Lipoxygenase-like Function of Some Binuclear Iron(III) Complexes

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Some binuclear iron(III) complexes with μ-phenoxo and μ-alkoxo bridges have been found to show high ability for the peroxidation of linolenic and linolic acid under aerobic conditions. The possibility of dioxygen activation in a substrate–metal complex–O_2 intermediate is discussed.

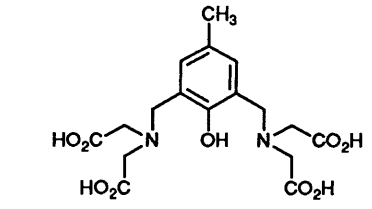
Non-haem iron centres have been identified as the catalytically active sites in a large number of enzymes whose mechanism may involve interactions of dioxygen and a substrate. These iron proteins may be characterized by the number of interacting iron centres in the active sites.[1] Mononuclear iron sites containing a single metal ion co-ordinated to the protein have been observed in the enzymes iron superoxide dismutase,[2] soy bean lipoxygenase,[3] phenylalanine hydroxylase,[4] catechol dioxygenases,[5] *etc.* A second class of non-haem sites contains a bridged binuclear iron complex and is found for example in the oxygen-carrier protein haemerythrin, where dioxygen binds reversibly to the binuclear iron(II) unit.[6]

Lipoxygenases are atypical mononuclear non-haem iron dioxygenases which catalyse peroxidation by dioxygen of fatty acids containing a 1,4-diene unit.[3] In contrast to the more familiar intradiol catechol dioxygenases,[5] soy bean lipoxygenase contains an iron with a relatively high reduction potential[7] and no phenolate ligands, and it is known that the iron is in a rhombically distorted, roughly octahedral, six-co-ordinate structure,[8] probably with four imidazole ligands.[9] Although the fatty acid binds very close to the iron,[10] there is no evidence for co-ordination of either the fatty acid or dioxygen.[11] The available data seem to be consistent with a mechanism involving oxidation and deprotonation of the diene, yielding a radical species that may react directly with dioxygen.[12] This oxidation reaction would not require co-ordination of the substrate to the iron; however, the observed regio- and stereospecificity of the reaction may imply the existence of an iron–alkyl intermediate.[13] However, direct evidence supporting the above mechanism is scarce.

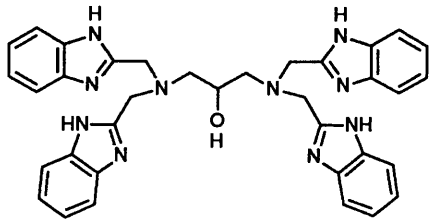
In this study we have observed that some binuclear iron(III) compounds with μ-phenoxo and μ-alkoxo bridges exhibit a high ability for the formation of 2-thiobarbituric acid (2-thioxopyrimidine-4,6-dione)-active compounds in the reaction with unsaturated fatty acids such as linolic (octadeca-9,12-dienoic) and linolenic (octadeca-9,12,15-trienoic) acids. This implies that the iron(III) compounds show lipoxygenase-like function, and thus we have attempted to elucidate the key step for the peroxidation of unsaturated fatty acids. A part of this work has previously been reported.[14]

Experimental

Materials.—The binuclear iron(III) complexes used are $[Fe_2L^1(CH_3CO_2)_2]^-$ and $[Fe_2L^2(NO_3)_5]$, the chemical structures of the ligands being illustrated below.[14] Mononuclear complexes, Na[Fe(edta)][15] and [Fe(acen)Cl],[16] were obtained according to the published methods, where H_4edta and H_2acen represent ethylenediamine-*N,N,N',N'*-tetra-acetic acid and ethylenebis(acetylacetoneimine), respectively. The commercial linolenic acid and linolic acid (Nakarai Chemicals, Ltd.) were used without purification. The contents of hydroperoxides as an impurity was evaluated by the Fe^{2+}–NH_4NCS method,[17] and



H_5L^1



HL^2

are 1.5 and 1.3% for linolenic acid and linolic acid, respectively. 2-Thiobarbituric acid and 4-methyl-2,6-di-t-butylphenol(butylated hydroxytoluene) were obtained from Tokyo Kasei Co. Ltd.

