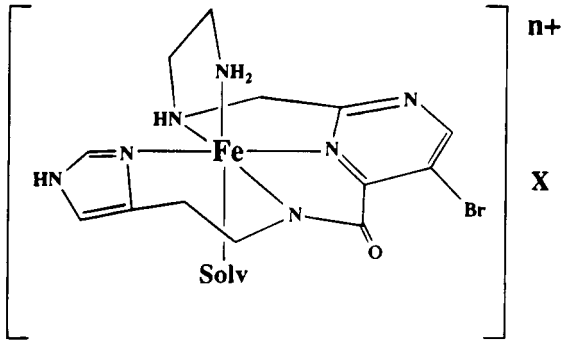
complex mechanisms for the autoxidation of polyunsaturated lipids,[20,21] (c) uncertainties related to the role of iron in radical-mediated lipid peroxidation,[22,23] and (d) the controversy regarding the exact formulation of activated bleomycin do not allow one to directly associate the process of lipid peroxidation to BLM-mediated pulmonary fibrosis.

In our research toward elucidation of the coordination structures and functions of the M-BLMs, we have synthesized a ligand, PMAH (**2**; H is the dissociable amide H), that mimics



**PMAH, 2**

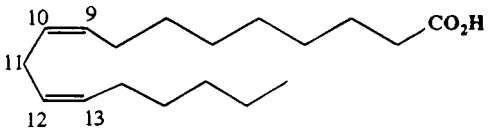
the metal-chelating domain of BLM. The structures of the Cu(II)[24,25] and the Co(III) complexes[26-28] of this tailored ligand have established the coordination structures of the corresponding M-BLMs. Also, the results of the spectroscopic and kinetic studies, as well as DNA cleavage reactions[25-28,29] by these complexes, have provided insight into the mechanisms of the oxidative and photolytic DNA damage by M-BLMs. Very recently, spectral parameters of the Fe(II) and Fe(III) complexes of PMAH, namely $[Fe^{II}(PMA)]Cl\cdot MeOH$ (**3**) and $[Fe^{III}(PMA)](NO_3)_2\cdot DMSO$ (**4**) allowed us to predict the coordination



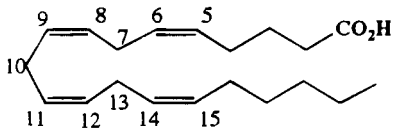
$[Fe^{II}(PMA)]^+$, X = Cl (**3**)
$[Fe^{III}(PMA)]^{2+}$, X = $(NO_3)_2$ (**4**)

structures of the Fe-BLMs.[30] In addition, these two complexes afford an oxygenated species which exhibits an EPR spectrum identical to that of activated bleomycin. Since the results of physicochemical studies confirm the formulation $[(PMA)Fe^{III}-O-OH]^+$ (low-spin) for the oxygenated species, we have suggested that activated bleomycin is a low-spin {hydroperoxo}iron(III) complex of BLM with five N donors (from the pyrimidine ring, $\beta$-hydroxyhistidine, and the $\beta$-aminoalaninamide moiety) ligated to the metal center.[30,31] Complexes **3** and **4** mimic all of the spectroscopic and chemical properties of the Fe-BLMs, including sequence-specific DNA cleavage (with concomitant production of base propenals) and stereospecific oxo transfer to olefinic substrates. Consequently, the low-spin {hydroperoxo}iron(III) complex $[(PMA)Fe^{III}-O-OH]^+$ has been implicated to be the active intermediate in both the DNA strand scission and the oxo transfer reactions.[30]

The fact that **3** and **4** can activate $O_2$ prompted us to try lipid peroxidation by these complexes. In this paper, we report that (a) $[Fe^{II}(PMA)]Cl\cdot MeOH$ (**3**) catalyzes peroxidation of lipids in presence of dioxygen much like $Fe^{II}$-BLM and (b) peroxidation of linoleic acid and arachidonic acid by **3** affords mainly



**Linoleic Acid**



**Arachidonic Acid**

the 13-OOH and the 15-OOH positional isomer, respectively. The two model complexes, $[Fe^{II}(PMA)]Cl$ (**3**) and $[Fe^{III}(PMA)](NO_3)_2\cdot DMSO$ (**4**), also promote peroxidation of linoleic acid and arachidonic acid by $H_2O_2$. In these lipid peroxidation reactions, both **3** and **4** afford the same predominant products as one obtains in enzymatic peroxidations with soybean lipoxygenase. Our results indicate that a low-spin {hydroperoxo}iron(III) intermediate could be involved in the process of lipid peroxidation by the Fe-BLMs.

## Experimental Section

**Materials.** $[Fe^{II}(PMA)]Cl\cdot MeOH$ (**3**) and $[Fe^{III}(PMA)](NO_3)_2\cdot DMSO$ (**4**) were synthesized by published procedures.[30] Linoleic acid, sodium linoleate, arachidonic acid, sodium arachidonate, and Tween 20 were purchased from Sigma. Lipoxidase, type 1-B from soybean, was purchased from Aldrich. The spin-trapping agent 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) was obtained from Aldrich and purified as described in the literature.[32] All solvents used were purified by standard procedures.

