Oxygenation of Nucleosides by Peroxide Adduct of Binuclear Iron(III) Complex with a μ-Oxo Bridge

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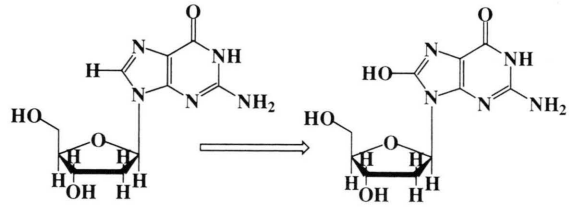
The (μ-oxo)(μ-carbonato)diiron(III) complex with H_2(tfda) (H_2(tfda) = 2-aminomethyltetrahydrofuran-N,N-diacetic acid) exhibited high activity for hydroxylation of 2′-deoxyguanosine in the presence of hydrogen peroxide, giving 8-hydroxydeoxyguanosine, but its hydroxylation activity towards other nucleosides such as 2′-deoxyadenosine, adenosine or thymidine was found negligible. In the case of the Fe(III)-(eda) complex (H_2(eda) = 2-methoxyethylamine-N,N-diacetic acid), hydroxylation occurred mainly at the sugar site, converting 2′-deoxyguanosine to guanosine. Based on the spectroscopic and structural properties of these iron(III) compounds, it seems most likely that an intrinsic active species for hydroxylation should be an electrophilic peroxide adduct of the (μ-oxo)diiron(III) core with η^1-coordination mode, while the contribution of OH· to the hydroxylation reaction of nucleosides is ruled out.

Oxidative damage rate to DNA occurs as part of normal metabolism. In each rat cell the steady-state level is estimated to be about 10^6 oxidative adducts and 10^5 new adducts are formed daily (Ames and Gold, 1991). Apparently this endogenous DNA damage is a major contributor to aging and the degenerative diseases of aging, such as cancer (Kuchino *et al.,* 1987; Shibutani *et al.,* 1991). Four endogenous processes leading to significant DNA damage are likely to be oxidation, methylation, deamination, and depurination (Ames and Gold, 1991; Saul and Ames, 1986). Measurements of DNA adducts by new methods shows that DNA damage produced by oxidation could be the most significant endogenous damage.

The most important and a more easily assayed product of oxidative DNA damage is 8-hydroxydeoxyguanosine (hereafter abbreviated as 8-OH-dG, see figure below) (Aruoma *et al.,* 1989; Floyd *et al.,* 1988) and it has been suggested that the 8-OH-dG is formed *in vivo* by OH· radicals (Floyd *et al.,* 1988).

Very recently we have reported that remarkable formation of 8-OH-dG is observed in the solution containing Fe(III) complex with (tfda) and hydrogen peroxide, (Nishida and Ito, 1995a) but its



deoxyguanosine 8-hydroxy-deoxyguanosine

formation is negligible in the solutions of iron(III) complex with (pac) and (eda), where (tfda), (pac) and (eda) are the tetradentate chelates as illustrated in Fig. 1. Seemingly the OH· radical is not an active species for formation of 8-OH-dG in the solution of the Fe(III)-(tfda) and hydrogen peroxide. In this study, we will show details on the structural and spectral features of these iron(III) chelates, and their reactivity towards nucleosides in the presence of hydrogen peroxide.

Experimental Section

Materials

The chelates used in this study were prepared according to published methods (Berchet, 1966).

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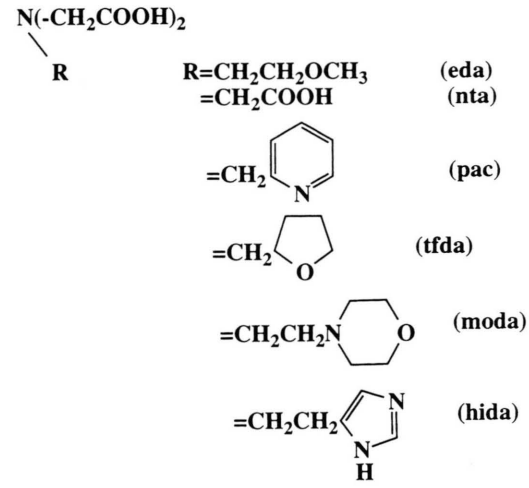


Fig. 1. Structures of ligands cited in this paper.

2′-Deoxyguanosine, 8-OH-dG, and other nucleosides and nucleic acids were purchased from Wako Chemicals.

