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Model Reactions for the Formation of Iron Deposition

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Abstract

Iron depositions, one of the non-transferrin-bound iron (NTBI), are frequently observed for the patients with hemochromatosis and several neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, etc. In this article, we have showed that iron deposition formation on the albumin proceeds in two different ways, one of them contains the participation of hydrogen peroxide, and the another case occurs without hydrogen peroxide, in addition to the case induced by zinc(II) ions. The present results are clearly consistent with the previous idea that iron deposition contains the di-μ-oxo bridged dimeric Fe(III) cores and proteins, which are supported by the DFT calculations.

Keywords: iron deposition, hydrogen peroxide, di-μ-oxo bridged dimeric Fe(III) cores, DFT calculations

1. NTBI and Iron Deposition

Plasma iron is normally bound to the iron transport protein transferrin (Dresow,Peterson, Fischer, & Nielsen, 2008). When excess chelates (amino acids derivatives, small peptides or citrate, etc.) are present in the plasma, the water-insoluble hemosiderin which contains polymeric iron(III) ions with oxo-bridges may dissolve with forming the water-soluble iron(III) chelates with amino-acids or citrates. These iron ions not associated with transferrin is generally termed as non-transferrin-bound iron (NTBI). NTBI is detected in the plasma of patients with hemochromatosis and several neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases and aceruplasminemia (Gaeta & Hider, 2005; Yoshida et al., 2000; Stankievicz et al., 2007; Roberts et al. 2012), and is present at concentration up to 10 μM. It should be noted here that the water-soluble NTBI has been thought to play a crucial role in iron induced cell damage with resultant peroxidation of cell membrane lipids and other biomolecules, and such oxidative damage is implicated as an important contributor in the pathogenesis of cancer, cardiovascular disease, aging and neurodegenerative diseases.(Gaeta & Hider, 2005; Nishida, 2004; 2012b, 2012c)

In addition to these water-soluble NTBI, it is well known that iron deposition, which is water-insoluble NTBI, is frequently observed for the patients with hemochromatosis and several neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, etc. (Yoshida et al., 2000; Stankievicz et al., 2007; Roberts et al. 2012). Despite numerous studies over the last 30 years since plasma NTBI was first postulated to exist, it is still poorly characterized. The inability thus far to characterize NTBI most likely reflects both its heterogeneous nature and the likelihood that the different forms will exist and vary with the concentration of the chelates such as amino-acids, peptides, and citrate, etc.

Very recently, Nishida have proposed that the iron deposition should be aggregation of the di-μ-oxo bridged dimeric Fe(III) complex based on the several observed facts (Nishida, 2012a, 2012b, 2012c), and in this article we will give several experimental evidences to support the Nishida's proposal.

2. Experimental

2.1 Materials

Albumin (Bovine serum albumin) was purchased from bibco. Tris-buffer solutins were prepared by diluting the commercial Tris-solution (1M, pH=7.6) to 10 mM solution. Iron(III) chelates were obtained according to the reported procedures; $K[Fe(ida)_2]3H_2O$, $K_4[Fe_2O(ida)_4]$ $10H_2O$, $K_4[Fe_2O(nta)_2(CO_3)]$, $K[Fe_2O(CO_3)(pac)_2]$,

$K_2[Fe_2O(CO_3)(edda)_2]$, $Fe(dpa)Cl_2$, and $Fe(dpal)Cl_2$, (Schimitt et al., 2002; Nishida & Ito, 1995; Sutoh, Okawamukai, Nishino, & Nishida, 2006) the chemical structures and their abbreviations of several ligands cited in this paper being illustrated in Figure 1.