Evaluation of Activity.—Typically the iron(III) complex (10 cm^3) (in water or in ethanol, 1×10^{-3} mol dm^{-3}) was mixed with an ethanol solution (5 cm^3) of an unsaturated fatty acid (1×10^{-2} mol dm^{-3}), and the resulting solution was stirred at room temperature. After several minutes, acetic acid (5 cm^3) was added and then 2-thiobarbituric acid (10 mg) and butylated hydroxytoluene (10 mg) were added to 5 cm^3 of the above solution and heated to 90 °C for 20 min.[18–22] The absorbance at 532 nm was recorded after addition of ethanol (2 cm^3).

Results and Discussion

In Figure 1 the time courses of the absorbance at 532 nm of reaction mixtures containing an iron(III) compound (in water) and linolenic acid (in ethanol) treated with 2-thiobarbituric acid is shown. It is known that the monomeric iron(III)–nitrilotriacetic acid complex is unable to peroxidize linolenic acid,[23] which is consistent with our present result, *i.e.* negligible ability of monomeric $[Fe(edta)]^-$ to form a 2-thiobarbituric acid-active compound in the reaction with linolenic acid. It should be noted that the abilities of the binuclear complexes to form such active compounds are much higher than those of the monomeric complexes, and the high ability of the $[Fe_2L^2]^{5+}$ complex is noteworthy. A similar result was observed for the system in ethanol solution (*cf.* Figure 2). In Figure 3 the result obtained for the iron(III) complex (in water) and linolic acid

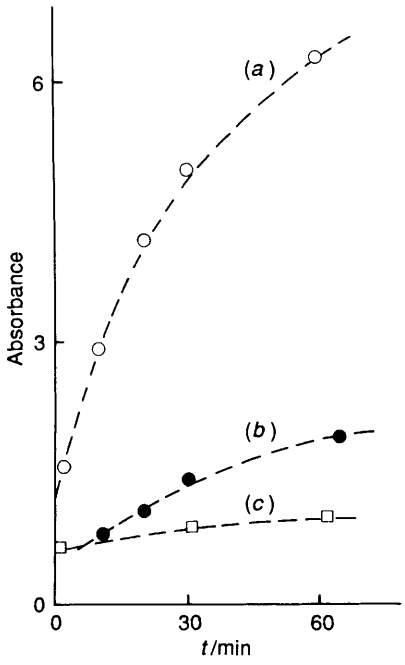


Figure 1. Time course of the absorbance at 532 nm of reaction mixtures of iron(III) (in water) and linolenic acid (in ethanol) treated with 2-thiobarbituric acid: (*a*) $[Fe_2L^2]^{5+}$, (*b*) $[Fe_2L^1(CH_3CO_2)_2]^-$, and (*c*) Na[Fe(edta)]

(ethanol) is illustrated, and it also shows that only the binuclear complexes exhibit high ability for the formation of 2-thiobarbituric acid-active compounds in the reaction with linolic acid.

It is known that the monohydroperoxide of the fatty acid, L–O_2H, which is the first product of peroxidation, is rather unstable and easily decomposes into other products as illustrated below.[18] According to recent work by Kikugawa and co-workers,[19] not only malonaldehyde but also several other

L–O_2H → (1) Chain cleavage and recurrent oxidation products: n-alkanols, 2-alkenals, 2,4-alkadienals, malonaldehyde, alkatrienals, α-hydroxyaldehydes, saturated and unsaturated ketones, alkanes, *etc.*

L–O_2H → (2) Rearrangement and consecutive products: hydroxyacids, ketoacids, ketohydroxyacids, dihydroxyacids, *etc.*

aldehydes react with 2-thiobarbituric acid, yielding substances which exhibit an absorption band at 532 nm. This suggests that the thiobarbituric acid test is not a conclusive method to determine the yield of peroxidation of fatty acids, but we can discuss the relative ability of the metal compounds for peroxidation in terms of the absorbance at 532 nm of the reaction mixture treated with 2-thiobarbituric acid. In the case of linolic acid, we have observed that both the products obtained from the reaction with $[Fe_2L^1(CH_3CO_2)_2]^-$ and native lipoxygenase (Tokyo Kasei, Co. Ltd.) give the same t.l.c. behaviour, indicating that the binuclear iron(III) complex exhibits a lipoxygenase-like function.[24]

As stated above, several mechanisms have been proposed for the peroxidation of fatty acids. Some authors have pointed out that it proceeds through a free-radical reaction.[12] The identity of the initial free radical is unknown, and many possible candidates including OH$^\bullet$ have been suggested. Since we have observed that the peroxidation of linolenic acid occurs in ethanol solution the participation of OH$^\bullet$ may be ruled out in