**Instrumentation.** Electronic spectra were recorded on a Perkin-Elmer Lamba 9 spectrophotometer. The EPR spectra of the radical adducts were obtained at 20 °C while the EPR spectrum of $[(PMA)-Fe^{III}-O-OH]^+$ was monitored at −173 °C. A Bruker ESP-300 spectrometer was used to record these spectra at X-band frequencies. The lipid hydroperoxides were detected by HPLC at 234 nm. The

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HPLC system consisted of Spectra Physics SP 8800 ternary HPLC pump, a 250 mm Econosphere 5 $\mu$m $C_{18}$ reverse phase column, Spectra 200 wavelength detector, and Chromjet integrator.

**Determination of the Extent of Lipid Peroxidation by Thiobarbituric Acid (TBA) Assay.** Malondialdehyde (MDA) formed by acidic treatment of peroxidized linoleic or arachidonic acid[20] was measured by colorimetry, after reaction with thiobarbituric acid.[33] A typical reaction mixture for lipid peroxidation contained 0.7 mM $[Fe^{II}(PMA)]^+$ complex and 30 mM lipid in 0.4 mL of cacodylate buffer (pH 7.8). Reactions were run with or without radical scavengers (ascorbic acid, vitamin E, etc.) or singlet oxygen scavengers (e.g. guanosine). The mixture was incubated at 37 °C for 25 min. To terminate the reaction, 50% trichloroacetic acid (0.02 mL) was added at 0 °C. Next, 0.2 mL of 1% TBA was added and the reaction mixture was stirred at 37 °C for 30 min to develop the color. Finally, 1 mL of butanol was added, and the mixture was vigorously shaken and centrifuged. The absorbance of the butanol layer at 532 nm was measured.

**Detection of Conjugated Dienes by Ultraviolet Spectroscopy.** A batch of 140 mg (0.5 mmol) of linoleic acid was introduced into a Schlenk tube (10 mL) at 0 °C. Cacodylate buffer (5mL, 10 mM, pH 7.8) was then added along with two drops of Tween 20, and the mixture was gently stirred. The reaction was initiated by the addition of 4 mg (0.008 mmol) of **3** and purging of the mixture with pure dioxygen. The temperature was kept between 0 and 4 °C during the whole reaction. Progress in the formation of the conjugated dienes[20] was followed by spectrophotometry.[33] Aliquots of the reaction mixture were taken out and diluted with ethanol (20 $\mu$L/5 mL), and absorbances were measured at 234 nm.

**Analyses of Specific Lipid Hydroperoxides by HPLC: Peroxidation under Dioxygen.**[34] To a solution of 0.013 mmol of the desired lipid (4 mg of sodium linoleate or 4.24 mg of sodium arachidonate) in 0.4 mL of cacodylate buffer (10 mM, pH 7.8) was added a solution of 1 mg (0.002 mmol) of **3** in 0.1 mL of the same buffer. When linoleic acid or arachidonic acid was used as the substrate, two drops of Tween 20 were added to the reaction mixture at this point. The reaction mixture was then stirred for 30 min under a pure dioxygen atmosphere. An aliquot was taken out and filtered through a Gelman Nylon Acrodisc filter (0.45 $\mu$m, Fisher Scientific), and the filtrate was injected into the HPLC column. Unlike some previous studies, the sample was not subjected to hydroxylation and/or methylation (which may cause isomerization of the unstable hydroperoxides) prior to HPLC analysis. The mobile phase for the detection of (9Z,11*E*)-13-(hydroperoxy)-9,-11-octadecadienoic acid (13-HPODE) was methanol/water/acetic acid (76:24:0.1, v/v/v) containing 30 mM lithium acetate. (5Z,8Z,11Z,13*E*)-15-(hydroperoxy)eicosatetraenoic acid (15-HPETE) was detected with methanol/water/triethylamine/acetic acid (80:20:0.05:0.1, v/v/v/v) as the mobile phase.[35] The flow rates for the two systems were 1.5 and 1.0 mL/min, respectively. The various reaction products were identified by the elution times of authentic standard compounds and by standard additions.

**Analyses of Specific Lipid Hydroperoxides by HPLC: Peroxidation with $H_2O_2$.** To a solution of 0.026 mmol of the desired lipid (8 mg of sodium linoleate or 8.5 mg of sodium arachidonate) in 1 mL of cacodylate buffer (10 mM, pH 7.8) was added 1 mg (0.0016 mmol) of **4** followed by 100 $\mu$L of 30% aqueous $H_2O_2$. The reaction mixture was stirred for 25 min and subjected to HPLC analysis, as described above.

Control reactions with the substrates and simple Fe salts, but no PMAH, were also performed. The lipid samples were analyzed prior to peroxidation reactions for initial contaminations of lipid hydroperoxides. Control reactions of the lipids with simple Fe(II) salts and $H_2O_2$ afforded very little lipid hydroperoxides (~2%) under these experimental conditions. Conditions for the enzymatic reactions can be found in ref 33.