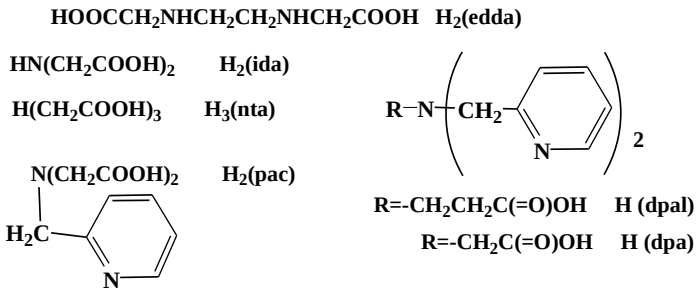

Figure 1. Structures of the ligands cited in this paper

2.2 Formation of Iron Deposition

The equi-volume solutions of albumin（1.5g/100 mL buffer solution）and iron(III) complex (0.05 M in buffer solution) were mixed. The formation of brown iron deposition was observed for only the $K[Fe(ida)_2]3H_2O$, but any another iron(III) complexes gave no precipitation (see Figure 2). When the dilute solution of $K[Fe(ida)_2]3H_2O$ (0.015 M in buffer solution) was mixed with the albumin under the same condition, no formation of precipitation occurred. But, when hydrogen peroxide solution (3%, commercial hydrogen peroxide solution (30 %) was diluted with distilled water) was added to the clear solution containing albumin and $K[Fe(ida)_2]3H_2O$ (0.015 M), iron deposition appeared *immediately*. The similar iron deposition was observed *gradually* when hydrogen peroxide was added to the solution containing albumin and other iron(III) chelates, such as with H(dpa), H(dpal), etc.

2.3 DFT Calculations

DFT calculations were performed according to the published methods. (Sakiyama、Kazama, Suzuki, & Nishida. 2009; Sakiyama, Oshima, Suzuki, & Nishida, 2009)

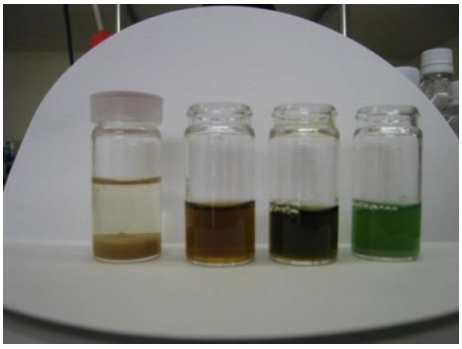

Figure 2. Solutions containing albumin and iron(III) complexes (0.05 M solution). From the left: $K[Fe(ida)_2]$, $Fe(dpa)Cl_2$, $K_2[Fe_2O(CO_3)(edda)_2]$, $K_2[Fe_2O(CO_3)(pac)_2]$

3. Results and Discussion

3.1 Iron Deposition Formation between Albumin and Fe(ida) Complex in the Absence of Hydrogen Peroxide

As described in the Experimental section, iron deposition occurred only when the $K[Fe(ida)_2]3H_2O$ (0.05 M) solution was added to the albumin solution (1.5g/100mL) .(see Figure 2); albumin, the major protein in plasma, is present at a concentration of about 40g/L (Evans, et al., 2008). The $K[Fe(ida)_2]3H_2O$ complex is green in the crystalline state, but its color changed to orange when it dissolved in the buffer solution. The orange color thus found is frequently observed for the iron(III) species with μ-oxo bridged dimeric Fe(III) cores (Ito et al., 1996), and this color is very similar to that of $K_4[Fe_2O(ida)_4]10H_2O$, its μ-oxo bridged dimeric core being confirmed by the crystal structure determination (Mizuno et al., 2006, and see Figure 3). These are implying that the μ-oxo bridged Fe(III) cores in $K[Fe(ida)_2]3H_2O$ complex solution is different from that in the $K_4[Fe_2O(ida)_4]10H_2O$ complex, because the latter complex did not give brown iron deposition in the reaction with albumin. Our previous study demonstrated that two structures are possible for μ-oxo bridged dimeric iron(III)-(ida) complex;

one should be of a μ-oxo bridged dimeric Fe(III) core with one (ida) ligand for each iron(III) atom, $[Fe_2O(ida)_2(H_2O)_4]$ (Nishida, Ito, & Satoh, 2007; Nishida, 2009), which is different from $K_4[Fe_2O(ida)_4]$ where two molecules of (ida) are coordinated to the iron(III) atom (Figure 3), and the latter complex cannot interact with other molecules because two iron(III) ions are fully occupied by the chelating atoms.

The optimized structure of $[Fe_2O(ida)_2(H_2O)_4]$ calculated by DFT method is illustrated in Figure 4 (Abe, Sakiyama, & Nishida, *in press*), where *two of the* four water molecules are located in *cis*-position, suggesting that $[Fe_2O(ida)_2(H_2O)_4]$ can interact with another molecule at two iron atoms simultaneously. This is exemplified by DFT calculations, for examples, tetraglycine can bind with two iron(III) atom simultaneously as shown in Figure 5. (Abe, Sakiyama, & Nishida, *in press*). In the case of $[Fe_2O(edda)_2(H_2O)_2]$ complex,two water molecules are located in the *trans* position.

