MHR

REVIEW ◇ HYPOTHESIS

Y. NISHIDA [2004] MED HYPOTHESES RES 1: 227-245.

OXIDATIVE STRESS AND NEURODEGENERATION

YUZO NISHIDA*

CHEMICAL INSTITUTE FOR NEURODEGENERATION, YAMAGATA UNIVERSITY, YAMAGATA 990-8560, JAPAN.

ABSTRACT. WE HAVE INVESTIGATED the chemical mechanisms underlying the pathogenesis of neurodegenerative diseases based on our new concept for the so-called "oxidative stress" induced by the metal ions. The following data were discussed in this paper: (i) Reactivity of the metal-OOH, hydroperoxo-metal speices of Fe(III) or Cu(II), is dependent on the interactions with peripheral groups and substrate, and is frequently highly reactive to oxidation of cellular constituents, such as lipids, proteins, and DNA, which leads to mutations and misfoldings of proteins. (ii) In some iron-containing enzymes such as phenylalanine hydroxylase or tyrosine hydroxylase, Fe(II) can activate O_2 without change of its oxidation state, and in this case weak interaction between the unpaired electrons of Fe(II) ion and O_2 is necessary to activate the O_2. The other metal ions such as Al(III) or Mn(II) cannot activate O_2 by the same mechanism, and thus the accumulation of Al(III) or Mn(II) ions in brain will lead to the deficiency of dopamine and other neurotransmitters, and also to the elevated iron levels in the brain. (iii) Elevated iron levels in brains and abnormalities in brain iron metabolism (so-called iron-overload syndrome) have been detected for Alzheimer's and Parkinson's diseases, and these iron ions exist as a soluble polymeric species through chelate formation with amino acids or small peptide. Soluble polynuclear metal chelates of Fe(III) or Cu(II) exhibit unique reactivity toward O_2, readily giving hydrogen peroxide in the presence of reducing agents. Thus the hydrogen peroxide produced can be an intrinsic origin for the "oxidative stress" in the presence of metal ions such as Fe(III) or Cu(II). Based on these results, we would like to propose that one of the most risky factors for the neurodegenerative diseases is accumulation of exogenous metal ions such as Al(III) or Mn(II) in brain, and thus, the following points must be noted to protect the sporadic neurodegenerative diseases: (i) to remove metallic aluminum from the instruments for storage of food and from the manufactures for food productions (e.g., dry milk and beer, etc); (ii) to remove the risk factors to induce "acid rain"; and (iii) to stop the use of methyl cyclopentadienyl manganese tricarbonyl in gasoline.

*ADDRESS ALL CORRESPONDENCE TO: DR. YUZO NISHIDA, CHEMICAL INSTITUTE FOR NEURODEGENERATION, YAMAGATA UNIVERSITY, YAMAGATA 990-8560, JAPAN.
E-MAIL: yuzo@sci.kj.yamagata-u.ac.jp

● MEDICAL HYPOTHESES AND RESEARCH ● THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH ●

1. INTRODUCTION

Between 1980 and roughly 1996, about 750,000 cattle infected with BSE (bovine spongiform encephalopathy) were slaughtered for human consumption in Great Britain, and it is now clear that BSE, also known as "mad cow disease" is not merely a UK phenomenon, nor is it merely an economic nuisance. In fact, it may be an impending world-wide health crisis, and in recent months several other European countries have found BSE in their cattle herds, and over the past few years about 100 mostly young individuals have fallen victim to a fatal condition known as new variant Creutzfeld-Jacob disease (vJCD) [1-3]. BSE and vJCD are among the transmissible spongiform encephalopathies (TSEs or prion diseases) which are a group of fatal neurodegenerative diseases that include BSE, vJCD, scrapie of sheep, chronic wasting diseases (CWD) of mule deer and elk, as well as Gestmann-Straussler-Scheinker disease (GSS) and fatal familial insomnia (FFI) of humans [4,5]. At present it is generally recognized that BSE may have originated from a scrapie agent infecting small ruminants, which have been recycled through cattle and disseminated through the use of contaminated meat and bonemeal. Compelling evidence links vCJD to exposure to beef infected with BSE prions, and recent studies have suggested that blood-borne transmission of CJD is possible.

Some 250 years ago, a sheep disease that presented with excitability, itching, ataxia and finally paralysis and death was recognized and this is known today as scarpie in English-speaking countries, "the trembles" in France, "trotting disease" in Germany and "itching disease" in Japan, reflecting the gamut of its symptoms. The first major advance in scrapie research took place in 1936 when Cuille and Chelle succeeded in transmitting the disease to sheep and goats by inoculating them with lumbar cord of diseased animals. Subsequently, transmission to mice and hamsters provided more-convenient experimental models. It was soon recognized that the transmissible agent had quite extraordinary properties, such as unusually long incubation periods, measured in months to years, and uncommon resistance to high temperature, formaldehyde treatment and UV irradiation. Enriching fractions from Syrian hamster (SHa) brain for scrapie infectivity led to the discovery of the prion protein (PrP), and at present it is generally accepted that the central event in TSEs is the post-translational conversion of the normal cellular prion protein (PrP^{C}) into an abnormal form called scrapie PrP (PrP^{Sc}) that has a high β-sheet content and is associated with transmissible diseases [6]. These misfodled prions (PrP^{Sc}) ultimately kills neurons and leaves the brain riddled with holes, like a sponge, and the 1997 Nobel Prize in Physiology and Medicine was awarded to Professor S. Prusiner of the University of California, San Francisco, for his contributions towards the identification of the infectious agent that causes TSEs.

PrP^{C} is a glycoprotein expressed on the surface of many cell [5-7] and the fact that the protein is expressed in neurons at higher levels than in any other cell types suggests that PrP^{C} has special importance for neurons. At present it is clear that PrP^{C} not only binds copper (Cu) within the octarepeat region located in the unstructured N-terminus, but under certain specific circumstances may bind along the C-terminal structured domain of protein fragments. Furthermore, recombinant PrP^{C} can also bind other metals such as manganese at both the octarepeats and the C-terminal sites [8]. Indeed, accumulating evidence suggests that metallochemical alterations may play a role in the pathogenesis of prion diseases and other neurodegenerative diseases. It has been demonstrated that both recombinant and brain-derived PrP have superoxide dismutase (SOD)-like activity when Cu is bound to the octarepeat region resulting in conformational changes to the protein. Brown et al. have reported [9] that it loses the SOD-like activity when Cu is replaced with Mn in recombinant PrP, and also that Cu binding to PrP purified from sporadic CJD was significantly decreased while the binding of Mn and Zn was markedly increased.

Recent investigations of scrapie, CJD, and chronic wasting disease clusters in Iceland, Slovakia and Colorado, respectively, have indicated that the soil in these regions is low in copper and higher in manganese, and Brown et al. observed striking elevation of manganese ion accompanied by significant reduction of copper ion bound to purified PrP in all sCJD (sCJD = sporadic CJD) variants [9]. These results suggest that altered metal-ion

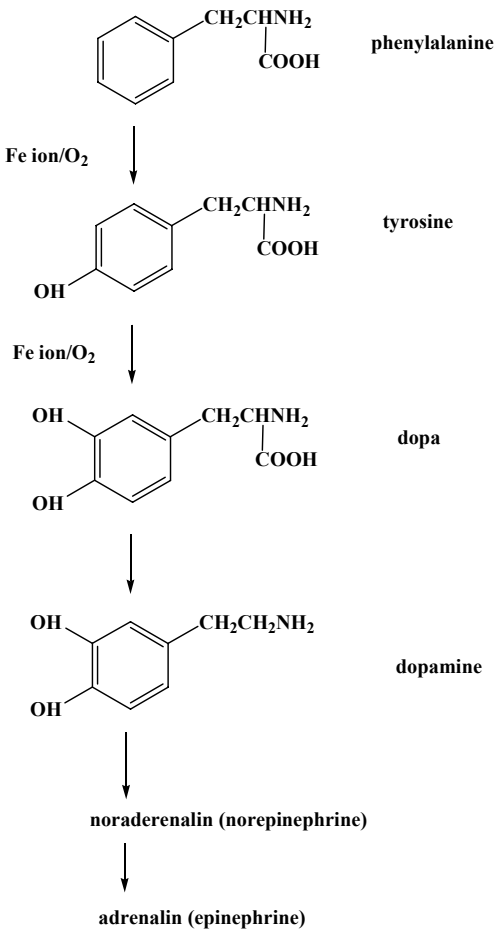


FIGURE 1. BIOSYNTHESIS OF ENDOGENOUS CATECHOLAMINE NEUROTRANSMITTERS.

occupancy of PrP plays a pivotal role in the pathogenesis of prion diseases. The possibility that imbalances in environmental cations entering the food chain may induce conditions favoring the formation of proteinase-resistant PrP is a controversial but intriguing possibility.