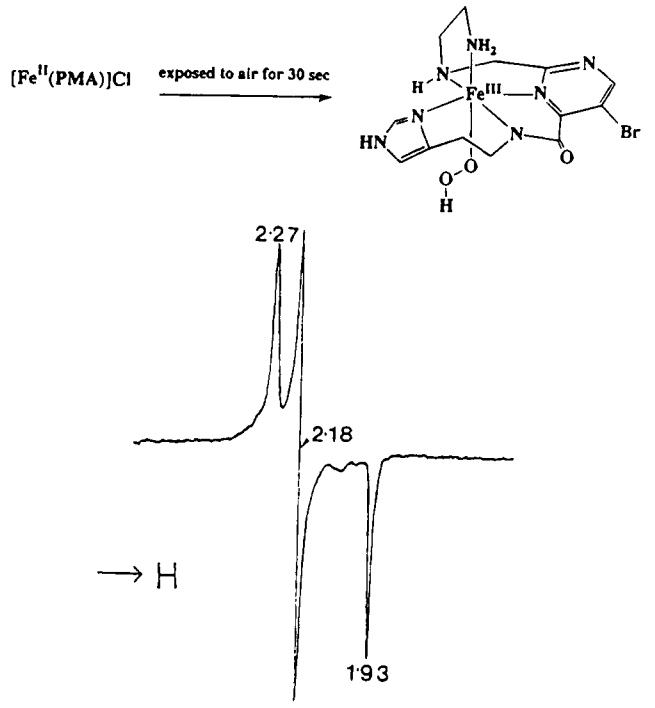


**Figure 1.** X-band EPR spectrum of $[(PMA)Fe^{III}-O-OH]^+$ in methanol glass (−173 °C). This EPR spectrum is identical to that of the "activated bleomycin". Spectrometer settings: microwave frequency, 9.43 GHz; microwave power, 13 mW; modulation frequency, 100 kHz; modulation amplitude, 2G.

In the present work, the yields of the various lipid hydroperoxides were determined within 30 min to avoid further decomposition of the lipid hydroperoxides into hydrocarbons and other carbonyl products.

**Spin-Trapping Experiments: Detection of the DMPO−Radical Adducts.**[13] The sample for the detection of the DMPO−radical adduct-(s) contained 36 mM sodium linoleate, 1 mM **3**, and 0.3 M DMPO in 0.55 mL of oxygen-saturated 0.1 M Tris-HCl buffer (pH 7.5). After the ingredients were mixed together, the reaction mixture was incubated in air. Aliquots were taken out at 1 min intervals and immediately transferred into capillary tubes and their EPR spectra recorded at 20 °C. Instrumental parameters: microwave frequency, 9.442 GHz; scan range, 200 G; modulation amplitude, 1 G; scan time, 2 min; time constant, 1.0 s; microwave power, 10 mW; receiver gain, $10^5$.

**Effect of Lipid Substrate on the EPR Signal of the Low-Spin {Hydroperoxo}iron(III) intermediate.** A solution of 6.5 mg (0.013 mmol) of **3** and 4 mg (0.013 mmol) of sodium linoleate in 0.3 mL of methanol was exposed to dioxygen for 20 s. The sample was frozen immediately, and its EPR spectrum was recorded. Next, the sample was thawed and 0.5 equiv of sodium linoleate was added. Following a short period of shaking, the mixture was frozen again and its EPR spectrum was monitored. This process was repeated three times. The characteristic EPR spectrum of the $[(PMA)Fe^{III}-O-OH]^+$ intermediate disappeared by the end of the third addition.

## Results and Discussion

In methanolic solution, $[Fe^{II}(PMA)]Cl\cdot MeOH$ (**3**) readily reacts with $O_2$ to yield the low-spin {hydroperoxo}iron(III) species $[(PMA)Fe^{III}-O-OH]^+$, which is readily identified by its characteristic EPR spectrum (Figure 1). The same species is obtained from the reaction of $[Fe^{III}(PMA)](NO_3)_2\cdot DMSO$ (**4**) with $H_2O_2$. As mentioned earlier, the EPR spectrum of $[(PMA)-Fe^{III}-O-OH]^+$ is identical to that of the "activated bleomycin". $[(PMA)Fe^{III}-O-OH]^+$ is stable for several hours at liquid $N_2$ temperature (at least for 3 h at 0 °C) but eventually decomposes into a brown precipitate. The strongly oxidizing nature of this low-spin {hydroperoxo}iron(III) intermediate is evidenced by (a) formation of $^{\bullet}CH_2OH$ radical (detected by spin trapping,

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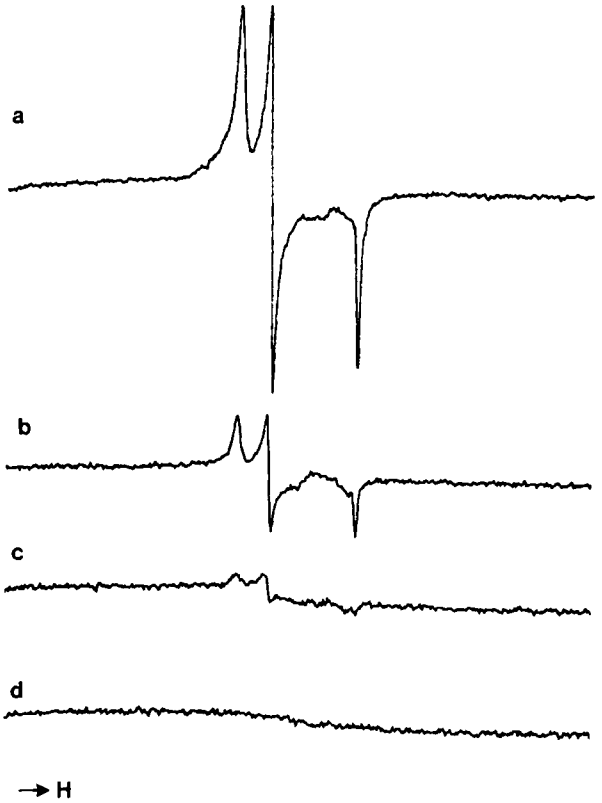


