

# Role of Zinc(II) Ion for the Formation of Iron Deposition in Human Body and Its Significance

Yuzo Nishida<sup>1</sup>

<sup>1</sup> Medical Research Institute, Kanazawa Medical University, Uchinada-machi, Japan

Correspondence: Yuzo Nishida, Medical Research Institute, Kanazawa Medical University, Uchinada-machi, Ishikawa Prefecture 920-0293, Japan. Tel: +81-76-225-8405. E-mail: nsd-2210@kanazawa-med.ac.jp

Received: August 22, 2012 Accepted: September 14, 2012 Online Published: October 15, 2012

doi:10.5539/ijc.v4n6p1

URL: <http://dx.doi.org/10.5539/ijc.v4n6p1>

## Abstract

Iron depositions, one of the non-transferrin-bound iron (NTBI), are frequently observed for the patients with thalassemia, hemochromatosis and other iron-overloading disorders. In this article, we have pointed out that zinc(II) ion and hydrogen peroxide play a critical role in the formation of the iron deposition, and that the formation of iron deposition by zinc(II) ion should be one of the important method to protect the oxidative stress by water-soluble NTBI. This implies that the zinc(II) ions contribute to depress the oxidative stress by NTBI.

**Keywords:** iron deposition, NTBI, Zinc(II) ion, hydrogen peroxide, antioxidant

## 1. NTBI and Iron Deposition

Plasma iron is normally bound to the iron transport protein transferrin. 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 thalassemia, hemochromatosis and other iron-overloading disorders, and is present at concentration up to 10  $\mu$ M (Dresow, Peterson, Fischer, & Nielsen, 2008; Fernaeus & Land, 2005; Gaeta & Hider, 2005). It should be noted here that NTBI has been thought to play an important 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.

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. At present, one of the most definitely definable NTBI should be iron deposition, which is frequently observed for patients of hemochromatosis and other iron-overloading disorders, and aceruplasminemia (Yoshida et al., 2000). The structure and role of the iron deposition is not known at present, which at present is only considered to be a signal to tell that the human body is iron-overlord state. In this article, we will demonstrate the several chemical models for NTBI and for the iron deposition, and propose the important role of zinc(II) ions to give iron deposition.

## 2. Formation and Structural Properties of Iron Deposition

As demonstrated above, the structure of the iron deposition is not clear at present. Here, I would like to propose that structure of the iron deposition should be similar to those of the models for hemosiderin, the structure of one of the hemosiderin models being illustrated in Figure 1 (Hearth & Powell, 1992).

Above compound is the water-insoluble iron(III) complex with  $H_3(hida)$  (for the structure of this chelate, see Figure 2), and it should be noted here that 1) central seven iron(III) ions are surrounded by ten oxo anions ( $O_2^{2-}$ ), 2) six oxo anions among the ten are coordinated to three iron atoms, and 3) the central part of this compound is the aggregation of eight *di- $\mu$ -oxo-diiron(III) species*. These are implying that the oxo anions play an important role for the formation of the model of hemosiderin, and also for the formation of iron deposition. When excess  $H_3(hida)$  was added to the solution containing above water-insoluble compound, the solid dissolved to form a clear solution, and from the solution the following compound,  $[Fe_2(hida)_2(H_2O)_2]$  was obtained (Hearth &

Powell, 1992), the structure of this complex being illustrated in Figure 3.

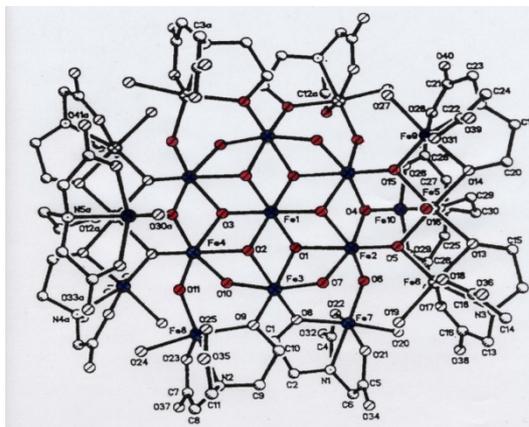


Figure 1. Structure of model compound of hemosiderin with  $H_3(\text{hida})$ . In the center circle (Fe, black; oxygen, red), seven iron(III) ions are surrounded by ten oxo anions forming the aggregation of eight di- $\mu$ -oxo-diiron(III) species

