Negligible Ability of Oxygen and Peroxide Ion Activation by Al(III) Ion Is Essential for Al(III)-Induced Neurodegeneration

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Al(III)-Induced Neurodegeneration, Oxygen Activation, Tyrosine Hydroxylase

The electron densities of the atoms in Fe(II)- or Al(III)-tyrosine hydroxylase (THO) containing oxygen and pterin were calculated by the DFT (Density-functional theory) method. The results obtained are consistent with our previous proposal that oxygen activation in THO proceeds through the formation of an intermediate derived from Fe(II), oxygen, and pterin. Electron donation from substrate to the oxygen molecule is important to cleave the O-O bond, and to give the hydroxylated product. Based on these results, it was concluded that hydroxylation of the aromatic ring does not proceed in the Al(III)-containing THO, and a relationship exists between Al(III) ion and neurodegeneration.

Introduction

It is generally recognized that aluminum is neurotoxic in both experimental animals and certain human diseases. (Yang *et al.*, 1998; Rao *et al.*, 1998; Savory *et* al., 1996) Minute quantities injected intracerebrally into rabbits induce severe neurological symptoms and neuropathological features of neurodegeneration. There are many other examples of Al-induced neurotoxicity, however, the question is still the subject of debate as to whether Al presents a health hazard to humans as a contributing factor to Alzheimer's disease. Several lines of evidences are presented that have formed the basis of the debate concerning the possible pathogenic role for Al in Alzheimer's disease. Important evidence for an Al-Alzheimer's causal relationship is the observation by laser microprobe mass analysis (LMMS) of the presence of Al in neurofibrillary tangles. There is another evidence that exposure to Al from drinking water might result in cognitive impairment and increased incidence of Alzheimer's disease. However, these epidemiological studies have inherent problems that must be scrutinized to determine if an association really does exist. (Savory *et al.*, 1996)

Several years ago we pointed out that the establishment of a chemical mechanism on the Al(III)-induced neurodegeneration is necessary in order to prevent the humans from the Al-induced neurodegenraiton, (Nishida and Ito, 1995a; Nishida, 1999) such as Alzheimer's, amyotrophic lateral sclerosis, etc. As it has been shown that transferrin, one of the chief iron transport protein in vertebrates, binds specifically Al(III) ions with a high affinity, (Battistuzzi *et al.*, 1995) it seems likely that the iron ion in phenylalanine hydroxylase or tyrosine hydroxylase, is replaced by Al(III) ion. (Nishida and Ito, 1995a; Nishida, 1999) The enzymes, phenylalanine hydroxylase or tyrosine hydroxylase (hereafter abbreviated as THO), plays an important role to synthesize dopamine, and it is well known that the deficiency of dopamine is the origin for Parkinson's disease. (Haavik and Toska, 1998)

In previous papers, we have reported that the addition of hydrogen peroxide to the solution of Fe(III)-(bdza) complex (H_2(bzda)=benzylamine-N,N-diacetic acid) induces the hydroxylation of the (bzda)-ligand, to give the phenol derivative (see Fig. 1 (A)), but such reaction does not proceed in the Al(III)-(bzda) compound. (Nishida and Ito, 1995a; Nishida, 1999) The addition of the unsaturated fatty acid to the iron(III)-(bzda) solution promotes the formation of the TBARS (= 2-thiobarbituric acid reactive substance, see Fig. 1 (B)), (Nishida and Ito, 1995b) which is due to high activity by the iron(III) compound for oxygen activation. (Nishida and Yamada, 1990; Nishida and Tanaka, 1994) In this study, we have observed that Al(III)-(bzda) complex exhibits no oxygen activation in the presence of unsaturated fatty acid,

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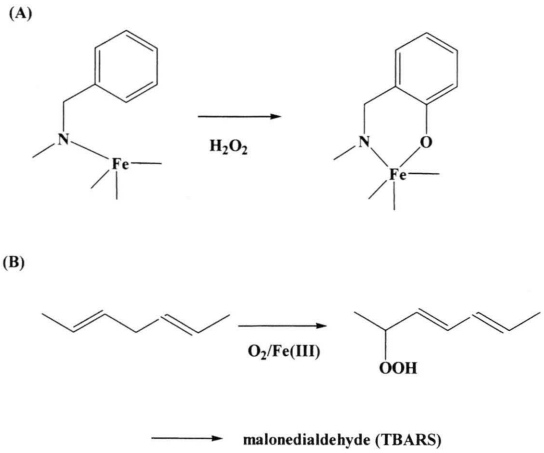

Fig. 1. Schematic illustration of the reactions. A hydroxylation of benzene ring by Fe(III)/H_2O_2 system, (B) peroxidation of unsaturated fatty acid and formation of malonedialdehyde by Fe(III)/O_2 system.

demonstrating that the Al(III) complex exhibits negligible activity for both the oxygen and peroxide ion activation.

