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REVIEW

The chemical process of oxidative stress by copper(II) and iron(III) ions in several neurodegenerative disorders

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Abstract The variety of factors and events involved in neurodegeneration renders the subject a major challenge. Neurodegenerative disorders include a number of different pathological conditions, which share similar critical metabolic processes, such as protein aggregation and oxidative stress, both of which are associated with the involvement of transition metal ions. In this review, amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and prion disease are discussed, with the aim of identifying the common trends underlying these devastating neurological conditions by elucidating the chemical process of the oxidative stress due to copper(II) and iron(III) ions.

Keywords ALS · SOD · Hydrogen peroxide · NTBI · Binuclear iron(III) species

Introduction

Neurodegeneration is a complex and multifaceted process leading to many chronic disease states. A conventional definition implies a progressive neuronal death, which usually affects a specific population of nerve cells, the vulnerability of which determines the clinical manifestations of a particular neurodegenerative disease.

A classification of neurodegeneration can be achieved on the basis of the principal neuropathological changes, characterized by the presence of abnormal protein components, which accumulate in the brain. There is extensive evidence for the association between protein aggregation and neurodegeneration in many neurodegenerative disorders [1–5]. Interestingly, metals such as iron and copper appear to play an important role in oxidative stress and are therefore likely to provide a link between the two pathological processes of protein aggregation and oxidative damage, as demonstrated below.

Friedreich's ataxia, the most common hereditary ataxia accounting for approximately 50% of all cases of hereditary ataxia, is an autosomal recessive degenerative disease, characterized by the development of muscle weakness. It is caused by the expansion of a GAA triplet located within the first intron of the frataxin gene that encodes frataxin, a mitochondrial protein that plays a role in iron homeostasis. Deficiency of frataxin results in mitochondrial iron accumulation, defects in specific mitochondrial enzymes, enhanced sensitivity to oxidative stress, and eventually cell death [6, 7].

Post-mortem studies in Parkinson's disease (PD) brains indicate that a wide range of molecules undergo oxidative damage, including lipids, proteins, and DNA. In fact, significant neurochemical, physical, histochemical, and biochemical evidence confirm the hypothesis that oxidative stress generates the cascade of events, which is responsible of the preferential degeneration of melanized dopaminergic neurons in the substantia nigra pars compacta (SNc) in PD [8–10]. In Parkinsonian brains several phenomena have been observed, but we would like to emphasize the elevated level of iron in microglia, astrocytes, oligodendrocytes, and dopaminergic neurons of SNc, and also changes in the normal iron and antioxidant concentration in SNc of PD patients [11–13].

Transmissible spongiform encephalopathies (TSEs) or prion diseases form a group of fatal neurodegenerative disorders that have the unique property of being either infectious or sporadic or genetic in origin. They share

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many pathologic features with the other neurodegenerative disorders, including the importance of oxidative damage of the brain, accumulation of aggregated proteins, and neuronal cell loss. The exact pathogenic mechanism in TSEs remains uncertain, but it is believed that oxidative stress plays a central role, because in the last 10 years the role of metal ions, the copper(II) ion in particular, has been shown to have a critical function in the physiopathology of prion diseases and in the process leading to the abnormal form from the normal prion protein [4, 14].

The loss of neurons in amyotrophic lateral sclerosis (ALS), which is one of the most common neurodegenerative disorders after Alzheimer's disease (AD) and Parkinson's disease (PD), results from a complex interplay of oxidative injury, excitotoxic simulation, aggregation and/or dysfunction of critical proteins, and genetic factors [4, 15, 16]. Degeneration of cortical and spinal motor neurons is the typical feature of ALS occurring both sporadically and as a familial disorder, with inherited cases accounting for about 10% of patients; in the latter cases participation of the mutant SOD enzyme, which contains the copper(II) ion, has been pointed out [15, 16].

Oxidative stress and transition metal ions

Oxidative stress has been defined as an imbalance between the production of oxidants and the ability of the cell to defend against them through a set of antioxidants and detoxifying enzymes. When this imbalance occurs, oxidatively modified molecules accumulate in the cellular compartment causing dysfunction. In the case of very sensitive cells such as neurons, the lack of control of defense systems may eventually lead to cell death.

As the plausible candidates for the oxidants described above, superoxide, hydroxyl radical, and nitric oxide have been postulated; all these molecules are referred to as reactive oxygen species (ROS). Among them, the hydroxyl radical (OH·), which may be generated by reaction between Fe(II) ion and hydrogen peroxide, was believed to be the most important; however, the formation of the hydroxyl radical seems to be completely negligible in the human body, because the concentration of free Fe(II) ion, i.e., $[Fe(H_2O)_6]^{2+}$, is very low.

It is clear that one of the main risk factors in most neurodegeneration is age and this should be directly linked to oxidative stress (lipid peroxidation, protein oxidation, DNA and RNA oxidation), which increases in the brain with age and plays a central role in the pathogenic mechanism of neurodegeneration. On the basis that abnormally high levels of iron in the brain have been demonstrated in a number of neurodegenerative disorders including PD and AD as described in the "Introduction," we concluded that the oxidative stress increasing with age should be closely related to the increased accumulation of iron levels in the brain with age. In order to elucidate the relationship between oxidative stress and accumulated iron ions, we investigated the chemical mechanism of H_2O_2 activation by several copper(II) and iron(III) complexes in vitro, and found that hydrogen peroxide can be activated to show reactivity similar to that of singlet oxygen ($^1\Delta_g$), and that this activated hydrogen peroxide should be a main risk factor for the oxidative stress to induce all the neurodegenerative disorders [17–20]. In addition, we also showed that the iron ions not associated with transferrin, generally termed as non-transferrin-bound iron (NTBI), facilely produce hydrogen peroxide in the presence of reducing agents [20].

