

Comparison on Reactivity of Fe(III) and Al(III) Compounds in the Presence of Hydrogen Peroxide: Its Relevance to Possible Origin for Central Nervous System Toxicity by Aluminum Ion

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Iron(III), Aluminum(III), Hydrogen Peroxide

The iron(III) compounds with several aminocarboxylate chelates containing an aryl or furan substituent exhibit high activity in enhancement of the reactivity of hydrogen peroxide, leading to facile hydroxylation at benzene ring, and to degradation of furan ring, but no such activity was observed for the corresponding Al(III) compounds. These results were interpreted in terms of the molecular orbital consideration, and lack of the activity of the Al(III) complexes was attributed to lack of electrophilic nature of the peroxide adduct due to the absence of a d-orbital; this may explain the fact that there were no tumors in Al-NTA(nitri-lotriacetic acid)-treated rats. Based on the facts observed in this study, the decreased function of iron(III) ions for synthesizing neurotransmitters in the brain was assumed to be one of the possible origin for the neurotoxicity by injection of the Al(III) salts *in vivo*.

Introduction

There is a hypothesis that an elevated increase of cancer is associated with chronic iron overload. (Uchida *et al.*, 1995). In fact, the risk for primary hepatocellular carcinoma in idiopathic hemochromatosis is more than 200 times greater than that of the control population. Awai *et al.* (1979) originally developed an experimental model of iron overload using ferric ion chelated with nitrilotriacetate (NTA, see Scheme 1). Intraperitoneal injections of Fe-NTA induced significant iron deposition in rat hepatic cells (Uchida *et al.*, 1995). Repeated intraperitoneal injections of Fe-NTA were reported to induce acute and subacute renal proximal tubular necrosis and a subsequent high incidence of renal adenocarcinoma in male rats and mice (Li *et al.*, 1987).

In 1984, Okada *et al.* examined Wistar male rats for nephrotoxicity and carcinogenicity after administration of Al-NTA, and found that there were no tumors in Al-NTA-treated rats, but injection of Al(III)-NTA gave central nervous system toxicity (Ebina *et al.*, 1984). Aluminum(III) ion is well known to be neurotoxic at present, although the molecular basis of its toxicity is unknown (Itzhaki, 1994). The possible involvement of alumi-

num in Alzheimer's Disease (AD) pathogenesis was proposed because of its neurotoxicity and because rabbits injected intracerebrally with aluminum salts were found to display neurofibrillary degeneration, impaired memory, and lower capacity for learning. Gutteridge *et al.* have reported that aluminum(III) salts does not themselves stimulate peroxidation of ox-brain liposomes, but they greatly accerelate the peroxidation induced by iron(II) salts at acidic pH values, and also that desferioxamine decreases the peroxidation reaction (Gutteridge *et al.*, 1985).

Because the solution chemistries of aluminum and iron are very similar (Jyo *et al.*, 1990), the origin for the above facts remains unknown at present. In this study, we will show that the reactivity of several Fe(III) compounds with amino-carboxylates in the presence of hydrogen peroxide is completely different from that of the corresponding Al(III) compounds, and will elucidate origin for the above facts. Based on these facts, we will propose one of the possible origin for Alzheimer's Disease by accumulation of aluminum ion in the brain.

Materials and Methods

Preparation of Al(III)-NTA complex

The pH of an aqueous solution (100 ml) containing aluminum chloride hexahydrate (2.41 g)