Preparation of solution of iron(III) chelate

The pH of the solution containing ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$ (270 mg, 0.001 mol) and the chelate(0.002 mol) was adjusted to 7.0 by $KHCO_3$; the concentration of the solution was 1/50 M for Fe(III) when the volume of the above solution is diluted to 50 ml.

Evaluation of formation of 8-OH-dG by HPLC method

To an aqueous solution of iron(III) chelate (50 ml, 1/50 M) containing 2′-deoxyguanosine (20 mg) was added hydrogen peroxide solution (10 ml, 1/10 M), and the formation of 8-OH-dG was detected in terms of HPLC according to method by (Kasai and Nishimura (1984) and quantified by a standard sample. In our experiments, the $(H_2O_2)/(Fe^{3+})$ ratio was one.

Crystal structure determination of Fe(III)-(pac) compound

The Fe(III)-(pac) complex, $Cs_2Fe_2O(pac)_2(CO_3) \cdot 6H_2O$ was obtained according to Nishida and Ito (1995b). Crystal data: monoclinic, space group C2/c, a = 2.2524(2), b = 1.0589(2), c = 1.5778(2) nm, β = 107.95(1)°, V = 3580.0(8) nm^3, D_x = 1.866 gcm^{-3}, Radiation Mo Kα (λ = 0.71073 Å). The data were collected by Rigaku AFC-5 four-circle diffractometer using ω-scan technique, and were corrected for absorption. The Cs atom shows positional disorder. The structure was solved by the direct method, and non-hydrogen-atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on the 2893 observed reflections ($|F_o| > 3\sigma(F_o)$) and 237 variables parameters and converged with unweighted agreement factor of R = 0.080. Experimental details on the structure determination have been de-

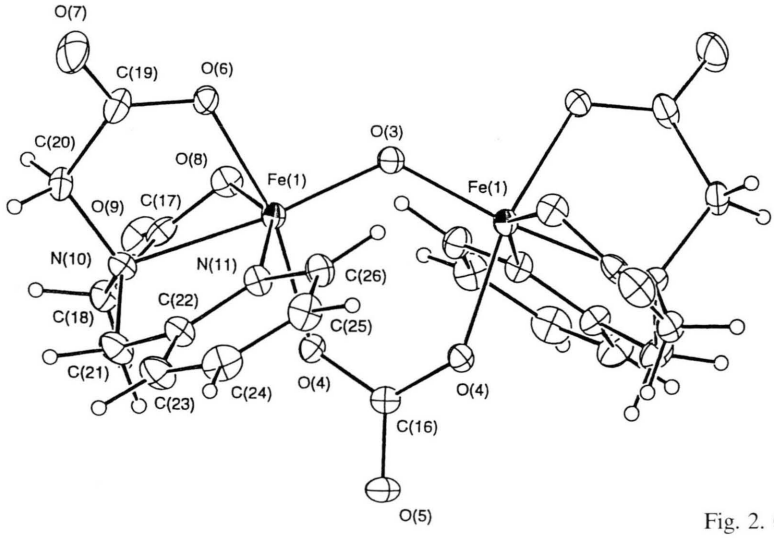


Fig. 2. ORTEP drawing of $Fe_2O(pac)_2(CO_3)^{2-}$.

posited as Supplementary data, which may be obtained from the senior author (Y. Nishida).

Measurements

Absorption spectra were measured with a Shimadzu spectrophotometer model UV-2200 at 295 K. HPLC were obtained with a Hitachi HPLC model D-7500 using Cosmosil Packed Column, 5C18-AR (Waters).

Results and Discussion

Structural properties of iron(III) compounds

In Fig. 2, ORTEP drawing of the complex anion $Fe_2O(pac)_2(CO_3)^{2-}$ is shown; the complex has a crystallographic twofold axis passing through O3, C16 and O5 atoms. The dimensions of the (μ-carbonato)(μ-oxo)diiron(III) core and the distorted octahedral geometries around the Fe(III) atom in the complex are very similar to those of the corresponding (nta) complex, $Fe_2O(nta)_2(CO_3)^{4-}$ (Fujita *et al.*, 1994). Two aliphatic amine nitrogen atoms are located at the trans-position of the μ-oxo group. As seen in Table I the Fe–O3(oxo) and Fe–O4(carbonato) bond distances of the (pac)-compound are shorter by 0.002–0.003 than those in the (nta)-complex, and this may be due to higher Lewis acidity of the Fe(III) ion induced by the di-negative (pac)-chelate than by that of tri-negative (nta)-ligand. The spectral properties (positions and intensities of the absorption bands (470–620 nm region), which are characteristic for binuclear structure with μ-oxo bridge) of Fe(III) chelates are essentially the same to each other (Nishida and Ito, 1995b, 1995c) indicating that the iron(III) chelates used in this study are predominantly existing in a dimeric form with a μ-oxo-μ-