On the basis of our results we have proposed that the intrinsic oxidant to induce the oxidative stress should be a copper(II) or an iron(III) complex containing hydrogen peroxide, and have given reasonable answers to the following key questions: why do iron levels increase abnormally in some regions of the brain, and what is the origin of toxicity due to iron ions accumulated in the brain [17, 18]? This review describes the details we have elucidated about the chemical process of the oxidative stress by copper(II) and iron(III) ions in several neurodegenerative disorders.

Amyotrophic lateral sclerosis (ALS) and mutant superoxide dismutase (SOD)

ALS is a progressive paralytic disease characterized by selective degeneration of the upper and lower motor neurons [4, 21, 22]. Although ALS is predominantly a sporadic disease, ~10% cases are inherited in an autosomal dominant manner [familial ALS (fALS)] and a subset of the fALS cases are caused by mutations in the SOD1 gene [15, 16, 23, 24]. The gene product of SOD1, cytoplasmic Cu,Zn-superoxide dismutase (SOD1), is a ubiquitously expressed enzyme that catalyzes the disproportionation reaction of superoxide radicals (Eqs. 1 and 2) [25]. The crystal structure of the SOD(Cu/Zn) has already been determined, its dimeric structure being illustrated in Fig. 1 (PDB, 1spd_x). The copper and zinc ions are bridged by an anionic form of the imidazole ring of histidine.

$$O_2^- + Cu(II) \rightarrow O_2 + Cu(I) \quad (1)$$

$$Cu(I) + O_2^- + 2H^+ \rightarrow H_2O_2 + Cu(II) \quad (2)$$

There are several lines of evidence that SOD1 mutations result in a gain, rather than loss of function that causes ALS. For instance, some fALS-associated mutant SOD1s retain full enzymatic activity [26]. In addition, SOD1 knockout

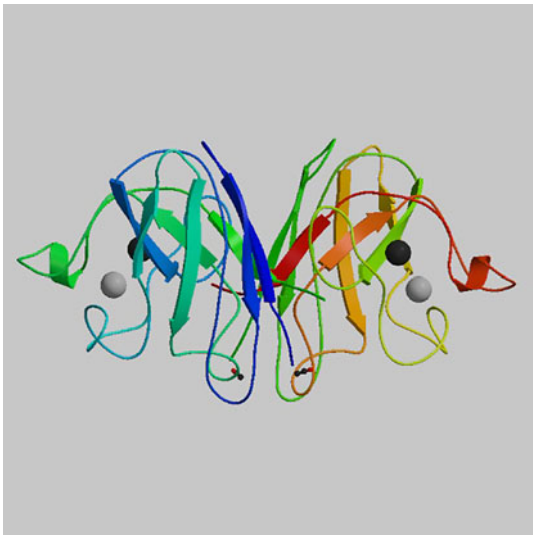


Fig. 1 Dimeric structure of the SOD molecule. Two copper and zinc ions are illustrated as *colored circles*

mice lack ALS symptoms, whereas transgenic mice expressing the fALS-associated mutant G93A SOD1 develop ALS-like symptoms despite expression of endogenous mouse SOD1. Lastly, overexpression of human wild-type SOD1 fails to alleviate symptoms in this transgenic mouse model for ALS [27].

One hypothesis about the "gain of function" of SOD1 is that misfolding of the mutant alters the catalytic mechanism to allow production of oxidants such as peroxynitrite [28] and possibly hydrogen peroxide [29]. Another major hypothesis is that toxicity is caused by intracellular aggregation of SOD1. SOD1 inclusion bodies, which also react with anti-ubiquitous antibodies, are a common pathological finding in motor neurons and neighboring astrocytes of ALS patients [30]. Although SOD1 aggregates may be inherently toxic or cause motor neuron toxicity by sequestering chaperons and blocking proper functioning of the proteasome, origin of toxicity by SOD1 aggregates has not been elucidated.

Lindberg et al. [31] compared the folding behavior of 15 ALS-associated SOD mutants with survival times ranging from 1 to 17 years after the onset of the first symptom. They found that the folding pattern of the ALS-associated mutants shows different and characteristic features that can be linked to their respective disease progression rates. Common to all mutations is a shift of the folding equilibrium toward the denatured monomer; however, the magnitudes and the mechanistic origins of this shift are found to vary. In order to rationalize the above facts, we must consider the effects by oxidative stress due to the copper(II) ion; this point is omitted in the study by Lindberg et al. In 1997, Yim et al. [32] reported that an fALS mutant (Gly93Ala = G93A) exhibits an enhanced free radical-generating activity, while its dismutation activity is identical to that of the wild-type enzyme. They reported that the free radical-generating activity of the mutant, as measured by a spin-trapping method at low H_2O_2 concentration, is enhanced relative to that of the wild type and G93A, wild type < G93A < A4 V, but the reason for the above fact has not been clarified.