BSE may have been spread among cattle by the feeding of infected offal but the majority of cases of naturally occurring prion diseases arise sporadically with no known cause. Thus, the most important problem to be solved is to elucidate the intrinsic origin, i.e., the chemical mechanism of the prion diseases which arise sporadically. The sporadic neurodegenerative diseases are in general endemic; many years ago ALS (amyotrophic lateral sclerosis) patients were collectively found in the New Guinea and Papua islands, and its origin has been attributed to the drinking subterranean water, which contains much Al^{3+} and Mn^{2+} ions, and in these region many patients of Alzheimer's and Parkinson's diseases were found [10], and increased aluminum levels were reported in the hippocampus of patients with Alzheimer's disease [11]. In Alzheierm's disease specific regions such as the hippocampus and motor cortex contain elevated iron levels relative to the normal, whereas the occipital cortex contains decreased levels of iron, and abnormalities in brain iron metabolism have been described for several neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease. These facts clearly indicate that the neurodegenerative diseases described above are closely related with the function of metal ions, and thus in this article we will clarify the mechanism of the "oxidative stress" induced by the metal ions such as copper, manganese, and iron, etc, in order to obtain the endogenous origin of the prion diseases and other neurodegenerative diseases which include ALS, Alzheimer's and Parkinson's diseases.

2. BIOCHEMISTRY OF PARKINSON'S DISEASE: DEFICIENCY OF NEUROTRANSMITTERS AND NEURAL CELL DEATH

In October 2000, the Nobel Assembly awarded the Nobel Prize in Physiology and Medicine to Arvid Carlsson of the University of Gothernburg in Sweden and to two pioneers in the study of nerve cell communications, Paul Greengard of Rockefeller University and Eric Kandel of Columbia University in New York. Greengard figured out how dopamine and other neurotransmitters trigger target neurons when they bind at the synapse, the junction between two nerve cells, and Kandel built upon these insights to demystify some aspects of learning and memory. Carlsson overturned conventional wisdom by proving that dopamine, once thought to be merely a precursor in the synthesis of the neurotransmitter norepinephrine (see FIG. 1), is an important nervous system messenger in its own right. They gave rabbits a drug that depletes norepinephrine in the brain, putting the animals into a temporary stupor. Carlsson found that the rabbits could be roused with injections of L-dopa, which the brain converts to dopamine. Later they disco-

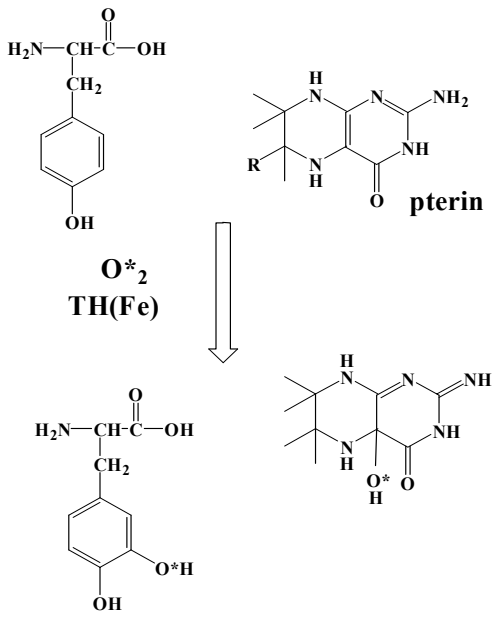


FIGURE 2. FUNCTION OF TYROSINE HYDROXYLASE (TH).

vered that Parkinson's disease resulting from degeneration of dopamine-producing neurons in the brain involved movement control. That finding led to the use of L-dopa as a therapy for Parkinson's patients.

Parkinson's disease is a common neurodegenerative disorder that is clinically characterized by tremor, bradykinesia, rigidity, and loss of postural reflexes. It is generally believed that the major symptoms of Parkinson's disease are caused by a striatal dopamine (DA) deficiency, secondary to degeneration of nigrostriatal dopaminergic neurons and possibly a decreased DA-biosynthetic capacity in the surviving neurons [12,13]. Although the DA loss is most pronounced, norepinephrine, serotonine, and melanin pigments are also decreased, whereas cholinergic activity seems to be increased. The selective loss of specific neurons in the central nervous system (CNS) is a characteristic feature for Parkinson's disease and other common neurodegenerative disorders, such as Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis (ALS), and also "mad cow disease". Although originally discounted, hereditary factors have emerged as the focus of research in Parkinson's disease; recent studies suggest that hereditary factors play an important role in sporadic Parkinson's disease, and two genes are clearly associated with the diseases; α-synuclein and parkin, and as a third, gene ubiquitin C-terminal hydroxylase L1 [14].

Let us at first consider the biochemical synthesis of dopamine. The chemical mechanism of dopamine synthesis has been elucidated by the biochemists, and the result is illustrated in FIG. 1. Dopamine is synthesized from phenylalanine and tyrosine, one of the 20 essential amino-acids through the oxygenation reaction at the benzene ring by the enzymes, phenylalanine hydroxylase (PAH) or tyrosine hydroxylase (TH). It should be noted here that the oxygenation at the benzene ring does not occur in the air without the catalyst, and thus it is necessary for us to know the detail chemical mechanism of the enzymes, TH or PAH.

TH is a non-heme iron protein that uses one molecule of dioxygen to hydroxylate its amino acid and tetrahydropterin substrates to hydroxy-amino acids and 4α-hydroxytetrahydropterins, respectively (see FIG. 2) [15,16]. As the 4α-hydroxytetrahydropterins subsequently dehydrates and is regenerated by the NADH-dependent enzyme dihydropteridine reductase, it is frequently termed a cofactor for the peteridine-dependent hydroxylases. The cofactor (BH_4) is the most abundant of the unconjugated tetrahydropterins in mammalian tissues and is considered to be the naturally tetrahydropterin substrate for these enzymes. The active site structure and catalytic mechanism of the aromatic amino acid hydroxylases have been investigated by kinetic and spectroscopic techniques, as well as by site-directed mutagenesis. The iron is necessary for catalytic turnover, and the tetrahydropterin and amino acid substrates bind close to the Fe(II) center, but probably without a direct coordination to the metal center.

Increased brain iron concentrations have been described in several neurodegenerative diseases, most notably in those diseases characterized by nigral degeneration, such as Parkinson's disease (see Introduction). The cause of nigral cell death in these disorders in unknown, but considerable experimental evidence supports the hypothesis that the cellular degeneration observed results from oxidative stress [13,17-19]. Oxidative stress manifests itself as an increased oxidation of cellular

constituents (lipids and proteins) and DNA damage. Lipid peroxidation and protein damage have been observed in the SN of Parkinson's disease patients, which suggests that oxidative stress is involved in the pathogenesis of this disease.

The increase in iron observed in these disorders is suggested to be critical because of its assumed role in catalyzing the production of the so-called oxygen free radicals via the metal dependent reduction of hydrogen peroxide. This reaction, sometimes referred to as the Fenton reaction, may involve the reaction of hydrogen peroxide with ferrous ion to produce the potentially damaging hydroxyl radical (OH•), but it should be noted that free intracellular ferrous iron concentration have been calculated to be very low, below 10^{-8} M [20], and nobody has succeeded in confirming its formation by the reliable chemical process.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH\bullet$$

Above discussions suggest that "oxidative stress hypothesis of Parkinson's disease" [6] seems to be valid, but we cannot understand it without the precise knowledge on the chemistry of oxygen and transition metal ion such as Fe(II).

3. MOLECULAR APPROACHES TO AMYOTOPHIC LATERAL SCLEROSIS (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive, devastating syndrome that affects both upper and lower motorneurons and results in limb and facial motor weakness, atrophy, and death [21,22]. The age-adjusted world-wide incidence of ALS is 0.5-3 per 100,000 person years (without obvious race-related differences). Older males and postmenopausal females are most typically related. Familial ALS (fALS) accounts for less than 10 % of diagnosed cases, with sporadic ALS (sALS) comprising the remainder of diagnoses. Although the pathogenesis of ALS remains unknown, notable progress has been made in identifying molecular processes potentially involved in ALS-mediated motor neuron injury.

A significant discovery in ALS research was the identification of a genetic defect associated with 10–15% of fALS kindreds. The involved gene, SOD1, encodes a cytosolic from of superoxide dismutase (SOD), and identified mutations in exons, 1, 2, 4, and 5 of the SOD1 gene all appear to reduce Cu/Zn-SOD stability [23]. There are several SOD enzymes, which contains manganese ion or iron ion, but in this chapter only the SOD which contains copper and zinc ions is restricted [24,25]. Two such point mutations (Ala4Val and Gly41Ser) produce an enzyme with less than 5% of normal Cu/Zn SOD activity, and death of affected patients occurs after less than one year of symptom onset.

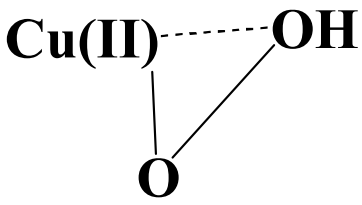
Siddique et al. have determined the crystal structures of human SOD, along with two other SOD structures, and have established that the fALS mutations do not change any active-site residues involved in the electrostatic recognition of the substrate, the ligation of the metal ions or the formation of the active-site channel, but only the slight change in the neighborhood around the Cu(II) ion is detected. On the basis of rigorous studies defining the structural and energetic effects of conserved hydrophobic packing interactions in proteins, six of the fALS mutations would be expected to destabilize the subunit fold or the dimer contact. The most frequent fALS mutations would disrupt both, the subunit fold and the dimer interface [23,26].

In 1997, Yim et al. have reported that a fALS mutant (Gly93Ala = G93A) exhibits an enhanced free radical-generating activity, while its dismutation activity is identical to that of the wild-type enzyme [27]. These are indicating that fALS symptoms are not associated with the reduction in the dismutation activity of the enzyme. They reported that the mutant and wild-type enzymes contain one copper ion per subunit have identical dismutation activities, however, the free-radical generating activity of the mutant, as measured by spin-trapping method at low H_2O_2 concentration, is enhanced relative to that of the wild-type and G93A, wild-type < G93A < A4V. To understand ALS pathogenesis, we must understand how altering SOD activity can induce cell injury. The SOD's are well-known enzymes that catalyze the disproportionation reaction of superoxide ion [24], which is considered to be one of the reaction oxygen species (ROS), into oxygen and hydrogen peroxide.