It seems likely that the above feature of the Al(III) ion may be a major origin for neurotoxicity by Al(III) ion in the brain, because by replacement of iron in the enzymes by Al(III) ions gives the enzymes cannot catalyze the oxygenation reaction of phenylalanine or tyrosine hydroxylase, leading to deficiency of dopamine. (Nishida and Ito, 1995a; Nishida, 1999) To confirm the validity of our consideration, in this study the change in electron density of oxygen and pterin molecules in THO with Fe(II) or Al(III) were investigated in terms of the DFT (Density-functional theory) calculation.

Results and Discussion

Mechanism of O_2-acivation by Ni(II), Mn(II) and Co(II) ions in the presence of substrate

We have reported previously that Ni(II), Mn(II), and Co(II) compounds can activate O_2 in the presence of aliphatic aldehyde, and this system exhibits a high reactivity for TCPN (=tetraphenylcyclopentadienone), one of the singlet oxygen ($^1\Delta_g$)-scavenger. (Nishida *et al.*, 1994a; Nishida *et al.*, 1995) On the basis of the detailed study, we have proposed that this reaction proceeds via complex formation derived from metal complex, oxygen, and substrate; the interaction between the former two species has been confirmed in terms of the cyclic voltammogram, (Nishida *et al.*, 1994b) and this is due to weak interaction between two unpaired electrons in the d-orbital of a metal ion and p-orbital of the oxygen molecule.

Under this condition, the electronic property of the oxygen molecule coordinated to a metal ion is quite different from that of the free oxygen molecule, because the molecular orbital derived from the d-orbital and p-orbital of the oxygen molecule is vacant (see Fig. 2). Thus, the oxygen molecule in this compound exhibits singlet-oxygen character, reacting with TCPN, to degrade it; the latter has been confirmed experimentally. (Nishida *et al.*, 1994a; Nishida *et al.*, 1995) Of course, the interaction with the occupied orbital of the organic substrate occurs via the interaction with this vacant orbital, which induces the stabilization of energy of the total system. In the case of Al(III) ion, stabilization of the total system as described above is unlikely, because Al(III) has no d-electron, and the oxygen molecule is always in the triplet state throughout the reaction, and this situation will retard the complex formation derived from the three molecules proposed for Ni(II) and another systems. (Nishida *et al.*, 1994a; Nishida 1998) Above discussions are consistent with the fact that Zn(II) complex cannot activate the oxygen molecule under the same experimental conditions, (Nishida *et al.*, 1994a) and the fact that no TBARS was detected in the solution containing Al(III)-(bzda)

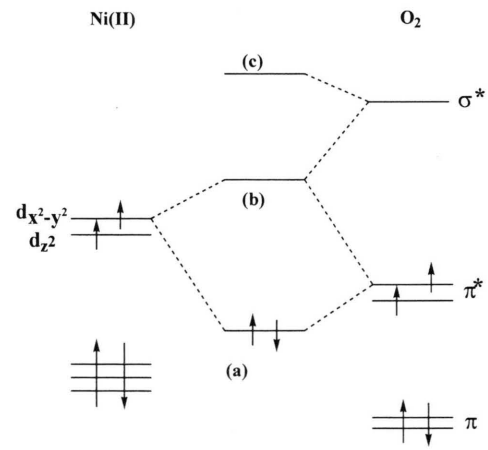

Fig. 2. Molecular orbital scheme of Ni(II)-O_2 system. The orbital (b) interacts with HOMO of the substrate.

and linolenic acid, and other Al(III) compounds such as a binuclear compound, $Al_2(HPTP)(OH)Cl_2(ClO_4)_2$.

Our mechanism for phenylalanine or tyrosine hydroxylases (Nishida 1998) is illustrated in Fig. 3, which is essentially the same as that proposed for epoxidation of olefin by $Ni(II)(acac)_2/O_2$/aldehyde system, where H(acac) represents acetylacetone. In this scheme oxygen activation is promoted through the interaction with unpaired d-electron and interaction with the pterin molecule. After electron transfer from the pterin and the substrate (in this case aromatic ring of the substrate) to the oxygen molecule, cleavage of the O-O bond gives the oxygenated substrate and pterin, i.e., hydroxylated product and hydroxy-pterin.