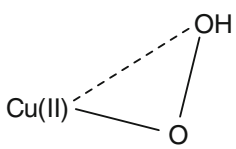
To understand ALS pathogenesis, we must clarify how altering the SOD molecule can induce cell injury. To carry out such an investigation, we have started to clarify the origin of the gain of function by the mutant SOD molecule. The reaction mechanism of SOD1 enzyme has been investigated by many authors. Very recently Nishida et al. pointed out the importance of formation of a copper(II)-OOH species (Scheme 1) as an intermediate in the second step (2) above, and this hydrogen peroxide produced is immediately removed from the wild-type enzyme because of the negligible interaction among hydrogen peroxide, the copper(II) ion, and the surrounding organic groups.

Origin of gain of function in mutant SOD enzyme and the unique reactivity of copper(II)-hydroperoxide adduct

In order to obtain a comprehensive solution for the correlation between the structural change in mutant SOD and pathogenesis of fALS, we studied the reactivity of copper(II)-OOH, proposed as an important intermediate in the SOD reaction. For this purpose, we synthesized many copper(II) compounds with ligands containing an *N*,*N*-bis(2-pyridinylmethyl)amine moiety as illustrated in Fig. 2 [33]. The structural features of all the copper(II) compounds are essentially similar to each other (as an example, the crystal structure of $[Cu(bdpg)Cl]^+$ is illustrated in Fig. 3). In the presence of hydrogen peroxide, formation of a peroxide adduct as shown at the right side of Fig. 3 was anticipated, and this was consistent with the results of the reaction with cyclohexane [34]; we also found that the reactivity of the peroxide adduct of the copper(II) compound is highly dependent on the R of the ligand system (Fig. 2), i.e., slight structural change around the copper(II) ion due to the different R moieties strongly controls the reactivity of the copper(II)-peroxide adduct.

We measured the ESR spectra of solutions containing a copper(II) complex and spin-trapping reagent, such as PBN (α-phenyl-*N*-*t*-butylnitrone) and TMPN (2,2,6,6-tetramethyl-4-piperidinol), specific reagents for OH· radical and singlet oxygen ($^1\Delta_g$) (Scheme 2), respectively [35].

Scheme 1



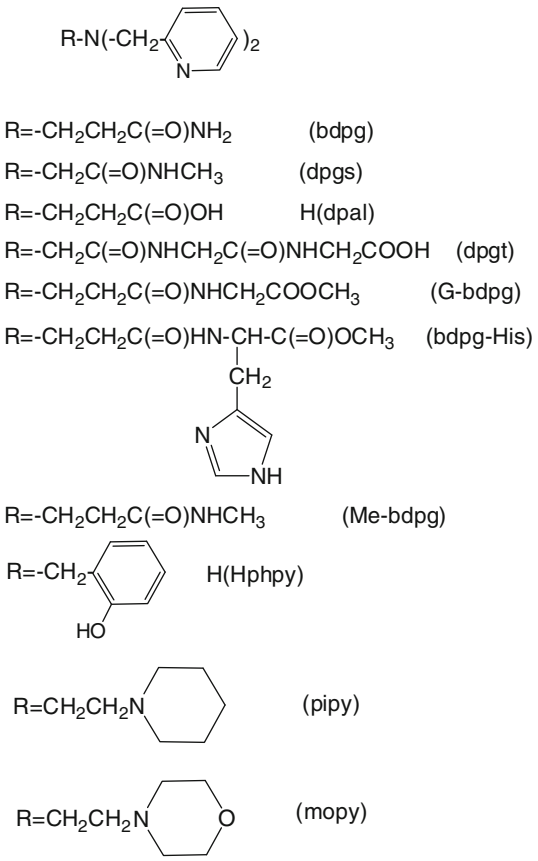
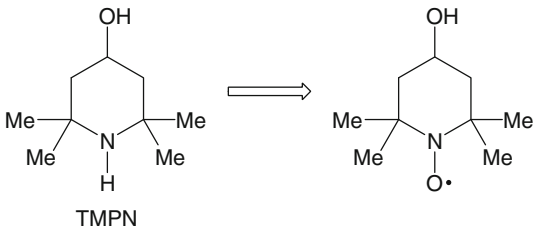


Fig. 2 Chemical structures of the ligands used in our study

No ESR signal due to the formation of a PBN radical was detected when the copper(II) complexes with tpa (tris(2-pyridinylmethyl)amine) or bdpg were mixed with H_2O_2 and PBN. However, strong peaks due to nitrone



Scheme 2

radical formation of the corresponding TMPN (Scheme 2) were detected in the solution with the Cu(tpa) complex, but not with the Cu(bdpg) complex.