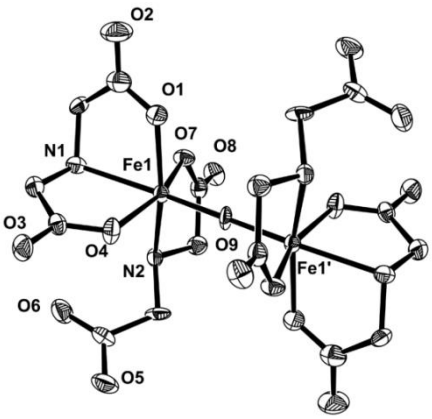

Figure 3. Structure of $[Fe_2O(ida)_4]^{4-}$ complex in the crystalline state

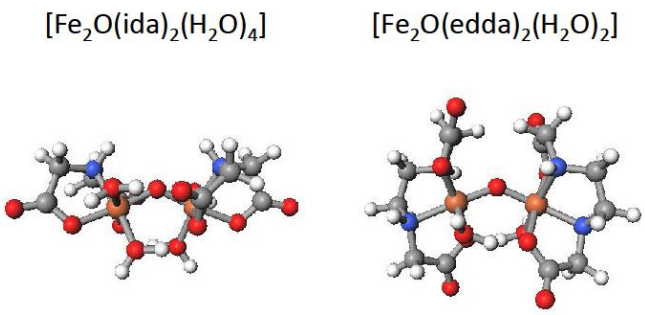

Figure 4. Optimized structure of Left: $[Fe_2O(ida)_2(H_2O)_4]$ and Right $[Fe_2O(edda)_2(H_2O)_2]$ (Orange, Fe; Blue, nitrogen; Red, oxygen; Gray, carbon; White, hydrogen)

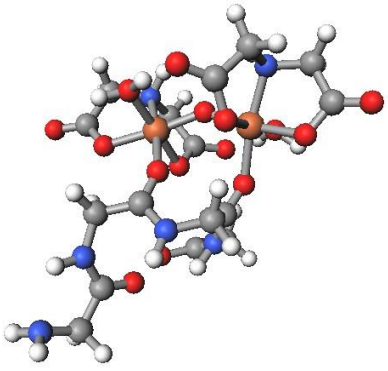

Figure 5. Optimized structure of $[Fe_2O(ida)_2(H_2O)_2(tetraglycine)]$

(Orange, Fe; Blue, nitrogen; Red, oxygen; Gray, carbon; White, hydrogen)

Based on the above discussions, it seems reasonable to assume that the spontaneous formation of the iron deposition by $K[Fe(ida)_2]$ $3H_2O$ complex (0.05 M) and albumin may proceed as described in Figure 7; in the first step two-point interaction between the two iron(III) ions of dimeric $[Fe_2O(ida)_2(H_2O)_4]$ and oxygen atoms of the albumin occurs (see also Figure 6), and in the next step the formation of di-μ-oxo bridged dimeric Fe(III) complex may occur through the attack by the iron(III) atom of the another iron(III) chelate. Since the strong *trans-effect* operatess in these compounds due to strong Fe-O(oxo) bonds (Sutoh, Okawamukai, Nishino & Nishida, 2006), the dissociation of the (ida) ligands from the iron(III) atoms may proceed, leading to the aggregation of di-μ-oxo bridged dimeric Fe(III) complex, as depicted in Figure 6. The second step is impossible for *all the other* iron(III) complexes such as $K_4[Fe_2O(nta)_2(CO_3)]$, $K[Fe_2O(CO_3)$ $(pac)_2]$, $K_2[Fe_2O(CO_3)$ $(edda)_2]$, and $[Fe_2O(ida)_4]^{4-}$ complex, because the ligands of the first three iron compounds are tetradentate, and this will explain the fact that no spontaneous precipitate formation was observed in the presence of these iron(III) compounds.