**Figure 2.** X-band EPR spectra (−173 °C) of the active intermediate $[(PMA)Fe^{III}-O-OH]^+$ formed in (a) the reaction mixture of **3** and sodium linoleate (1:1) in methanol (reactants mixed, exposed briefly to air, and frozen), (b) the same reaction mixture with an additional 0.5 equiv of sodium linoleate (mixture thawed, substrate added, stirred for 10 s, and frozen), (c) the same reaction mixture with one more addition of 0.5 equiv of sodium linoleate (mixture thawed, substrate added, and frozen after 10 s of stirring), (d) the same reaction mixture with the third addition of 0.5 equiv of sodium linoleate (substrate added after thawing, stirred for 10 s, and frozen). Spectrometer settings: same as for Figure 1.

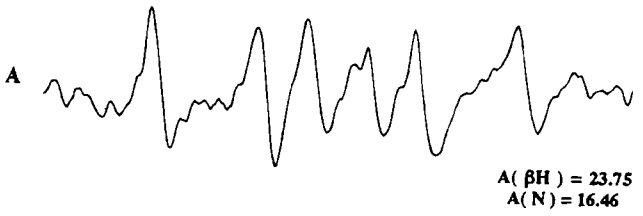


**Figure 3.** X-band ESR spectrum (20 °C) of the DMPO+C-based radical spin-adduct formed under aerobic conditions in the reaction mixture of **3**, sodium linoleate, and excess DMPO. Selected coupling constants are indicated. For spectrometer settings, see text.

$A^H = 22.7$ G, $A^N = 16$ G[36]), (b) production of formaldehyde (detected by Nash reagent[37]), and (c) appearance of the EPR signal of the peroxy radical $^{\bullet}OOCH_2OH$ ($g = 2.22$ and 2.05) within minutes. Reaction of $O_2$ with $^{\bullet}CH_2OH$ (derived from $CH_3OH$ via H atom abstraction by the oxidizing intermediate) gives rise to this peroxy radical in the reaction mixture. Further evidence of the oxidizing power of $[(PMA)Fe^{III}-O-OH]^+$ comes from its capacity for DNA cleavage with the formation of base propenals.[30] Since the oxidative damage of DNA is initiated by H atom abstraction from the sugar moieties, the DNA strand scission by $[(PMA)Fe^{III}-O-OH]^+$ demonstrates the ability of the low-spin {hydroperoxo}iron(III) species to

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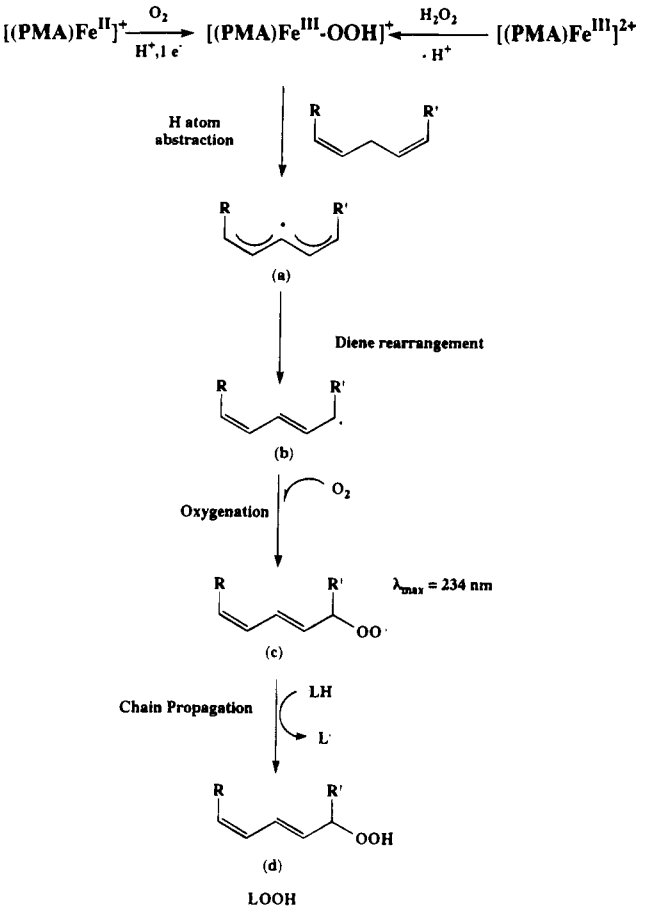
**Scheme 1**




abstract H atoms from organic substrates.[38,39] Finally, the fact that both **3** and **4** promote rapid oxo transfer to olefinic substrates in the presence of $O_2$, $H_2O_2$, and PhIO[30] also indicates that H atom abstraction from olefinic substrates can be readily initiated by $[(PMA)Fe^{III}-O-OH]^+$.

