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Heme-coordinated histidine residues form non-specific functional "ferritin-heme" peroxidase system: Possible and partial mechanistic relevance to oxidative stress-mediated pathology in neurodegenerative diseases☆

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ABSTRACT

Ferritin is a giant protein composed of 24 subunits which is able to sequester up to 4500 atoms of iron. We proposed two kinds of heme binding sites in mammalian ferritins and provided direct evidence for peroxidase activity of heme-ferritin, since there is the possibility that "ferritin–heme" systems display unexpected catalytic behavior like heme-containing enzymes. In the current study, peroxidase activity of heme-bound ferritin was studied using TMB[1], L-DOPA, serotonin, and dopamine, in the presence of H_2O_2, as oxidant substrate. The catalytic oxidation of TMB was consistent with first-order kinetics with respect to ferritin concentration. Perturbation of the binding affinity and catalytic behavior of heme-bound His-modified ferritin were also documented. We also discuss the importance of the peroxidase-/nitrative-mediated oxidation of vital molecules as well as ferritin-induced catalase inhibition using *in vitro* experimental system. Uncontrollable "heme–ferritin"-based enzyme activity as well as up-regulation of heme and ferritin may inspire that some oxidative stress-mediated cytotoxic effects in AD-affected cells could be correlated to ferritin-heme interaction and/or ferritin-induced catalase inhibition and describe its contribution as an important causative pathogenesis mechanism in some neurodegenerative disorders.

1. Introduction

Iron, as an essential element for living organisms is the most abundant transition metal in the brain. Since free iron is able to generate reactive oxygen species (ROS) such as hydroxyl radical via Fenton reaction [1], to prevent iron-induced oxidative damages, free iron in the cells either enters mitochondrion for utilization in metabolic processes, such as synthesis of heme or is stored within cytosolic and mitochondrial ferritin core [2,3]. Ferritin is a 450 kDa protein composed of 24 subunits (usually 12 nm in diameter with an 8-nm-diameter cavity) which is able to sequester up to 4500 atoms of iron [4]. This huge protein, in all three kingdoms of life, controls the intracellular concentration of Fe^{2+} (or Fe II) in a dynamic fashion, and stores excess iron as a ferrihydrite-like ($Fe_2O_3{\cdot}9H_2O$) mineral inside its cavity protecting the cell from potential iron-dependent formation of reactive oxygen species (ROS) and allowing the release of the metal according to cellular

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; β-Lg, β-lactoglobulin; BSA, bovine serum albumin; DEPC, diethylpyrocarbonate; DMSO, dimethyl sulfoxide; DOPA3,4, dihydroxyphenylalanine; F, ferritin; H, heme; HO-1, hemeoxygenase-1; HRP, horse radish peroxidase; ND, neurodegenerative disorders; NT, nitrated tyrosine residues; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; 5-HT, 5-hydroxytryptamine (serotonin); SN, substantia nigra; TMB,3,3;5,5; tetramethylbenzidine; TNBS, 2, 4, 6, trinitrobenzenesulfonic acid.

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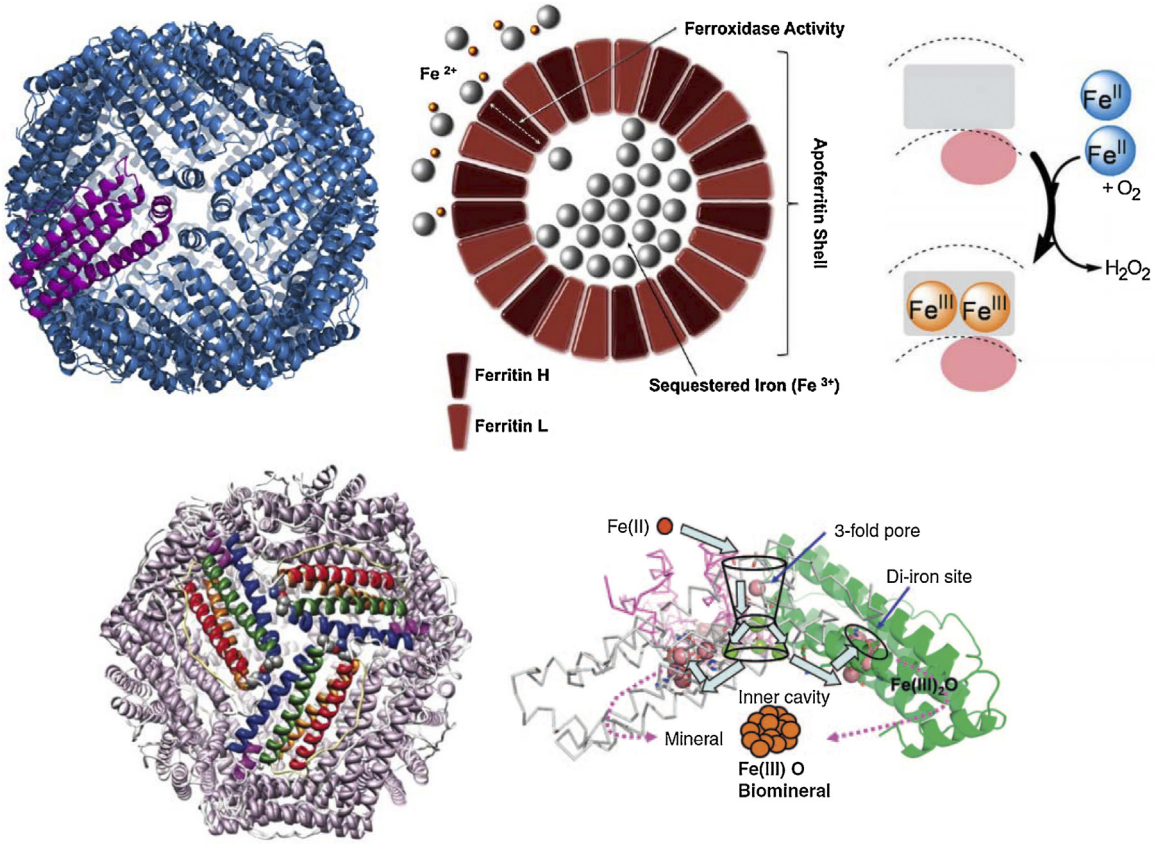

