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The Role of Ferritin in Iron Homeostasis

Moammar Azeez

Biochemistry Department University of Health Sciences Lahore, Pakistan.

ABSTRACT

Ferritin, a major iron storage protein, is essential to iron homeostasis and is involved in a wide range of physiologic and pathologic processes. In clinical medicine, ferritin is predominantly utilized as a serum marker of total body iron stores. In cases of iron deficiency and overload, serum ferritin serves a critical role in both diagnosis and management. Elevated serum and tissue ferritin are linked to coronary artery disease, malignancy, and poor outcomes following stem cell transplantation. Ferritin is directly implicated in less common but potentially devastating human diseases including sideroblastic anemias, neurodegenerative disorders, and hemophagocytic syndrome. Additionally, recent research describes novel functions of ferritin independent of iron storage. Iron is an essential micronutrient that is problematic for biological systems since it is toxic as it generates free radicals by interconverting between ferrous (Fe2+) and ferric (Fe3+) forms. Additionally, even though iron is abundant, it is largely insoluble so cells must treat biologically available iron as a valuable commodity. Thus elaborate mechanisms have evolved to absorb, re-cycle and store iron while minimizing toxicity. Focusing on rarely encountered situations, most of the existing literature suggests that iron toxicity is common. A more nuanced examination clearly demonstrates that existing regulatory processes are more than adequate to limit the toxicity of iron even in response to iron overload. Only under pathological or artificially harsh situations of exposure to excess iron does it become problematic. In this article, we review ferritin and its role in iron homeostasis.

Keywords**:** Ferritin, Toxicity, Oxidative stress, Free radicals, Amino acid.

INTRODUCTION

Iron is a very abundant atom accounting for approximately 5% of the earth's crust, and due in part to its ability to exist in a wide range of oxidation states (−2 to +6) it has some very interesting properties [1]. For biological systems, the characteristic of iron to stably interconvert between its most common oxidative forms, Fe2+ and Fe3+, has been extensively exploited [2] [3] [4]. Iron is indispensable for most life forms and is widely used in a variety of different proteins to carry out a number of functions [5] [6] [7]. For example, iron is critical for the formation of heme that serves as a functionally critical prosthetic group for oxygen binding proteins like hemoglobin and myoglobin [8]. It is also critical for the formation of iron-sulfur clusters that mediate redox and electron transfer reactions for a large number of proteins including cytochromes, ferrodoxins and dehydrogenases. A number of other proteins can directly bind iron [9]. For example, the ability to form structures that can bind and store large amounts of iron is the main function of ferritins [9] [10]. On the other hand, a number of the other sub-familymembers of ferritin-fold containing proteins like the small subunit of ribonucleotide reductase use iron as a direct cofactor [11].

The use of iron in biological systems nevertheless comes at a price. Free iron is toxic since it can donate or accept an electron from neighbouring molecules to cause damage to cellular components or to generate reactive oxygen species (ROS) that are themselves toxic to the cell. In effect, the Fenton and Haber-Weiss

reactions ((1) Fe2+ +- H2O2→Fe3++HO·+OH−and (2) Fe3++H2O2→Fe2++HOO·+H+; net=·O2−+H2O2 →·OH+OH−+O2) describe the reactions of free iron with free radicals within a cell in order to generate even more toxic radicals [13] [14]. Another commonly encountered problem is the scarcity of biologically useful forms of iron [15] [16]. In effect, although iron is very abundant,most forms are insoluble and are thus not easily accessible biologically [17] [18] [19]. For example, the most abundant form of iron, ferric (iron III or Fe3+) hydroxide, is soluble at around 10 − 18M in neutral solutions [20]. This low solubility extends to most common salts of the ferric ion [21]. On the other hand most ferrous (iron II or Fe2+) salts of iron are much more soluble that is in the low 10−2 M range [22]. The limited solubility of iron is a challenge as well as an advantage since it limits its toxicity (see also Section 5).

Structure of Ferritin

Conservation of ferritin protein sequence, folding, tertiary and quaternary structure among plants and animals is very high, emphasized by the use of an animal sequence (frog) to clone the first plant (soybean) ferritin. In addition, structures of plant and animal ferritin are superimposable. Ferritin in contemporary bacteria diverges considerably in sequence, but not in secondary, tertiary and quaternary structure, suggesting evolutionary convergence with eukaryotic ferritins.

Ferritin is a large protein (12-nm diameter, 480,000 Da) with a large cavity (256 nm3) for the mineral that is created by the spontaneous assembly of 24 ferritin polypeptides folded into four-helix bundles bound to each other by hydrogen and salt (ionic) bonds [23]. The structure appears to have evolved as a patchwork of other proteins such as non-heme di-iron oxygenases that share with ferritin the binding of Fe and O2. Iron in the dioxygenases is a cofactor, but iron in ferritin is a substrate. Ion channel/pore proteins share properties with ferritin Fe entry and exit sites and may be progenitors of ferritin pores. Protein mineralization surfaces are shared among ferritin and other proteins in matrices that form biominerals.

