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STRUCTURES AND REACTIVITIES OF SEVERAL IRON(III) COMPLEXES IN THE PRESENCE OF HYDROGEN PEROXIDE: RELEVANCE TO INDUCTION OF TISSUE DAMAGE CAUSED BY IRON(III) CHELATES IN RATS

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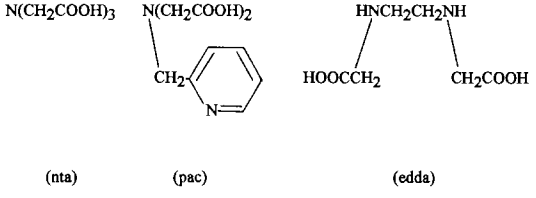
Abstract—Although the structural features of iron(III) complexes with nitrilotriacetic acid (nta), ethylenediamine-*N*,*N*′-diacetic acid (edda) and 2-aminomethyl-pyridine-*N*,*N*-diacetic acid (pac) are very similar, the reactivity of the iron(III) chelate in the presence of hydrogen peroxide is quite different in each case; the Fe^{III}–nta/H_2O_2 system is highly active for the degradation of deoxyribose, and the Fe^{III}–edda/H_2O_2 system is active for the hydroxylation reaction of 2′-deoxyguanosine, but Fe^{III}–pac/H_2O_2 is completely inactive for both reactions. The above facts have been elucidated in terms of the structural characteristics of the peroxide adducts, and the origin of the severe tissue damage caused by the iron(III)–nta complex in rats is discussed based on the present results.

Iron carries out a wide range of biological functions; it is responsible for the transport of dioxygen and the activation of both molecular nitrogen and dioxygen (nitrogenase and a variety of oxygenases).[1] However, iron ions participate in the conversion of molecular oxygen to highly reactive and damaging species, such as hydroxyl radical, which are believed to form by Fenton or Fenton-like chemistry.[2] Iron sequestration, i.e. chelating iron in inactive form, has been considered to be one of the most important ways to inhibit the damage by the reactive species, and successful examples for the above purpose are the uses of desferrioxamine and several steroids.[3–5]

Unfortunately, not all iron compounds act as inhibitors for formation of reactive oxygen species. Some of them are more active promoters for the formation of dangerous species than the ferric ion itself. One of the most famous examples is ferric nitrilotriacetate (Fe^{III}–nta), which causes acute renal proximal tubular necrosis[6–10] and renal adenocarcinoma.[11] Very recently, Liu and Okada[12] have investigated the effects of several iron(III) chelates in rats, and observed that tissue damage by the administration of the iron(III) compounds is highly dependent on the chelates used (see Table 1). It should be noted here that the tissue damage by the Fe^{III}–pac chelate (pac = 2-amimomethylpyridine-*N*,*N*-diacetic acid; see structures) is almost negligible,[13] the chemical structure of the pac being very similar to that of the nta ligand.



(nta) (pac) (edda)

In order to elucidate the result shown in Table 1, we have determined the crystal structures of two iron(III) compounds, Fe^{III}–nta and Fe^{III}–pac,[13] and reported that the structural features of the two compounds, which consist of a dimeric unit with μ-oxo-μ-carbonato bridge, are very similar to each other.[13,14] This suggests that a more detailed study on the chemical reactivity of these iron(III) chelates

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Table 1. Tissue damage by iron(III) chelates [administration: 10 mg Fe/kg (weight) in Wistar rat]

Fe^{III}–nta	renal proximal-tubular cell death, severe
Fe^{III}–edda	renal proximal-tubular cell death, mild to moderate
Fe^{III}–pac	no cell death

is necessary. Thus, in this study we have investigated and compared the chemical reactivity of the iron(III) complexes with nta, edda (ethylenediamine-*N*,*N*′-diacetic acid), and pac, attempting to clarify the origin of the severe tissue damage caused by the Fe^{III}–nta chelate.

EXPERIMENTAL

Preparation of solution of iron(III) chelate

The pH of the solution containing ferric chloride, $FeCl_3 \cdot 6H_2O$ (270 mg, 0.001 mol) and the chelate (0.001 mol) was adjusted to be 7.0 by the addition of $KHCO_3$; the concentration of the solution is 1/50 M for iron(III) ion if the volume of the solution is diluted to 50 cm^3.

