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Model Compounds for Purple Lipoxygenase

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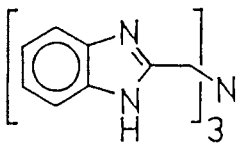
We have found that some iron(III) compounds with tripodal-like ligands react with t-butyl hydroperoxide to yield a purple species, whose spectroscopic properties are very similar to those observed for the purple lipoxygenase. Based on these facts the origin of the purple color in the native enzyme was discussed.

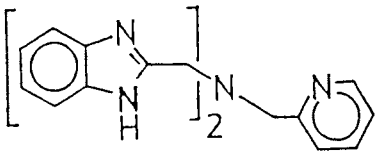
Lipoxygenase(linoleate:oxygen oxidoreductase, EC 1.13.11.12) is a non-heme iron dioxygenase which catalyzes the dioxygenation of polyunsaturated fatty acids containing a 1Z,4Z-pentadiene system.[1] Interest in the mechanism of lipoxygenase has been stimulated these last years by the discovery of the implication of lipoxygenase in the biosynthesis of leukotrienes which have an important role in inflammation and immediate hypersensitivity.[2] A still unsolved mechanistic question is whether lipoxygenation proceeds via free-radical or organoiron intermediate formed during rate-limiting C-H bond cleavage.[3] Native lipoxygenase-1 from soybean is cololess(Fe^{2+}) and virtually ESR-silent. Addition of an equivalent amount of 13(S)-hydroperoxy-9Z,11E-octadienoic acid(abbreviated as 13(S)-HPOD) to the ferrous state yields an active yellow enzyme species and ESR signal around g=6, stemming from high spin Fe^{3+}. Treatment of this ferric form with another 13(S)-HPOD results in the formation of a meta-stable purple enzyme.[1] This form reverts slowly to the native ferric enzyme with release of 12,13-epoxy-11-hydroxy-9-octadecanoic acid, suggesting that it represents an intermediate complex on the isomerization pathway. However, the origin of the purple color has never been adequately explained.[4] In this article we have obtained the first model compounds for the purple lipoxygenase; i.e., iron(III) compounds which react with alkylhydroperoxide, yiedling a purple species

whose spectroscopic (absorption and ESR) properties are very similar to those observed for native enzyme.

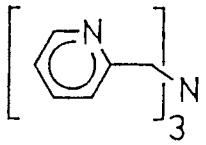
The iron(III) compounds used in this study are those with tripodal-like ligands, Fe(ntb)Cl_3, Fe(pb2)Cl_3, and Fe(tpa)Cl_2ClO_4, and the chemical structures of the ligands are illustrated below.



(ntb)



(pb2)



(tpa)

When the orange dmso (N,N-dimethylsulfoxide) solution of Fe(ntb)Cl_3 was treated by the dmso solution of t-butyl hydroperoxide, notable color change was not observed. Addition of dmso solution of triethylamine to the above solution has lead to immediate purple coloration with absorption maximum at 580 nm with ε =1850 (cf. Fig. 1). This purple species is rather unstable at room temperature, decomposing gradually to the original orange solution, as shown in Fig. 1. Similar color change was also observed for the dmso solution of Fe(pb2)Cl_3 (λ_{max}=580 nm, ε =1840), but new absorption band was not observed in the visible region for the case of Fe(tpa)Cl_2ClO_4 under the same experimental conditions. As shown in Fig. 2, the addition of t-butyl hydroperoxide also caused the change in the ESR

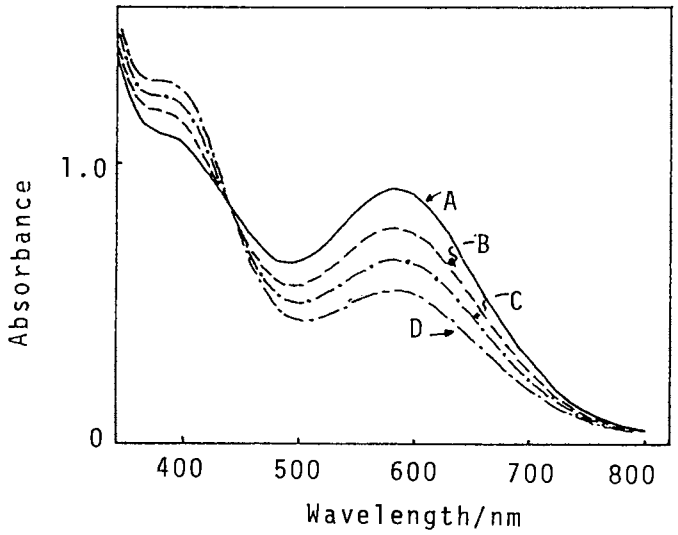
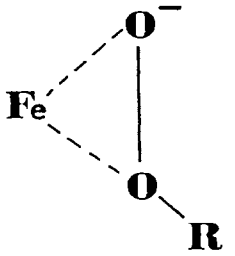