The comparison between the $Cu(pipy)Cl^+$ and the $Cu(mopy)Cl^+$ is very interesting [35]. Structural features of the two compounds are essentially the same, the only difference being that the oxygen atom of the morpholine ring of the $Cu(mopy)Cl^+$ complex is replaced by a $–CH_2–$ in the $Cu(pipy)Cl^+$ complex. In the case of $Cu(pipy)Cl^+$, no formation of the nitrone radical was observed in the presence of hydrogen peroxide; in contrast, high activity for the radical formation by the $Cu(mopy)Cl^+$ complex was detected as illustrated in Fig. 4. Similar high activity for TMPN radical formation was also observed for the copper(II) complex $[Cu(Hphpy)Cl]^+$. In this case, similar to the $Cu(mopy)Cl^+$ complex, the addition of the H_2O_2 to the copper(II) solution does not induce the change in ESR spectrum due to the copper(II) ion; but the addition of TMPN leads to the dramatic change in the ESR signals attributed to the copper(II) species (i.e., the change of hyperfine structure values due to the copper atom). These are all comprehensively elucidated on the assumption that the complex formation of copper(II), hydrogen peroxide,

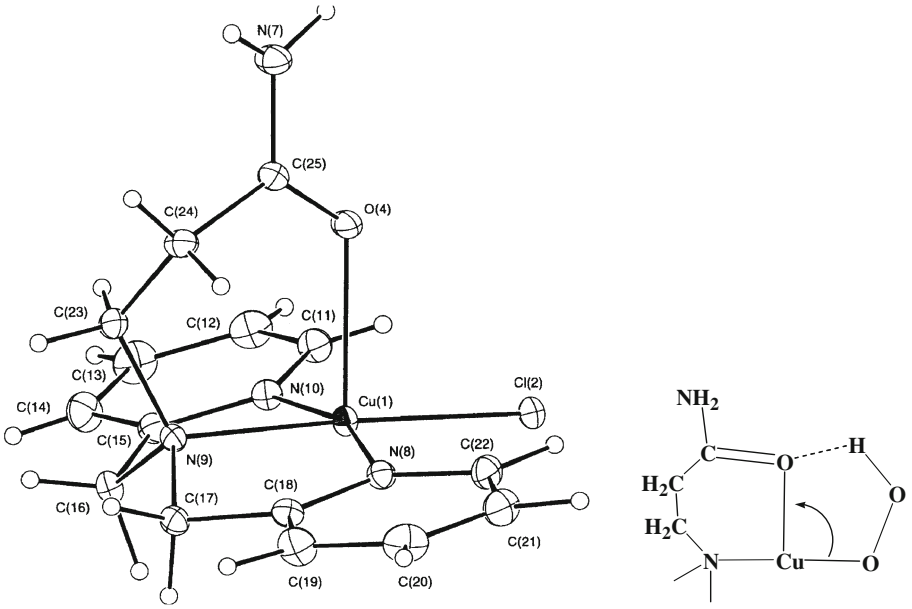


Fig. 3 *Left* crystal structure of $[Cu(bdpg)Cl]^+$ [34]. *Right* assumed structure of the peroxide adduct $[Cu(bdpg)(OOH)]^+$

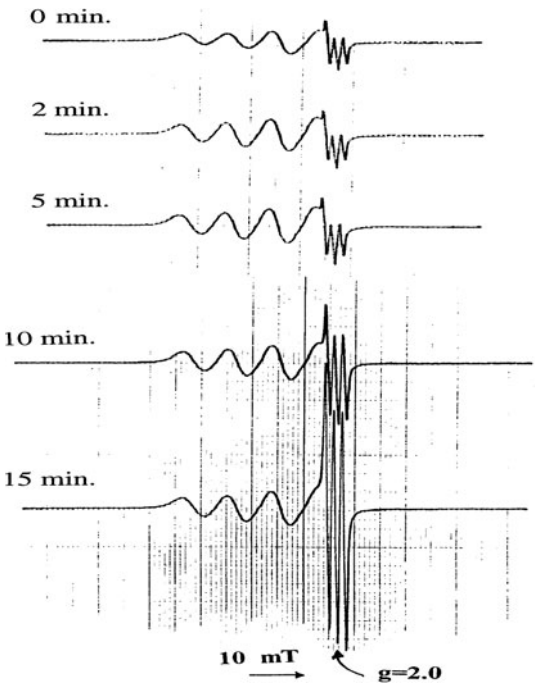


Fig. 4 Time course of ESR spectra of the solutions containing $[Cu(mopy)Cl]^+$, H_2O_2, and TMPN

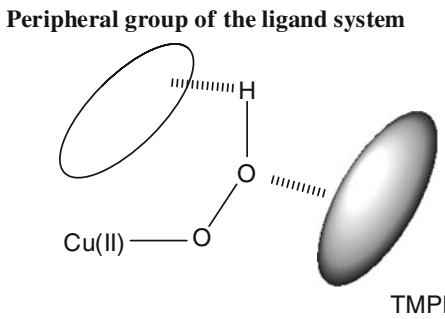
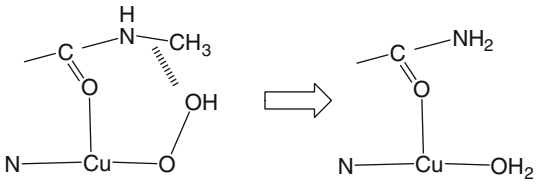


Fig. 5 Assumed intermediate among copper(II) chelate, H_2O_2, and TMPN



Scheme 3

and TMPN occurs only when three reagents are present in the solution (Fig. 5), and the unique reactivity of hydrogen peroxide observed is detected only when the intermediate is formed in the solution.