Preparation of crystal of iron(III)–pac chelate

The iron(III) compound with pac was obtained as crystals in this study. The crude crystals, which were obtained by adding ethanol to the Fe^{III}–pac solution neutralized by Cs_2CO_3, were recrystallized from an ethanol–water mixture. Found: C, 24.6; H, 3.5; N, 5.2. Calc. for $Cs_2Fe_2O(pac)_2(CO_3) \cdot 7H_2O$: C, 24.6; H, 3.4; N, 5.5%. Unfortunately, we could not obtain crystals of Fe^{III}–edda suitable for X-ray analysis.

Evaluation of activity for peroxidation of linolenic acid by the iron(III) chelates

Aqueous iron(III) chelate solution (5 cm^3 of 1/20 M) and linolenic acid solution [2 cm^3 of ethanol solution (80 cm^3) containing 1 cm^3 of linolenic acid] were mixed, and the formation of 2-thiobarbituric acid-reactive substance (TBARS)[15] was evaluated by measuring the absorbance at 532 nm of the solution treated in the usual way as described in the literature.[16]

Evaluation of activity for degradation of 2′-deoxyribose by iron(III) chelate in the presence of hydrogen peroxide

To an aqueous solution of iron(III) chelate (50 cm^3, 1/50 M) containing 2′-deoxyribose (20 mg) was added hydrogen peroxide solution (10 cm^3, 1/10 M solution), and the resulting solution was immediately treated using the TBARS method as described above.[15] The degradation of ribose can be detected by measuring the content of TBARS in the solution.[9]

Evaluation of activity for hydroxylation at the 8-position of deoxyguanosine by iron(III) chelate in the presence of hydrogen peroxide

To an aqueous solution of iron(III) chelate (50 cm^3, 1/50 M) containing 2′-deoxyguanosine (20 mg) was added hydrogen peroxide solution (10 cm^3, 1/10 M), and the formation of 8-hydroxy deoxyguanosine (8-OH-dG) was detected in terms of HPLC.[17]

Evaluation of activity of iron(III) chelate towards 2,2,6,6-tetramethyl-4-piperidinol[18] *in the presence of hydrogen peroxide*

In this case, the iron(III) solution was prepared by the use of a crystalline sample and the solvent was water–methanol (1:1). The time dependence of the ESR spectra was measured for the solution mixture of iron(III) chelate (1 cm^3, 1/500 M), piperidinol (1 cm^3, 1/2.5 M) and hydrogen peroxide (2 cm^3, 1/10 M) at 295 K.

Instruments

Absorption spectra were measured with a Shimadzu spectrophotometer model UV-2200 at 295 K. ESR spectra were obtained with a JEOL ESR apparatus model RE-2X by using an X-band at 295 K. HPLC was recorded with a Hitachi High Performance Liquid Chromatograph at room temperature.

RESULTS AND DISCUSSION

Absorption spectra of iron(III) chelates

In Figs 1 and 2, the absorption spectra of an aqueous solution (pH 7.0) of iron(III) chelates are shown. These spectra are specific in the sense that there are two bands around the 600–700 and 450–500 nm regions. The structures of Fe^{III}–nta and Fe^{III}–pac have been determined to be of a dimeric

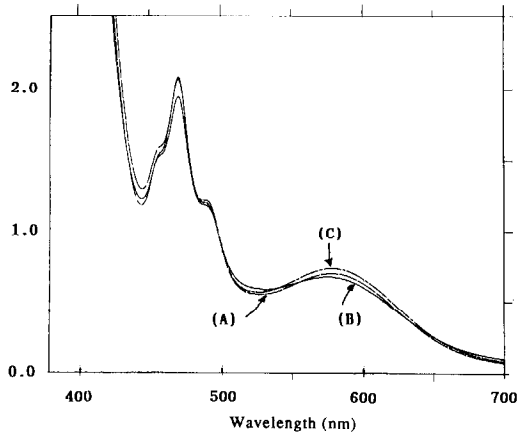


Fig. 1. Spectral data of Fe^{III}–nta chelate (in water, pH 7.0). (A) Fe^{III}–nta solution ($[Fe^{3+}] = 1/60$ M). (B) Fe^{III}–nta solution containing H_2O_2, measured immediately after addition of H_2O_2 ($[Fe^{3+}] = 1/60$ M, $[H_2O_2] = 1/60$ M). (C) Fe^{III}–nta solution containing H_2O_2, measured 15 min after addition of H_2O_2.