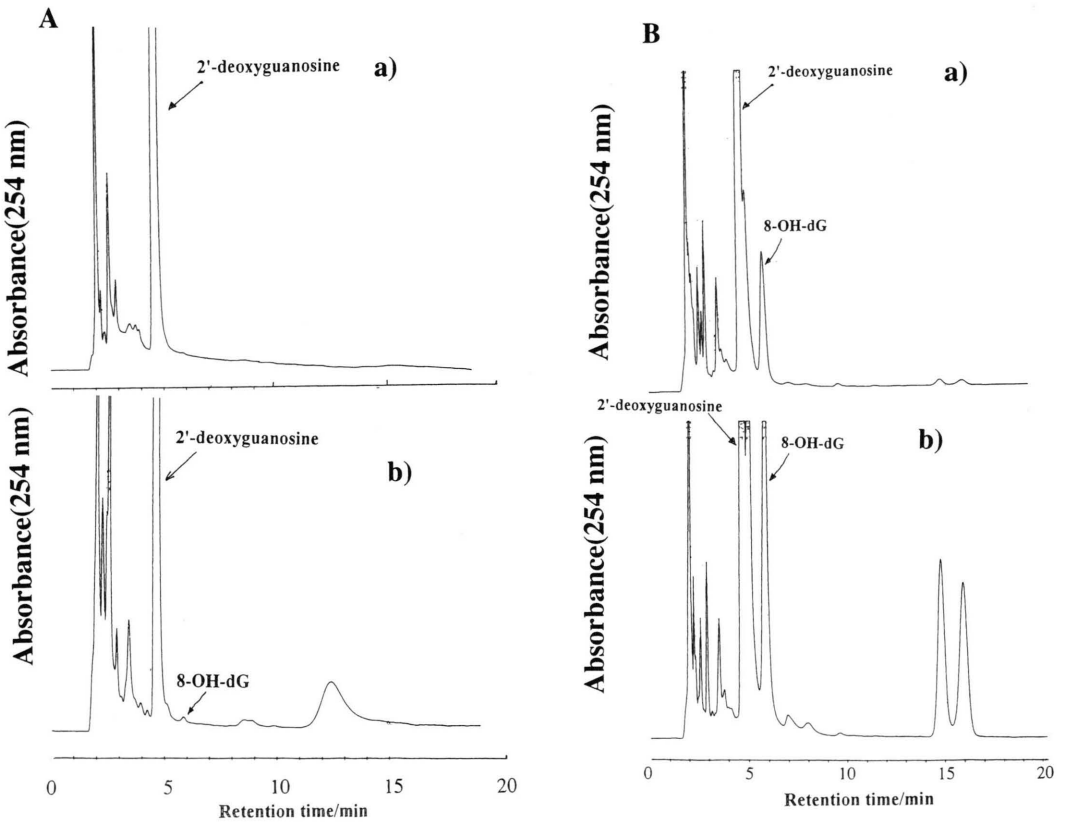


Fig. 3. HPLC of the solution of A) Fe(III)-(pac), 2′-deoxyguanosine (20 mg), and H_2O_2, and B) Fe(III)-(tfda), 2′-deoxyguanosine (20 mg), and H_2O_2. A: a) 2 min after addition of H_2O_2 to solution containing Fe(III)-(pac) and 2′-deoxyguanosine; b) 90 min after addition of H_2O_2. B: a) 2 min after addition of H_2O_2 to the Fe(III)-(tfda)/2′-deoxyguanosine solution; b) 60 min after addition of H_2O_2.

Table I. Comparison of dimensions between Fe(III)-(pac) and (nta) complexes (bond lengths [Å] and bond angles [°]).