The numbers of functional sites in ferritin are: one mineral cavity in the protein center, eight entry and exit pores on the outer surface, 12 mineral attachment sites on the protein cavity surface and a variable number of catalytic ferroxidase sites in the center of each subunit, depending on the number of H and L subunits per ferritin molecule. H-ferritin subunits have an active ferroxidase site and occur in multiple forms in humans, animals, plants and bacteria. L-ferritin subunits have a degenerate ferroxidase site and the gene duplication to encode L-ferritin subunits is found only in vertebrate animals. H- and L-ferritin subunit expression is set by gene transcription during cell differentiation. Changes in ferritin H/L ratios are known to occur in animals or cells responding to very high levels of iron or growth factors and to hypertransfusion in humans with sickle cell disease and *b*-thalassemia [24], [25].

Function of Ferritin

Ferritin is required, not a luxury, as shown by the lethality of ferritin gene deletion during mouse embryonic life and presence even in strictly anaerobic bacteria. In humans, the only diseases related to ferritin mutations were discovered very recently and are relatively benign or appear late in life. The human ferritin gene mutations that are compatible with survival modulate expression or change the protein structure in a variable region, which suggests the lethality of mutations in major ferritin features. Understanding the multiple functions of ferritin requires defining and studying each type of functional site (Fe entry, oxidation, translocation, mineralization and exit).

How each functional site interacts with the others of the same type (eight Fe entry sites, three to 24 Fe oxidation sites, 24 Fe translocation sites, 12 Fe mineral attachment/mineralization sites and eight Fe exit sites) and of the different types adds a unique type of complexity to

understanding ferritin function. However, many lessons can be learned from oxygenases and pore proteins [26]. When Fe^{2+} enters the ferritin protein pores, it rapidly (in milliseconds) readies the ferroxidase site buried within each H-ferritin subunit. The ferroxidase site activity that oxidizes Fe^{2+} and forms diferroxo-mineral precursors (Fe31-O-Fe31) is the function best characterized in ferritin. Mossbauer, resonance Raman and EXAFS spectroscopies have been used to analyze the fast ferroxidase reaction intermediates (in milliseconds) by rapid mixing and freezing to trap enough of a blue (A650nm) diferric peroxo-transition state for the spectroscopic studies.

Trapping the diferric complex in protein crystals has not been possible. The peroxo intermediate also forms in di-iron peroxo oxygenases. Iron is a substrate converted to the diferric peroxo intermediate in ferritin and is released into the interior of the protein as a diferric-oxo mineral precursor. (Note that in di-iron oxygenases, the iron is retained at the active site as a cofactor, and O is activated for insertion into substrates such as methane, to make methanol).

Ferritin releases H_2O_2 and diferric-oxo mineral precursors, leaving behind an active site that is altered for a fairly long time, presumably to allow peroxide to diffuse away before binding the next Fe(II) substrate atoms. In bacteria, the iron site in ferritin has a variety of Fe amino acid ligands. Heme is also a cofactor and mineralization does not produce peroxide. Other bacterial ferritin-like molecules do not bind oxygen, but rather bind peroxide and convert it to water [27]. H_2O_2 production, when iron and oxygen enter the protein to form the iron mineral, will reflect the H/L subunit ratio. When the amount of extra iron in a cell is far above normal, as in transfusional iron overload or hereditary hemochromatosis, the combination of extra iron in the ''labile iron pool'' and extra peroxide from mineralizing iron may outstrip the activities of catalase, glutathione peroxidase and superoxide dismutase [28].

In a number of cases during iron overload in animals or cultured cells, the H/L ferritin subunit ratio decreased, likely to minimize tissue damage from reaction of labile iron and peroxide. Recently, a correlation between liver fibrosis and H/L ferritin subunit ratio was observed in humans with transfusional iron overload [29], [30]. Protein pores are the sites in ferritin where Fe^{2+} enters the protein from the cell or solution on the way to the ferroxidase site. In addition, Fe^{2+} leaves the protein as the mineral dissolves (reduction and rehydration) through eight pores formed at the junction of three ferritin subunits. Crystal structures of all L- and all H-ferritin subunits show the pores to be similar.

Conserved amino acids important for iron entry, Asp 127 and Glu 131, were identified by amino acid substitution where decreases in oxidation rates were up to 1003. Fe^{2+} exit is controlled by a different set of conserved amino acids, Arg 72, Leu 110, Asp 122 and Leu 134, which have small to no effects on Fe oxidation but large effects (up to 303) on iron exit rates triggered by NADH/FMN and detected by formation of colored chelator complexes outside the protein. Identification of a ''gate'' in the ferritin pore occurred when the crystal structure of the –L134 mutated protein was examined. The entire helix-loop-helix of each subunit around the pore, amino acids 110–134, was completely disordered (unfolded), although the remainder of the subunit had folded and assembled normally, which was associated with enhancing the rate or reduction and chelation of iron in the ferritin mineral. When the ferritin polypeptide chain was extended by an insertion of five amino acids between 120 and 125 at the pore, the effect on Fe^{2+} exit was the same as the amino acid substitutions.