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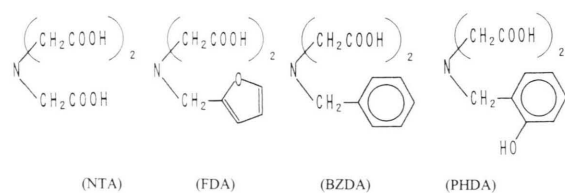
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and nitrilotriacetic acid (2.00 g) was adjusted to 7.0 by Cs_2CO_3 , and the resulting solution was evaporated to 20 ml. The white precipitates obtained by adding ethanol to the solution were recrystallized from an ethanol/water (=1/1) mixture. Found: C 16.32; H 2.34; N 2.84%. Calcd for $\text{Cs}_3\text{Al}_2(\text{OH})(\text{CO}_3)(\text{NTA})_2 \cdot 3\text{H}_2\text{O}$: C 16.27; H 1.99; N 2.92%. By the same method, the corresponding potassium and rubidium salts were obtained as white needles. Found: C 25.32; H 2.08; N 4.45%. Calcd for $\text{K}_3\text{Al}_2(\text{OH})(\text{CO}_3)(\text{NTA})_2$: C 25.00; H 2.10; N 4.49%. Found: C 18.51; H 2.58; N 3.14%. Calcd for $\text{Rb}_3\text{Al}_2(\text{OH})(\text{CO}_3)(\text{NTA})_2 \cdot 4\text{H}_2\text{O}$: C 18.68; H 2.53; N 3.35%.

TBA(2-thiobarbituric acid) assay for degradation of 2'-deoxyribose by Fe(III)-NTA and Al(III)-NTA compounds in the presence of hydrogen peroxide

To an aqueous solution (20 ml) containing metal(III) chloride (0.001 mol) and NTA (200 mg) neutralized by KHCO_3 (pH=7.0) was added 2'-deoxyribose (50 mg) and hydrogen peroxide solution (0.1 M solution, 10 ml), and the resulted solution (4 ml) was treated immediately by dilute hydrochloric acid solution (1N, 1 ml) containing TBA (2-thiobarbituric acid, 20 mg) and heated to 90 °C for 15 min, and the absorbance at 532 nm of the solution was recorded (Nishida and Yamada, 1990).

By the same ways, the TBA-assays for the Fe(III)-FDA (2-aminomethylfurfurylamine-N,N-diacetic acid, see Scheme 1) and Al(III)-FDA were examined in the presence of hydrogen peroxide; in this case, 2'-deoxyribose is absent in the solutions. A solution containing metal chloride (M=Fe(III) and Al(III)) and FDA was treated by hydrogen peroxide, and TBA as described above; concentrations of Fe(III) and Al(III) ions are 0.01 and 0.02 M, respectively.



Scheme-1

Formation of 8-OH-dG in the reaction mixture of metal-NTA compound and 2'-deoxyguanosine in the presence of hydrogen peroxide

To an aqueous solution (50 ml) containing metal chloride (0.001 mol) and NTA (200 mg, 0.001 mol) neutralized by KHCO_3 (pH=7.0) was added 2'-deoxyguanosine (20 mg), and hydrogen peroxide (0.1 M solution, 10 ml), and the formation of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) was determined by HPLC (Kasai and Nishimura, 1984).

Reactivity of hydrogen peroxide in the presence of metal compounds with benzylamine-N,N-diacetic acid

Benzylamine N,N-diacetic acid (BZDA, see Scheme 1) was obtained by the usual method from benzylamine and monochloroacetic acid. *o*-hydroxyphenylglycine was prepared as follows; to a methanol solution containing equimolar of sodium glycinate and salicylaldehyde was added sodium borohydride, and the resulted solution was acidified by dilute hydrogen chloride solution. The white precipitates obtained were once recrystallized from a methanol/water mixture. By reacting *o*-hydroxyphenylglycine and mono-chloroacetic acid, N-(*o*-hydroxyphenyl)-iminodiacetic acid (PHDA, see Scheme 1) was obtained as white powder. Found: C 53.29; H 5.33; N 5.56%. Calcd for the compound with semi-hydrate: C 53.22; H 5.68; N 5.64%.

To an aqueous solution (100 ml) containing ferric chloride hexahydrate (270 mg) and (BZDA, 223 mg) a hydrogen peroxide solution (0.1 M solution, 10 ml) was added and the absorption spectra of the resulted solution were measured.