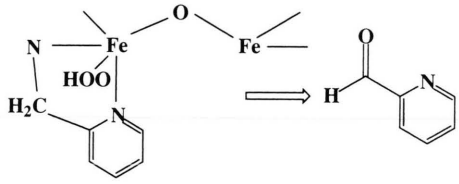
Fe-Complexes	(pac)	(nta)
Fe–Fe	3.190(2)	3.188(1)
Fe–O3(oxo)	1.801(5)	1.830(2)
Fe–O4(carbonato)	1.988(6)	2.005(3)
Fe–O6(carboxylate)	2.057(7)	2.025(3)
Fe–O8(carboxylate)	2.028(7)	2.020(3)
Fe–N10(amine)	2.235(7)	2.246(4)
Fe–N11(pyridine)[a]	2.174(7)	–
Fe–O10(carboxylate)[a]	–	2.082(3)
Fe–O3–Fe	124.6(5)	121.1(2)
O3–Fe–N10(amine)	172.1(4)	175.4(1)
O3–Fe–O4	100.1(3)	98.7(1)
O3–Fe–O6	92.7(3)	95.5(1)
O3–Fe–O8	102.2(2)	103.8(1)
O3–Fe–N11(pyridine)[a]	104.1(3)	–
O3–Fe–O10(carboxylate)[a]	–	102.5(1)
O4–Fe–O6	165.5(3)	165.5(1)
O4–Fe–O8	91.4(3)	88.1(1)
O4–Fe–N10(amine)	87.7(3)	85.6(1)
N10–Fe–N11(pyridine)[a]	76.7(3)	–
N10–Fe–O10(carboxylate)[a]	–	76.4(1)

[a] Nitrogen atom N11(pyridine) in the (pac)-complex corresponds to O10 (carboxylate) of the (nta)-complex.

carbonato unit and the chelates acts as a tetradentate ligands; the coordination of ethereal oxygen atom to an iron(III) atom was already confirmed (Ito *et al.*, 1997).

Formation of 8-OH-dG catalyzed by binuclear iron(III)/hydrogen peroxide system

In Figs 3, 4, and 6, the results of HPLC are illustrated. As shown in Fig. 3, the iron(III) complex with (pac) shows negligible ability to give 8-OH-dG in the presence of 2′-deoxyguanosine and hydrogen peroxide; appearance of the peak at *ca.* 5.8 min is due to formation of 8-OH-dG under our experimental conditions. The new peak observed at ca. 12–13 min is attributed to the formation of pyridine-2-aldehyde, which was verified by the use of the authentic sample, and this may be derived from the oxidative degradation of the ligand system by a peroxide adduct shown below; facile replacement of the carbonato ion in the original compound by peroxide ion has been confirmed in several cases (Nishida and Ito, 1995b).



The Fe(III) compounds with (moda) and (hida) also gave no 8-OH-dG under the same experimental conditions (data not shown). In contrast to this, iron(III) with (tfda) chelate exhibited abnomally

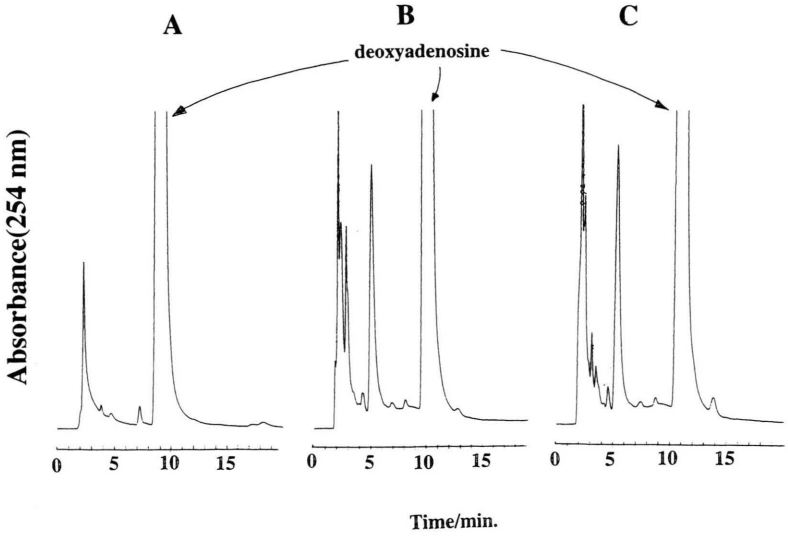


Fig. 4. HPLC of the solution of Fe(III)-(tfda), 2′-deoxyadenosine (20 mg), and H_2O_2. A: Fe(III)-(tfda) and 2′-deoxyadenosine; B: 4 min after addition of H_2O_2 to the Fe(III)-(tfda)/2′-deoxyadenosine solution; C: 120 min after addition of H_2O_2.