The above facts indicate that the reactivity of Cu(II)-OOH is controlled by the structural properties of the intermediate (Fig. 5), i.e., by the chemical interactions among copper(II)-OOH species, peripheral groups, and substrate [17, 18]. It should be noted here that although hydrogen peroxide was believed to be relatively inert and not toxic to cells, our present results clearly show that some copper(II) chelates can activate the hydrogen peroxide to exhibit high reactivity similar to that of singlet oxygen ($^1\Delta_g$).

In order to get further information about the reactivity of a copper(II)-OOH species, we measured ESI mass spectra of the solutions of copper(II) compounds and hydrogen peroxide. When hydrogen peroxide was added to Cu(Me-bdpg)Cl solution (for Me-bdpg, see Fig. 2), the formation of [Cu(bdpg)Cl], not [Cu(dpal)], was detected by ESI–MS [36]. This clearly indicates that Cu(II)-OOH species can cleave the peptide at the C–N bond oxidatively, not hydrolytically, because the hydrolytic cleavage may give Cu(dpal) species from the Cu(Me-bdpg) compound (Scheme 3).

We also found that some copper(II) complexes exhibit high activity to oxygenate the methionine residue of amyloid beta-peptide(1–40) at the sulfur atom [37, 38], and decompose several proteins in the presence of hydrogen peroxide [39]. All these facts may suggest that the gain of function of the mutant SOD is due to formation of a long-lived highly reactive copper(II)-OOH as an intermediate in the process of SOD reaction. The chemical structures around the copper(II) in the mutant SOD are slightly changed, and this gives an unexpected effect on the reactivity of copper(II)-OOH as observed in our papers. In the mutant SOD the C–N bond cleavage by Cu(II)-OOH may give great changes in the surface of SOD, leading to destabilization of the dimer contact of the SOD enzyme [40]. Thus, it is quite likely that formation and existence of a highly reactive Cu(II)-OOH species is an intrinsic origin of oxidative stress in the pathogenesis of fALS, which may be consistent with the recent studies on the destabilization of the dimer contact of the SOD enzyme [41, 42].

Dissociation of dimeric SOD molecule into monomers

As stated before, it is widely recognized that protein aggregations are common pathological features of many neurological disorders, including Huntington's, Alzheimer's, and Parkinson's diseases, and that SOD1 aggregates may be inherently toxic or cause motor neuron toxicity by sequestering chaperons and blocking proper functioning of the proteasome.

In 2004, Rakhit et al. [43] reported that SOD1, normally a dimeric enzyme, dissociates to monomers prior to aggregation for both wild-type and mutant proteins. They used the dynamic light scattering (DLS) method to detect the dissociation of dimeric SOD to monomers. Very recently we reported that the capillary electrophoresis

method (CE) is very suitable to investigate the conformational change of the proteins and aggregation states of the proteins in solution [19].

We observed that a drastic decrease of the peak strength due to the dimeric SOD molecule occurs when copper(II)/ascorbic acid solution was added to the SOD molecule [44]; our experimental system was the same as that reported by Rakhit et al. This clearly shows that the dissociation of the dimeric SOD molecule can be readily detected by the CE method. We also found that the presence of excess hydrogen peroxide induces the dissociation of the dimeric structure of the wild-type SOD molecule, because a drastic decrease of the peak height due to the dimeric structure was observed (Fig. 6) [45]. Thus, it seems quite likely that the oxidant in the system, the copper(II)/ascorbic acid solution, used by Rakhit et al. should be hydrogen peroxide, and that sporadic ALS should be closely related to the presence of excess hydrogen peroxide [19]; the same discussion may be applied to the elucidation of sporadic prion diseases (see later).

By using antibody methods to rapidly purify SOD1 and coupling this with mass spectrometry, Sato et al. [16] measured the relative accumulated levels of wild-type and mutant SOD1 in erythrocytes of 29 SOD1-mutated fALS patients. They observed that the patients with undetectable SOD1 mutant had the shortest disease durations. Although age at disease onset was found to be uncorrelated with the amount of mutant SOD1, the evidence convincingly shows a strong inverse correlation between disease duration and mutant accumulation. Said another way, an accelerated disease course is found for mutants that are less stable. This surprising discovery implies that it is the misfolded unstable forms of SOD1 mutants that contribute to toxicity

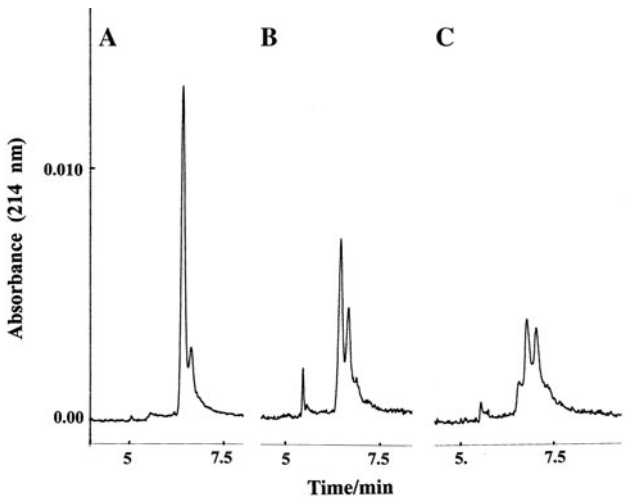


Fig. 6 CE profiles of the solutions. *A* Wild-type SOD (1 mg/cm^3), *B* measured immediately after H_2O_2 was added, *C* measured 60 min after addition of H_2O_2

underlying disease progression, and that despite its apparent importance for progression, SOD1 mutant stability is not correlated with disease onset. Thus, dissociation of the dimeric SOD1 molecule to misfolded monomers should be an essential important process for APS pathogenesis. As it has become apparent that hydrogen peroxide plays an important role in the formation of SOD1 monomers [45], we should pay attention to the formation of excess hydrogen peroxide in the human body, especially due to the reaction between a dimeric iron(III) species and the glutathione cycle and other related systems [18, 20].