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

$$Cu(I) + O_2^- \rightarrow O_2H_2 + Cu(II) \quad (2)$$

The reaction mechanism of this enzyme has been investigated by many authors. Very recently, Nishida et al. have postulated new mechanism for this enzyme based on the results used by the model compounds [28,29]. We have pointed out the importance of formation of a Cu(II)-OOH species as an intermediate (see SCHEME I) in the second step (2) above, and this hydrogen peroxide produced immediately is moved from the wild-type enzyme because of the negligible interaction between hydrogen peroxide and the Cu(II) ion which is due to a distorted square pyramidal structure of the Cu(II) ion in the enzyme, and destroyed into water and oxygen.

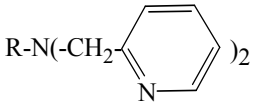


SCHEME I

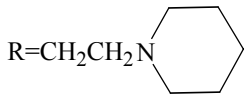
In order to obtain the comprehensive solution for the correlation between the structural change in mutations and pathogenesis of ALS, we have studied the reactivity of a Cu(II)-OOH, proposed as an important intermediate in the SOD reaction. For this purpose, we have synthesized many Cur(II) compounds with the ligands which contain N,N-bis(2-picolylmethyl)amine moiety, as illustrated in FIG. 3 [28].

We have measured the ESR spectra of the solution containing a Cu(II) complex and spin-trapping reagent, such as PBN (α-phenyl-N-t-butylnitrone) and TMPN (N,N,N',N'- tetramethyl-4-piperidinol), specific reagents for OH• radical and singlet oxygen ($^1\Delta g$) (SCHEME II), respectively [29].

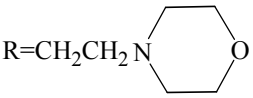
We have found that no ESR signal due to the formation of radical of PBN was detected when the Cu (II) complex was mixed with H_2O_2 and PBN.



R=-$CH_2CH_2C(=O)NH_2$ (bdpg)
=-$CH_2C(=O)NH_2$ (dpgs)
=-CH_2CH_2COOH H(dpal)
=-$CH_2C(=O)NHCH_2C(=O)NHCH_2COOH$ (dpgt)
=-$CH_2CH_2C(=O)NHCH_2COOCH_3$ (G-bdpg)

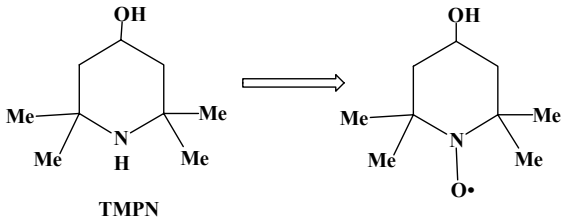


(pipy)



(mopy)

FIGURE 3. CHEMICAL STRUCTURES OF THE LIGANDS USED IN OUR STUDY.

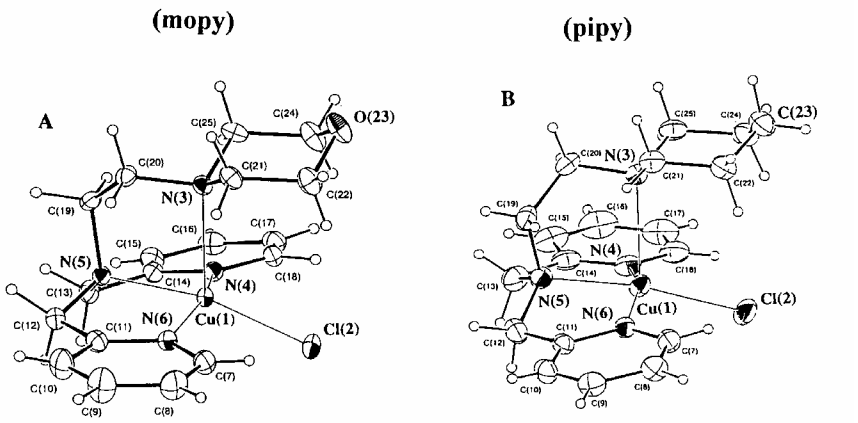


TMPN

SCHEME II

However, strong peaks due to nitron radical formation of the corresponding TMPN (SCHEME II) was detected in some cases; especially comparison between the Cu(pipy)Cl^+ and the Cu(mopy)Cl^+ is interesting. Structural features of the two compounds are essentially the same, instead the difference of the oxygen atom on the morphorin ring of Cu(mopy)Cl^+ complex is replaced by the $–CH_2$ in the Cu(pipy)Cl^+ complex (see illustration).

In the case of Cu(pipy)Cl^+, no formation of the nitron radical was observed; on contrast to this, high activity for the radical formation by the Cu(mopy)Cl^+ complex. In this case, similar to those of the Cu(mopy)Cl^+ complex, the addition of the H_2O_2 to the Cu(II) solution does not induce the change in ESR spectrum due to the Cu(II) ion; but



the addition of TMPN leads to the dramatic change in the ESR signals attributed to the Cu(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 Cu(II), hydrogen peroxide, and TMPN occurs only when three reagents are present in the solution (see the FIG. 4), and unique reactivity of the hydrogen peroxide observed is detected only when the intermediate is formed in the solution. Our present results clearly show that some Cu(II) chelates can activate the hydrogen peroxide to exhibit high reactivity similar to that of the singlet oxygen ($^1\Delta g$) [29].

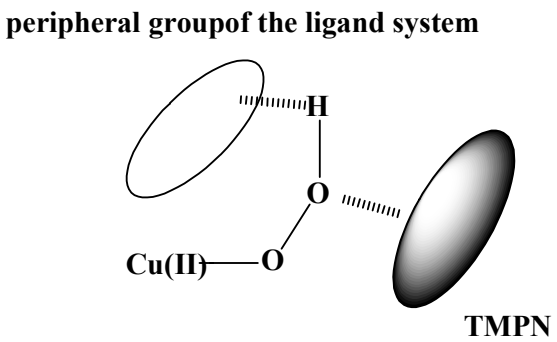
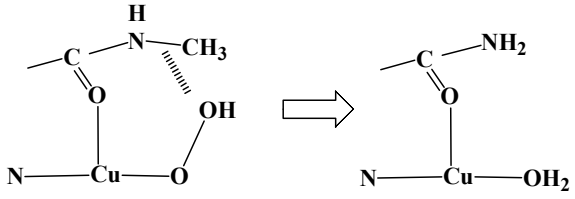


FIGURE 4. ASSUMED INTERMEDIATE AMONG CU(II) CHELATE, H_2O_2 AND TMPN.

In order to get further information on the reactivity of a Cu(II)-OOH species, we have measured the ESI-Mass spectra of the solutions of Cu(II) compounds and hydrogen peroxide. When hydrogen peroxide was added to the Cu(Me-bdpg)Cl solution, the formation of [Cu(bdpg)Cl], not [Cu(dpal)], was detected by ESI-Mass spectra [30]. These are clearly indicate 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.



All these facts may indicate that the "gain-of-function" of the mutant SOD is due to highly reactive Cu(II)-OOH formed as an intermediate in the process of mutant SOD reaction; the chemical structures around the Cu(II) in the mutant SOD is slightly changed, and this gives an unexpected effect on the reactivity of a Cu(II)-OOH as observed in our papers. In the mutant SOD the C-N bond cleavage by the Cu(II)-OOH may give great changes in the

surface of SOD, leading to destabilizing of the dimer contact of the SOD enzyme [31].

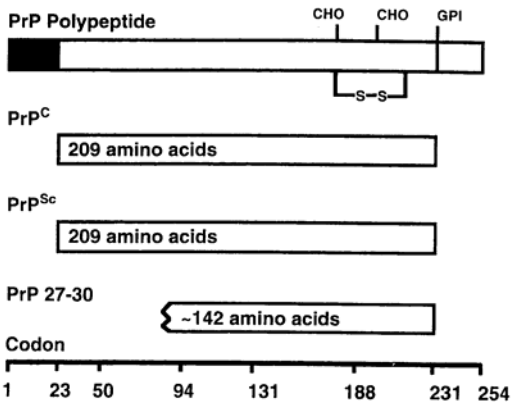
It has been generally believed that hydrogen peroxide is relatively inert and not toxic to cells, but damage occurs when hydrogen peroxide interacts with the reduced forms of transition metal ions, e.g., Fe(II) or Cu(I), and decomposes to highly reactive hydroxyl radical. Our data clearly shows that hydrogen peroxide exhibits high activity when it interacts with the metal ions such as Cu(II) or Fe(III) (see later chapter), and that a Cu(II)-OOH species can be a highly reactive and toxic species in the biological system without formation of hydroxyl radical [32,33]. It should be noted here that the reactivity of the Cu(II)-OOH is determined by the structural properties of the intermediate, depending upon the chemical interactions of Cu(II)-OOH species with peripheral groups and substrate (see FIG. 4) [34].