The discussion described above suggests that Al(III)-replaced phenylalanine hydroxyrase or tyrosine hydroxylase cannot activate the oxygen molecule even in the presence of pterin, and this seems to be consistent with the calculated results as described below.

DFT calculations on the Fe(II)-replaced tyrosine hydroxylase

In this study we have performed the DFT calculations on the THO, where the metal ion is either Fe(II) , Fe(III) or Al(III). The structural features are obtained from X-ray analytical data. (Goodwill *et al.*, 1997) DFT calculations were done by the use of Q-Chem (Q-Chem Inc., Pittsburgh, 1998; basis set 3–21G, hybrid LYP) for the high-spin state of the Fe-containing systems.

At first we investigated the effect of the oxygen molecule approaching the system at the coordination site of a metal ion; there are two possible sites for binding of oxygen molecule, but the site-(A) seems unlikely because of the steric repulsion of

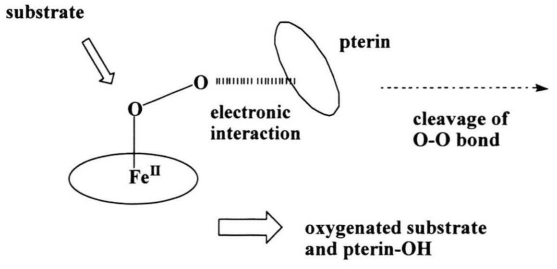

Fig. 3. Nishida's mechanism for tyrosine hydroxylase (Nishida, 1998).

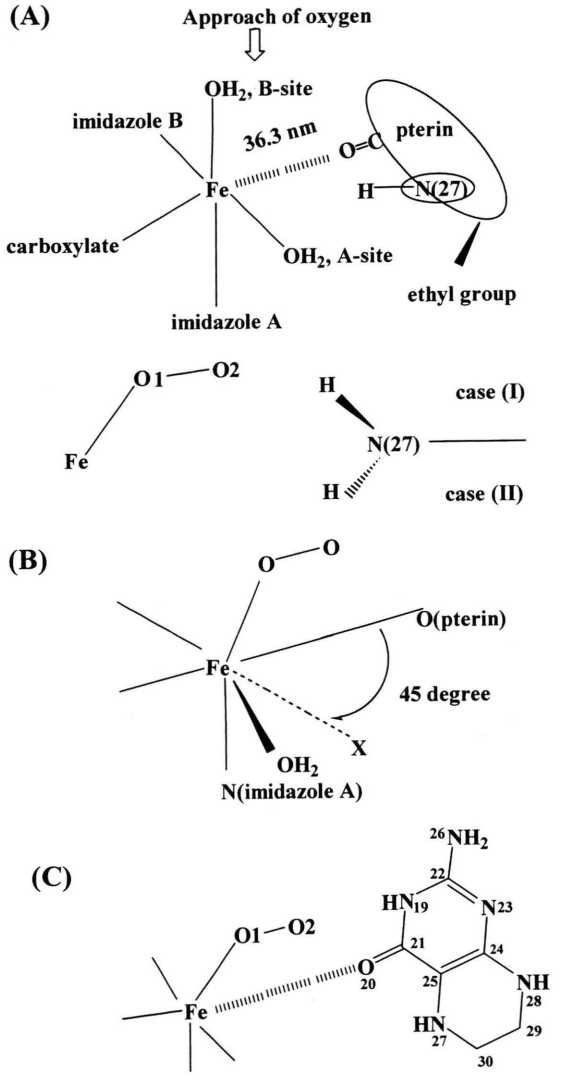

Fig. 4. Geometrical features of the system used for DFT calculations.

the ethyl group of the pterin molecule (see Fig. 4 (A)). Thus, we have mainly performed the calculation for the system where the oxygen molecule approaches the metal ion at the (B) site. There are two possible states for the direction of hydrogen atom at the N (27) of the pterin molecule, and we thus have calculated for the two cases, (I) and (II), as illustrated in Figure 4 (A). A metal-oxygen complex can be characterized by several parameters like: metal-O1(O_2 molecule) distance (22–25 nm), O-O distance(12.6–14.6 nm), angles of (N(imidazole A)-Fe-O1), Fe-O1-O2, X-Fe-O1(X

is the atom on the line bisecting O(OH_2, A)-Fe-O(pterin), torsion angle O2-O1-Fe-X, 45°; see Figure 4 (B)), and Fe1-O1-O2, and torsion angle O2-O1-Fe-O(pterin), etc.

