Iron(III) Complexes with Hydrogen Peroxide which Can Discriminate Two Reaction Types; Oxidation (H-Atom Abstraction) and Oxygenation Reaction

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Iron(III)-NTA (nitrilotriacetic acid) solution shows high activity for oxidative degradation of 2’-deoxyribose in the presence of hydrogen peroxyde, whereas its activity of Fe(III)-TFDA (2-amnimethyltetrahydrafuran-N,N-diacectie acid) is negligible under the same experimental conditions; however the latter solution exhibits abnormally higher reactivity for oxygenation reaction at 8-position of 2’-deoxyguanosine than other iron(III) chelates examined. These results suggest that oxidative degradation of deoxyribose and the oxygenation of deoxy­ guanosine are caused by a different iron(III)-peroxide species.

Introduction
Oxidative damage to DNA, proteins, lipids, sugars, and other macromolecules have been postulated to be a major cause of endogenous damage leading to aging and age-related diseases (Ames et al., 1993). Epidemiological as well as clinical studies have confirmed that the high incidence of hepatocellular carcinoma (HCC) in patients with primary hematocromatosis, a hereditary disease which leads to high level of iron accumulating in the body (Deugnier et al., 1993). There are many clinical evidences to favor the direct involvement of iron in the development of HCC (Kew, 1990), however the mechanism by which iron triggers carcinogenesis is not yet known. In 1982, Okada and Midorikawa have reported that Fe-NTA (nitrilotriacetic acid) can produce renal cell carcinoma in mice and rats after repeated intraperitoneal administration (Okada and Midorikawa, 1982). It is well known that Fe-NTA is a very effective iron chelate to induce the iron-overload status in rats (Awai et al., 1979), and very recently it has become apparent that the tissue damage caused by Fe-NTA is observed only for the place where formation of thiobarbituric acid-reactive substance (TBARS) (Halliwell and Gutteridge, 1985) is found (Liu and Okada, 1995). Uemura et al. (1990) have demonstrated that Fe-NTA attacks kidney DNA to result in the significant increase in 8-hydroxydeoxyguanosine (8-OH-dG); it is generally accepted that 8-OH-dG is a suitable marker for measuring the level of oxidative DNA damage both in vitro and in vivo (Kasai and Nishimura, 1984). In our previous paper, we have presented strong evidence that high damaging effect by the Fe-NTA complex should be due to its dimeric structure and the presence of hydrogen peroxide, and the formation of TBARS by Fe-NTA in vivo should be due to degradation of 2’-deoxyribose (Nishida et al., 1994a). In this article, we will show that two kinds of oxidative damage by the iron(III) chelates in the presence of hydrogen peroxide, that is, degradation of deoxy­ ribose and formation of 8-OH-dG, should be caused by a different iron(III)-peroxide adduct.

Experimental
The chemical structures of the ligands cited in this paper are illustrated below; these are all containing an ethylar oxygen atom in the chelate. The iron(III) solution was prepared by adjusting the pH of the solution containing ferric chloride and ligand of twice equivalence to be 7.0 in terms of KHCO₃. The formation of TBARS by the iron(III) chelate and hydrogen peroxide was detected as follows: to 20 ml of iron(III) chelate solution ([Fe³⁺] = 5 x 10⁻² mol/l) containing 2’-deoxyribose (50 mg) was added 10 ml of hydrogen

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peroxide solution \((1\times10^{-1}\ \text{mol/l})\), and the resulted solution was treated with TBA as described in the literature (Nishida and Yamada, 1990). In the present cases, TBARS is undoubtedly malondialdehyde; this reacts with TBA to give pink compound which shows strong absorption band at 532 nm (Halliwell and Gutteridge, 1985). The formation of 8-OH-dG was followed as below; to 50 ml iron(III) chelate solution \([\text{[Fe}^{3+}\text{]} = 2\times10^{-2}\ \text{mol/l}]\) containing 2'-deoxyguanosine \((20\ \text{mg})\) was added 10 ml of hydrogen peroxide solution \((1\times10^{-1}\ \text{mol/l})\); in the case of Fe-TFDA, the concentrations of hydrogen peroxide solution was \(1\times10^{-2}\ \text{mol/l}\), and the formation of 8-OH-dG was evaluated in terms of HPLC by the use of authentic sample (Kasai and Nishimura, 1984).

\[
\text{N(CH}_2\text{COO})_2 \quad \text{N(CH}_2\text{COO})_2 \quad \text{N(CH}_2\text{COO})_2
\]

\[
\text{CH}_2\text{CH}_2\text{OCH}_3 \quad \text{CH}_2\cdot \quad \text{CH}_2\text{CH}_2\text{OCH}_3
\]

\(\text{FDA} \quad \text{EDA} \quad \text{TFDA}\)