Results

In Fig. 1, the absorption spectra of the iron(III) complexes with (BZDA) and (PHDA) are shown. In the case of Fe(III)-(BZDA), the solution is yellow and there is no absorption band, on the other hand, the solution of Fe(III)-(PHDA) is dark violet color, showing a strong absorption band at 510 nm; this band is known to be characteristic for the presence of Fe(III)-phenol bonding (Yan *et al.*, 1988). When hydrogen peroxide is added to the Fe(III)-(BZDA) solution, the solution became dark violet within a few minutes; it

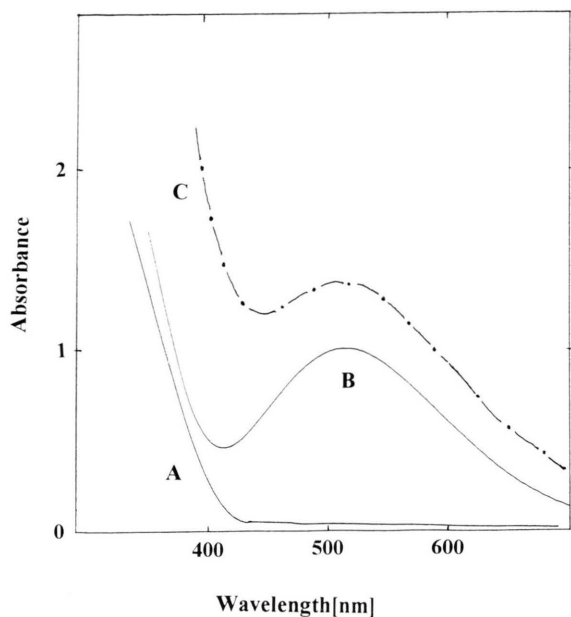
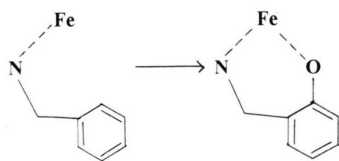


Fig. 1. Absorption spectra of iron(III) chelates ($[\text{Fe}^{3+}] = 1 \times 10^{-3} \text{ mol dm}^{-3}$ in water) A: Fe(III)–(BZDA); B: Fe(III)–(PHDA); C: Fe(III)–(BZDA) and hydrogen peroxide ($[\text{Fe}^{3+}] = 4 \times 10^{-3} \text{ mol dm}^{-3}$).

should be noted here that the absorption spectrum of the violet species formed in the Fe(III)–(BZDA) solution is completely the same as that of Fe(III)–(PHDA). This demonstrated that hydroxylation of the *ortho*-position of benzene ring of (BZDA) occurred in the presence of hydrogen peroxide as shown below (Scheme 2). Under our experimental conditions, about 40% of the benzene ring was hydroxylated within an hour.



Scheme-2

If an iron(III) salt such as ferric nitrate or ferric chloride was added to a reaction mixture of Al(III) chloride (BZDA) and hydrogen peroxide, we could not observe the coloration due to the Fe(III)–phenol bonding. This implies that the hydroxylation reaction observed for the iron(III)

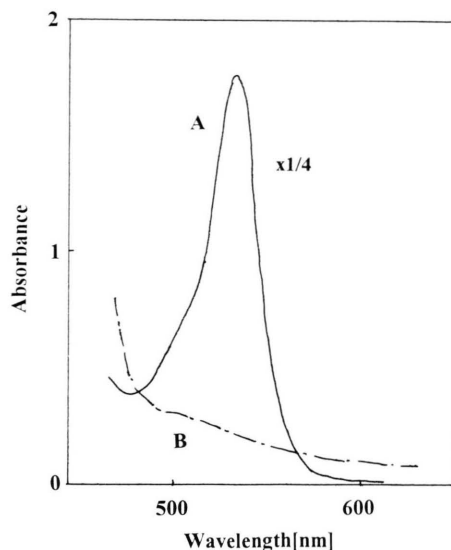
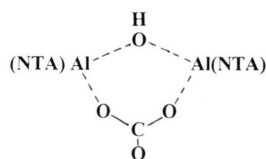


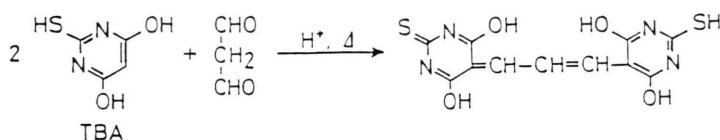
Fig. 2. Absorption spectra of metal(III)-NTA solution containing hydrogen peroxide and 2'-deoxyribose treated by TBA. A: Fe(III)–NTA; B: Al(III)–NTA.

compound does not occur in the case of Al(III)–(BZDA)/ H_2O_2 solution.