Fig. 1. Absorption spectra (in dmso, 25 °C)
A(——):mixture of Fe(ntb)Cl_3(1 ml, 1/500 M), t-butyl hydroperoxide(2 ml, 1/50 M) and triethylamine (1 ml, 1/500 M)
B(– – –): after 5 minutes
C(—·—): after 10 minutes
D(—··—): after 20 minutes

spectra. In the absence of t-butyl hydroperoxide, the ESR spectrum of Fe(ntb)Cl_3 in dmso is very similar to that of lipoxygenase-1,[5] e.g., there are several signals at g=7.5, 5.5, and 4.3. Addition of the

hydroperoxide has lead to the loss of signals at g=7.5 and 5.5, as shown in Fig. 2. This ESR spectral change by the addition of the hydroperoxide is also very similar to that observed for the native enzyme.[1] These facts are demonstrating that some changes occurred in the environment around the iron atom, and implying that the purple color of the present model systems should be due to the formation of a chelated species of t-butyl hydroperoxide, as illustrated below, and the origin of the purple lipoxygenase-1 may be the same as that described above; this may be supported by the fact that the peroxide adduct of Fe(III)-edta complex with side-on configuration is violet.[6] In the case of dmf(N,N-dimethyl-formamide) solution, the purple species once formed turned immediately to an orange species (λ_{max}=470 nm) at room temperature and this species decomposed slowly within a day.



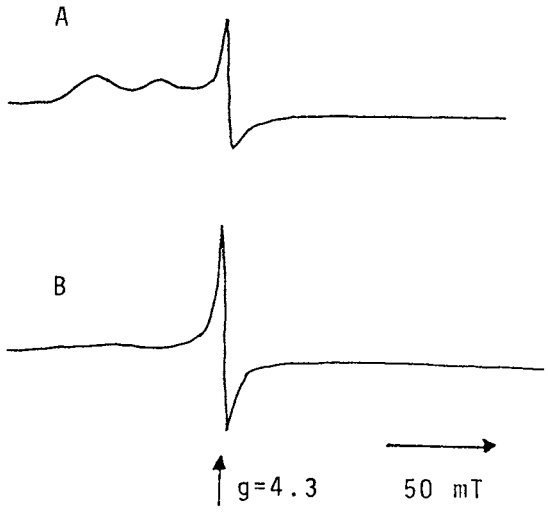


Fig. 2. ESR spectra(X-band, 77 K, in dmso)
A: $Fe(ntb)Cl_3$
B: mixture of $Fe(ntb)Cl_3$ (1 ml, 1/500 M), t-butyl hydroperoxide(1 ml, 1/50 M) and triethylamine(1 ml, 1/500 M)

A similar reddish-violet coloration was also observed for the dmso solutions of $Fe(Bz\text{-}dbz)Cl_3$ and $Fe(Et\text{-}dbz)Cl_3$ by addition of t-butyl hydroperoxide, where (Bz-dbz) and (Et-dbz) represent N,N-bis(2-benz-imidazolylmethyl)-benzylamine and -ethylamine, respectively. It should be noted here that the formation of purple species by the addition of t-butyl hydroperoxide occurs only when the iron(III) complexes with tripodal-like ligands are used. In the previous paper,[7] we have reported that the iron(III) compounds with tripodal-like ligands react with superoxide anion, yielding a colored iron(III)-peroxide adduct, whereas the formation of a peroxide adduct was not observed for the iron(III) compounds with

planar tetradentate Schiff base ligands such as Fe(salen)Cl and Fe(acen)Cl. Present results seem to be consistent with the above result, that is, the reactivity of the iron(III) complexes toward superoxide anion or butyl hydroperoxide is highly dependent on the coordination geometry and steric requirement around the iron atom.

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