high activity for oxygenation of deoxyguanosine, forming much 8-OH-dG (see Fig. 3B). Based on the peak area calculation, it was found that about 6% of the added 2′-deoxyguanosine was converted into 8-OH-dG in the Fe(III)-(tfda)/H_2O_2 system within 2 hours and the order of activity of the complex for hydroxylation of 2′-deoxyguanosine is (tfda) ≫ (eda) > (edda) > (moda), (pac), (hida) ~0. Similar high activity for oxygenation by the Fe(III)-(tfda) complex was also detected for hydroxylation of guanosine(data not shown; in this case 8-hydroxyguanosine (8-OH-G) is formed). Interestingly, this high activity toward oxygenation reaction of nucleosides by the Fe(III)-(tfda) complex/H_2O_2 system is not found for other nucleosides, such as 2′-deoxyadenosine, adenosine, cytidine, or thymidine, etc. (see Fig. 4; in these cases, degradation of nucleoside/or nucleic acid is negligible; HPLC patterns exhibited no change with time). This is demonstrating that the Fe(III)-(tfda)/H_2O_2 system complex can recognize only the guanine moiety among the four nucleic acids. In 1991, Sies *et al.* have reported that singlet oxygen ($^1\Delta_g$) is highly active for hydroxylation of guanine base at 8-position, but a corresponding 8-hydroxy derivative is not formed from deoxyadenosine (Devasagayam *et al.*, 1991). Their results are quite similar to our results described here, and thus indicating that Fe(III)-(tfda)/H_2O_2 system shows similar reactivity towards nucleic acids as that of singlet oxygen ($^1\Delta_g$). According to the theoretical calculations, it is known that guanine base has the highest HOMO among the four DNA nucleic acids, i.e., it is the most readily oxidizable base (Sugiyama and Saito, 1996). The present results and also the fact reported by Sies *et al.* seem to be consistent with the calculated results.

In addition to the peak due to formation of 8-OH-dG, we have observed that another two intense peaks have appeared at 14.8–16 minutes in HPLC of Fe(III)-(tfda)/H_2O_2/2′-deoxyguanosine, and their peak areas have increased with time (see Fig. 3B). According to the measurements by mass spectroscopy (Liquid chromatography mass spectroscopy; LC/MS)), the molecular weights of these two compounds are the same (see Fig. 5), m/z = 366 (positive ion), m/z = 364 (negative ion). We also observed that this component isolated separately from chromatography, is not stable, and decomposes into 2′-deoxyguanosine, demonstrating that these compounds observed at 14.8–16 minutes in Fig. 3B, contain 2′-deoxy-guanosine moiety. Based on these facts, it was assumed that the compound is a Schiff base ($C_{14}H_{19}N_5O_6$; MW = 365; see Scheme 1) derived from 2′-deoxyguanosine and tetrahydrofuran-2-hydroxy-4-aldehyde (see Scheme 2). Tetrahydrofuran-2-hydroxy-4-aldehyde may generate from direct hydroxylation of the ligand system (Nishida *et al.*, 1997) and subsequent oxidative degradation of –N–CH_2– bond of (tfda) ligand (see Scheme 2); the latter process may be supported by formation of pyridine-2-aldehyde as described in the Fe(III)-(pac)/H_2O_2 system. The presence of two peaks in