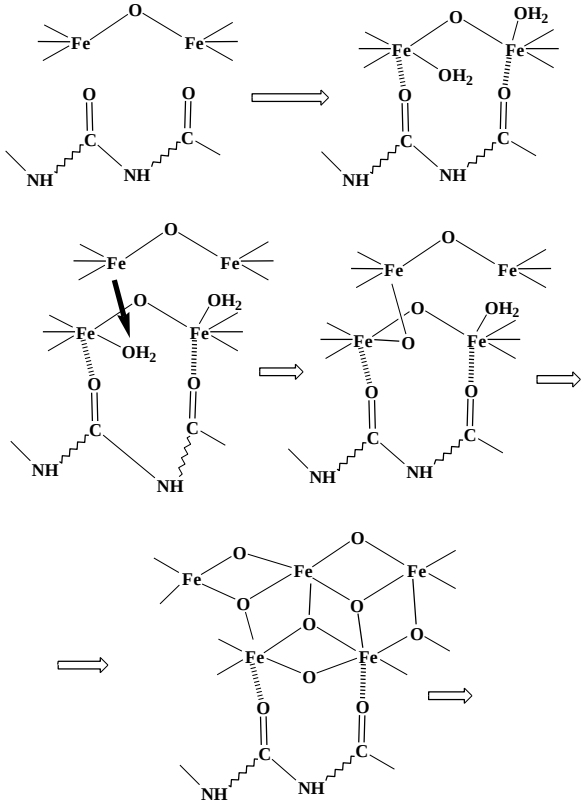

Figure 6. Assumed mechanism for iron deposition formation between albumin and $Fe_2O(ida)_2(H_2O_2)_4]$ complex in the absence of hydrogen peroxide (Four water molecules of $Fe_2O(ida)_2(H_2O)_4$ complex are omitted)

3.2 Iron Deposition Formation between Albumin and Fe(ida) Complex in the Presence of Hydrogen Peroxide

In the presence of H_2O_2, it seems quite likely that the coordination of H_2O_2 to the iron ion should occur, as illustrated in Figure 7. As it is known that hydrogen peroxide gives facilely a di-μ-oxo bridged dimeric Fe(III) species in the reaction with a Fe(III) chelate through the formation of a binuclear Fe(III)-peroxide adduct (Sutoh, Okawamukai, Nishino & Nishida, 2006), it seems reasonable to assume that the peroxide adduct in the Figure 7 should give the formation of a di-μ-oxo bridged dimeric Fe(III) species, leading to the aggregation of di-μ-oxo bridged dimeric Fe(III) complex, as depicted in Figure 6. In this process, some oxidative damages should occur in the albumin, probably oxidations of phenol or alcoholic groups of the albumin.

Immediate formation of the iron deposition in the solution containing $K[Fe(ida)_2]$ $3H_2O$ complex (0.015 M) and albumin solution by H_2O_2 addition strongly supports that the structure of the iron deposition formed contain di-μ-oxo bridged dimeric Fe(III) cores as proposed (Nishida, 2012a), and also is consistent with the suggestion that alpha-synuclein acts in concert with iron and dopamine to induce formation of Levy body pathology in Parkinson's disease (PD) and cell death in PD. (O-Golts et al, 2000), and also gives reasonable explanation for the fact that α-syn-Fe(III) generated from the oxidation of α-syn-Fe(II) by O_2 with H_2O_2 as a co-product, is a short-lived, dissociates to hydrolyze to ferrihydrite gel. (Peng et al. 2009)

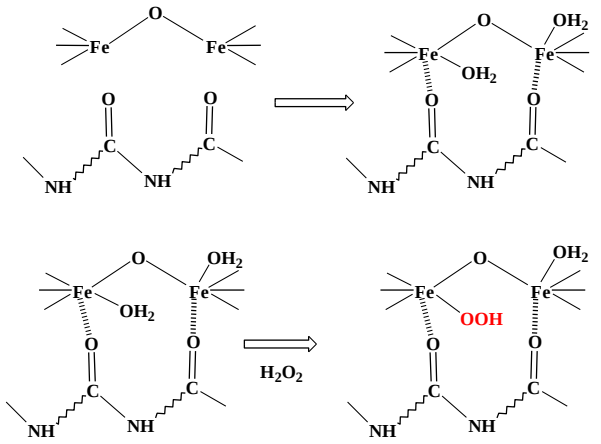

Figure 7. Assumed mechanism for iron deposition formation between albumin and $[Fe_2O(ida)_2(H_2O_2)_4]$ complex in the presence of hydrogen peroxide (Four water molecules of $Fe_2O(ida)_2(H_2O)_4$ complex are omitted)