Peroxidation of the methylene-interrupted diene lipids, such as linoleic acid or arachidonic acid, is initiated by facile H atom loss from the methylene moiety flanked by the double bonds with concomitant production of stable pentadienyl radicals.[19−21] Since $[(PMA)Fe^{III}-O-OH]^+$ exhibits an unusually high capacity for H atom abstraction from various organic substrates, the $[Fe(PMA)]^{n+}$ complexes could promote rapid peroxidation of such lipids, in the presence of $O_2$ and $H_2O_2$. We therefore decided to study the course of the peroxidation of linoleic acid and arachidonic acid with the $[Fe(PMA)]^{n+}$ complexes. With $O_2$ and $H_2O_2$ as the source of oxygen, *only* $[(PMA)Fe^{III}-O-OH]^+$ is detected as the intermediate in these systems. For

(38) Alternatively, a hypervalent iron−oxo ($Fe^{IV}=O$, ferryl, or $Fe^{V}=O$, perferryl) species, formed from the $Fe^{III}-O-OH$ intermediate via O−O bond scission, could be responsible for H atom abstraction. However, we have not been able to obtain any direct evidence to confirm the presence of such an intermediate in this study (or any study) so far. Though the existence of such a high-valent iron−oxo intermediate has been demonstrated spectroscopically in the case of some heme systems, there is absolutely no proof of such active species in non-heme iron chemistry. In absence of any stabilization from a porphyrin ligand, attainment of an oxidation state higher than 3 by iron in a medium containing water is also questionable by the first principles of chemistry.

(39) Along the same line, in a recent paper (Leising, R. A.; Kim, J.; Perez, M. A.; Que, L., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 9524), Que and co-workers have pointed out that "there is as yet no direct spectroscopic evidence for the participation of a high-valent iron−oxo species in the cytochrome P450 reaction, though such a species enjoys wide acceptance as a plausible intermediate"!