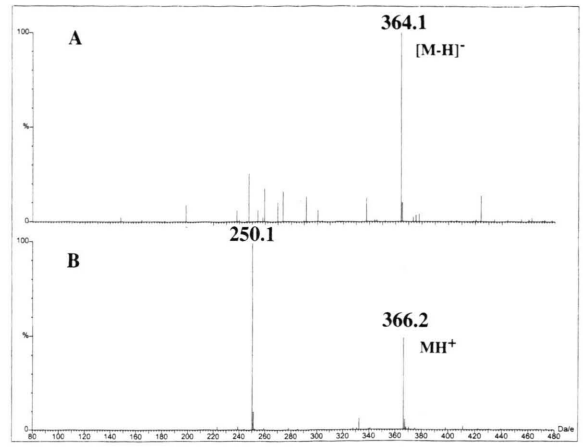
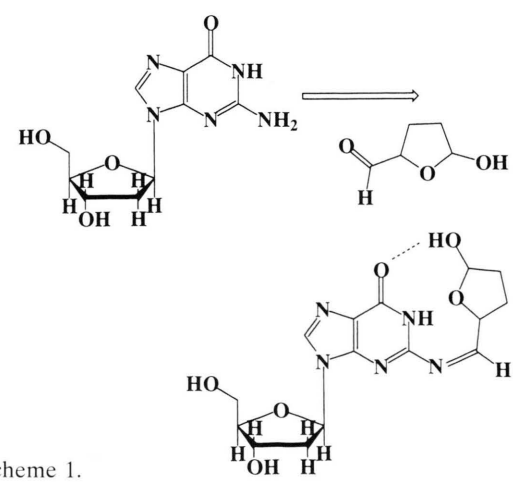
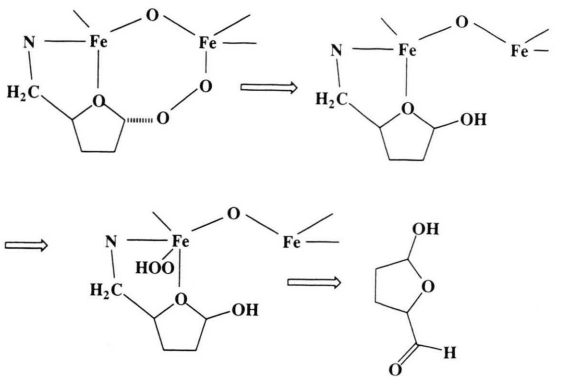


Fig. 5. Mass specrtra of the compound of the peak at 14.8 min (see Fig. 3B). A: negative pattern; B: positive pattern.



Scheme 1.

the range 14.8–16 minute may be due to keto-enol isomerism of the Schiff base in Scheme 1.



Scheme 2.

In the case of the (eda)-complex, the formation of 8-OH-dG is much low compared to that of the (tfda)-complex (see Fig. 6). But in this case, another different compounds formed in the reaction course. As seen in Fig. 6, the increase of the peak intensities at 3.6 and 4.6 min. is noteworthy. The peak at 4.6 should be attributed to the formation of guanosine, which was confirmed by the authentic sample. The molecular weight of compound at 3.5 min. was shown to be m/z = 284 (positive ion), indicating the one oxygen atom is incorporated into sugar moiety of 2′-deoxyguanosine(4′- or 1′-position) , similar to the formation of guanosine at 4.6 min. These are suggesting that Fe(III)-(eda) compound promotes the incorporation of oxygen atom into sugar moiety of the deoxyguanosine in the presence of hydrogen peroxide.

Active species for formation of 8-OH-dG

Until now, it has been proposed that formation of 8-OH-dG in the biological systems occurs via OH· radicals (Floyd *et al.*, 1988). This mechanism, however cannot be applied to the present case, because it is well known that OH· attacks adenine, cytosine and also 2′-deoxyguanosine, to give oxidized products (Hiraoka *et al.*, 1990; Vieira and Steenken, 1990). This is highly inconsistent with our present results, i.e., Fe(III)-(tfda)/H_2O_2 system attacks guanine moiety only, and not attack on adenine or other nucleic acids as described before.

By the addition of hydrogen peroxide to the binuclear μ-oxo-μ-carbonato-diiron(III) complex solutions, there may be a formation of the peroxide adducts, as shown below. Formation of **Adduct I** has been confirmed for the Fe(III)-(nta) solution (Nishida and Ito, 1995b, 1995c).

It is quite apparent that a different peroxide adduct formation from **Adduct I** should occur in the