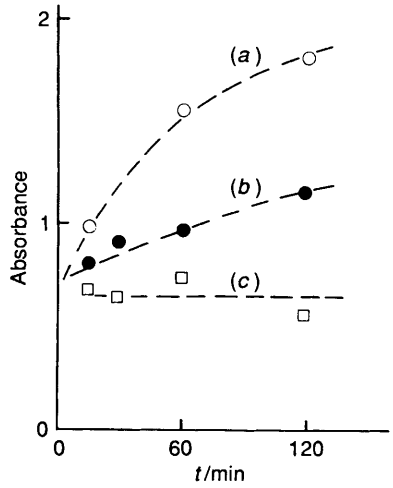


Figure 2. Time course of the absorbance at 532 nm of reaction mixtures of iron(III) (in ethanol) and linolenic acid (in ethanol) treated with 2-thiobarbituric acid: (*a*) $[Fe_2L^2]^{5+}$, (*b*) $[Fe_2L^1(CH_3CO_2)_2]^-$, and (*c*) [Fe(acen)Cl]

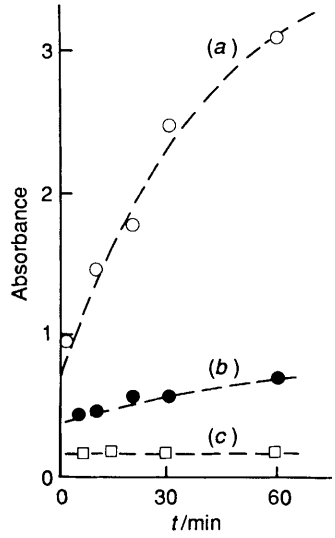
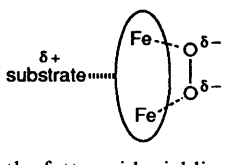


Figure 3. Time course of the absorbance at 532 nm of reaction mixtures of iron(III) (in water) and linolic acid (in ethanol) treated with 2-thiobarbituric acid. Key as in Figure 1

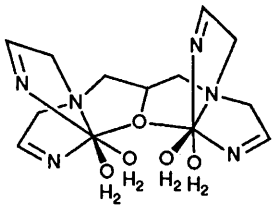
the present system. Corey and co-workers[13] proposed an alternative mechanism including an iron–alkyl intermediate. Since their mechanism cannot explain the present results that only the binuclear iron(III) compounds exhibit high ability for the formation of thiobarbituric acid-active compounds in the reaction with unsaturated fatty acids, it seems necessary to consider another mechanism.

We would like to propose a new mechanism as described below; the activation of O_2 may occur in a substrate–metal complex–O_2 intermediate (illustrated below) and the activated



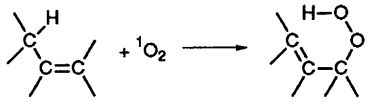
O_2 molecule attacks the fatty acid, yielding a hydroperoxide of the latter. For the formation of the above intermediate, space allowing the O_2 molecule to approach the two iron atoms is necessary. In the case of the $[Fe_2L^2]^{5+}$ complex the co-ordination environment around the metal ion is more flexible,

because only four co-ordination positions are fixed by the ligand system (two benzimidazoles, one amine nitrogen, and one alcoholic oxygen atom) and the two other positions may be occupied by water molecules, as depicted below. Thus, the



approach of the O_2 molecule may be facile. On the other hand, six-co-ordination around the iron atoms is fixed by the ligand system in the case of $[Fe_2L^1(CH_3CO_2)_2]^-$, which will more or less resist the approach of O_2. This will explain the lower lability of the latter complex compared with that of $[Fe_2L^2]^{5+}$. In order to verify the above assumption a study on the complex $[Fe_2L^2(CH_3CO_2)_2]^{3+}$ (ref. 25) is desirable. Unfortunately the latter complex is scarcely soluble in water and ethanol.

It is well known that the two-electron reduction of O_2 is thermodynamically more favourable than the one-electron reduction,[26] and this will explain the high ability of the binuclear complexes compared with those of the mononuclear ones. In previous papers[27] we have pointed out that the O_2 molecule in the intermediate assumed above may contain more or less singlet oxygen ($^1\Delta_g$) character, and it is known that singlet oxygen reacts with olefenic compounds facilely to yield a hydroperoxide as shown below.[28] Thus, we might explain the



lipoxygenase-like function observed for the binuclear iron(III) complexes in terms of the dioxygen activation in a substrate–metal complex–O_2 intermediate, as described above.

Acknowledgements

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