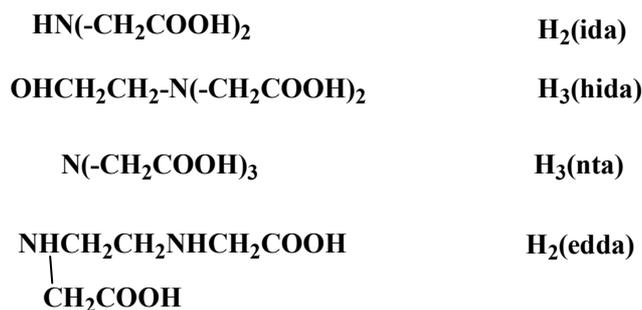
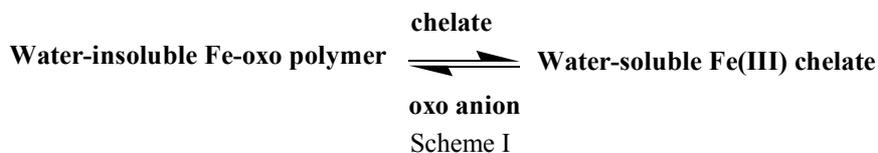


Figure 2. The structures of the ligands cited in this paper

Figure 3 clearly indicates that the exclusion of the oxo anions around the iron(III) ion leads to the formation of water-soluble species, which again supports that oxo anions are closely related with the formation of iron deposition. Similar facts are also observed for the case of  $\text{H}_2(\text{ida})$  chelate (see Figure 2). These facts are demonstrating that there is an equilibrium between the water-insoluble and water-soluble iron(III) species in the solution (see Scheme I), which is controlled by the concentrations of oxo-anion and chelates. Both the water-insoluble and water-soluble species are called as NTBI, but NTBI that play an important role in iron induced oxidative stress should be water-soluble ones (Nishida, 2009).



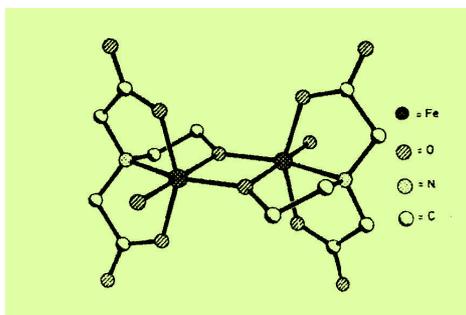


Figure 3. Structure of  $[\text{Fe}_2(\text{hida})_2(\text{H}_2\text{O})_2]$

Two water molecules of two iron(III) ions are located to the *trans*-site to each other.

### 3. Participation of $\text{H}_2\text{O}_2$ and Zinc(II) Ion for Formation of Iron Deposition

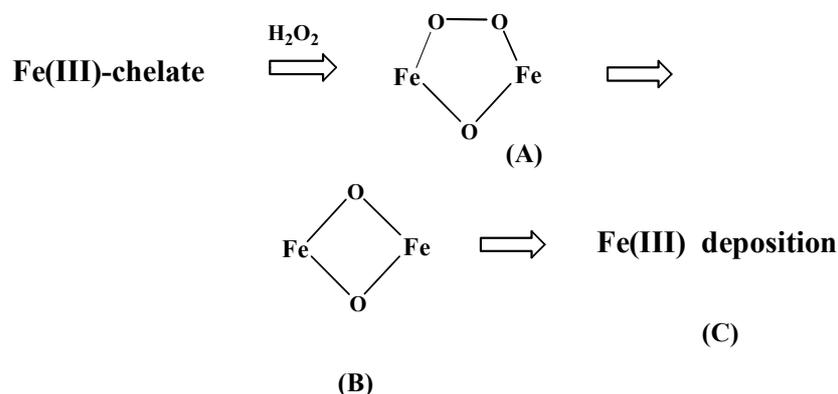
#### 3.1 $\text{H}_2\text{O}_2$ in the Patients of Aceruloplasminemia

As stated above, it is clear that oxo anions play an important role to the formation of iron deposition, and in this section we will give the clear evidence that  $\text{H}_2\text{O}_2$  plays a critical role to give the oxo anions.

Aceruloplasminemia, originally called familial apoceruloplasmin deficiency, is an iron metabolic disorder where ceruloplasmin deficiency is caused by a lack of apoceruloplasmin biosynthesis and copper metabolism is not disturbed (Yoshida et al., 2000). Ceruloplasmin is expressed in the central nervous system as well as in visceral organs and functions in brain iron metabolism. This disease is characterized by marked iron accumulation in the brain as well as visceral tissue despite low serum iron levels. These findings distinguish clearly aceruloplasminemia from hereditary hemochromatosis.