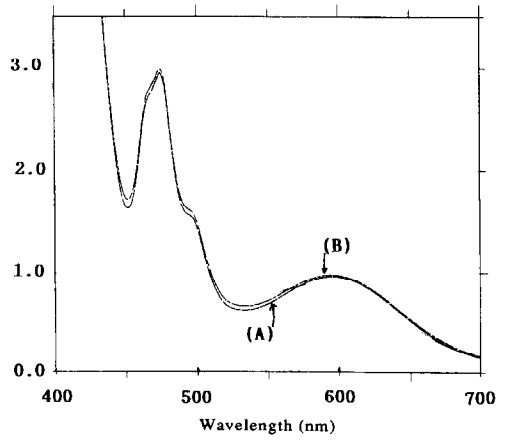


Fig. 2. Spectral data of Fe^{III}–pac chelate (in water, pH 7.0). (A) Fe^{III}–pac solution ($[Fe^{3+}] = 1/60$ M). (B) Fe^{III}–pac solution containing H_2O_2 ($[Fe^{3+}] = 1/60$ M, $[H_2O_2] = 1/60$ M).

nature with a μ-oxo-μ-carbonato bridge.[13,14] According to the discussion by Que and True,[19] these two absorption bands are characteristic of binuclear iron(III) compounds with an oxo and another bridging group, such as carbonato or acetate ions, and are assigned to the charge transfer bands from the oxo oxygen atom to an iron(III) ion. Although the crystal structure determination was not performed for the Fe^{III}–edda complex, we can estimate that this complex also has a dimeric structure with oxo and carbonato bridges, on the basis of the absorption spectrum shown in Fig. 3. As the spectral data of the solid sample are exactly the same as that of the solution spectra,[20] it seems

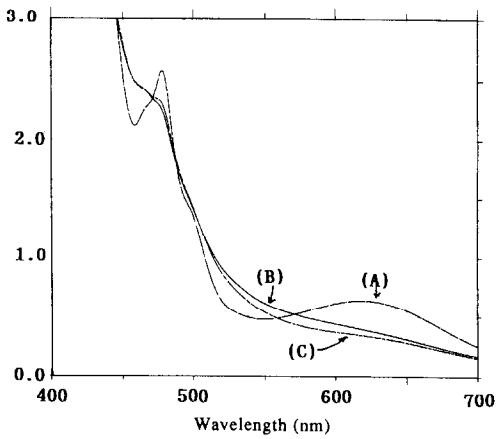
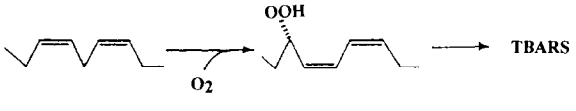


Fig. 3. Spectral data of Fe^{III}–edda chelate (in water, pH 7.0). (A) Fe^{III}–edda solution ($[Fe^{3+}] = 1/60$ M). (B) Fe^{III}–edda solution containing H_2O_2, measured immediately after addition of H_2O_2 ($[Fe^{3+}] = 1/60$ M, $[H_2O_2] = 1/60$ M). (C) Fe^{III}–edda solution containing H_2O_2, measured 15 min after addition of H_2O_2 ($[Fe^{3+}] = 1/60$ M, $[H_2O_2] = 1/60$ M).

reasonable to assume that a dimeric iron(III) species exists in aqueous solution.

Relationship between structure of iron(III) chelate and tissue damage

As shown in Table 1, it is noteworthy that the tissue damage caused by the iron(III) chelates in the Wistar rats is highly dependent on the chelate used.[12] Since the structural features of the Fe^{III}–nta and Fe^{III}–pac compounds are essentially the same,[13,14] there should be an another origin for the severe tissue damage observed for the Fe^{III}–nta system. According to Okada[12] and Uemura *et al.*,[21] one of the most prominent effects of the administration of Fe^{III}–nta chelate to animals is the increase of TBARS in the kidney, whereas formation of TBARS is negligible in the case of the Fe^{III}–pac complex, and it should also be noted that severe tissue damage by the Fe^{III}–nta complex is observed only in places where the formation of TBARS is detected.[12] We have already reported that some binuclear iron(III) complexes can catalyse the peroxidation reaction of unsaturated fatty acids, leading to the formation of TBARS (see Scheme 1).