Typical examples are (M=Fe(II), Fe(II), Al(III)):

Case-A: M-O1, 22.0 nm, M-O(pterin), 36.3 nm; M-O1-O2, 120°, O-O, 12.6 nm, N(imidazole A)-Fe-O1, 180°, torsion angle (O2-O1-Fe-O(pterin)), 0–90°, carboxylate: acetate, and case (II).

Case-B: M-O1, 25.0 nm, M-O(pterin), 36.3 nm; M-O1-O2, 120°, O-O, 12.6 nm, N(imidazole A)-Fe-O1, 180°, torsion angle (O2-O1-Fe-O(pterin)), 0–90°, carboxylate: acetate, and case (II).

Case-C: M-O1, 22.0 nm, M-O(pterin), 36.3 nm; M-O1-O2, 120°, O-O, 12.6 nm, N(imidazole A)-Fe-O1, 180°, torsion angle (O2-O1-Fe-O(pterin)), 0–90°, carboxylate: formate, and case (I).

Case-D: M-O1, 25.0 nm, M-O(pterin), 36.3 nm; M-O1-O2, 120°, O-O, 12.6 nm, N(imidazole A)-Fe-O1, 180°, torsion angle (O2-O1-Fe-O(pterin)), 0–90°, carboxylate: formate and case (I).

Case-E: M-O1, 22.0 nm, M-O(pterin), 36.3~80.0 nm; M-O1-O2, 120°, O-O, 12.6 nm, angle X-Fe-O1, 70–90°, torsion angle (O2-O1-Fe-O(pterin)), 45°, carboxylate: formate and case (I). The smaller the angle X-Fe-O1, the smaller the distances between O2 and H(HN(29)), and oxygen molecule and pterin molecule become. When the angle X-Fe-O1 is 90°, it corresponds to the Case-C, where torsion angle (O2-O1-Fe-O(pterin)) is 45°.

Case-F: M-O1, 25.0 nm, M-O(pterin), 36.3~80.0 nm; M-O1-O2, 120°, O-O, 12.6 nm , angle X-Fe-O1, 70–90°, torsion angle (O2-O1-Fe-O(pterin)), 45°, carboxylate: formate and case (I).

At first we have compared the electronic structure of the resting state, i.e., there is no oxygen in the system. As shown in Table I (for the numberings of the atoms in pterin molecule, see Fig. 4 (C)), the oxidation state on a metal ion gives a sizable effect on the electron densities of the atoms in the system. The electron density at the Al(III) is the lowest, and the highest for the Fe(II) ion. When oxygen approaches at a metal ion, the changes of electronic densities on the oxygen atoms occur, but this change is highly dependent on a metal ion and many structural parameters as described above (Table II ~ Table IV), and results obtained are summarized as follows:

Table I. Electron densities of the atoms in the THO-H_2O (THO=tyrosine hydroxylase) system.

	M= Fe(III)	= Fe(II)	= Al(III)
1 C	0.349156	0.336325	0.349472
2 N	−0.691775	−0.665143	−0.699684
3 C	−0.059041	−0.055339	−0.054692
4 C	0.057519	0.036201	0.060472
5 N	−0.706068	−0.713461	−0.707309
6 H	0.309034	0.29661	0.312676
7 H	0.238796	0.237381	0.265157
8 H	0.309214	0.281582	0.325393
9 H	0.396447	0.376648	0.406364
10 C	0.318593	0.303479	0.3277
11 N	−0.717132	−0.676638	−0.727998
12 C	−0.042463	−0.045674	−0.032946
13 C	0.044964	0.023915	0.047931
14 N	−0.70653	−0.713403	−0.706831
15 H	0.333509	0.320648	0.336825
16 H	0.242282	0.230362	0.255282
17 H	0.309021	0.27996	0.325029
18 H	0.396061	0.375866	0.407162
19 N	−0.798429	−0.803061	−0.803194
20 O	−0.446965	−0.480805	−0.519601
21 C	0.720532	0.695397	0.69115
22 C	0.802825	0.777726	0.784911
23 N	−0.615892	−0.649574	−0.632706
24 C	0.569817	0.537634	0.549264
25 C	0.121572	0.090237	0.094735
26 N	−0.708626	−0.73588	−0.724676
27 N	−0.647509	−0.673263	−0.682179
28 N	−0.66445	−0.687393	−0.680284
29 C	−0.275764	−0.260774	−0.264698
30 C	−0.295833	−0.27839	−0.282519
31 H	0.280002	0.239001	0.254055
32 H	0.381974	0.356905	0.360236
33 H	0.339192	0.312913	0.320774
34 H	0.367068	0.340626	0.354331
35 H	0.371497	0.33663	0.35251
36 H	0.261245	0.229909	0.238223
37 H	0.274125	0.228351	0.249274
38 H	0.240217	0.207069	0.207965
39 H	0.317203	0.291098	0.264775
40 O	−0.632867	−0.639065	−0.61087
41 C	0.3921	0.369636	0.371021
42 H	0.241922	0.187541	0.272188
43 O	−0.434434	−0.444696	−0.39264
44 H	0.466991	0.437665	0.464791
45 H	0.44806	0.42834	0.457411
46 H	0.468183	0.446226	0.474895
47 H	0.411448	0.387071	0.42119
48 O	−0.617036	−0.604986	−0.609053
49 O	−0.704994	−0.685489	−0.692413
50 Metal	0.985239	0.814078	1.22113