Iron is incorporated into metabolically active compounds or sequestered within ferritin. Ferritin is the principal protein for iron storage and detoxification (Harrison & Arosio, 1996). Almost all cells contain ferritin, which functions both as a safe storage site for iron and as a readily accessible reserve for iron acquired by the cell in excess of its intermediate metabolic needs. The heavy subunit has a ceruloplasmin site which functions in the oxidation of ferrous ion to a ferric form with formation of water, where the ferrous ion released from the ferritin should be chelated compound with amino acids, peptide, or citrate. Thus, the most important problem to be solved is to clarify how the ferrous ion is oxidized to a ferric ion in the absence of ceruloplasmin. Very recently, we have observed that apo-transferrin exhibits oxidase-like function towards several metal chelates (Sato, Abe, & Nishida, 2012), suggesting that in the patients of aceruloplasminemia the ferrous ions are oxidized to a ferric state by apo-transferrin with the formation of hydrogen peroxide. In the catalytic oxidation of Fe(II) to Fe(III) by ceruloplasmin, hydrogen peroxide does not form, and thus it should be noted here that *much quantity of hydrogen peroxide formation occurs* in the patients of aceruloplasminemia.

We have reported that hydrogen peroxide catalyzes the formation of di- $\mu$ -oxo-diiron(III) species (B) in Scheme II from several iron(III) chelates with amino acid or citrate in the presence of reducing agents such as phenol or alcohol (Sato, Okawamukai, Nishino, & Nishida, 2006), and that this species is derived from ( $\mu$ -peroxo)( $\mu$ -oxo)-diiron(III) species (A) in Scheme II formed in the reaction mixture, which turned to the di- $\mu$ -oxo-diiron(III) species through oxidizing the organic molecules such as alcohol or phenols. It should be remembered that in the model of hemosiderin the central part of the compound illustrated in Figure 1, is made from the di- $\mu$ -oxo-diiron(III) skeleton. Thus, it seems quite likely that the further aggregation of the di- $\mu$ -oxo-diiron(III) species may proceed to give the iron deposition. As these iron(III) ions in the polymeric compounds are not transferred to apo-transferrin (Nishida, Itoh, & Sato, 2007), above discussion explains the marked iron accumulation including iron deposition in the brain as well as visceral tissue despite low serum iron levels observed (Yoshida et al., 2000), and the fact that highly toxic ( $\mu$ -peroxo)( $\mu$ -oxo)-diiron(III) species (A) in Scheme II generated under the presence of hydrogen peroxide, can degrade the peripheral proteins or DNA, etc. (Nishida, 2004; 2009) is highly consistent with the oxidative damages observed for the patients of aceruloplasminemia (Yoshida et al., 2000).

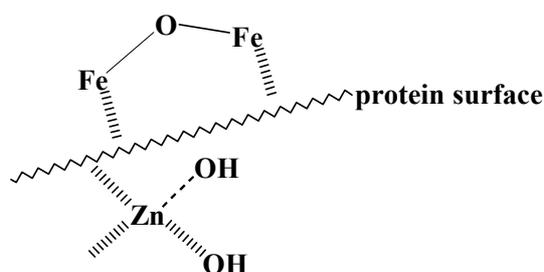


Scheme II

### 3.2 Zinc(II) Species Act as $\text{OH}^-$ -Transporter to Give Iron Deposition

We have found that deposition of the iron(III) hydroxide occurs readily on the aggregates of amyloid beta-peptide ( $\text{A}\beta$  (1-40)) when zinc(II) chloride solution is added to the solution ( $\text{pH}=7.4$ ) containing iron(III) compounds with (nta), (edda), and other amino acid derivatives, and  $\text{A}\beta$  (1-40) (Okawamukai, Sutoh, & Nishida, 2006). We have observed that the similar iron deposition has occurred on several proteins such as albumin or transferrin, indicating that iron deposition by zinc(II) ions are not specific for the amyloid proteins.