Oxidative stress by copper(II)-OOH in sporadic prion diseases

Between 1980 and roughly 1996, about 750,000 cattle infected with BSE (bovine spongiform encephalopathy, one of the TSEs) were slaughtered for human consumption in Great Britain, and at present it is accepted that the central event in TSEs is the post-translational conversion of the normal cellular prion protein (PrP^C) into an abnormal isoform of called scrapie PrP (PrP^{Sc}) that has a high β-sheet content [46]. It is generally recognized that PrP^C is a copper-containing protein (at most four copper ions are present within the octarepeat region located in the unstructured N-terminus). Analysis of recombinant mouse and chicken PrP^C led to the discovery of an important gain of function following the formation of the PrP^C copper complex; PrP^C has been shown to contribute directly to cellular SOD activity.

The misfolded prions (PrP^{Sc}) ultimately kill neurons and leave the brain riddled with holes, like a sponge. In addition to PrP^{Sc}, another protease-resistant PrP of 27–30 kDa, which is called PrP27-30, was extracted from affected brains. It should be noted here that PrP27-30 is derived from only PrP^{Sc} (not from PrP^C), and no difference in amino acid sequence between PrP^C and PrP^{Sc} has been identified. On the basis of these facts we may assume that the chemical environment around the copper ion in the PrP^{Sc} should be different from those in the PrP^C; this situation is similar to the difference observed between environments around copper(II) ions in the wild-type and mutant SOD enzyme. Thus, it is most likely that the gain of function in the PrP^{Sc} due to a "highly reactive" Cu(II)-OOH formation may occur as described for the mutant SOD molecule, which leads to the cleavage of the peptide bonds around the copper ion (at about site 90), giving dangerous PrP27-30; the latter protein may behave like the misfolded SOD monomer. In addition to this, it seems quite likely that the copper(II) ions in PrP^c and also PrP^{Sc} may react with hydrogen peroxide to yield a Cu(II)-OOH species, which may exert serious effects on the PrP^C such as oxygenation at methionine residues, conformational

change (i.e., formation of PrP^{Sc}), and degradation of protein in the presence of hydrogen peroxide (Scheme 4). Several experimental facts observed for the native prion proteins [47–54] seem to be consistent with our discussions. All these findings support our proposal that hydrogen peroxide, which may derive from the SOD function of PrP^{Sc} and/or from NTBI [20], should be one of the serious origins of the oxidative stress in sporadic prion diseases.

Structural characteristics of iron(III) chelates that induce tissue damage and renal carcinoma: the chemical origin of iron toxicity

It is generally recognized that iron, the most abundant transition metal ion in mammalian systems, is a necessary trace element and is required for normal metabolic processes spanning molecular oxygen transport, respiratory electron transfer, DNA synthesis, and drug metabolism. In fact, iron deficiency leads to the deficiency of neurotransmitters such as dopamine and serotonin in the brain, inducing several mental diseases such as Parkinson's disease, depression, and schizophrenia [18]. Thus, the ancient Greeks concocted potions of iron filings dissolved in vinegar, hoping that drinking this liquor would empower them with the properties of the element.

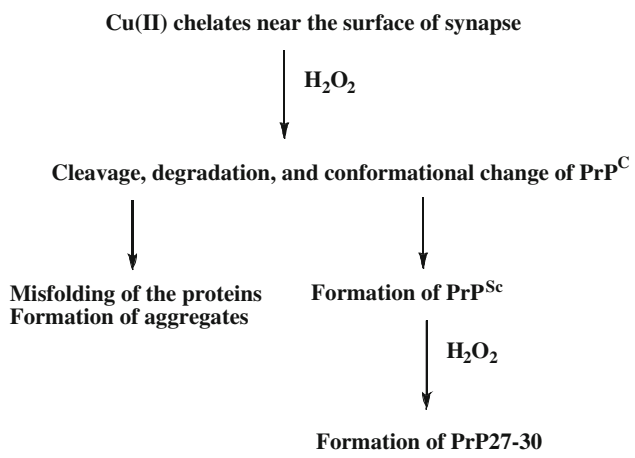
In the human body, we have no real mechanism for the elimination of excess iron and, as a result, cells continuously store excess absorbed iron in a complex with the protein ferritin. Under conditions of iron excess some of it is shunted into another storage form known as hemosiderin [55, 56] in which the excess iron is deposited as ferrihydrate structures. Hemosiderin is typically insoluble, but when amino acid derivatives or small peptides are present in the plasma, the precipitated ferrihydrate in hemosiderin may dissolve forming an iron(III) chelate. These iron ions, or NTBI, are detected in the plasma of patients with thalassemia, hemochromatosis, and several neurodegenerative disorders, and are thought to play an important role in iron-induced cell damage as pointed out in the "Introduction." Despite numerous studies over the last 30 years since plasma NTBI was first postulated to exist, little is understood about the chemical composition of NTBI and the origin of toxicity due to NTBI at present. In this section, we discuss the chemical origin of iron toxicity in the human body due to NTBI, and propose a technique to eliminate the NTBI safely.