Scheme 1.

In Fig. 4, the time dependence of TBARS formation in the reaction mixture of linolenic acid and the binuclear iron(III) chelate is shown; the results indicate that all the binuclear iron(III) chelates used in this study can catalyse the peroxidation of linolenic acid. Thus, the formation of TBARS observed in rats by the injection of Fe^{III}–nta solution should not be due to the lipoxygenase-like function of the Fe^{III}–nta chelate added, because no formation of TBARS was observed in the case of Fe^{III}–pac solution *in vivo*.

Generally, three reasons have been considered for the origin of the formation of TBARS *in vivo*:

(a) decomposition product of oxidized unsaturated fatty acids;[9]

(b) decomposition product of sugars such as ribose;[22]

(c) decomposition product of nucleic acids.[22]

As we have shown it to be very unlikely that a binuclear iron(III) species reacts directly with substrates such as sugar or nucleic acid to give TBARS, the experimental facts observed by Okada[12] and Uemura *et al.*[21] clearly indicate that there should be another important chemical compound which induces the formation of TBARS in the presence of Fe^{III}–nta *in vivo*. Since it is known that Fe^{III}–nta can decompose DNA in the presence of hydrogen peroxide,[23] we next consider the role of hydrogen peroxide for the formation of TBARS in the presence of an iron(III) chelate.

Decomposition of ribose in the presence of hydrogen peroxide and iron(III) *chelate*

As shown in Fig. 5, the Fe^{III}–nta/hydrogen peroxide system is very specific for the formation of

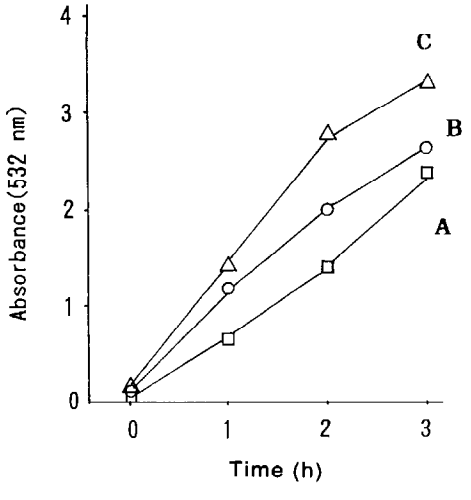


Fig. 4. Time dependence of absorbance at 532 nm of the solution containing iron(III) chelate and linolenic acid, treated with 2-thiobarbituric acid (*cf.* Experimental section). (A) Fe^{III}–nta. (B) Fe^{III}–pac. (C) Fe^{III}–edda.

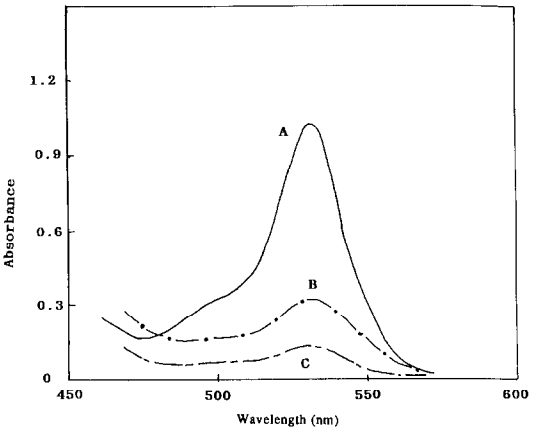
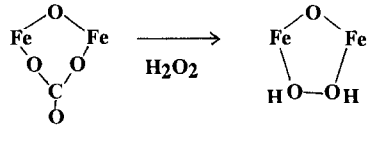


Fig. 5. Absorbance at 532 nm of solution containing iron(III) chelate, hydrogen peroxide and 2′-deoxyribose, treated with 2-thiobarbituric acid. (A) Fe^{III}–nta. (B) Fe^{III}–edda. (C) Fe^{III}–pac.