It seems quite likely that the formation of iron deposition observed above proceeds according to the Scheme III: zinc(II) complex which contains hydroxide ion ( $\text{OH}^-$ ) (Nishino et al., 2006) approaches to the protein, where the iron(III) complexes bind with the protein through two-point interaction (Scheme III) (Nishida, Itoh, & Satoh, 2007), and then the transfer of the hydroxide ion from the zinc(II) to the iron(III) ion occurs, to lead to the formation of di- $\mu$ -oxo-iron(III) species, and finally to iron deposition as illustrated in Scheme II. In this reaction the zinc(II) ions are operating as  $\text{OH}^-$ -transporter to give di- $\mu$ -oxo-iron(III) species on the protein. 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 aceruplasminemia patients (Yoshida et al., 2000), it seems reasonable to assume that zinc(II) ions play an important role on the formation of the iron deposition in the aceruplasminemia 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, which is consistent with the fact that amyloid deposits generally contain much quantities of iron(III) and zinc(II) ions (Bush, 2003).



Scheme III

## 4. Transferrin Rigorously Distinguishes and Recognizes the Structure of the Iron(III) Chelates

Transferrins are group of iron-binding proteins, that include serum transferrin, lactoferrin and ovotransferrin; they are all glycoproteins that have a molecular mass of about 80 kDa, and bind two  $\text{Fe}^{3+}$  per molecule with high affinity. Serum transferrin has a specific role as an iron transporter, delivering the bound iron to target cells via receptor-mediated endocytosis. But, the detailed mechanism of up-take of iron-ion from the solution by apo-transferrin remains unclear at present.

In order to get information on this problem, we investigated the interaction between many iron (III) chelates and apo-transferrin, where the formation of holo-transferrin was evaluated by the use of absorption spectroscopic, ESR and capillary electrophoresis methods (Nishida, 2009; Nishida, Itoh, & Satoh, 2007). As the results, it has become apparent that the transfer of the iron(III) ion from the iron(III)-chelates to apo-transferrin is highly dependent on the structure of the iron(III)-chelates; demonstrating that not all the iron(III) ions in plasma are transferred to apo-transferrin. For example, for the two alkoxo-bridged binuclear iron(III) complexes,  $\text{Fe}_2(\text{hida})_2(\text{H}_2\text{O})_2$  and  $\text{Fe}_2(\text{HPTP})\text{Cl}_4$  (see Figure 4) (Nishino, Kobayashi, Kunita, Matsushima, & Tokii, 1999), the iron(III) ions of only the latter complex are readily transferred to apo-transferrin, but the those of the former complex, are not. Our results on many iron(III) complexes have lead to the conclusion that formation of Intermediate shown in Figure 5, where the two iron(III) ions and the protein are interacting *via* two-point interaction, is necessary for the facile transfer of the iron(III) ions from the iron(III) chelates to apo-transferrin. In the case of  $\text{Fe}_2(\text{hida})_2(\text{H}_2\text{O})_2$ , two iron(III) ions cannot interact with the protein *via* two-point interaction as described in Figure 5 because of the steric requirement of this complex (see Figure 3), and thus apo-transferrin cannot capture the iron(III) ions from this complex. On the other hand the two-point interaction is possible for the  $\text{Fe}_2(\text{HPTP})\text{Cl}_4$  complex, because the four chloride ions are labile in the aqueous solution, the facile transfer of iron(III) occurs from this complex to apo-transferrin, similar to the case of  $\text{Fe}(\text{III})\text{-(nta)}$  complex (see Figure 5). Thus, the iron(III) ion in the monomeric  $\text{Fe}(\text{III})\text{-(edta)}$  chelate, is not transferred to the apo-transferrin ((edta)= ethylenediamine-*N,N,N',N'*-tetraacetato anion). This unique property of apo-transferrin is very important to understand the fact that the marked iron accumulation in the brain as well as visceral tissue despite low serum iron levels in the aceruplasminemia patients (Yoshida et al., 2000) as described in Section 3.1.

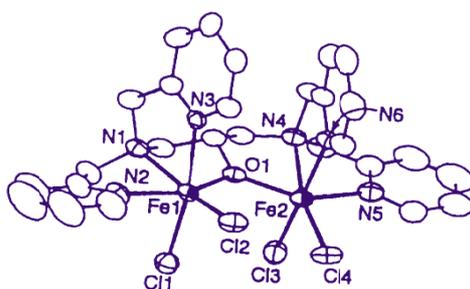


Figure 4. Structure of  $[\text{Fe}_2(\text{HPTP})\text{Cl}_4]^+$

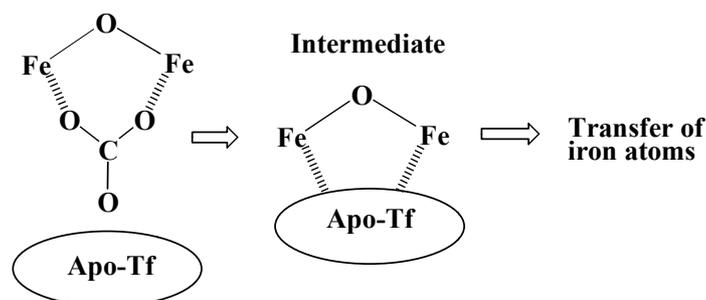


Figure 5. Schematic illustration of iron(III) atom transfer from the  $\text{Fe}(\text{III})\text{-(nta)}$  chelate to apo-transferrin