Ferritin in Iron Homeostasis

Ferritin, an iron storage protein, is the primary iron storage mechanism and is critical to iron homeostasis. Ferritin makes iron available for critical cellular processes while protecting lipids, DNA, and proteins from the potentially toxic effects of iron. Alterations in ferritin are

seen commonly in clinical practice, often reflecting perturbations in iron homeostasis or metabolism. It is increasingly recognized that ferritin also plays a role in a multitude of other conditions, including inflammatory, neurodegenerative, and malignant diseases.

In humans, the majority of iron is integrated within the globin proteins that facilitate the transport of oxygen throughout the body. Iron is also critical in converting oxygen into useable cellular energy by serving as a key component in the electron transfer chain. In addition to its' role in respiration, iron is also utilized as an enzymatic co-factor in numerous other reactions. One such reaction is the conversion of ribose nucleotides to deoxyribose nucleotides, an iron dependent process catalyzed by ribonucleotide reductase that is necessary for DNA replication and cell division.

Despite iron's integral role within the body, it also has the potential to be highly toxic by facilitating the formation of free radicals. Thus, carefully regulated mechanisms have evolved to transport iron across biological membranes, distribute it throughout the body, and store it in inert form until needed. Regulation of systemic iron balance occurs exclusively at the site of absorption, as there is no physiologic process present to excrete excess iron. The majority of iron absorption occurs via enterocytes in the proximal small intestine. Here, iron is transported across the cellular membrane by the divalent metal transporter, DMT1, a member of the N ramp family that transfers iron (and other divalent metals) across the apical membrane and into the cell through a proton-coupled process.

Prior to transport, the iron must be in the ferrous state (Fe2+). The conversion of dietary inorganic non-heme iron to Fe2+ is facilitated by brush border ferrireductases. The absorption of heme iron is less well understood, although heme transporters have recently been discovered. DMT1 levels are upregulated in response to systemic iron deficiency, thereby increasing cellular uptake. Though some absorbed iron remains present within the enterocyte as ferritin, the majority is transported to other sites within the body. Ferroportin is a newly identified iron efflux pump that mediates the export of iron from the enterocyte.

Prior to the transport of iron outside the cell, intracelluar iron must be converted to Fe3+. This is facilitated by either hephaestin or ceruloplasmin, both of which have ferroxidase activity (Fe2+ → Fe3+). Within the intestine, both of these proteins are active, whereas in the liver (a major storage site for iron) ceruloplasmin is the primary workhorse [31] [32]. Iron is then loaded onto transferrin, the primary transporter of iron in the circulation. When bound to transferrin, Fe3+ is soluble and nonreactive, thereby allowing it to enter the circulatory system. The primary consumer of iron is the bone marrow, where red cells require large amounts of iron to meet demand for the production of iron-containing hemoglobin [33].

Within the bone marrow, erythroid precursors express transferrin receptors (TfRs) on their surfaces. Upon binding of the iron-saturated transferrin to its receptor, the complex is endocytosed. The acidic environment of the endosome prompts the release of iron from transferrin. The unbound iron is subsequently reduced to its ferric/ferrous form (Fe2+) by Steap 36 and transported out of the endosome into the cytoplasm by DMT1. The empty transferrin and transferrin receptors are returned to the cell's surface where they dissociate at a neutral pH, and re-enter the circulation [34].

Interestingly, the transport of iron into non-hematopoietic cells does not require the transferrin receptor. This is highlighted in mouse experiments, where a disrupted TFR gene leads to lethal anemia at an early stage of development, while other non-hematopoietic tissues contain normal amounts of intracellular iron. The constant turnover of red cells demands recycling of the iron contained within hemoglobin. This recycling process is accomplished primarily within the macrophage, which is capable of

phagocytosing erythrocytes, which are then lysed [35]. The iron is liberated from the phagolysosome heme via hemoxygenase. Unstored iron within the macrophage is then exported in a process thought to be dependent on ferroportin.

The dynamics of the transport, distribution, and recycling of iron are tightly regulated within humans. While much remains to be discovered regarding the control of iron balance, hepcidin, a recently discovered 25 amino-acid protein, is believed to be critical to this process. Hepcidin serves as a negative regulator, and when elevated, results in reduced intestinal iron absorption and macrophage iron release. Hepcidin often increases in response to inflammation, a process that is thought to be responsible for much of the iron abnormalities that are a hallmark of anemia of chronic disease. The impact of inflammation on hepcidin has led some to theorize that hepcidin evolved as a method of host defense, decreasing available iron for invading pathogens and malignant cells to reproduce. Conversely, the deficiency of hepicidin, as seen in juvenile hemochromatosis, may lead to pronounced and toxic iron overload [36] [37].

CONCLUSION

Iron metabolism is carefully balanced by complex networks to meet the daily iron demands and to prevent iron overload. Ferritin is considered crucial for iron homeostasis and their regulatory mechanisms are increasingly recognized. Iron is an essential nutrient that plays a critical role in life sustaining processes. Due to its ability to gain and lose electrons, iron works as a cofactor for enzymes involved in a wide variety of oxidation-reduction reactions This function makes iron an essential nutrient.

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