Atom number: 1–18, two imidazole molecules; 19–39; pterin molecule; 40–43, formic acid; 44–49, two water molecules.

(1) change in electron density at Al(III) ion is much smaller than those observed for Fe(II) and Fe(III) ions,

Table II. Electron densities of the atoms in the THO-OH_2 system. (Case-D; M-O1 = 25 nm, torsion angle(O2-O1-Fe-O(pterin) = 45°).

	M= Fe(III)	= Fe(II)	= Al(III)
	M=Fe(III)	=Fe(II)	=Al(III)
1 C	0.348705	0.344613	0.351444
2 N	−0.685677	−0.679454	−0.700391
3 C	−0.060489	−0.054816	−0.054755
4 C	0.054772	0.044173	0.060069
5 N	−0.706587	−0.710167	−0.707642
6 H	0.309991	0.303621	0.316164
7 H	0.225316	0.245635	0.251387
8 H	0.304762	0.292439	0.321516
9 H	0.395007	0.38424	0.405603
10 C	0.333034	0.332494	0.33859
11 N	−0.722274	−0.713623	−0.725055
12 C	−0.029463	−0.025539	−0.022332
13 C	0.043487	0.034409	0.045835
14 N	−0.708351	−0.711754	−0.708801
15 H	0.33381	0.331743	0.336303
16 H	0.236541	0.239681	0.246259
17 H	0.303173	0.290323	0.317266
18 H	0.393418	0.384377	0.403174
19 N	−0.790156	−0.801685	−0.79574
20 O	−0.387739	−0.497869	−0.460333
21 C	0.742645	0.701066	0.716846
22 C	0.82278	0.7822	0.807234
23 N	−0.593818	−0.648252	−0.612924
24 C	0.58936	0.542151	0.573214
25 C	0.162787	0.091093	0.12961
26 N	−0.691418	−0.736502	−0.707869
27 N	−0.623527	−0.678449	−0.654099
28 N	−0.6453	−0.685835	−0.661139
29 C	−0.285896	−0.260172	−0.275597
30 C	−0.308499	−0.278624	−0.29621
31 H	0.30103	0.238453	0.278932
32 H	0.403083	0.359236	0.384019
33 H	0.359754	0.316314	0.343161
34 H	0.380774	0.342036	0.368908
35 H	0.389871	0.337858	0.372993
36 H	0.285493	0.233186	0.264683
37 H	0.29633	0.22908	0.274934
38 H	0.267626	0.205576	0.238381
39 H	0.352481	0.28917	0.308903
40 O	−0.619135	−0.6326	−0.594663
41 C	0.420272	0.408382	0.403736
42 H	0.242191	0.219067	0.270699
43 O	−0.462292	−0.426822	−0.423153
44 H	0.463139	0.454206	0.465307
45 O	−0.710343	−0.702897	−0.700182
46 H	0.470718	0.479301	0.489379
47 O1	−0.078937	−0.106007	−0.118173
48 O2	−0.069282	−0.062163	−0.055476
49 metal	0.946831	0.957106	1.189988

Atom number: 1–18, two imidazole molecules; 19–39, pterin molecule; 40–43, formic acid; 44–46, water molecule

Table III. Electron densities of the atoms in THO-O_2 systems. Case-C, Fe-O1, 22 nm, A is the torsion angle (O2-O1-Fe-O(pterin).