4. THE MOLECULAR APPROACH TO SCRAPIE AND BOVINE SPONGEFORM ENCEPHALOPATHIES

As described before, PrP^C is a glycoprotein expressed on the surface of many cell types. PrP^C is linked to the cell membrane by glycosylphophatidylinositol (GPI) anchor. It has one or two sugar chains that are closely linked to the C-terminus and also exists in a non-glycosylated form. The genetic code of the prion protein PrP^C was identified only after the isolation of an abnormal isoform, PrP^{Sc} from brains of mice that were infected with the disease scrapie [4-8,35].

As indicated in Introduction, it is generally recognized that PrP^C not only binds Cu within the octarepeat region located in the unstructured N-terminus, but under certain specific circumstances may bind along the C-terminal structured domain of protein fragments. Furthermore, recombinant PrP^C can also bind other metals such as manganese at both the octarepeats and the C-terminal sites. The copper at the synapse is released in vesicles and studies of copper concentration have suggested that the level can reach 250 mM locally. The copper released in this way appears to be taken up rapidly by the neurons, and deployed within 30 minutes of this process. It is unknown in what from this copper is bound, however it is probable that the copper is chelated to some peptide chaperones or amino acids because there is little free copper found in the body [36]. The exact role of copper in the synapses remains elusive.

Since 1996 there has been increasing evidence that PrP^C increases cellular resistance to oxidative stress. Cerebelle neurons and astrocytes from PrP^C knowkout mice are more sensitive to superoxide toxicity, whereas cells with higher levels of PrP^C expression are more resistant to oxidative stress [8]. Analysis of recombinant mouse and chicken PrP^C has lead to the discovery of an important "gain-of-function" following the formation of the PrP^C copper complex. Recombinant PrP^C that has as least two atoms of copper bound specifically has an activity similar to that of superoxide dismutase. PrP^C has been shown to contribute directly to cellular SOD activity. This implies that PrP^C might act as to detoxify superoxide.



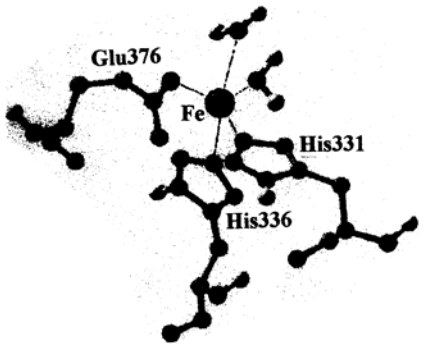
The protease-resistant PrP extracted from affected brains was of 27-30 kDa and became known as PrP^{27-30} (see the illustration above). PrP^{27-30} is derived from a large molecule of 33-35 kDa, designated PrP^{Sc}, and no difference in amino acid sequence between PrP^C and PrP^{Sc} have been identified. PrP^{Sc} is derived from PrP^C by a posttranslational process, and it is linked to polymorphism in codon 129 of the prion gene. It should be noted here that the chemical environment around the copper ion in the PrP^{Sc} should be

different from those in the PrP^{C}, which is similar to the difference observed between the Cu(II) ion in the wild-type and mutant SOD enzyme. Thus, it is most likely that the "gain-of-function" in the PrP^{Sc} may be due to a "highly reactive" Cu(II)-OOH formation, which leads to the cleavage of the peptide bonds around the Cu ion (near at about 90 site), giving $PrP^{27\text{-}30}$. Several experimental facts support our idea [37,38].

PrP^{Sc} is extracted from affected brains as highly aggregated, detergent-insoluble materials that is not amenable to high-resolution structural technique. PrP^{Sc} is covalently indistinguishable from PrP^{C}. During infection, the underlying molecular events that lead to the conversion of PrP^{C} to the scrapie agent remain ill defined. A central question at the molecular level is: what induces the conversion of PrP^{C} to PrP^{Sc} or other abnormal TSE-associated forms of PrP [7,8,39]? The prediction from theoretical models is that PrP^{Sc} itself induces the conversion. Indeed, in vitro experiments have shown that when PrP^{C} was taken out of the context of the membranes, it can binds selectively to PrP^{Sc} and be converted to a protease-resistant state that is indistingushabe from that of PrP^{Sc} itself. A schematic model of this two-step process is suggested [7,8,39]. Although this PrP^{Sc}-induced conversion reaction was originally demonstrated under the cell-free conditions containing non-physiological denaturants, it has been adapted to much more physiologically compatible conditions. In fact, in situ conversion reactions have been demonstrated using intact, TSE-infected brain slices, revealing that both amyloid plaque and diffuse deposits of PrP^{Sc} have the ability to induce conversion. The PrP^{Sc}-associated converting activity correlates with scrapie infectivity in guanidine hydrochloride denaturation studies, and further studies based on the protein-protein interaction should be necessary for the purposes.

5. MECHANISM OF TYROSINE HYDROXYLASE AND O_2 ACTIVATION

As shown before, the mammalian aromatic amino acid hydroxylases (phenylalanine, tyrosine, and tryptophan hydroxylases; PAH, TH, and TPH, respectively) are a unique class of monooxygenases in their use of tetrahydropterins as obligatory cofactors [15,16]. These enzymes play important roles in mammalian metabolism: PAH initiates the detoxifications of high level of phenylalanine. Phenylalanine hydroxylase catalyzes the formation of tyrosine; mutations in the enzyme are the most common cause of phenylketonuria. Tyrosine hydroxylase catalyzes the formation of dihydroxylphenylalanine, the first step in the biosynthesis of the catecholamine neurotransmitters, including dopamine. Tryptophan hydroxylase catalyzes the formation of 5'-hydroxytryptophan, the first step in the biosynthesis of the neurotransmitter serotonin. These enzymes are monooxygenases, incorporating one atom of oxygen from molecular oxygen into the substrate and reducing the other atom to water. The two electrons required for the reduction of the second atom to water are supplied by the tetrahydrobiopterin (BH_4) substrate. Phenyalanine hydroxylase is present at relatively abundant levels in liver. Both tyrosine and tryptophan hydroxylases are found in the central nervous system. TH is also present in the aderenal gland, a common source of the naturally occurring enzyme.



The structure of the iron site in TH is shown (see the illustration right above) [15,16]. There are thee amino acid ligands to the metal, histidine 331 and 336 and glutamate 376. In addition, there are two solvent molecules 2.0 Å from the iron, resulting in square pyramidal geometry with histidine 331 as the axial ligand.

In the absence of bound substrates or inhibitors, the active site can be identified from the location of the iron atom. This assignment is supported by the

results of Maitines, who used the paramagnetic effects of the metals in TH and PAH to show that both the aromatic ring of the amino acid substrates ate the tetrahydropterin bind closely to the metal. In both structures the iron is located in a hydrophobic cleft 10 A from the surface.

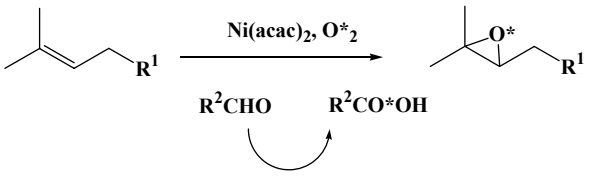
As shown in FIG. 2, one of the products of the reactions catalyzed by all three hydroxylases is a 4α-hydroxypterin. The oxygen atoms in both the amino acid and the pterin products have been shown to come from molecular oxygen. The formation of this compound establishes that there must be a reaction between molecular oxygen and tetrahydropterin during catalysis. It has been accepted that 4α-peroxytetahydropterin is the intermediate results of the reaction of the tetrahydropterin and molecular oxygen. Based on the model studies with both flavins and pterin, this reaction was assumed to occur in two steps; initial slow, single-electron transfer to form superoxide and the pterin cation radical followed by rapid combination of the two radicals. The 4α-peroxypterin may be considered as a candidate for the hydroxylating intermediate, since the analogous 4α-peroxyflavin is thought to be the hydroxylating intermediate in the flavin phenol hydroyxlases. Fitzpatrick et al. have proposed [16] that the range of the reaction resembles the reaction catalyzed by cytochrome P450-dependent hydroxylases [40], in which the hydroxylating intermediate has been believed to be a high-valence iron-oxo species, and thus it seems likely that a more reactive species containing the iron, an iron-oxygen species is involved.

Dix and Benkovic reported [41] that the amount of hydroxylated amino acid formed agreed well with the amount of 4-hydroxypterin, suggesting that the oxygen-oxygen bond is not cleaved unless hydroxylation occurs, and no hydrogen peroxide was detected. Iron is clearly required for activity, and the mutation of the ligands to the iron results in the loss of detectable activity. Electron paramagnetic resonance spectrum of enzyme frozen at 7K during turnover does not show any ESR-detectable species, and this can be rationalized by formation of the hydroxylating intermediate being much slower than its substitution reaction. An additional potential role for the iron atom would be to assist in the reaction of tetrahydropterin and molecular oxygen to form the peroxypterin. The ^{18}O kinetic isotope effects determined by Francisco et al. are consistent with either a single-electron transfer from tetrahydropterin to oxygen, or with equilibrium binding of O_2 to the iron atom followed by single-electron transfer from the tetrahydropterin to the iron-bound oxygen. Dix et al have suggested the peroxypterin-iron complex as a reasonable intermediate, and pointed out that heterolytic cleave of the oxygen-oxygen bond of the species would generate the peroxypterin and the iron-oxo hydroxylating species directly.

I cannot believe that high-valent iron-oxo species is an active species in the cytochrome P450 [42], and there is no reasonable explanation for the peroxopterin formation proposed by Dix et al., we proposed quite different mechanism from those published before, and we would like to point out that only our mechanism can elucidate the mechanism of the hydroxylses reasonably, and give a reasonable explanation for the correlation between the neurodegenerative diseases and metal ions.

