DNA Cleavage by Binuclear Iron(III)–Peroxide Adducts

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(Received February 28, 1989; revised May 11, 1989)

Nature has utilized structurally similar metalloprotein sites for reversible oxygen binding and for oxygen activation; myoglobin and cytochrome P-450 constitute one such carrier/activator which contains a mononuclear heme site [1], while hemocyanin and tyrosinase have a binuclear copper site [2]. In preceding papers, Nishida *et al.* [3, 4] have shown that the peroxide ion trapped in metal complexes (both mononuclear and binuclear) exhibits a very different reactivity from that of free H_2O_2, and that this may be due to the activation of the peroxide ion through coordination to the metal ion(s). This implies that the origin of activation of the peroxide ion is similar for both the mononuclear and the binuclear metal compounds, and also that the activation of peroxide ion is not dependent on the number of metal ions. In this study we have investigated the reactivity of the peroxide adducts of binuclear iron(III) complexes toward DNA, in order to obtain more detailed knowledge on the relationship between the DNA cleavage and activation of the dioxygen molecule, which seems to be important in the development of new antitumor metal compounds.

The iron(III) complex $Fe_2(L)^{5+}$ and its peroxide adduct (illustrated below) were obtained according to the published method [5]. DNA (calf thymus) was purchased from Tokyo Kasei Co. Ltd. The binuclear iron(III) complex $Fe_2(L)(NO_3)_5$ is fortunately soluble in water, yielding an orange solution. When H_2O_2 was added to this solution, it turned to violet (cf. Fig. 1), indicating formation of the peroxide adduct, similar to cases observed in organic solvents [5]. The aqueous solution (100 ml) containing DNA (40 mg), 5 ml H_2O_2 (1×10^{-2} mol) and 25 ml $Fe_2(L)^{5+}$ (2×10^{-3} mol) was allowed to stand at room temperature. After the disappearance of the violet color (about 1–2 days), 2-thiobarbituric acid (TBA, 20–30 mg) was added to the solution, which was heated at 90 °C for 20 min [6]. The formation of pink crystals was detected, which show an absorption peak at 532 nm in dmf [6, 7]. These facts clearly indicate that the peroxide adduct of $Fe_2(L)^{5+}$ can cleave DNA, since no pink coloration was observed in the blank experiments where no iron(III) was present under the same conditions.

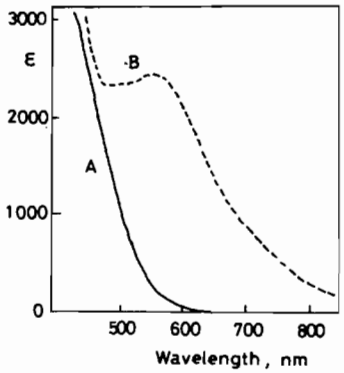

Fig. 1. Absorption spectra (in water): (A) $Fe_2(L)(NO_3)_5$; (B) $Fe_2(L)(NO_3)_5 + H_2O_2$ ($[H_2O_2]/[Fe^{3+}] = 10$).

The quantitation or characterization of the products produced is now in progress. The peroxide adducts of the binuclear iron(III) complexes prepared by Que *et al.* [8] (illustrated below) also gave TBA positive materials in the reaction with DNA. These peroxide adducts showed activity for the degradation of 1,3-diphenylisobenzofuran, one of the singlet oxygen ($^1\Delta_g$) scavengers, as observed for $Fe_2(L)^{5+}$–H_2O_2 [3].

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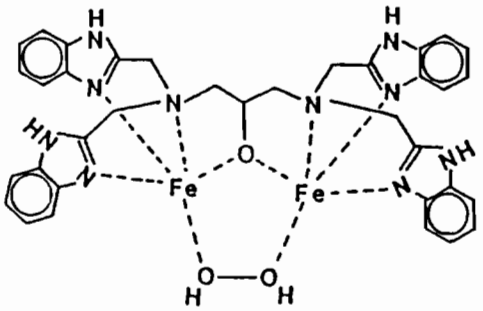
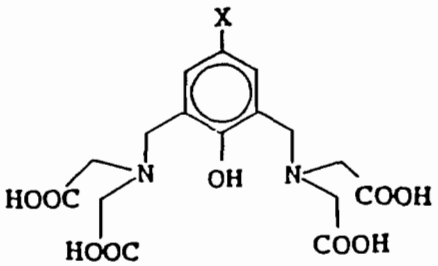


Peroxide adduct of the $Fe_2(L)^{5+}$ complex



X	Abbreviation	Dimeric Fe(III) complexes
CH_3	$H_5(L\text{-}CH_3)$	$[Fe_2(L\text{-}CH_3)(OH)(H_2O)_2]$
Cl	$H_5(L\text{-}Cl)$	$[Fe_2(L\text{-}CH_3)(CH_3COO)_2]^-$
		$[Fe_2(L\text{-}Cl)(OH)(H_2O)_2]$

0020-1693/89/$3.50

A number of investigations in the field showed that both OH· and O_2^- are generated by Fe(II)–BLM (BLM = bleomycins) and O_2, and proposed that these active oxygen species may be responsible for degradation of DNA [9]. However, Rodriguez and Hecht [10] indicated that tert-butylphenylnitrone does not inhibit DNA degradation and that the amount of hydroxyl radical spin-trapped adduct produced was about 1/40 that observed with comparable amounts of Fe(II) and H_2O_2, a known OH· generator. In addition, studies with known OH· scavengers, such as dimethyl sulfoxide, indicated no effect on the rate of DNA cleavage. They also showed that a small molecule superoxide dismutase catalyst, tetrakis(4-*N*-methylpyridyl)porphyrinatoiron(III) had no effect on BLM-mediated DNA degradation.

In the previous papers, we have proposed that the peroxide ion trapped in the binuclear iron(III) complex contains some degree of singlet oxygen ($^1\Delta_g$) character [3], and this has been confirmed by several other systems such as oxyhemocyanin model compounds [4] and some mononuclear peroxide adducts [3]. Thus, it is very likely that the initial stage of DNA cleavage, i.e., H atom abstraction at the 4′-position [9], is caused by the activated peroxide ion in the binuclear iron(III) complexes and also in the activated BLM [11]. This gives a reasonable explanation for the presence of 'caged' hydroxyl radicals [12] (or crypto-OH· [13]), i.e., the activated peroxide ion will react with the H atom (abstracted from the organic compound) as below

$$Fe^{3+}\text{–}H_2O_2 + H \longrightarrow Fe^{3+} + H_2O + OH\cdot$$

$$\text{or} \quad \longrightarrow Fe^{4+}\text{–}OH + H_2O$$

and thus this reaction is not inhibited by any OH· scavenger. Our assumption seems to be consistent with the results by Stubbe and co-workers [14], theoretical considerations by Nagata and Aida [15], and those by Tero-Kubota *et al.* [16] who used the spin-trapping method.

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