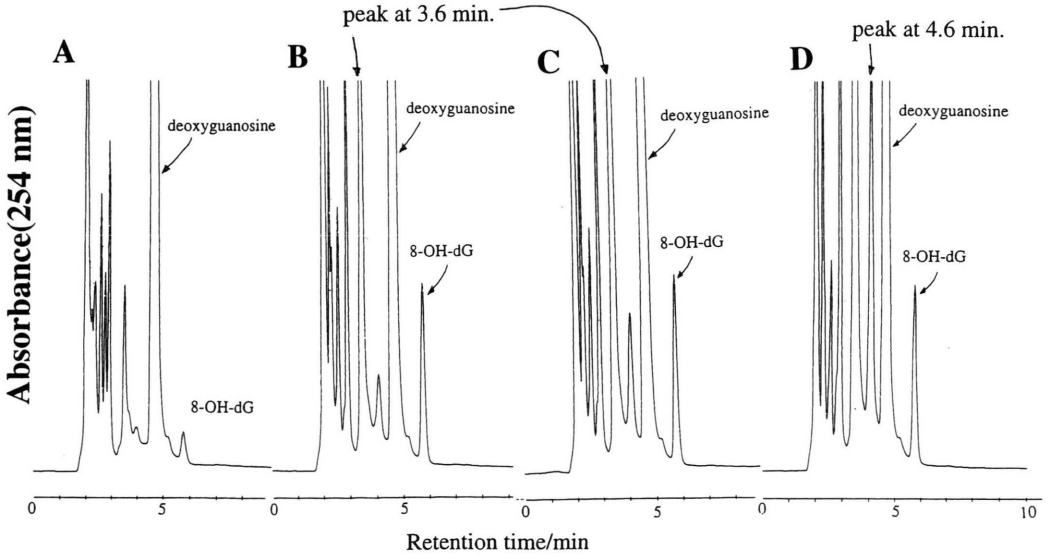
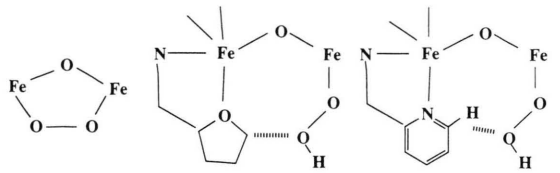
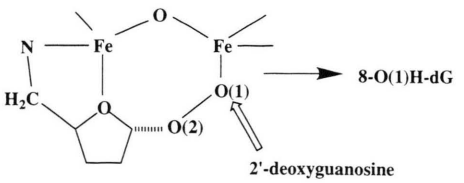


Fig. 6. HPLC of the solution of Fe(III)-(eda), 2′-deoxyguanosine and H_2O_2. A: 2 minutes after addition of H_2O_2 to Fe(III)-(eda) and 2′-deoxyguanosine; B: 30 minutes after addition of H_2O_2; C: 60 minutes after addition of H_2O_2; D: 150 minutes after addition of H_2O_2.



solution of Fe(III)-(tfda) /H_2O_2 system. The absorption spectra of the solution containing hydrogen peroxide (ratio of $(H_2O_2)/(Fe^{3+}) = 1$, is the same to the cases of HPLC experiments, see Experimental section) are essentially the same as those without hydrogen peroxide(data not shown), supporting the presence of a species with bent μ-oxo diiron(III) core in solution. In our previous paper (Ito *et al.*, 1997; Nishida *et al.*, 1997) we have reported that the presence of tetrahydrofuran ring can activate the peroxide adduct of Fe(III) with η^1-coordination mode through the electronic interaction between them (see **Adduct II**), but this situation is unfavorable for the cases of pyridine (see **Adduct III**), imidazole, and morphorin rings, because of the electronic and steric reasons (Ito *et al.*, 1997). Based on these facts and discussion, we would like to propose that an electrophilic peroxide adduct of **Adduct II** forms in the Fe(III)-(tfda)/H_2O_2 system, where the peroxide ion is more activated through electronic interaction with tetrahydrofuran ring of the (tfda)-ligand, and hydroxylation reaction proceeds at O(1) atom through concerted heterolytic O–O bond cleavage as shown below (Ito *et al.*, 1997; Nishida *et al.*, 1997).



If 2′-deoxyguanosine was not added to the solution, the green solution turned to be clear after one day with precipitation of iron(III) hydroxide. However, when substrate, for example 2′-deoxyguanosine or cyclohexane, is present in the solution, the solution exists as a green state several days. This suggest that ligand degradation through hydroxylation at 2-position of the tetrahydrofuran ring (incorporation of O(2) atom into the tetrahydrofuran ring) may cause the production of iron(III) hydroxide, and the presence of substrate prevents the hydroxylation of the tetrahydrofuran ring, because the substrate itself is hydroxylated instead of the ligand system as shown above. The same situation, i.e., interaction between C–H bond and hydroperoxide adduct, may be possible for the Fe(III)-(eda) complex (Nishida *et al.*, 1997). In this case, however the steric requirement of the ligand system may induce the approach of the sugar ring to the oxygen atom of the peroxide ion, leading to facile oxygenation at the carbon atoms of the sugar ring.

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