5.1. NISHIDA'S MECHANISM FOR THE HYDROXYLASES

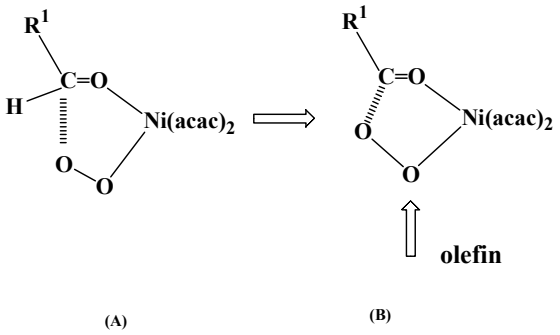
In 1991, Mukaiyama et al. have reported that in the presence of a catalytic amount of bis(1,3-diketonato)Ni(II) complex, trisubstituted and exo-tetrminal olefins or norbornene are smoothly monooxygenated into the corresponding epoxides in high to quantitative yields on treatment with aldehyde under an atmosphere pressure of oxygen at room temperature [43].



This report have attracted much attention for the chemists, because it is generally recognized that the Ni(II) complexes are in generally in the oxidation state II, and redox reactions concerning the Ni(II) ion are almost rare. In the previous papers, we already postulated that in some metal compounds a

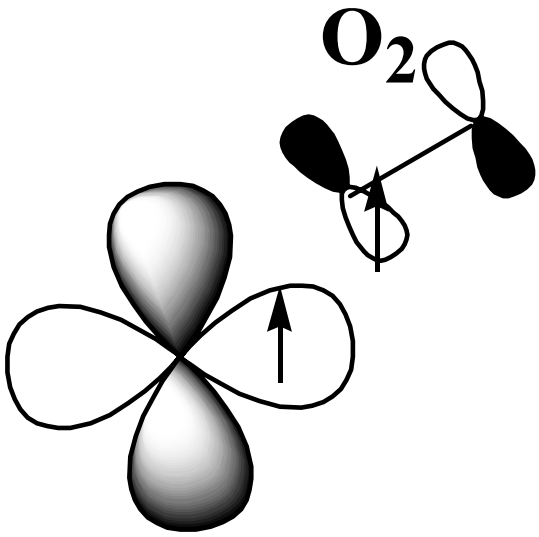
transition metal ion such Fe(III) or Cu(II) can activate oxygen without change the oxidation state of the metal ions, [44-47] which is quite against the common sense of general chemists.

According to the Nishida's mechanism, the epoxidation by the Mukaiyama reaction can be explained as follows: in the reaction mixture, an intermediate among six-coordinate Ni(II) species of acetylacetone, oxygen, and aldehyde, is formed as illustrated (see [A] in the illustration right below), and this intermediate reacts with olefin to give the epoxide and the corresponding acid through the heterolytic cleavage of the O-O bond (see [B]) [45].



In this scheme, the oxidation state of the Ni(II) is unchanged throughout the reaction. The formation of the intermediate is promoted through the interaction of unpaired electrons in the d-orbital of the Ni(II) ion and that of the oxygen molecule (see SCHEME III; this weak interaction between the unpaired electrons of d-electron and oxygen was confirmed experimentally [48,49]), and is also promoted by the interaction with the aldehyde molecule. In this process, it is anticipated that the oxygen coordinated to the Ni(II) is activated to behave as singlet oxygen ($^1\Delta g$) which was confirmed by us experimentally (see also FIG. 5), [45-47] and the same mechanism was also applied to elucidate the reaction of lipoxygenase [42].

It should be noted here that the Mukaiyama reaction is very similar to those of the hydroxylases, such as TH, i.e., the aldehyde used in the Mukaiyama reaction is replaced by pterin in TH. Thus, we may elucidate the reaction mechanism of the hydroxylases by the use of scheme described for



SCHEME III

the Mukaiyama reaction as shown in SCHEME IV, that is the oxygen in the intermediate is activated through interaction with pterin and d-electron of the Fe(II) ion, to react with the substrate, giving the hydroxylated product and 4α-hydroxytetrahydropterin (see also FIG. 2). In this latter process, it is likely that the substrate also activates the coordinated

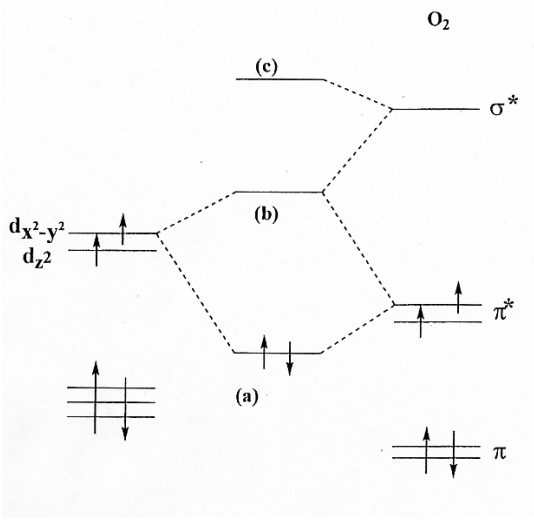
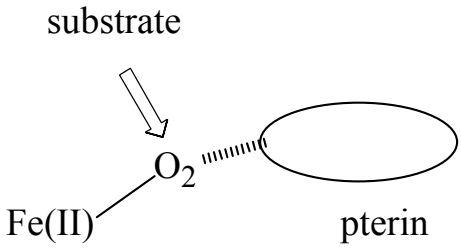


FIGURE 5. MO SCHEME FOR NI(II)-O_2 SYSTEM. The formation of orbital (b) may be responsible for the appearance of reactivity similar to singlet O_2 ($^1\Delta g$).



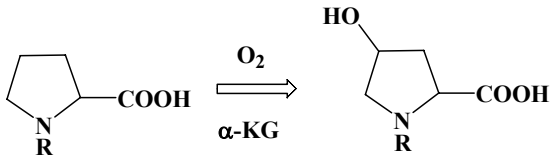
substrate:phenylalanine, tyrosine

SCHEME IV
Nishida's mechanism for hydroxylases

oxygen, as proposed for monooxygenases [42]. It is quite likely that the displacement of the iron in TH for Al^{3+} should lead to the deficiency of dopamine, because the Al^{3+} ion has no unpaired electron.

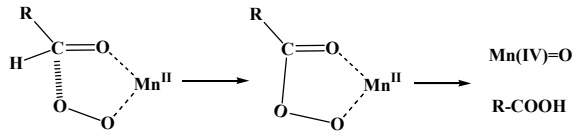
5.2. DIFFERENCE IN Fe(II) AND Mn(II) IONS IN OXIDATION REACTION

The α-keto acid-dependent enzymes are distinguished from other non-heme iron enzymes by requirement of an α-keto acid cofactor as well as Fe(II) and O_2 for reactivity [50]. They now constitute a large class of enzymes which are essential in the biosynthesis of many biological compounds. Examples of these enzymes are illustrated below: The reactions catalyzed by these enzymes involve the oxidation of an unactivated C-H bond to give either hydroxylated products.



Prolyl hydroxylase

In general, these enzymes require 1 equiv of Fe(II), and α-keto acid (usually α-ketoglutarate), and ascorbate for full activity. Substitution with other divalent metal ions [Zn(II), Mn(II), Co(II), Mg(II), and Ni(II)] results in the complete loss of enzymatic activity, which is attributed to competitive binding of these ions at the active site [50]. These facts are clearly show that the oxidation activity by the Fe(II) ion is completely different from those of other metal ions, especially Mn(II). This should be due to that Mn(II) compounds are in favour of the "two-electron oxidation" reaction and readily oxidized to an Mn(IV) ion, whereas "one-electron reaction", for the Fe(II) species [51]. In fact, it was already reported that a Mn(IV) species facilely forms in the reaction mixture of a Mn(II) complex and an aliphatic aldehyde, generally considered to be a reducing agent.



These are suggesting that in the biological iron-containing enzymes such as phenylalanine hydroxylase or tyrosine hydroxylase, the replacement of the iron ion by Mn(II) ion will give rise serious damages on the synthesis of neurotransmitters such as dopamine, etc. It should noted here that the recent investigations of scrapie, CJD, and CWD clusters in Iceland, Slovakia, and Colorado have indicated the soil in these regions is low in copper and higher in manganese. Similar facts were also known, that is, ALS and Parkinson's and Alzheimer's patients were collectively found in the New Gunia and Papua island, and its origin has been postulated to the drinking subterranean water, which contains much concentrations of Al^{3+} and Mn^{2+}, and increased aluminum levels were reported in the hippocampus and the motor cortex patients with Alzheimer's disease. Thus, it seems likely that the elevated concentration of these exogenous Al^{3+} and Mn^{2+} ions is a one of the serious origin of these endemic neurodegerative diseases; which should lead to the loss of activity of the enzymes which

play a central role in the synthesis of neurotransmitters.

5.3. "MANGANISM" AND ITS ORIGIN

As a nutrient, manganese is an essential component of several enzymes; a deficiency can lead to heart and bone problems and in children, stunted growth. However, it has been known since 1837 that workers in manganese mines can develop manganism, a dreaded illness marked by Parkinson's-like tremors, violent outbursts, and hallucinations [52]. Victims have lesions in the globus pallidus and striatum of the basal ganglia, a part of the brain involved in fine muscle control. When manganese (this should be manganese oxide) is inhaled, blood ferries it from the lungs to the brain, where it can readily cross the blood-brain barrier. As stated above, excess manganese ions in the brain may lead to the deficiency of "dopamine", and this should give the most reasonable explanation for the "manganism" observed.