**Results and Discussion**

In Fig. 1, the absorption spectra of two iron(III) chelates are illustrated. It should be noted here that the chelates containing ethereal oxygen atom, such as FDA and TFDA, can dissolve iron(III) ion at pH 7.0 in the presence of carbonato ion, while formation of ferric hydroxide occurs when KHCO\(_3\) is added to the solution of iron(III)-iminodiacetic acid system at pH 5; this is indicating that the coordination of ethereal oxygen atom to an iron(III) ion plays an important role in the complex formation of these chelates; in fact the coordination of ethereal oxygen atom to an iron(III) was confirmed by the crystal structure determination of analogous iron(III) complex with N,N-bis(2-pyridylmethyl)-2-aminomethyltetrahydrofuran. As shown in Fig. 1, there are two absorption bands in the visible region, which is characteristic for binuclear iron(III) compounds with \(\mu\)-oxo-\(\mu\)-carbonato bridges as exemplified in the previous paper (Nishida et al., 1994a), demonstrating that these iron(III) chelates exist as a dimeric compound in the solution.

We have found that formation of much quantity of TBARS occurs in the solution containing Fe-NTA, hydrogen peroxide and 2'-deoxyribose, however the formation of TBARS was much smaller in the cases of Fe-TFDA and Fe-EDA systems (see Fig. 2). The formation of TBARS in the present case is assumed to occur through the oxidative degradation of ribose via hydrogen atom abstraction at 4'-position, as shown below (Stubbe and Kozarich, 1987).

On the other hand, Fe-TFDA solution exhibits much higher activity for the formation of 8-OH-dG in the presence of hydrogen peroxide and 2'-deoxyguanosine than those of Fe-NTA, Fe-EDA, Fe-FDA, and Fe-EDDA (ethylenediamine-N,N'-

**Fig. 1.** Absorption spectra of the iron(III) chelates. \([\text{[Fe}^{3+}\text{]} = 2\times10^{-3}\ \text{mol/l}]\). A: Fe(III)-NTA; B: Fe(III)-TFDA.

**Fig. 2.** Absorption spectra of the solutions treated by TBA (2-thiobarbituric acid; see Experimental section). A: Fe-NTA; B: Fe-TFDA; C: Fe-PAC (2-aminomethylpyridine-N,N-diacetic acid); D: Fe-EDDA.
detected in the absorption spectrum of Fe-NTA complex by the addition of hydrogen peroxide and also the fact that peroxide adduct of the binuclear iron(III) complex, Fe$_2$(HPTP)(OH)(NO$_3$)$_4$ (Nishida et al., 1994a) with $\eta^2$-type coordination type is highly active for the formation of TBARS in the reaction mixture of 2'-deoxyribose; high activity of the latter peroxide adduct for H-atom abstraction was also demonstrated in the reaction with phenols (Nishida et al., 1991). Thus, it seems reasonable to assume that the structure of the peroxide adduct in Fe-TFDA complex is quite different from that of Fe-NTA. In the cases of iron(III) chelates with TFDA, EDA, and EDDA, two absorption bands in the visible region disappear by the addition of hydrogen peroxide, and reappear with the decomposition of hydrogen peroxide added. This is demonstrating that coordination of hydrogen peroxide to an iron(III) atom occurs with the dissociation of carbonato ion in the solution. Based on the fact as described above.
and also the fact the formation of 8-OH-dG is much smaller in the case of Fe-EDA and hydrogen peroxide, it seems reasonable to assume that there should be three different peroxide adducts in the present systems as shown in Scheme I illustrated above; adduct-I was already assumed for a peroxide adduct of Fe-NTA compound (Nishida et al., 1994a). Since it is known that degradation of furan ring proceeds in the solution of Fe-FDA and hydrogen peroxide (Nishida and Ito, 1995) and also that cyclohexane reacts with hydrogen peroxide to give a hydroperoxide adduct (Fish et al., 1991), it seems quite likely that the structure of hydrogen peroxide adduct in the Fe-TFDA system is of a \( \eta^1 \)-type, adduct-III in Scheme I; formation of a hydroperoxide adduct between peroxide ion and tetrahydrofuran ring may occur, and this intermediate may react readily with deoxyguanosine, to give 8-OH-dG. Adduct-II may be considered for the cases of Fe-EDDA and Fe-EDA systems. Above assumption may be coincident with our recent theoretical investigations on the reactivities of these peroxide adducts (Nishida et al., 1994b, c).

In this article we have obtained the strong evidences to support that oxidative degradation of deoxyribose and the oxygenation reaction of deoxyguanosine are caused by a different iron(III)-peroxide adduct, and the present results may give important key to elucidate the mechanism of oxidative DNA damage by the iron(III) chelate in vivo.