In Fig. 2, the absorption spectra of the iron(III)-NTA and aluminum(III)-NTA compounds containing 2'-deoxyribose treated with TBA are shown. The structure of the Fe(III)–NTA was already determined to be of a dimeric compound with μ -oxo- μ -carbonato bridge (Fujita *et al.*, 1994). Based on the analytical data obtained for the Al(III) compounds, we can assume that Al(III)–NTA compound is of a dimeric unit with μ -hydroxo- μ -carbonato bridge, similar to that of the iron(III) complex (see Scheme 3). It is well known that TBA reacts with malondialdehyde or alkylaldehyde (these are called TBARS, TBA-reactive substances), yielding a colored species, as shown below (Scheme 4); the compound derived from malondialdehyde shows strong absorption band at 532 nm (Gutteridge and Halliwell, 1985). Accord-



Scheme-3



Scheme-4

ing to Aruoma *et al.* we can estimate the activation of hydrogen peroxide by a metal compound in terms of degradation of 2'-deoxyribose, which can be detected by the TBA assay (Aruoma *et al.*, 1989).

As shown in Fig. 2, Fe(III)–NTA/H₂O₂ system exhibits high activity for degradation of 2'-deoxyribose; on the other hand, no degradation of the ribose was detected in the mixture of Al(III) complex and hydrogen peroxide. This implies that Al(III)–NTA complex cannot activate hydrogen peroxide.

In Fig. 3, the absorption spectra of Fe(III)–FDA and Al(III)–FDA solutions treated by TBA are shown. In the case of Fe(III)–FDA/H₂O₂ system, strong absorption around 540 nm was observed, indicating the presence of aldehydes in the solution, which should be due to degradation of furan ring in the FDA chelate, whereas no formation of the aldehydes was detected in the Al(III)–FDA/H₂O₂ and Fe(III)–NTA/H₂O₂ systems. As shown in our previous papers, some iron(III) com-

plexes gave 8-OH-dG in the reaction mixture containing 2'-deoxyguanosine and hydrogen peroxide (Nishida and Ito, 1995). In this study we could not detect the 8-OH-dG in the reaction mixture of Al(III)–NTA, 2'-deoxyguanosine, and hydrogen peroxide (not shown).

Discussion

The crystal structure determinations on the several compounds (Jyo *et al.*, 1990) suggest that the structures of Fe(III)–FDA and Al(III)–FDA in acidic solution should be similar to each other, as shown in Scheme 5, (a): the coordination of an etheric oxygen atom to a metal(III) ion was confirmed by crystal structure determinations on the analogous iron(III) compounds (Nishida *et al.*, 1995). By the addition of hydrogen peroxide, there may be formation of a peroxide adduct, illustrated in Scheme 5, (b). The formation of TBARS was detected in the reaction mixture of Fe(III)–FDA and hydrogen peroxide, but not in the solution of Fe(III)–NTA, clearly indicates that degradation of furan ring occurred in the former solution. The degradation of furan is known to be catalyzed by the methane monooxygenase (MMO) (Lee *et al.*, 1993) and thus our results suggest that there is an activated-dioxygen molecule in the Fe(III)–FDA/H₂O₂ system, whose reactivity should be very similar to that in MMO. It is known that furan reacts with singlet oxygen (¹Δ_g) to yield dialdehyde (see Scheme 6), (Acheson, 1976) and thus the activated