Many countries in the world completed phasing out leaded gasoline (gasoline containing tetraethyl-lead), paving the way for widespread use of a manganese-based compound, MMT (methyl cyclopentadienyl manganese tricarbonyl), in gasoline. The additive increases octane level, which boosts engine performance and enables fuel to be burned more evenly. This means that manganese oxide, one of the famous chemical carcinogens [53], is widely spread in the sky, and the human inhale the manganese oxide every day, which is stored in the lung. The use of MMT should be stopped as soon as possible.

6. POLYNUCLEAR METAL SPECIES AS A SERIOUS ORIGIN IN "OXIDATIVE STRESS"

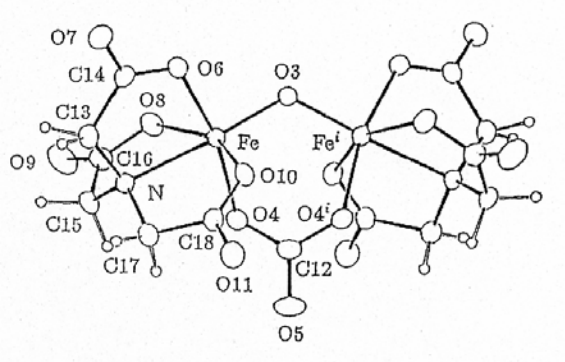
Human iron metabolism and absorption has been the subject of a recent review. Normal human males contain 3-5 g of iron (often less in females) and of this, 2/3 is in circulating red cells as hemoglobin and 15-25% in storage in ferritin and hemosiderin [20]. The remaining iron is in muscle myoglobin (~8%) and in cytochromes and iron-containing enzymes. Plasma transferrin accounts for only 3 mg of Fe, but the daily exchange if iron through plasma transferring is ten times this account. Transferrin therefore plays a central role in iron distribution. Iron delivery by transferring to etythroid and many non-erythroid cells involves interaction of transferring with specific receptors followed by endocyosis and recycling of apotransferrin and receptor. These receptors are present in low amounts on phagocytosis cells which receive their iron from degraded red cell hemoglobin. In the case of hepatocytes it has been proposed that iron is released by reduction at the plasma membrane rather than receptor-mediated endocytosis, but there is no evidence for a diferric reductase in rat liver plasma membrane. Under pathological conditions (e.g., iron overload or serum transferring deficiency) the amount of iron not bound to transferring may become important. This is distributed to organs in an inappropriate manner leading to iron overload in parenchymal organs such as liver and pancreas. Fe(III) bound citrate, nitriloacetate (nta, see the next page) or other complexes is efficiently taken up by cultured cells. Ferritin may also carry significant amounts of iron between liver Kupffer cells and hepatocytes.

Genetic haematochromatosis (either idiopathic or hereditary) is one of the most common genetic disorders in western populations, particularly among Celtic peoples. This disease is associated with greatly increased deposits (sometimes as high as 50-fold) of storage iron, predominantly as haemo-siderin, in the liver and other tissues due to abnormally high absorption from the gut, although there seems to be little or no ferritin in the duodenal absorption cells. The excess iron cannot be eliminated and must be stored. Elevated body iron leads to increased iron in storage (and not to increases in hemoglobin, myoglobin, etc. except in the treatment of iron deficiency anaemia). Under normal iron loading, ferritinis the major storage form, but in diseases of iron overload, the capacity to synthesize ferritin levels off and haemosiderin becomes predominant.

As shown in Introduction, a decrease in substantia nigra copper content was shown in Parkinson's disease. The elevation of zinc concentration in the substantia nigra appears specific to Parkinson's disease, whereas increased aluminum levels were reported in the hippocampus of patients with Alzheimer's disease. In Alzheimer's disease, specific regions such as the hippocampus

and the motor cortex contain elevated iron levels. In contrast to iron levels, ferritin levels were differentially altered in the brain of patients with Alzheimer's disease [11]. The ratio of ferritin to iron decreases by over one-third in the superior temporal gyrus with both age and Alzheimer's disease, whereas there is little change in the ratio in the motor cortex and an actual increase (three times) in the occipital cortex. This suggests that iron-over load syndrome may be induced through the accumulation of exogenous metal ions such as Al^{3+} and Mn^{2+}; as stated before, the accumulation of the metal ions, Al(III) and Mn(II) in brain will lead to the deficiency of dopamine and other neurotransmitetrs, and under this circumstances the brain will order the transferrin to transport the iron ion to the enzyme, but the transported metal ions including Fe(III) and Al(III) should be discarded to the tissue, because the enzyme is loaded with the metal ion, Al(III); this is consistent with the fact observed.

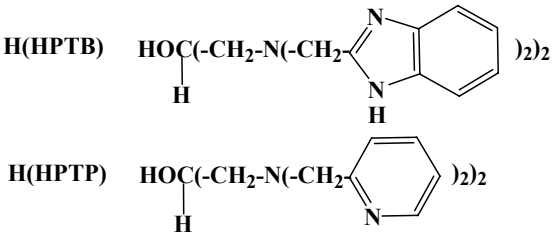
Normal cells store iron mainly in ferritin molecules, but under conditions of iron overload some of it is shunted into another source of storage form known as haemosiderin. Several data support the hypothesis that haemosiderin is a degradation product of ferritin, and this has been confirmed for phytosiderin, an insoluble iron-containing product from pea seed, whch was found to contain a peptide derived from the ferritin subunit. These findings indicated that ferritin is taken up by lysosomes, and that subsequent processing involved a partial dissolution of the core, and degradation to siderosomal ferritin and to the insoluble haemosiderin. Thus the haemosiderin from various iron-loaded animals were consistently found to have ferrihydrite-like iron cores similar to ferritin, and haemosiderin is typically insoluble, as isolated, in contrast to the soluble ferritin, but it can be solubilized by treatment with alkali and detergents, and also with the several amino acids or small peptides. It is quite likely that some Fe(III) ions exist in brain as soluble dimeric (or polymeric) compounds with amino acids or peptides, exemplified such as $Fe_2(HPTP)Cl^{4+}$, or with nta; the latter complex is of a μ-oxo bridged dimeric structure, $[Fe_2O(nta)_2(CO_3)]^{2-}$, as illustrated on the right [54-56].



6.1. UNIQUE REACTIVITY OF BINUCLEAR Fe(III) SPECIES IN THE PRESENCE OF HYDROGEN PEROXIDE AND/OR REDUCING AGENTS

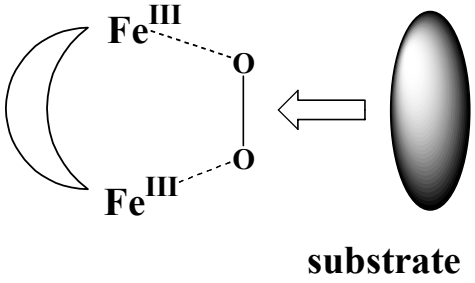
We have reported that dimeric or polymeric Fe(III) (including binuclear Cu(II) and Co(II)) complexes show unique reactivity: (i) high ability to form hydrogen peroxide in the presence of O_2 and reducing agents, (ii) high ability to oxygenate linoleic acid or DMPO in the presence of O_2, and (iii) high ability to activate hydrogen peroxide.

For example, the oxidation of the TMPD, one of the famous one-electron donor, is greatly accelerated in the presence of the binuclear Fe(III) complex, $Fe_2(HPTP)Cl^{4+}$ or binuclear Cu(II) complex [57,58], to give much quantity of hydrogen peroxide.



We have found that the binuclear iron(III) complexes with H(HPTB) or H(HPTP) (structures shown above) give much TBARS in the reaction with linoleic acid in the air [59,60]; here TBARS are malondialdehyde (which gives pink products with TBA; λmax = 532 nm) or monoaldehyde derivatives

(gives orange products), which are formed from the peroxidation of linoelic acid. (TBA = 2-thiobarbituric acid) [61]. Above results have been elucidated on the assumption that oxygenation of O_2 into the linoleic acid proceeds without the change of oxidation state of Fe(III) through forming an intermediate containing two Fe(III) ions, O_2, and linoleic acid (substrate), as illustrated in SCHEME V; this is very similar to that proposed for the elucidation of Mukaiyama reaction and lipoxygenase. In this case, the interaction between the two unpaired electrons of two Fe(III) atoms and O_2 is necessary to activate the O_2, which is also promoted via interaction with the substrate, linoleic acid.



SCHEME V

The binuclear Al(III) complex, $Al_2(HPTP)(OH)Cl_2(ClO_4)_2$ was also isolated, but the Al(III) complex exhibit no activity for the oxygenation of linoleic acid, and this can be attributed to the absence of unpaired d-electron in Al(III) complex, and this also supports the importance of role of unpaired d-electron in the activation of O_2.