The carbonato ion of the binuclear complex,  $\text{Fe}_2\text{O}(\text{nta})_2(\text{CO}_3)$  is labile, and it may readily dissociate from the complex in the reaction with apo-transferrin, and the transfer of iron(III) ions proceeds through the formation of Intermediate (Nishida, Itoh, & Satoh, 2007).

## References

- Bush, A. I. (2003). The metallobiology of Alzheimer's disease. *Trends Neuroscience*, 26, 207-214. [http://dx.doi.org/10.1016/s0166-2236\(03\)00067](http://dx.doi.org/10.1016/s0166-2236(03)00067)
- Dresow, B., Peterson, D., Fischer, R. & Nielsen, P. (2008). Non-transferrin-bound iron in plasma following administration of oral iron drugs. *BioMetals*, 21, 273-276. <http://dx.doi.org/10.1007/s10534-007-9116-5>

- Fernaues, S., & Land, T. (2005). Increased iron-induced oxidative stress and toxicity in scrapie-infected neuroblastoma cells. *Neuroscience Letters*, 382, 217-220. <http://dx.doi.org/10.1016/j.neule.2005.03.069>
- Gaeta, A., & Hider, R. C. (2005). The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy. *Brit. J. Pharm.*, 146, 1041-1059. <http://dx.doi.org/10.1038/sj.bjp.0706416>
- Harrison, P. M., & Arosio, P. (1996). The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim. Biophys. Acta*, 1275, 161-203.
- Hearth, S. L., & Powell, A. K. (1992). The trapping of iron hydroxide units by the ligand "heidi": Two new hydroxo(oxo)iron clusters containing 19 and 17 iron atoms. *Angew Chem Int. Ed. Engl.*, 31, 191-194.
- Nishida, Y. (2004). Oxidative stress and neurodegeneration. *Med. Hypothesis Res.*, 1, 227-245.
- Nishida, Y. (2009). Structural characteristics of iron(III) chelates to induce tissue damage and renal carcinoma: Chemical origin of the iron toxicity. *TCIMail*, No. 141, 2-15. Retrieved from <http://www.tciamerica.com/tcicemail/backnumber/article/141drE.pdf>
- Nishida, Y., Ito, Y., & Satoh, T. (2007). Origin of renal proximal tubular injuries by Fe(III)-nta chelate. *Z. Naturforsch.*, 62c, 608-612.
- Nishino, S., Kobayashi, T., Kunita, M., Matsushima, H., & Tokii, T. (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.
- Nishino, S., Kobayashi, T., Matsushima, H., Tokii, T., & Nishida, Y. (2006) Enhanced nucleophilicity and depressed electrophilicity of peroxide ion by zinc(II), aluminum(III), and lanthanum(III) ion. *Z. Naturforsch.*, 56c, 138-143.
- Okawamukai, Y., Sutoh, Y., & Nishida, Y. (2006). Deposition of iron(III) hydroxide on aggregations of several proteins. *Synth. React. Inorg. Metal-org. Nano-metal Chem.*, 36, 373-375.
- Satoh, T., Abe, K., & Nishida, Y. (2012). Oxidase-like function by apo-transferrin towards manganese(III) chelates. *Int. J. Chem.*, 4(3), 10-13. <http://dx.doi.org/10.5539/ijc.v4n3p10>
- Sutoh, Y., Okawamukai, Y., Nishino, S., & Nishida, Y. (2006). Structure of a new tertanuclear iron(III) complex with an oxo-bridge; Factors to govern formation and stability of oxo-bridged iron(III) species in the L-subunit of ferritin. *Z. Naturforsch.*, 61c, 149-154.
- Yoshida, K., Kaneko, K., Miyajima, H., Tokuda, T., Nakamura, A., Kato, M., & Ikeda, S. (2000). Increased lipid peroxidation in the brains of aceruplasminemia patients. *J. Neurol. Sci.*, 175, 91-95.