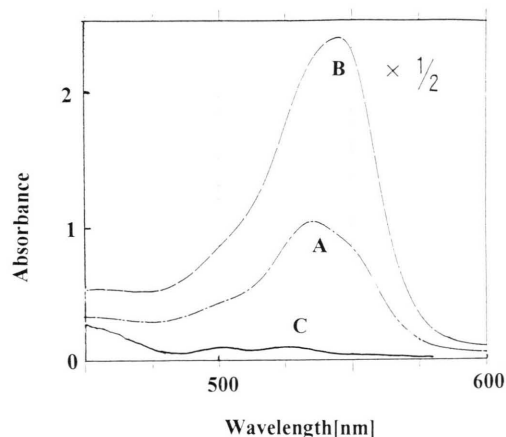
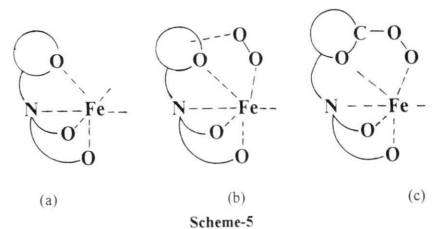
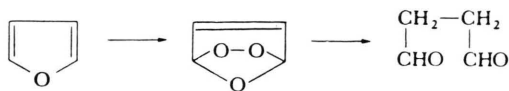


Fig. 3. Absorption spectra of metal(III)-FDA solution containing hydrogen peroxide treated by TBA. A: Fe(III)–FDA, 30 sec after addition of H₂O₂; B: Fe(III)–FDA, 10 min after addition of H₂O₂; C: Al(III)–FDA, 30 min after addition of H₂O₂.





Scheme-6

hydrogen peroxide observed in this system should be similar to the singlet oxygen in reactivity.

In our previous papers, we have investigated the reactivities of the metal peroxide adducts and reported that several metal-peroxide adducts act as a singlet oxygen; *i.e.*, exhibit high electrophilic nature toward organic compounds (Nishida and Takeuchi, 1987; Nishida *et al.*, 1991, 1992). Our consideration has been supported by several authors (Bach *et al.*, 1993; Wilks *et al.*, 1994). We can explain the high reactivity of the peroxide adduct of the Fe(III) complex in Scheme 5(b) as follows; as already proposed the dioxygen molecule coordinating to a nickel(II) ion can interact with HOMO of the aldehyde through the orbital interaction, because the antibonding orbital consisting of metal d-orbital and π^* -orbital of dioxygen molecule is not doubly occupied, and thus exhibits an electrophilic nature (Nishida *et al.*, 1994a). In the present cases, the same discussion as described above may be applied; *e.g.*, the peroxide anion coordinating to an iron(III) atom in Scheme 5(b), which is of an electrophilic nature, may interact with occupied bonding orbital of the furan ring of

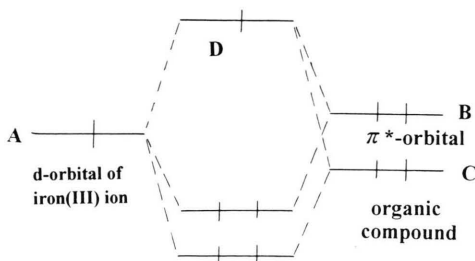


Fig. 4. Molecular orbital scheme for interacting between metal peroxide and organic compound. Bonding orbital of an organic compound, **C** and π^* -orbital of the peroxide ion, **B** are all doubly occupied, and thus no interaction occurs between them (Fleming, 1976). The coordination of peroxide ion to an iron(III) ion leads to formation of singly occupied orbital, **D**, and thus this orbital can interact with doubly occupied orbital **C**. This induces the interaction between π^* -orbital of peroxide ion, **B** and occupied orbital of organic compound, **C**, leading to formation of C-OO bond.

FDA (see also Fig. 4), yielding a peroxide adduct (see Scheme 5(c)), and the subsequent decomposition of the peroxide adduct may give TBARS, as observed. In the case of Al(III)-FDA complex, the formation of a peroxide adduct may occur, but this adduct does not interact with the furan ring, because of the lack of electrophilic nature due to the absence of d-orbital. The same discussion as described for the Fe(III)-FDA/H₂O₂ system may be applied for the case of Fe(III)-BZDA system; in this case the peroxide adduct of the benzene ring formed may decompose to yield a phenol.