Iron(III)-nta chelate as a renal carcinogen

Ferric nitrolotriacetate (Fe(III)-nta) is a well-known renal carcinogen (for nta, see Fig. 7), and Fe(III)-nta-injected animals have been used as a model of carcinogenesis [57–60]. When Fe(III)-nta is intraperitoneally injected into animals, lipid peroxidation and oxidative modification of proteins and DNA occur in renal proximal tubules, and tubular epithelial cells are damaged. Thiobarbituric acid reactive substance (TBARS) [61] has also been shown to increase in kidney, and increases in 4-hydroxy-2-nonenal (4-HNE)-modified proteins and 8-hydroxy-deoxyguanosine (8-OH-dG) were also demonstrated.

We have determined the crystal structures of several iron(III) compounds including nta, pac, and ida [62–64]. As shown in Fig. 8, the Fe(III)-(nta) complex is of a dimeric structure with oxo and carbonato bridges. It should be noted here that although the crystal structure of the Fe(III)-(pac) complex is of a dimeric structure similar to that of the nta compound, tubular injuries by the Fe(III)-(pac) compound are negligible and the Fe(III)-(pac) compound does not induce renal carcinoma [20, 62].

On the basis of the spectral studies on the solution containing an iron(III) compound and hydrogen peroxide, we found that in the presence of hydrogen peroxide the Fe-(nta) complex facilely gives a (μ–η^1:η^1)-peroxodiiron(III)-(nta) species shown in Scheme 5 [20, 62, 65], which



Scheme 4

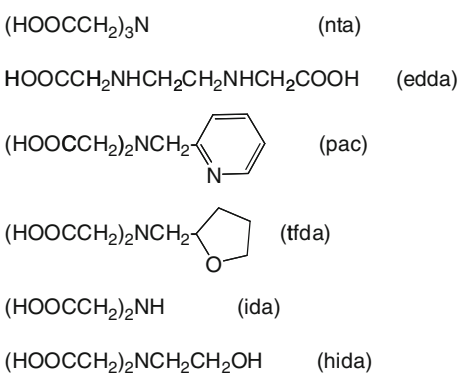


Fig. 7 Several chelates used in our study

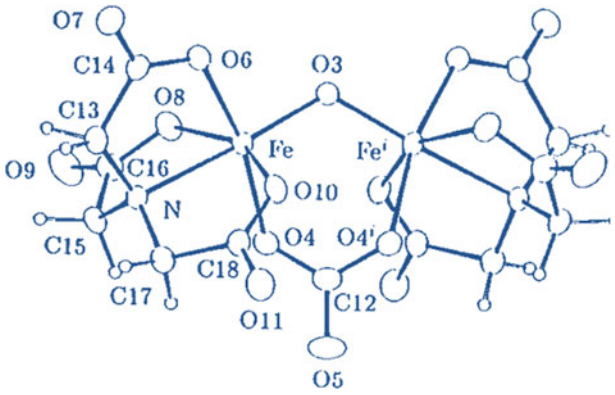
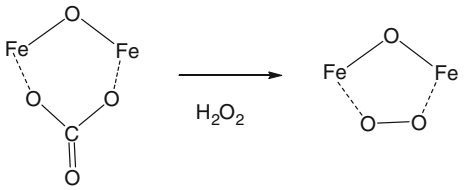


Fig. 8 Crystal structure of the $[Fe_2O(nta)_2(CO_3)]^{4-}$ ion



Scheme 5

exhibits high reactivity similar to that of singlet oxygen ($^1\Delta_g$) [66–68]. However, no formation of (μ–η^1:η^1)-peroxodiiron(III) species occurs in the solution of Fe-(pac) complex in the presence of hydrogen peroxide. In the case of the edda compound, the carbonato ion in the dimeric unit is readily replaced by the hydrogen peroxide, leading to the formation of a dimeric species with a linear Fe–O–Fe core which does not give a (μ–η^1:η^1)-peroxodiiron(III) species.

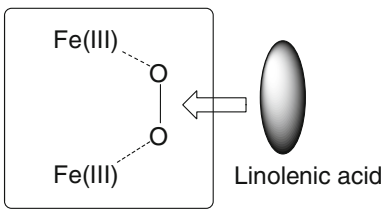
Why does the tissue damage occur only in the vicinity of renal proximal tubules?

It is clear that the proximal tubules necrosis induced by artificial iron(III) chelates in rat kidneys is highly dependent on the chelate structure (Fig. 7), and injuries such as lipid peroxidation and protein oxidation are observed mainly in the renal proximal tubules [63, 64], but no injury was observed in the distal position. It should be noted here that the glutathione cycle is highly active in the renal proximal position [69], and this may suggest that the glutathione cycle promotes the iron(III)-induced injuries.