When the DMPO, which is one of the famous spin-trapping agent for OH• radical, was added to the solution containing binuclear Fe(III) complex, $Fe_2(HPTP)(OH)(NO_3)^{22+}$; strong four signals which corresponds to the formation of DMPO-OH, as shown in FIG. 6 [62], whereas no such signal was detected by the addition of DMPO to the solutions of Fe(edta)- and $Al_2(HPTP)(OH)Cl_2(ClO_4)_2$. This clearly indicates that the formation of DMPO-OH is not due to the presence of OH• in the solution. Above mysterious fact was elucidated by the similar

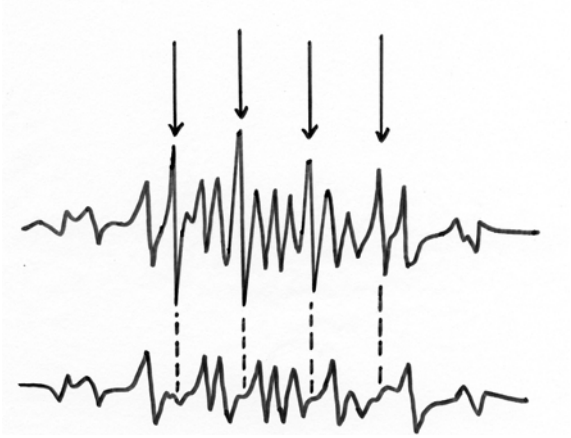
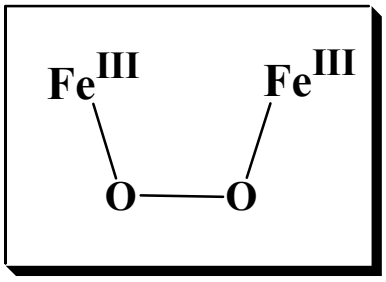
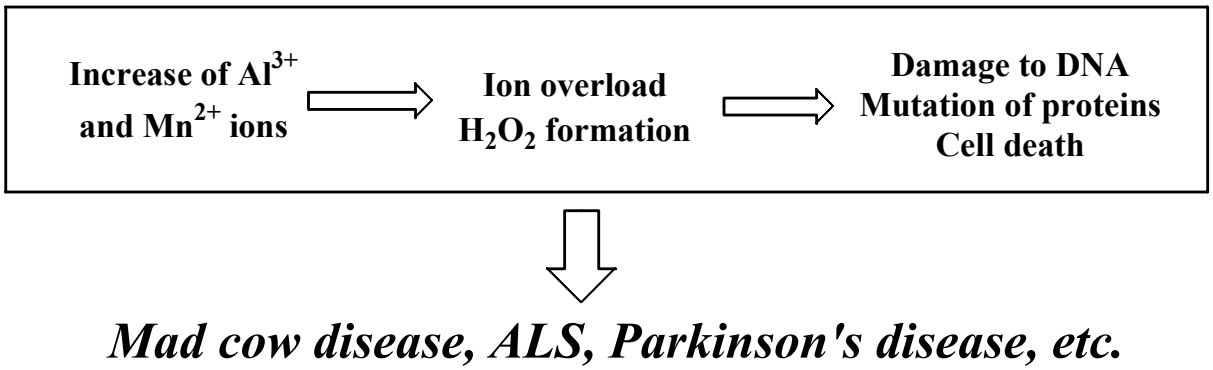


FIGURE 6. ESR SPECTRA OF THE SOLUTIONS CONTAINING Fe(III) COMPLEX AND DMPO [62].
Upper trace: $Fe_2(HPTP)(OH)(NO_3)_2^{2+}$
Lower trace: Fe(edta)Na

way as described for the oxygenation of linolenic acid through the formation of the intermediate similar to that assumed for the linoleic acid (SCHEME V), O_2 is activated to interact with the DMPO, leading to DMPO-OH formation. These demonstrate that the results obtained by the use of spin-trapping reagents, especially for the formation of OH• are doubtful, and the results reported hitherto should be re-investigated, and the presence of so-called Fenton reaction seems also to be doubtful.



The abnormal activation of hydrogen peroxide in the presence of a binuclear metal chelates observed in many cases, for examples, oxidative degradation of cellular constituents, such as lipids, proteins, sugars, and DNA, [54-56,60] was assumed to proceed via a peroxide adduct formation as



illustrated right: in addition to these the cell-death by the administration of Fe(III)-nta complex was reported by Okada et al. [56].

7. CONCLUSIONS

We have investigated the chemical mechanism of pathogenesis of neurodegenerative diseases based on our new concept for the so-called "oxidative stress" induced by the metal ions: the results are summarized below.

1) Reactivity of the metal-OOH, hydroperoxo-metal chelates of Fe(III) or Cu(II), is dependent on the interactions with peripheral groups and substrate, and is frequently highly reactive to oxidation of cellular constituents, such as lipids, proteins, and DNA, which leads to appearance of mutant proteins and misfolded proteins.
2) In some iron-containing enzymes such as phenylalanine hydroxylase or tyrosine hydroxylase, Fe(II) can activate O_2 without change of its oxidation state, and in this case weak interaction between the unpaired electrons of Fe(II) ion and O_2 is important to activate O_2; other metal ions such as Al(III) or Mn(II) cannot activate O_2 by the same mechanism, and thus the accumulation of Al(III) or Mn(II) ions in brain will lead to the deficiency of dopamine and other neurotransmitters, and also to the elevated iron levels in the brain.
3) Elevated iron levels in brains and abnormalities in brain iron metabolism (so-called iron-overload syndrome) have been detected for Alzheimer's and Parkinson's diseases, and these iron ions exist as a soluble polymeric species through chelate formation with amino acids or small peptide. Soluble polynuclear metal chelates of Fe(III) or Cu(II) exhibit unique reactivity toward O_2, and readily gives hydrogen peroxide in the presence of reducing agents, and the hydrogen peroxide thus produced can be a intrinsic origin for the "oxidative stress" in the presence of metal ions such as Fe(III) or Cu(II) as described above in POINT-1.

Based on these results, we would like to propose that one of the most risky factors for the neurodegenerative diseases is the accumulation of exogenous metal ions such as Al(III) or Mn(II) in brain. Thus, the sudden and explosive increase of scrapie and BSE in the last decade may be partially due to "acid rain", because the acid rain makes Al(III) and Mn(II) ions soluble in the subterranean aquifers.

REFERENCES

[1] CHANI AC, FERGUSON NM, DONNELL CA, AND ANDERSON RM [2000] Predicted vCJD mortality in Great Britain. Nature 406: 583-584.

[2] BEALE AJ [2001] BSE and vCJD: what is the future? J Roy Soc Med 94: 207-209.

[3] HOUSTON F, FOSTER JD, CHONG A, HUNTER N, AND BOSTOCK CJ [2000] BSE and transmission through blood. The Lancet 356: 955-956.

[4] COHEN FE AND PRUSINER SB [1998] Pathologic conformations of prion proteins. Ann Rev Biochem 67: 793-819.

[5] COLLINGE J [2001] Prion diseases of humans and animals: Their causes and molecular basis. Ann Rev Neurosci 24: 519-550.

[6] PRUSINER SB [1998] Prions. Proc Natl Acad Sci USA 95: 13363-13383.

[7] CAUGHEY B [2000] Interactions between prion protein isoforms: The kiss of death? Trends Biochem Sciences 25: 235-242.

[8] BROWN D [2001] Prion and prejudice: normal protein and the synapse. Trends Neurosciences 24: 85-90.

[9] WONG BS, CHEN SG, COLUCCI M, XIE Z, PAN T, LIU T, LI R, GAMBETTI P, SY MS AND BROWN DR [2001] Abberant metal biding by prion protein in human prion diseases. J Neurochemistry 78: 1400-1408.

[10] SHIRAKI H AND YASE Y [1991] "Handbook of Clinical Neurology", ed by Vinken PI, Bruyn W and Klawans HL Vol. 15: pp. 273-300.

[11] GERLACH M, SCHACHAR DB, RIEDERER P AND YOUDIM MB [1994] Altered brain metabolism of iron as a cause of neurodegenerative diseases? J Neurochem 63: 793-807.

[12] YOUDIM MB AND RIEDERER P [1997] Understanding Parkinson's diseases. Scientific American 52-59.

[13] HAAVIK J AND TOSKA K [1998] Tyrosine hydroxylase and Parkinson's disease. Mol Neurobiol 16: 285-309..

[14] KRUGER R, EBERHARDT O, RIESS O AND SCHULZ JB [2002] Parkinson's disease: one biochemical pathway to fit all genes? Trends Mol Med 8: 236-240..

[15] FITZPATRICK PF [1999] Tetrahydropterin-dependent amino acid hydroxylases. Ann Rev Biochem 68: 355-381.

[16] KAPPOCK TJ AND CARADONNA JP [1996] Pterin-dependent amino acid hydroxylases. Chem Rev 96: 2659-2756.

[17] HIRSCH EC [1994] Biochemistry of Parkinson's diseases with special reference to dopaminergic systems. Mol Neurobiol 9: 135-142.

[18] HAAVIK J, ALMAS B AND FLATMARK T [1997] Generation of reactive oxygen species by tyrosine hydroxylase: A possible contribution to the degeneration of dopaminergic neurons? J Neurochem 68: 328-332.

[19] FINKEL T AND HOLBROOK NJ [2000] Oxidants, oxidative stress and the biology of ageing. Nature 408: 239-247.

[20] HARRISON PM AND AROSIO P [1996] The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275: 161-203.

[21] SMITH RG AND APPEL SH [1995] Molecular approaches to amyotrophic lateral sclerosis. Annu Rev Med 46: 133-145.

[22] BROWN RH JR [1995] Amyotrophic lateral sclerosis: recent insights from genetic and transgenic mice. Cell 80: 687-692.