We have proposed the formation of a peroxide adduct with η^2 -type side-on structure in the reaction mixture of Fe(III)-NTA and hydrogen peroxide at pH-7.0 (Nishida *et al.*, 1994b) and reported that this species is highly reactive toward H-atom abstraction reaction (oxidation), which is consistent with the formation of much quantity of TBARS in the reaction with 2'-deoxyribose. Negligible activity of the Al(III)-NTA complex for degradation of 2'-deoxyribose and hydroxylation reaction at 8-position of 2'-deoxyguanosine in the presence of hydrogen peroxide may be explained in terms of the same discussion as described above *i.e.*, the peroxide adduct of the Al(III) compounds, which may be similar to that assumed for the corresponding Fe(III)-NTA does not exhibit an electrophilic nature because of the absence of d-orbital. It is well known that the peroxidation of unsaturated fatty acids by an iron(III) ion is closely related with the activation of dioxygen molecule (Nishida and Yamada, 1990); thus the fact that Al(III) salts themselves does not stimulate peroxidation of ox-brain liposomes (Gutteridge *et al.*, 1985) may be understood in terms of the discussion described above.

Okada *et al.* (1993) have shown that membrane lipid peroxidation, as seen by TBARS, is one of the basic mechanism of Fe(III)-NTA induced renal injury and is closely related with renal carcinogenesis. The facts observed in this study, namely that an Al(III) ion does not contribute to the activation of dioxygen and hydrogen peroxide, may elucidate that no carcinogenicity is induced by the Al-NTA complex. At present, however, the role of Fe(III)-NTA for formation of TBARS is not fully established. We have pointed out the importance of hydrogen peroxide for the formation of such substances (Nishida *et al.*, 1994b) and the re-

sults obtained in this study, *i.e.*, an peroxide ion coordinated to an iron(III) ion exhibits a high electrophilic nature toward several organic compounds, may give evidence to elucidate the reaction mechanism for the reactive aldehyde formation *in vivo* (Toyokuni *et al.*, 1994).

Effect of Al(III) Ion in Brain and Plasma

It is believed that most of the iron bound to NTA, which was injected to rats and rabbits, is transferred to transferrin (the chief iron transport protein in vertebrates) and taken up by hepatocytes, part of the iron appear in the kidney (Toyokuni *et al.*, 1994). The major aluminum(III) binding fraction of plasma has been shown to be transferrin(Tf). Tf has recently been shown to specifically bind Al(III) ion with a high affinity, approaching its affinity for iron(III) (Battistuzzi *et al.*, 1995). Thus, the Al(III) ion injected as Al(III)-NTA complex, may be readily transferred to transferrin, and also transported to brain (Roskams and Connor, 1990). In the brain, the Al(III) ion may accumulate at the several organs, as observed for an iron(III) ion.

Since the chemical features of the Al(III) and Fe(III) ions are quite similar, Al(III) ion may oc-

cupy the position of an iron(III) in several non-heme enzymes, such as phenylalanine or tyrosine hydroxylases. These non-heme iron enzymes play an important role to synthesize the neurotransmitters such as dopamine or adrenalin (Dix and Benkovic, 1988). As described in this study, Al(III) ion cannot activate dioxygen or hydrogen peroxide; this may support the assumption that in the brain rich in Al(III) ions, the formation of the neurotransmitters does not occur, leading to degeneration of the central nervous systems.

As stated before, Al(III) can bind transferrin rather strongly. Thus, injection of Al-NTA solution may induce the increasing of the iron(III) ion concentration in plasma. The presence of an iron(III) species in plasma leads to peroxidation of unsaturated fatty acids (Nishida and Yamada, 1990), which may explain the result reported by Gutteridge *et al.* (1985a). Very recently, we have observed that Fe(III)-desferioxamine complex does not give TBARS in the presence of ascorbic acid and 2'-deoxyribose under aerobic condition; this explains the results reported by Gutteridge *et al.* (1985b) *i.e.*, addition of desferioxamine decreases the peroxidation reaction in the presence of an Al(III) ion. All these findings may support the validity of our consideration as described above.

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