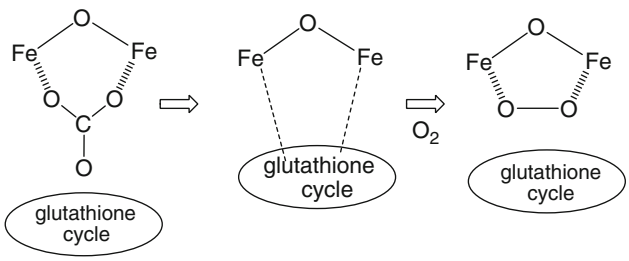
It has become apparent that some binuclear iron(III) compounds exhibit very unique reactivity towards reducing agents in the presence of oxygen. For example, the binuclear iron(III) complex with H(HPTB), $Fe_2(HPTB)(OH)(NO_3)_2$, exhibits high activity for oxygenation of linolenic acid in the presence of oxygen, and the two-electron transfer reaction to oxygen (formation of hydrogen peroxide) from TMPD (*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine) [70, 71]. This was elucidated on the assumption that a binuclear iron(III)-oxygen intermediate formation is promoted through interaction with the reducing agent, such as linolenic acid or TMPD (Scheme 6) [20].

On the basis of the above discussion, the tissue damage and renal carcinoma induced by the Fe(III)-(nta) chelate may be explained as follows (Scheme 7) [20, 63, 64]: when the binuclear Fe(III)-(nta) compound reacts with the glutathione cycle, the carbonato ions dissociate from the compound, leading to the interaction between two iron(III) atoms and the protein. At this stage, when oxygen is present, formation of peroxide ion is accelerated through the interaction with the glutathione cycle, and the peroxide adduct of the binuclear Fe(III)-(nta) complex formed shows high oxidative reactivity towards the proteins, leading to the tissue injuries and renal carcinoma. Thus, the tissue damage and renal carcinoma occur only in the renal proximal tubules where glutathione cycles are highly active. In the case of the Fe-(pac) complex, no formation of a (μ–η^1:η^1)-peroxodiiron(III) species occurs even in the presence of the glutathione cycle, which should be due to the stronger bonds in the Fe–O–Fe–CO_3 moiety of this complex [20].

Our conclusion on the active species inducing tissue injuries shown in Scheme 7 can explain comprehensively all the results concerning cell damage by the iron(III) chelates [20, 60, 63, 64]. In previous papers, the role of the hydroxyl radical in inducing tissue damage and renal carcinoma has been frequently pointed out, but this cannot explain the lower tissue-damaging activity exerted by the Fe(III)-(edda) chelate, nor the difference in the tissue damage exerted by the two alkoxo-bridged binuclear iron(III) complexes $Fe_2(hida)_2(H_2O)_2$ (non-toxic) and



Scheme 6



Scheme 7

$Fe_2(HPTP)Cl_4$ (highly toxic), or by Fe(III)-(nta) and Fe(III)-(pac) chelates [20, 63, 64].

Models of NTBI and the origin of its toxicity

We have found that deposition of iron(III) hydroxide occurs readily on the aggregates of amyloid beta-peptide (1–40) [abbreviated as Aβ(1–40)] by the addition of zinc(II) chloride to the solution containing iron(III) compounds with nta, edda, and other amino acid derivatives [72]. This implies that iron(III) compounds with amino acids or peptides in plasma may be an intrinsic iron(III)-ion carrier to induce the high level accumulation of iron(III) ions in the amyloid deposits.

Recently it was suggested that the toxicity of Aβ and other amyloidogenic proteins lies not in the insoluble fibrils that aggregate but rather in the soluble oligomeric intermediates, indicating that the soluble oligomers may be more important pathologically than are the fibrillar deposits [73, 74]. The origin of the high toxicity of the soluble oligomeric intermediates should be due to the iron(III) species bound in the oligomers, which may contain a dimeric iron(III) species with oxo bridges; the soluble iron(III) species in the oligomers may readily oxidize the proteins in the presence of hydrogen peroxide, leading to the aggregation of Aβ proteins and/or degradation of surrounding proteins. Thus, it seems quite reasonable to assume that several iron(III) compounds with amino acids or peptides including amyloid β-peptide in plasma are possible candidates for NTBI models. The participation of the copper(II) ion in the toxicity of Aβ and other amyloidogenic proteins has been discussed by many authors [4, 75].

The above discussion also supports the estimation that the formation of insoluble amyloid plaques including iron(III) ions promoted by zinc(II) ions may be one of the important methods to protect against oxidative stress by soluble oligomeric iron(III) compounds with amino acids or peptides in vivo [76]. Recently, the contribution of D-serine in the pathogenesis of ALS and other neurodegenerative disorders including schizophrenia and Alzheimer's disease was been pointed out [77]. The studies on the formation of D-serine have led to the assumption that NTBI and hydrogen peroxide should play an important role in the formation and accumulation of D-serine through its high oxidative ability to racemize the L-amino acids.

Very recently, we prepared new chelates which bind only with the iron ions in NTBI and remove them without showing any toxicity due to the iron ion, and thus these may contribute to advances in iron overload therapies including several neurodegenerative disorders [4, 78, 79].

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