TBARS in the presence of 2′-deoxyribose, since the absorbance at 532 nm of the solution treated by TBA is the highest. The absorbance at 532 nm of the TBA-treated solutions of Fe^{III}–edda and Fe^{III}–pac systems is much smaller than that of the Fe^{III}–nta system, demonstrating that the former two systems are almost inactive for the degradation of 2′-deoxyribose in the presence of hydrogen peroxide. This result is quite consistent with the result in Table 1. At present, many experimental facts support the above conclusion, i.e. that tissue damage by the iron(III) chelates in rats is closely related to the activity of the iron(III) chelate towards degradation of ribose in the presence of hydrogen peroxide.[24]

As shown in Figs 1 and 2, the absorption spectral change due to the addition of hydrogen peroxide is negligible in the case of the Fe^{III}–nta and Fe^{III}–pac systems. This suggests that the structure of the original binuclear unit with double bridges remains unchanged in the presence of hydrogen peroxide. In order to explain the high reactivity of the Fe^{III}–nta/H_2O_2 system, we have considered the formation of a peroxide adduct (see Scheme 2), whose structural features are essentially the same as those of the original one, and thus the result in Fig. 1 may be reasonably explained.



Scheme 2.

In the case of the Fe^{III}–pac system, we may assume that no displacement reaction between carbonato ion and hydrogen peroxide proceeds, the reason being developed in the later section.

In order to investigate the reactivity of the peroxide adduct illustrated in Scheme 2, we have examined the reaction between Fe^{III}–chelate/H_2O_2 system and 2,2,6,6-tetramethyl-4-piperidinol (hereafter abbreviated as TMPN), a spin trapping reagent for singlet oxygen ($^1\Delta_g$).[18] In previous papers, we have reported that the peroxide ion in the metal–peroxide adduct sometimes acts as singlet oxygen, exhibiting high reactivity towards singlet oxygen scavengers, such as tetraphenylcyclopentadienone or 1,3-diphenylisobenzofuran.[25] In this study, we first examined the reaction between TMPN and the peroxide adduct of the molybdenum(VI) compound[26] (shown below), and found that an increase of ESR signal strength due to the nitron radical (see Scheme 3) was observed in the reaction mixture (not shown).

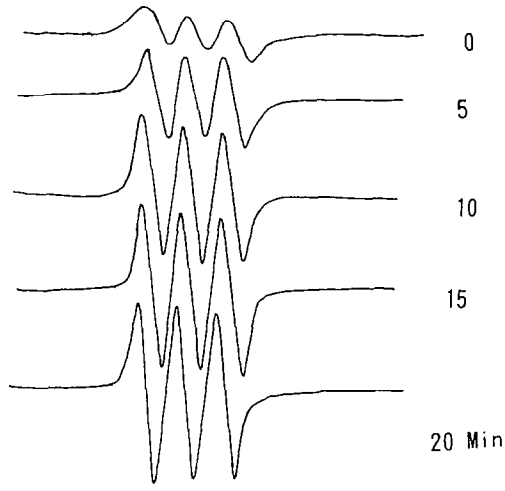
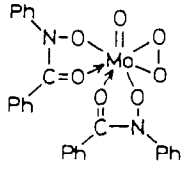
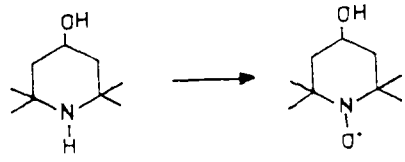


Fig. 6. ESR spectra (X-band, 295 K) of solution containing Fe^{III}–nta, H_2O_2 and 2,2,6,6-tetramethyl-4-piperidinol. The signal is due to the nitron radical illustrated in Scheme 3.



Mo(VI)-peroxide adduct



Scheme 3.

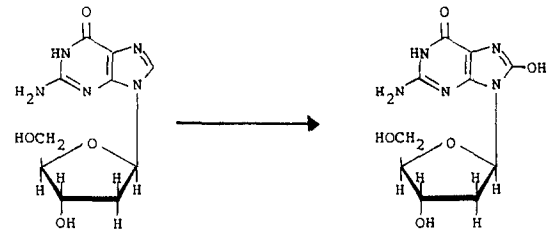
As shown in Fig. 6, the increase in formation of the nitron radical is also observed in the solution containing TMPN, Fe^{III}–nta complex and hydrogen peroxide, whereas no formation of the radical was observed in the mixture of Fe^{III}–pac and hydrogen peroxide (not shown). These are consistent with the previous assumption that peroxide adduct formation occurs in the solution of Fe^{III}–nta, but not for the Fe^{III}–pac solution. At present, the mechanism for formation of the nitron radical in the TMPN and Fe^{III}–nta/H_2O_2 systems is not clear; however, this method seems useful to confirm the formation of the peroxide adduct of metal complexes in solution. In the Fe^{III}–nta/H_2O_2 system, the formation of hydroxyl radical has been reported by the use of DMPO, a spin trapping reagent for ·OH,[12,27] but this method is questionable for detecting the hydroxyl radical, as pointed out in the previous paper.[28]