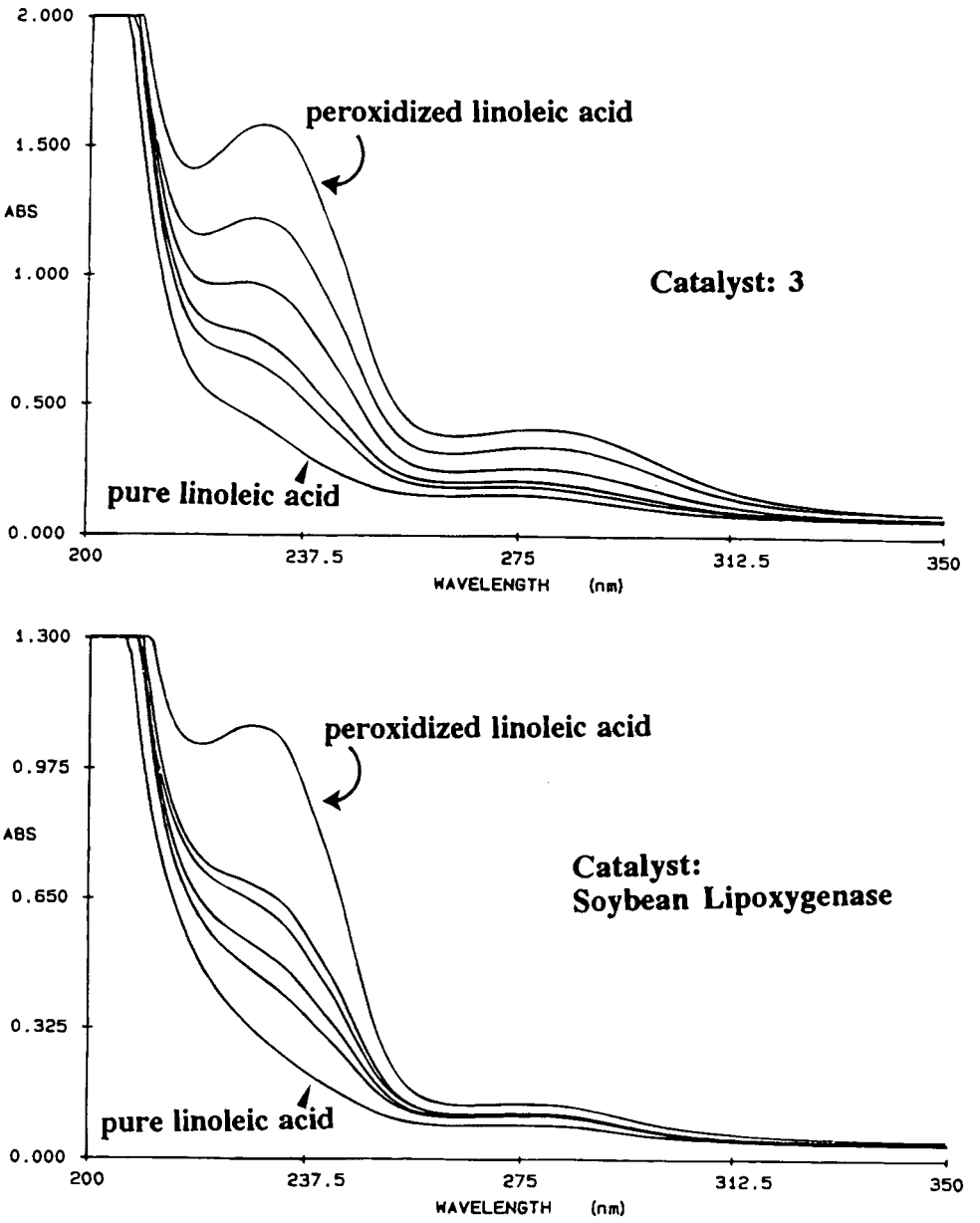



**Figure 4.** Progressive increase in the electronic absorption band at 234 nm recorded at 10 min intervals for oxygenated mixtures of **3** and linoleic acid (top panel) and soybean lipoxygenase and linoleic acid (bottom panel). The enzymatic reaction was run at much lower concentration (approximately 1:4) to keep the absorbance values within limits of the spectrophotometer.

example, when a mixture of **3** and sodium linoleate in methanol (or cacodylate buffer, 10 mM, pH 7.8) is briefly exposed to $O_2$ and frozen immediately, the EPR signal of $[(PMA)Fe^{III}-O-OH]^+$ is detected (top trace, Figure 2). The intensity of this signal rapidly diminishes as the reaction proceeds (Figure 2).

Studies by various groups have confirmed that the first step in a lipid peroxidation reaction is the loss of an H atom by the bis(allylic) methylene group to generate a C-based radical. Though such radicals are unstable, at least in two reactions, namely between (a) sodium linoleate and Fe(III)-BLM and (b) sodium linoleate and soybean lipoxygenase, a C-based radical has been detected by spin trapping (with DMPO as the spin trap, $A^{\beta H} = 23.8$ G, $A^N = 16.5$ G).[13] In the present study, the same C-based radical has been observed with the use of DMPO as the spin trap. As shown in Figure 3, incubation of a mixture of **3**, sodium linoleate, and excess DMPO under aerobic conditions readily affords the six-line ESR spectrum of the spin-adduct ($A^{\beta H} = 23.8$, $A^N = 16.5$). It is therefore evident that the $[(PMA)Fe^{III}-O-OH]^+$ intermediate readily abstracts one H atom from the methylene group situated between the two double bonds in the lipid to generate the cyclopentadienyl C-based radical **a** (Scheme 1).

In the next step toward the formation of lipid hydroperoxide by the $[Fe(PMA)]^{n+}$ complexes, **a** rearranges to a conjugated diene system **b** (Scheme 1). The rearrangement is conveniently monitored by the appearance of a strong absorption centered around 234 nm.[20,33] In Figure 4, the increase in the absorption at 234 nm recorded for a mixture of **3** and sodium linoleate under $O_2$ is shown in the top panel. The same kind of change, albeit to a much higher extent, is observed when soybean lipoxygenase is used instead of **3** (Figure 4, bottom panel). Reaction between **b** and $O_2$ results in the formation of the peroxy radical **c** in the reaction mixture (Scheme 1). The peroxy radical **c** takes part in the chain propagation reaction **c** → **d** to produce more lipid hydroperoxide.

Peroxy radicals such as **c** are known to form cyclic peroxides which, upon hydrolysis at elevated temperatures, afford malondialdehyde (MDA). In the present study, rapid formation of MDA in the oxygenated mixtures of lipids and **3** (detected by the thiobarbituric acid assay) confirms that a considerable

