CHEMISTRY LETTERS, pp. 195-198, 1990.

Remarkably High Activity of Binuclear Iron(III) Complexes for Formation of TBA-active Compounds in Reaction with Linolenic Acid

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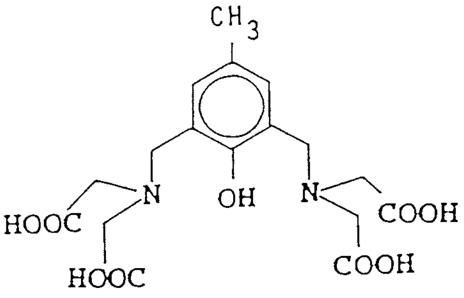
We have observed that some binuclear iron(III) complexes with μ-phenoxo or μ-alkoxo bridge exhibit remarkably high activity for the formation of TBA-active compounds in the reaction with linolenic acid, whereas no activity was observed for the binuclear complexes of copper(II), nickel(II), cobalt(II), and manganese(III) with the same ligands.

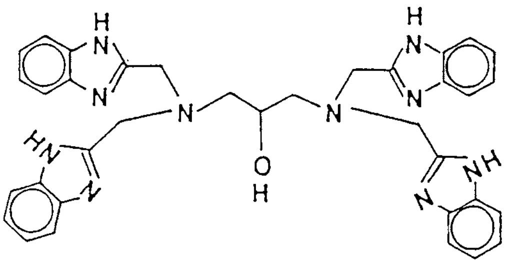
It is becoming increasingly evident that free-radical mediated peroxidation of cell membranes is a common pathway in the onset of several disease states, varying from drug-associated toxicity to postischemic reperfusion injury[1,2] and carcinogenesis.[3] Partially reduced forms of dioxygen, like superoxide(O_2^-) and its dismutation product hydrogen peroxide(H_2O_2), have been implicated in the initiation of lipid peroxidation.[4] However, it has been shown that neither O_2^- nor H_2O_2 or the uncatalyzed reaction of O_2^- with H_2O_2 can directly initiate lipid peroxidation.[5-7] The hydroxyl radical(OH·) is frequently proposed as the initiating species,[8] but the intrinsic ability of OH· to initiate membrane lipid peroxidation has been questioned by some investigators.[9] The criticism is in part a consequence of the realization that OH· is a short-lived radical which reacts with most organic compounds at nearly diffusion-controlled rates.[10] In agreement, many in vitro lipid peroxidation systems are not inhibited by catalase or OH· scavengers.[9] These observations provided a rationale for the hypothesis of alternate initiators, tentatively described as a complex between oxygen and different valence states of iron, and in this respect much experimental attention has been given to the ferryl ion,[11] perferryl ion[12] and a ferrous-dioxygen-ferric complex.[13] Very recently Aust et al.[14] studied the initiation of lipid peroxidation by Fe^{2+} and H_2O_2, and concluded that the oxidation of Fe^{2+} by H_2O_2 generates a strong oxidant which is capable of peroxidizing phospholipid liposomes, and the oxidant requires both Fe^{2+} and Fe^{3+}, the optimum activity being at approximately 1:1. However, it remains unclear that what species is the initiator to lipid peroxidation in these systems.

In this article we have observed that some binuclear iron(III) complexes with μ-phenoxo or μ-alkoxo bridge exhibit remarkably high activity for the formation of TBA(2-thiobarbituric acid)-active compounds in the reaction with linolenic acid, whereas no activity was observed for the binuclear complexes of copper(II), nickel(II), cobalt(II) and manganese(III) with the same ligand. The present results will give an important key to elucidate the reaction mechanism of lipid peroxidation in the biological systems.

The binuclear iron(III) complexes with $H_5(L_a)$ and $H(L_b)$(illustrated below) were prepared according to the published methods.15,16) These are



$H_5(L_a)$



$H(L_b)$

$[(n\text{-}bu)_4N][Fe_2(L_a)(CH_3COO)_2]$15) and $Fe_2(L_b)(NO_3)_5$.16) The binuclear complexes of copper(II), nickel(II), cobalt(II) and manganese(III) with $H_5(L_a)$ were prepared in this study.17) The activity of the mononuclear complex of H_3(nta) (=nitriletriacetic acid) was also compared.18)