Fig. 1. (Top) *Left*, Ribbon presentation of human ferritin (*PDB code: 3HX7*). *Middle*, schematic structure of ferritin. Apoferritin forms a roughly spherical container within which ferric iron is stored as a ferrihydrite mineral. Apoferritin refers to the iron free form of the protein; the iron-containing form is termed holoferritin or simply ferritin. Taken from Ref. [14]. *Right*, In eukaryotic H and M ferritins, one O_2 molecule is consumed for simultaneously oxidation of two Fe II ions in the ferroxidase center to form a blue intermediate (a peroxodiferric specie [15], which subsequently decays to the Fe III products and hydrogen peroxide. (Bottom) *Left*, Molecular model of the 3-fold channel with the three intersecting subunits represented as ribbons. Taken from Ref. [16]. *Right*, A cartoon of iron moving through ferritin external pores to Fe(II) ion channels and to oxidoreductase sites, then through the Fe(III)O nucleation channels into the mineralization cavity. Arrows are paths of iron through the cage: blue, Fe(II) and dashed-pink, Fe(III)O. Taken from Ref. [17]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demand [5]. To perform this vital function, the protein catalyzes O_2-mediated oxidation of Fe II to Fe III in a di-iron catalytic center, the ferroxidase center (Fig. 1). Mammalian apoferritin is composed of two different but very similar polypeptide subunits which share 55% amino acid sequence identity and are structurally homologous; H (heart or heavy) and L (liver or light) subunits, with molecular masses of 21 and 19 kDa, respectively [6] (Fig. 1).

On the other hand, heme is a complex of iron with protoporphyrin IX which serves as a prosthetic group in a number of hemoproteins such as catalases and peroxidases as well as the oxygen storage and transport molecules. Since free heme is lipophilic and toxic to cells, promoting lipid peroxidation and the production of ROS, the intracellular levels of free heme must be tightly controlled [7]. There are multiple levels of regulatory control for heme synthesis, storage and degradation [8]. For example, as judged from the K_m of hemeoxygenase-1 (HO-1) reaction (1 μM) [9], cellular level of free heme should be approximately lower than 1 μM (100 nM). Recent studies demonstrated that heme concentration is abnormal in brain of Alzheimer's disease (AD) patients [10] and increase of free heme also up-regulates expression of HO-1, which is responsible for heme degradation [11,12]. A resultant product of this regulatory mechanism is free iron [13].

Additionally, in AD as one of widespread neurodegenerative disorders (ND), it has been indicated that enhanced ferritin expression ensues due to increased iron concentration [15] as even a low increase in iron level leads to increase 3-fold of ferritin concentration [18]. Also it has been demonstrated that the ratio of H/L subunits of ferritin changes in the cortex and substantia nigra (SN) in ND patients compared to control subjects [19]. It is previously reported that "heme-Aβ" can induce ROS production and oxidative stress through peroxidase activity [10,20], so that differential affinity of heme to human and rodent Aβ peptide and related peroxidase activity have been proposed to account for the susceptibility of humans (not rodents) to AD [8]. Jaafari et al. [21] reported that heme tightly binds to ferritin and "heme-ferritin" system exhibits non-specific peroxidase activity, so that can potentially contribute to "heme sequestration"-mediated oxidative stress. However there is still not enough information on either the role of ferritin-heme interplay in neurodegenerative diseases or nature of catalytic sites within "ferritin-heme" complex.

His residues are generally involved in the catalytic site of some heme-peroxidases such as horse radish peroxidase (HRP) and play an important role in heme-binding as well as catalysis [22]. "Heme-albumin" binary system (methemalbumin), on the other hand, has been shown to exhibit a weak (pseudo-) peroxidase activity. However, by constructing an artificial heme pocket in human serum albumin (HSA) by replacing Ile^{142} and Leu^{185} by His, "mutant HSA-heme" showed a 17 fold enhancement in peroxidase activity relative to that of "wild HSA-heme" complex [23]. Crystallographic data also reveal that bound porphyrins in myoglobin and HSA are linked to some lysine residues (Lys^{45} in myoglobin and Lys^{190} of HSA) through salt bridges [23–25]. As earlier stated, although the interaction between heme and ferritin has been previously reported and His^{125} and His^{115} side chains around 3D pore of