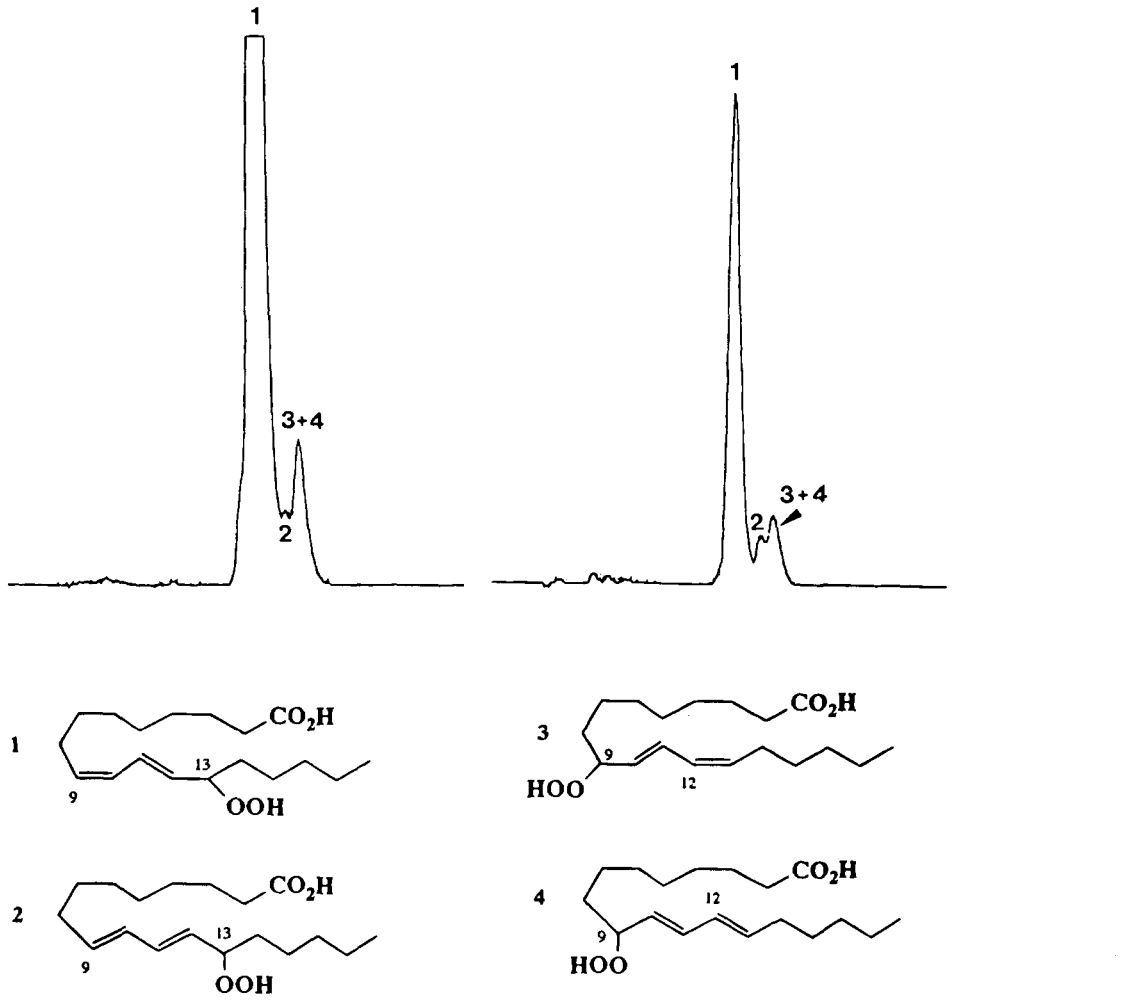



**Figure 5.** HPLC traces showing product distribution in the reaction of linoleic acid with soybean lipoxygenase (left panel) or **3** (right panel). The structures of the four products are also shown.

amount of the peroxy radical **c** is generated in the reaction mixtures. For example, a strong pink color of the MDA-TBA adduct is observed with a reaction mixture of arachidonic acid, **3**, and $O_2$ following an incubation period of 10 min. Use of simple iron salts in place of **3** in the same reaction mixture results in very little color.

Addition of oxygen at C9 and C13 of the pentadienyl radical of linoleate is expected to produce four products (for 9-HPODE 10*E*,12*Z* and 10*E*,12*E*; for 13-HPODE 9*Z*,11*E* and 9*E*,11*E*). Since arachidonic acid leads to three different stabilized pentadienyl radicals, twelve lipid hydroperoxides are possible from the addition of oxygen at C5, C8, C9, C11, C12, and C15 (with *EZ* and *EE* configurations for each hydroperoxide). However, it is known that oxidation of arachidonic acid produces the *EZ* conjugated diene hydroperoxides as the major products and only traces of the *EE* products are detected.[20] When peroxidation is achieved with lipoxygenases, the reactions are regio- and stereoselective and the preferred site of dioxygenation varies depending on the source of the enzyme. For example, with linoleic acid as the substrate, soybean lipoxygenase gives rise almost exclusively to 9*Z*,11*E* 13-HPODE (Figure 5, left panel) while with arachidonic acid 5*Z*,8*Z*,11*Z*,13*E* 15-HPETE is the predominant product. Interestingly, peroxidations of linoleic acid and arachidonic acid with the PMA complexes (**3** and **4**) also produce 9*Z*,11*E* 13-HPODE (Figure 5, right panel) and 5*Z*,8*Z*,11*Z*,13*E* 15-HPETE, respectively, as the prevalent (~90%) products. Although simple iron and copper salts, as well as heme proteins, promote regioselective peroxidation of linoleic acid,[40] the lipoxygenase-like selectivity of **3** and **4** is especially noticeable because of the much higher yields, faster reaction times, and no dependence on detergent.[41] For example, under the same reaction conditions, simple iron salts produce negligible amounts of 9*Z*,11*E* 13-HPODE and 5*Z*,8*Z*,11*Z*,13*E* 15-HPETE from linoleic acid and arachidonic acid, respectively, within a period of 30 min, while **3** or **4** affords the same products in 15–30% yields (based on consumed substrates). The yields of the major products of the peroxidation reactions are listed in Table 1. When calculated on the basis of the concentrations of the iron complexes employed, the yields of the lipid hydroperoxides indicate that these peroxidation reactions are catalytic. For example, **3** produces 200% of 9*Z*,11*E* 13-HPODE (as detected by HPLC) within 30 min. Since lipid hydroperoxides rapidly decompose to MDA and other products in the presence of metal salts/complexes,[42] the actual yields of the lipid hydroperoxides in these reactions are much higher than 200%.