Relationship between peroxide adduct formation and structural features of the iron(III) *chelates*

In Table 2, the structural parameters of the two compounds $Fe_2O(CO_3)(nta)_2^{4-}$ and $Fe_2O(CO_3)(pac)_2^{-}$ are summarized. According to the general discussion, it is estimated that the Lewis acidity of an iron(III) ion in the Fe^{III}–nta complex is lower than that in the Fe^{III}–pac one, because nta coordinates to the iron(III) atom as a tri-negative anion. This is supported by the fact that bond distances of Fe—O, oxo-oxygen atom and carbonato ion are shorter in the Fe^{III}–pac compound than those in Fe^{III}–nta. This demonstrates that the Fe—O bonding of the carbonato ion is weaker in the Fe^{III}–nta complex than that in the Fe^{III}–pac complex, which may support our assumption that formation of a peroxide adduct occurs in the Fe^{III}–nta solution, but not for the Fe^{III}–pac compound.

Table 2. Comparison of selected bond lengths (Å) of the compounds[13] $Fe_2O(CO_3)(nta)_2^{4-}$ and $Fe_2O(CO_3)(pac)_2^{2-}$

	Fe^{III}–nta	Fe^{III}–pac
Fe—Fe	3.188	3.186
Fe—N(aliphatic amine)	2.246	2.235
Fe—O(oxo-oxygen)	1.830	1.800
Fe—O(carbonato ion)	2.004	1.984
Fe—O(carboxylate)	2.025	2.061
Fe—O(carboxylate)	2.020	2.020
Fe—O(carboxylate)	2.082	
Fe—N(pyridine)		2.166



Scheme 4.

Iron(III)–edda and hydrogen peroxide system

This system is clearly different from the Fe^{III}–nta and Fe^{III}–pac systems. It should be noted that a drastic change in the absorption spectrum occurs by the addition of hydrogen peroxide, as shown in Fig. 3. This indicates that a remarkable structural change in the binuclear unit with double bridges proceeds in the presence of hydrogen peroxide. The formation of TBARS was also observed in the initial stage (see Fig. 5); however, the TBARS disappeared after 1 h. As shown in Fig. 7, the Fe^{III}–edda/H_2O_2 system can catalyse the hydroxylation reaction at the 8-position of 2′-deoxyguanosine, forming 8-OH-dG (see Scheme 4), the latter compound being known as a suitable marker for measuring the level of oxidative DNA damage *in vitro* and *in vivo*.[21]

The above result indicates that the formation of a peroxide adduct should occur, which must be different from that assumed for the Fe^{III}–nta/H_2O_2 system. Based on the absorption spectral data, we have proposed that the following structural change may occur in the Fe^{III}–edda/H_2O_2 system (see Scheme 5), and the final product should be the adduct **II**, which is similar to that estimated for oxy-haemerythrin.[29] In this adduct, two iron atoms are bridged by one oxo-oxygen atom. In order to confirm the above discussion, we have investigated the reactivity of a binuclear iron(III) compound with Hedta, whose structure has been determined to be a binuclear complex with a single oxo-bridge.[30] The result in Fig. 8, showing that Fe^{III}–Hedta can also catalyse the formation of 8-OH-dG in the presence of hydrogen peroxide, may support the assumption described for the Fe^{III}–edda/H_2O_2 system. No formation of 8-OH-dG was detected in the Fe^{III}–pac/H_2O_2/2′-deoxyguanosine solution, also consistent with the discussion described before. As the activity of the Fe^{III}–Hedta/H_2O_2 system for degradation of 2′-deoxyribose is very low, which is very similar to those of the Fe^{III}–edda and Fe^{III}–pac systems, it seems likely that the chemical reactivities of two peroxide adducts, adduct **I** and adduct **II** in Scheme 5, may differ; only the adduct **I**, which is assumed for the Fe^{III}–nta/H_2O_2 system, is active for degradation of deoxyribose. The short life-time of