System	A/deg= 0	= 30	= 45	= 60	= 90
Fe(II) system					
C(25)	0.0893	0.0944	0.0911	0.097	0.097
O2	−0.0176	−0.0364	−0.0498	−0.0829	−0.076
O1	−0.1764	−0.1229	−0.142	−0.115	−0.114
Fe(II)	0.9941	0.9305	0.9941	0.929	0.937
Al(III) system					
C(25)	0.1307	0.1352	0.1381	−0.1405	0.1421
O2	−0.03	−0.0689	−0.087	−0.1002	−0.106
O1	−0.197	−0.185	−0.179	−0.176	−0.175
Al(III)	1.224	1.226	1.228	1.23	1.23
Fe(III) system					
C(25)	0.1433	0.1612	0.1644	0.1625	0.1582
O2	0.0049	−0.038	−0.094	−0.0655	−0.1049
O1	−0.1461	−0.136	−0.144	−0.0969	−0.1583
Fe(III)	1.0347	0.9905	1.011	0.9478	1.0532

Table IV. Electron densities of the atoms in THO-O_2 systems. Case-E, X-Fe-O1 = 70o^0, R = distance between metal ion and O(pterin molecule).

	Fe(II) system			
	R = 36.3	= 40.0	= 50.0	= 80.0
C(25)	0.1096	0.1115	0.1069	0.1116
O2	−0.071	−0.072	−0.0783	−0.064
O1	−0.135	−0.135	−0.184	−0.144
Fe(II)	0.948	0.935	1.029	0.968
	Al(III) system			
C(25)	0.1573	0.1544	0.1502	0.1484
O2	−0.136	−0.132	−0.125	−0.115
O1	−0.2071	−0.2062	−0.209	−0.216
Al(III)	1.2647	1.2635	1.2625	1.262

(2) electron density at the Fe(II) ion is essentially the same as at the Fe(III) ion, indicating that the oxidation of the Fe(II) system is carried out by the electron transfer from the ligand system, mainly pterin, to the oxygen molecule,

(3) in the case of Al(III) system, the total electron density on the oxygen molecule is independent on the distance between Al(III) ion and pterin (see Table IV; in case-E, the distance between O2 and pterin molecule is shorter than that in case-A), suggesting that the accumulated electron on the oxygen molecule is due to the electron transfer to the oxygen molecule (metal ion with higher oxidation state may stabilize peroxide anion) and there is no chemical interaction among Al(III), the oxygen molecule and pterin molecule.

This is quite different from the Fe(II) ion (see Table IV) and in the latter case the calculated results clearly demonstrates the presence of chemical (or electronic) interaction among the Fe(II), oxygen, and pterin. This clearly suggests that Al(III)-replaced THO cannot catalyze the hydroxylation of aromatic compounds in the presence of pterin, because it was already shown that peroxide ion cannot hydroxylate the aromatic compounds in the presence of an Al(III) ion. (Nishida and Ito, 1995a; Nishida, 1998; Nishida, and Nishino, 2001)

(4) Calculated results shows that electron density at the oxygen in the Fe(II) system is generally lower than that in the Fe(III)- or Al(III) systems, and this clearly indicates that two conclusions proposed by Fitzpatrick (Ramsey *et al.*, 1996; Holly *et al.*, 2000), are not compatible, namely (1) an active center in THO should be an Fe(II) (Ramsey *et al.*, 1996) and (2) pterin radical formation is a very important process in the catalytic cycle of THO. (Holly *et al.*, 2000) Our result undoubtedly is coincident with the conclusion (1), and suggests that the electron donation from the substrate to the system is necessary to cleave O_2, leading to the hydroxylated substrate.

All calculated results are consistent with our proposal that oxygen activation in the THO proceeds through the formation of an intermediate in the scheme shown in Figure 3. Electronic interaction between Fe(II), O2, and pterin, and electron donation from substrate to the oxygen molecule are very important to activate the oxygen, and to give the hydroxylated product of the substrate. Based on these discussions, we would like to conclude that negligible ability of oxygen and peroxide ion activation by Al(III) ion is essential origin for the Al(III)-induced neurodegeneration, and to point out that association between Al(III) ion and neurodegeneration really does exist. (Nishida, 1999) The geometrical feature around the Fe(II) ion in THO is very similar to other Fe(II)-containing enzymes, such as isopenicillin N synthase, (Roach *et al.*, 1997) and thus the present results may give important information to elucidate the reaction mechanism of these enzymes, and the study of this problem is now in progress.

Supplemental data on DFT calculations may be obtained from the author on request.

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