In the typical run, 10 ml of the iron(III) complex(1×10^{-3} mol dm^{-3}) was mixed with 5 ml ethanol solution of linolenic acid(160 mg/50 ml ethanol)19) and the resulting solution was stirred at room temperature. After 10 min , 5 ml acetic acid was added to the solution. TBA(10 mg) and butylated hydroxytoluene (10 mg) were added to the 5 ml of the above solution, and the solution was heated to 90 °C for 20 min.20) Then the absorbance at 532 nm was recorded after addition of ethanol(2 ml).21) The representative results are shown in Fig. 1. The figure clearly indicates that a lot of TBA-active compounds are formed in the reaction mixture of the binuclear iron(III) complex, which is much larger than that of corresponding mononuclear complex, Fe(III)-nta. Figure 2 demonstrates that the TBA-active compounds in the reaction mixture with $[Fe_2(L_a)(CH_3COO)_2]^-$ increase with time. The absorbance at 532 nm was almost zero in the solutions of other binuclear complexes of copper(II), nickel(II), cobalt(II) and manganese(III), indicating that iron(III) is very specific for the formation of TBA-active compounds in the reaction with linolenic acid. The trinuclear complexes, such as $[Fe_3O(CH_3COO)_6(H_2O)_3]^+$ and its Cr(III) and Mn(III) derivatives are also inactive. This is indicating that the presence of two iron atoms is essentially important for the formation of TBA-active compounds. Similar high activity of the binuclear iron(III) complexes for the formation of TBA-active compounds was also found in the reaction with linolic acid, and the detailed studies on the reaction mechanism are now under progress.

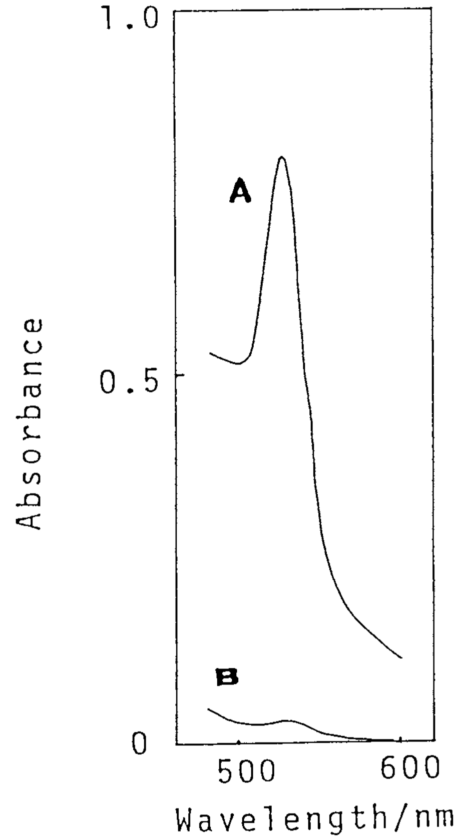


Fig. 1. Absorption spectra of reaction mixtures heated with TBA.

A: $[Fe_2(L_a)(CH_3COO)_2]^-$
B: Fe(III)-nta complex

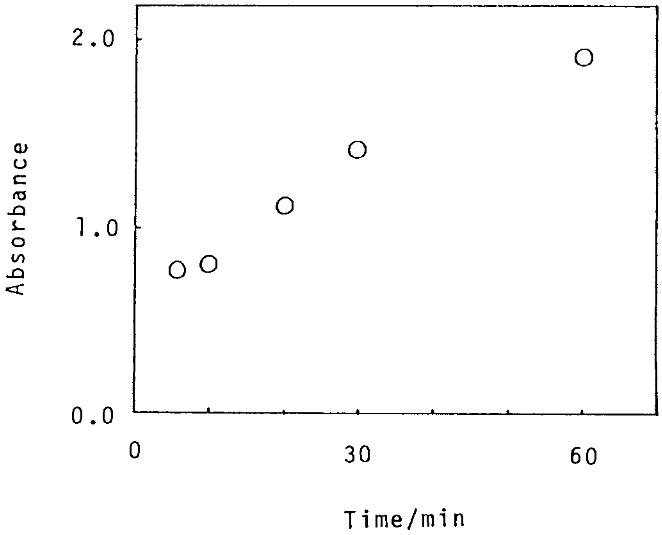


Fig. 2. Time course of absorption at 532 nm of reaction mixture heated with TBA for $[Fe_2(L_a)(CH_3COO)_2]^-$ and linolenic acid system.

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17) Found: C, 37.30; H, 3.84; N, 4.18; and Mn, 16.6%. Calcd for $Na[Mn_2(L_a)(CH_3COO)_2]\cdot 3/2H_2O$: C, 37.57; H, 3.90; N, 4.17; and Mn, 16.37%. Found: C, 35.03; H, 2.94; N, 4.80%. Calcd for $Na_2[Cu_2(L_a)(OH)]$: C, 34.99; H, 3.11; and N, 4.80%. Found: C, 34.41; H, 3.38; and N, 3.57%. Calcd for $Na_3[Ni_2(L_a)(CH_3COO)_2]\cdot 2H_2O$: C, 34.37; H, 3.71; and N, 3.82%. Found: C, 34.37; H, 3.46; and N, 3.87%. Calcd for $Na_3[Co_2(L_a)(CH_3COO)_2]\cdot 2H_2O$: C, 34.35; H, 3.71; and N, 3.82%.

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19) The commercial linolenic acid(Nakarai Chemicals, Ltd.) was used without purification.22) The content of hydroperoxide as an impurity was evaluated by Fe^{2+}-NH_4NCS method23) to be 1.5%.

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(Received October 20, 1989)