[23] DENG HX, HENTATI A, TAINER JA, IQBAL Z, CAYABYAB A, HUNG WY, GETZOFF EF, HU P, HERZFELDT B, ROOS RP, WARNER C, DENG G, SORIANO E, SMYTH C, PARGE HE, AHMED A, ROSES AD, HALLEWELL RA, P-VANCE MA AND SIDDIQUE T [1993] Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science 261: 1047-1051.

[24] FRIDOVICH I [1995] Superoxide radical and superoxide dismutase. Ann Rev Biochem 64: 97-112.

[25] TAINER JA, GETZOFF ED, RICHARDSON JS AND RICHARDSON DC [1983] Structure and mechanism of copper, zinc superoxide dismutase. Nature 306: 284-290.

[26] W-PAZOS M, GOTO JJ, RABIZADEH S, GRALLA EB, ROE JA, LEE MK, VALENTINE JS AND BREDESEN DE [1996] Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. Science 271: 515-518.

[27] YIM MB, KANG JH, YIM HS, KWAK HS, CHOCK PB AND STADTMAN EB [1996] A gain-of-function of an amyotrophic lateral sclerosis-associated Cu,Zn superoxide dismutase mutant. An enhancement of free radical formation due to a decrease in K_M for hydrogen peroxide. Proc Natl Acd Sci USA 93: 5709-5714.

[28] KOBAYASHI T, OKUNO T, SUZUKI T, KUNITA M, OHBA S AND NISHIDA Y [1998] DNA degradation by the copper(II) complex with tripodal-like ligands containing peptide group. Polyhedron 17: 1553-1559.

[29] NISHINO S, KOBAYASHI T, KUNITA M, ITO S AND NISHIDA Y [1999] Structural variety of copper(II)-peroxide adduct and its relevance to DNA cleavage. Z Naturforsch 54C: 94-99.

[30] NISHINO S, KUNITA M, KANI K, OHBA, S, MATSUHSIMA H, TOKII T AND NISHIDA Y [2000] Cleavage of C-N bond of a peptide group by a copper(II)-peroxide adduct with an η1-coordination mode. Inorg Chem Commun 3: 145-148.

[31] CIONI P, PESCE A, MOROZZO B, CASTELLI S, FALCONI M, PARRILLI L, BOLOGNESI M, STRAMBINI G AND DESIDERI A [2003] Active-site copper and zinc ions modulate the quaternary structure of prokaryotic Cu,Zn superoxide dismutase. J Mol Biol 326: 1351-1360.

[32] NISHIDA Y AND NISHINO S [1999] Contribution of a metal-peroxide adduct to neurodegeneration is due to its oxidative protease activity. Z Naturforsch 54C: 1107-1112.

[33] NISHIDA Y AND NISHINO S [2001] Electronic property and reactivity of (hydroperoxo)metal compounds. Z Naturforsch 56C: 144-153.

[34] NISHINO S, KISHITA A AND NISHIDA Y [2001] Alternative origin for gain-of-function of mutant SOD enzyme and for conformational change of normal prion protein. Z. Naturforsch 56C: 1144-1149.

[35] WESTAWAY D AND CARLSON GA [2002] Mammalian prion proteins: Enigma, variation and vaccination. Trends Biochem Sci 27: 301-307.

[36] RAE TD, SCHMIDT PJ, PUFAHL RA, CULOTTA VC AND O'HALLORAN TV [1999] Undetectable intracellular free copper: the requirement of a copper chaperon for superoxide dismutase. Science 284: 805-808.

[37] MAMAHON HEM, MANGE A, NISHIDA N, CREMINON C, CASANOVA D AND LEHMANN S [2001] Cleavage of the amino terminus of the piron protein by reactive oxygen species. J Biol Chem 276: 2286-2291.

[38] REQUENA JR, GROTH D, LEGNAME G, STADTMAN ER, PRUSINER SB, AND REVINE RL [2001] Copper-catalyzed oxidation of the recombinant SHa(29-231) prion protein. Proc Natl Acad Sci USA 98: 7170-7175.

[39] BEHRENS A AND AGUZZI A [2002] Small is not beautiful antagonizing functions for prion protein PrP^C and its homologue Dpl. Tresnds Neurosci 25: 150-154.

[40] SONO M, ROACH MP, COULTER ED AND DAWSON JH [1996] Heme-containing oxygenases. Chem Rev 96: 2841-2887.

[41] DIX TA AND BENKOVIC SJ [1985] Mechanism of "uncoupled" tetrahydropterin oxidation by phenylalanine hydroxylase. Biochemistry 24: 5839-5846.

[42] NISHIDA Y [1998] Important role of substrate in activation of dioxygen in biological systems. Trends Inorg Chem 5: 89-103.

[43] YAMADA T, TAKAI T, RHODE O AND MUKAIYAMA T [1991] Highly efficient method for epoxidation of olefins with molecular oxygen and aldehydes catalayzed by nickel(II) complexes. Chem Letters 1-4.

[44] NISHIDA Y, FIJIMOTO T AND TANAKA N [1992] Formation of singlet-oxygen like species in system containing nickel(II) complex and aliphatic aldehyde. Chem Letters 1291-1294.

[45] NISHIDA Y, TANAKA N, AND OKAZAKI M [1994] Chemical mechanism of olefin oxygenation catalyzed by bis(acetylacetonato)nickel(II) or cobalt(II) compounds in the presence of reducing agents. Polyhedron 13: 2245-2250.

[46] NISHIDA Y AND TANAKA N [1994] Chemical mechanism of dioxygen activation by trans-[$FeCl_2$(cyclam)] in the presence of reducing agents such as aliphatic aldehyde or linolenic acid. J Chem Soc Dalton Trans 2805-2809.

[47] NISHIDA Y, TANAKA N, YAMAZAKI A, HASHIMOTO H, IDE K AND TOKII T [1995] Novel reactivity of dioxygen molecule in the presence of a manganese(II) complex and reducing agents. Inorg Chem 34: 3616-3620.

[48] NISHIDA Y, TOKII T AND WATANABE I [1992] Reaction between superoxide anion and several oxovanadium(IV) compounds. Z Naturforsch 47b: 905-909.

[49] NISHIDA Y, WATANABE I, TAKAHASHI S, AKAMATSU T, YAMAZAKI A AND SAKAMOTO M [1994] Electrochemical evidence for weak interaction between oxovanadium(IV) conpounds and dioxygen molecule. Polyhedron 13: 2205-2210.

[50] QUE L JR AND HO RYN [1996] Dioxygen activation by enzymes with mononuclear non-heme iron active sites. Chem Rev 96: 2607-2624.

[51] NISHIDA Y [2004] Formation of a Mn(IV) species in the reaction mixture of a manganese(II) complex and analiphatic aldehydes. Syn React Inorg Metal-org Chem (in press).

[52] KAISER J [2003] Manganese: A high octane dispute. Science 300: 926-928.

[53] "Food, Nutrition and Prevention of Cancer: A global perspective", World Cancer Research Fund / American Institute for cancer Research, 1997.

[54] NISHINO S, KUNITA M, KOBAYASHI T, MATSUSHIMA H, TOKII T AND NISHIDAY [1999] Interaction between the peroxide adduct of binuclear iron(III) complex with (HPTP) anion and the sugar moiety of nucleosides. Z Naturforsch 54b: 1272-1276.

[55] ITO S, SASAKI Y. TAKAHASHI Y, OHBA S AND NISHIDA Y [1999] Oxygenation of nucleosides by peroxide adduct of binuclear iron(II) complex with a □-oxo bridge. Z Naturforsch 54c: 554-563.

[56] NISHIDA Y [1999] Structure and function of free iron ion in biological system and their model compounds. Recent Res Devel Pure Appl Chem 3: 103-122.

[57] NISHIDA Y, TAKEUCHI M, SHIMO H AND KIDA S [1985] Synthesis and reactivity of binuclear iron(III) complexes with ligands composed of two tridente chelating groups. Inorg Chim Acta 96: 115-120.

[58] NISHIDA Y, SHIMO H, MAEHARA H AND KIDA S [1985] Crystal structure and magnetic property of binuclear five-coordinate copper(II) complexes with a phenolate bridge and their catalytic functions in multielectron redox reactions. J Chem Soc Dalton Trans 1945-1951.

[59] NISHIDA Y, AND YAMADA K [1990] Lipoxygenase-like function of some binuclear iron(III) compounds. J Chem Soc Dalton Trans 3639-3641.

[60] NISHIDA Y AND ITO S [1995] Structures and reactivity of several iron(III) complexes in the presence of hydrogen peroxide: relevance to induction of tissue damage caused by iron(III) chelates in rats. Polyhedron 14: 2301-2308.

[61] HALLIWELL B AND GUTTERIDGE JMC [1985] "Free Radicals in Biology and Medicine", Oxford University Press, chapter 4.

[62] NISHIDA Y, NASU M AND AKAMATSU T [1992] Reaction between binuclear iron(III) compounds and DMPO. J Chem Soc Chem Commun 94-95.

ABBREVIATIONS:

ECS: endogenous cannabinoid system;

FAAH: fatty acid amide hydrolase;

CB1 (or CB2): cannabinoid 1 (or 2) receptor;

THC: delta-9-tetrahydrocannabinol;

GABA: gamma amino butyric acid;

CNS: central nervous system;

DSI: depolarization-induced suppression of inhibition;

LTD: long-term depression;

SOD: superoxide dismutase;

MMT: methylcyclopentadienyl manganese tricarbonyl.

RECEIVED 5-27-2004.

ACCEPTED 6-24-2004.