At least two groups have reported that Fe(III)-BLM catalyzes the decomposition of 13-HPODE and 15-HPETE to produce

(40) (a) Chan, H. W. S.; Newby, V. K. *Biochim. Biophys. Acta* **1980**, *617*, 353–362. (b) Chan, H. W. S.; Newby, V. K.; Levett, G. *J. Chem. Soc., Chem. Commun.* **1978**, 82–83.

(41) The positional selectivity, observed in peroxidation of linoleic acid by hemoproteins and transition metal ions in aqueous solutions, is abolished by the addition of Tween to the reaction mixtures.[40] This result has been explained in terms of specific conformation(s) of the lipid as the determinant of positional selectivity. Quite in contrast, in the present study, the positional selectivity (Table1) is retained in all reactions, with or without Tween.

(42) Halliwell, B.; Gutteridge, J. M. C. In *Free Radicals in Biology and Medicine*; Oxford University Press: New York, 1991; p 214.

**Table 1.** Yields of the Major Products Derived from the Peroxidation Reactions[a]

Substrate: Linoleic Acid

| oxidant | catalyst | % substrate consumed | % major product[b] **13-HPODE** | ref |
|---|---|---|---|---|
| $O_2$ | **[$Fe^{II}$(PMA)]Cl (3)** | **35** | **80** | this work |
| | $Fe^{III}$-peplomycin | 36 | 48 | 14 |
| | $Fe^{II}$-bleomycin | *c* | *c* | 13 |
| | $Fe^{III}$-bleomycin | *c* | *c* | 13 |
| $H_2O_2$ | **[$Fe^{III}$(PMA)]$(NO_3)_2$ (4)** | **36** | **75** | this work |

Substrate: Arachidonic Acid

| oxidant | catalyst | % substrate consumed | % major product[b] **15-HPETE** | ref |
|---|---|---|---|---|
| $O_2$ | **[$Fe^{II}$(PMA)]Cl (3)** | **20** | **80** | this work |
| | $Fe^{II}$-bleomycin | *d* | *d* | 16 |
| | [$Fe^{III}$-bleomycin | *d* | *d* | 16 |
| $H_2O_2$ | **[$Fe^{III}$(PMA)]$(NO_3)_2$ (4)** | **25** | **80** | this work |

[a] For reaction conditions, see text. [b] Percentage based on total substrate consumed. [c] 13-HPODE is formed but yield is not reported. [d] Only formation of MDA is reported.

small quantities of singlet oxygen[13,43] which, in turn, is capable of initiating further peroxidation of lipids.[42] Oxygen-based free radicals such as •OH can also stimulate lipid peroxidation by abstracting a H atom from a bis(allylic) methylene group. Since the extents of lipid peroxidations by **3** or **4** are not altered in the presence of $^1O_2(\Delta g)$-scavengers such as guanosine and •OH-scavengers such as D-mannitol, the present peroxidation reactions are not induced by $^1O_2(\Delta g)$ or •OH. Antioxidants such as ascorbic acid and cysteine (lipid hydroperoxide scavenger), however, slow the rate of lipid peroxidation by **3** or **4**. The peroxidation by **3** or **4** practically stops with the inclusion of α-tocopherol (vitamin E) in the reaction mixtures. This is expected, since vitamin E converts the chain-propagating lipid peroxy radicals (**c** in Scheme 1) into the terminating lipid hydroperoxides (**d** in Scheme 1). Vitamin E also reacts readily with $[(PMA)Fe^{III}-O-OH]^+$; the typical EPR spectrum of $[(PMA)Fe^{III}-O-OH]^+$ is rapidly replaced by the ESR spectrum of the α-tocopheryl radical when vitamin E is added to a sample of $[(PMA)Fe^{III}-O-OH]^+$. These results support Scheme 1 and indicate that $[(PMA)Fe^{III}-O-OH]^+$ could be the active intermediate in the lipid peroxidations by **3** and **4**. As mentioned earlier, $[(PMA)Fe^{III}-O-OH]^+$ is the only intermediate we have been able to detect in the peroxidation reaction mixtures.

## Summary and Conclusions

The two synthetic analogues **3** and **4**, which have been shown to be good models for the metalated cores of Fe-bleomycins, promote facile lipid peroxidations in the presence of $O_2$ and $H_2O_2$, respectively. These reactions are not induced by singlet oxygen or the •OH radical. Spectroscopic studies demonstrate the presence of the low-spin {hydroperoxo}iron(III) species $[(PMA)Fe^{III}-O-OH]^+$ as the active intermediate in the reaction mixtures. The product distributions observed in the lipid peroxidations by **3** and **4** are very similar to those noted with soybean lipoxygenase although the overall yields are higher in the case of enzymatic peroxidation. Lipid peroxidation by Fe-BLMs has been implicated in pulmonary fibrosis, the major side effect of BLM therapy. It is possible that a similar low-spin {hydroperoxo}iron(III) species is the active intermediate in lipid peroxidations by soybean lipoxygenase as well as the Fe-BLMs.

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(43) Kanofsky, J. R. *J. Biol. Chem.* **1986**, *261*, 13546–13550.