3.3 Iron Deposition Formation between Aβ (1-40) and Iron(III) Complex in the Presence of Zinc(II) ion

In our previous paper, we have showed that iron deposition occurs readily when zinc(II) chloride solution is added to the solution containing Aβ (1-40) and iron(III) compounds with tetradentate ligand (nta) or (edda) (Okawamukai, Sutoh, & Nishida,2006), and also by adding the iron(III) compound solution to the mixed solution of Zn(II) chloride and Aβ (1-40) containing the white Zn(II)/Aβ (1-40) precipitation. Nishida have proposed that the formation of iron deposition observed is induced by strong power of zinc(II) ion as a transporter of hydroxide ion (OH^-) to the iron(III) ions (Nishida, 2012a, 2012b). In this article we would like to propose the formation of iron deposition when iron(III) compound was added to the mixed solution of Zn(II) chloride and Aβ (1-40) containing the white Zn(II)-Aβ (1-40) precipitation as shown in Figure 8; zinc(II) ions act as a transporter of hydroxide ion (OH^-) to the iron(III) ion, leading to the formation of di-μ-oxo-iron(III) species, and to the dissociation of the chelate from the iron(III) ion, finally to the formation of iron deposition. The dissociation of the tetradentate ligands from the iron(III) ion depicted in Figure 9 may be attributed to the instability of di-μ-oxo-iron(III) species due to the *remarkable trans-effect* by the strong Fe(III)-oxo bond as exemplified by several compounds (Sutoh, Okawamukai, Nishino & Nishida, 2006). It should be noted here that the formation of the iron deposition by zinc ion proceeds for the iron(III) chelates with tetradentate ligands, which are not observed in the spontaneous process as observed for $Fe_2O(ida)_2(H_2O)_4$ (0.05 M solution).

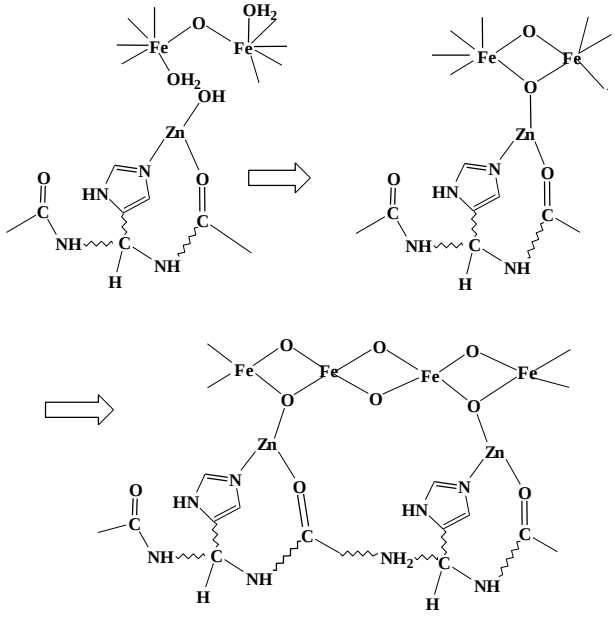

Figure 8. Assumed mechanism for iron deposition formation between amyloid-β peptide(1-40) and iron(III)-(edda) complex in the presence of zinc(II) ion

In addition to the above, we in this study found that zinc(II) ion leads to the iron deposition formation (see Figure 9) when the binuclear iron(III) complexes with an alkoxo-bridge $[Fe_2(HPTP)Cl_4]^+$ ion was added to the solution containing Aβ(1-40) and zinc(II), where H(HPTP) represents N,N,N',N'-tetra (2-pyridylmethyl)-1,3-diamino-2- propanol (Nishino et al. 1999). Remarkably strong power of zinc (II) ion to give iron deposition should be noted !

Figure 9. **I.** Zinc (II) chloride (10 μl, 1 M) was added to the Aβ (1-40) solution (100 μl, 0.25mg/ mL). **II.** $Fe_2(HPTP)Cl_4ClO_4$ solution (50 μl, 2 mg/mL) was added to the solution **I.**

Since the total zinc(II) concentration is relatively reduced compared with that of normal cases and massive iron deposition are observed in the brain and on several organs such as kidney or spleen of the patients of aceruplasminemia (Yoshida et al., 2000) and other neurodegenerative disorders (Grabrucker, Roman, & Garner, 2011), it seems reasonable to assume that zinc(II) ions play an important role on the formation of the iron deposition in these patients. Since the formation of the iron deposition means the deletion of toxic NTBI from the plasma, we can consider that zinc(II) ions act as an antioxidant in the patients of several neurodegenerative disorders. Thus, the amyloid deposition which frequently observed for the Alzheimer's patients, may be due to one of the antioxidative function by zinc(II) ion (Okawamukai, Sutoh, & Nishida, 2006; Nishida 2012a, 2012b,2012c).

4. Summary

This study clearly demonstrates *experimentally* that there are three different ways of the formation of iron deposition in the presence of proteins; the first way which proceeds *spontaneously*, and the another ways which occur in the presence of hydrogen peroxide *or* zinc(II) ion; in the case induced by H_2O_2 should be associated with simultaneous occurrence of oxidative damages. All these processes are quite consistent with the assumption that iron deposition contains the di-μ-oxo bridged dimeric Fe(III) cores and proteins, and this will give useful therapeutic methods to prevent the many disorders which are closely related with toxicity by the iron(III) ions.

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