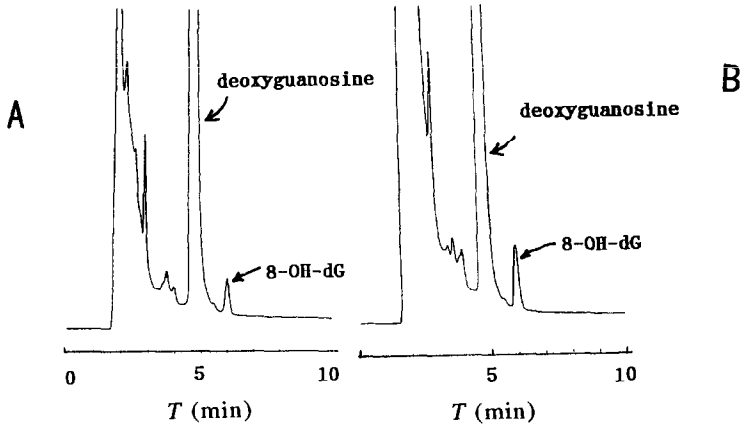


Fig. 7. HPLC of the solution containing Fe^{III}–edda, H_2O_2 and 2′-deoxyguanosine (see Experimental section). (A) After 3 min..(B) After 15 min.

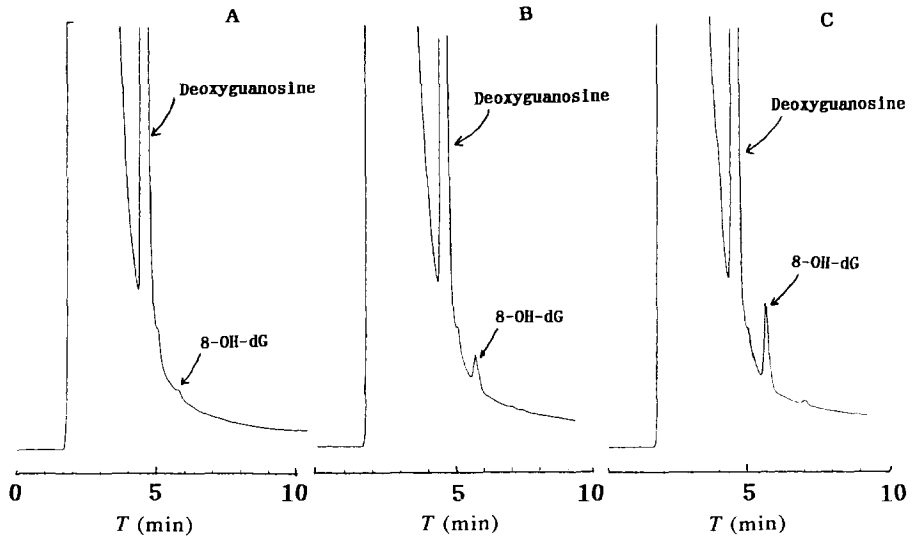
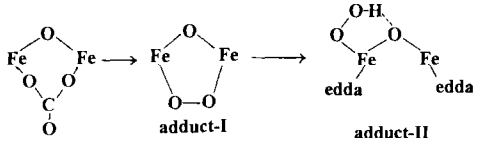


Fig. 8. HPLC of the solution containing Fe^{III}–Hedta, H_2O_2 and 2′-deoxyguanosine (see Experimental section). (A) After 20 min. (B) After 100 min. (C) After 200 min.



Scheme 5.

the adduct I in the Fe^{III}–edda/H_2O_2 system may explain the lower activity of this system for the degradation of deoxyribose, and may also elucidate the negligible tissue damage by the administration of Fe^{III}–edda solution in rats as shown in Table 2. This consideration may be consistent with the fact that the Fe^{III}–nta/H_2O_2 system is highly active for the cleavage of DNA, but Fe^{III}–Hedta/H_2O_2 system is almost inactive.[23]

Origin of severe tissue damage caused by the iron(III)–nta complex

Based on the above facts and discussion, we may conclude that severe tissue damage in rats is due to the presence of a dimeric Fe^{III}–nta species and hydrogen peroxide *in vivo*, and the reactive oxygen species should be a peroxide adduct of the iron(III) complex, which is highly reactive for degradation of sugars such as deoxyribose or mannitol.

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