EDITORIAL REVIEW

Low Molecular Weight Intracellular Iron Transport Compounds

By A. Jacobs

Evidence is presented for the existence of an intracellular pool of low molecular weight iron compounds which acts as an intermediate between extracellular iron and a wide variety of intracellular processes. It is in equilibrium with storage iron and iron enzymes and is of major importance in iron toxicity and chelation therapy.

IRON IS ESSENTIAL for all mammalian cells; despite the widely different functions of different tissues, there are certain basic similarities in their iron metabolism. All obtain iron from extracellular transferrin, and much of this is directed to mitochondria which are the site of heme synthesis either for mitochondrial cytochromes, for extramitochondrial cytochromes, such as cytochrome P450, or for specialized proteins such as hemoglobin or myoglobin. In addition, all cells contain nonheme iron enzymes, such as the iron-sulphur proteins which take part in mitochondrial electron transport, and iron-activated enzymes, such as ribonucleotide reductase and aconitase. The capacity to synthesize ferritin is universal in mammalian cells, though the extent to which this occurs depends on the intracellular iron load.

Despite the considerable amount of information available on all these aspects of cellular iron metabolism, there is remarkably little information available regarding the intermediate stage between the release of iron from the cell membrane and its incorporation into its final biochemical form. A review of evidence regarding this pathway indicates the presence of a labile intermediate pool, and a number of inferences can be made about its characteristics and functions.

ERYTHROID METABOLISM

The pathway of iron from the external cell membrane to heme synthesis has been extensively studied in erythroblasts and reticulocytes, where hemoglobin formation predominates over all other activities. It seems likely that mitochondria constitute the final point of the pathway as it is here that the enzyme ferrochelatase is located and it is here that excess iron accumulates in the sideroblastic anemias when there is reduced heme synthesis. Apart from mitochondrial iron-proteins the only well recognized non-heme iron compounds in

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the erythroblast are ferritin and hemosiderin, but no information is available regarding the nature of the iron released from the cell membrane and available for incorporation into ferritin or the iron released from ferritin and available for mitochondrial uptake.

A number of workers have found evidence for the existence of short-lived nonheme iron intermediates within the erythrocyte though none of these has been well characterized. Experiments both in vivo[1] and in vitro[2] have suggested the presence of an intermediate that is clearly distinct from ferritin and transferrin, but unfortunately in neither of these studies was the radioiron initially bound to transferrin. Zail et al.,[3] however, presented serum-bound ^{59}Fe to cultures of human marrow cells and found a nonferritin hemoglobin precursor from which iron was rapidly removed for heme synthesis. Iron accumulating as ferritin in the cells appeared to be in excess of metabolic requirements. In marrow cultures from iron-deficient patients there was transient labeling of the intermediate fraction before complete incorporation into hemoglobin. Ferritin was not found at any time, thus demonstrating that it did not form an essential precursor, but simply provided a storage form for iron, as in other cells.

Primosigh and Thomas[4] confirmed the presence of a soluble intracellular iron compound that served as a source of iron for both hemoglobin and ferritin. It had a molecular weight of less than 5000, it was dialyzable through cellophane, and it was precipitated by 8-hydroxyquinoline at pH 7.7. When heme synthesis was inhibited by isonicotinic acid hydrazide, iron derived from transferrin accumulated in a low molecular weight fraction, in ferritin, and in the mitochondria themselves.[5,6] It thus seems likely that within the developing red cell iron moves from the membrane, after its detachment from transferrin, into the cytosol, where it becomes available (presumably in complex form) for either heme or ferritin synthesis. The suggestion that the transferrin–iron complex may become internalized by a process of pinocytosis after attachment to the red cell membrane[7] implies that the detachment of iron occurs within the cell.

IRON METABOLISM IN NONERYTHROID CELLS

The pattern of intracellular iron metabolism is modified in different cell types. The small intestinal epithelial cell has a polar anatomy and, in addition to the entry and exit of iron via transferrin, iron can enter the cell from the luminal border either in the form of heme, which is split to release its iron, or as a mixture of iron complexes whose chemical form is completely unknown. A different pattern is seen in reticuloendothelial (RE) cells, which may gain much of their iron by the ingestion of effete erythrocytes with their subsequent digestion and heme catabolism. The iron load of such cells is extremely high in proportion to their metabolic requirements, but they adjust to this by increased ferritin synthesis and hemosiderin formation. The presence of an intermediate "labile iron pool" has been postulated both for RE cells[8] and intestinal epithelial cells,[9,10] though until recently no very clear evidence for its existence has been found in nonerythroid tissues.

Indirect evidence for a highly chelatable form of iron which is probably a normal metabolic intermediate in RE cells has been provided by a number of studies using the chelator desferrioxamine.[11] In six patients with megaloblastic

anemia, ferrioxamine excretion after administration of the chelator was raised. During the first week of specific therapy there was a 40% fall in iron chelation despite only minimal mobilization of iron stores for effective hemoglobin production. This change coincided with the period in which intramedullary hemolysis ceases.[12] Similarly, desferrioxamine-induced iron excretion increased 4–8 hr after the transfusion of nonviable erythrocytes but returned to normal after 20 hr despite the increase in storage iron.[13] A reduction in iron flow through the RE cell produced by suppression of erythropoiesis reduced the amount that could be chelated[14] and an increase in iron flow induced by hemolysis increased chelation.[15] Rat experiments[14] confirmed that ferritin and hemosiderin are not important direct donors of iron to desferrioxamine and that compounds on the pathway between storage compounds and transferrin are the probable immediate source.

Recent data regarding the intracellular labile iron pool in nonerythroid cells have been obtained from experiments using cultured Chang cells as a model system. Almost 30% of the iron taken up by the cells from transferrin is membrane bound and the remainder is found in the cytosol. Chang cells synthesize ferritin actively in response to an iron load,[16] and most of the cytosol iron is present in this form. However, about 35% of this iron is in a nonheme, nonferritin dialyzable form.[16] Gel filtration of the membrane-free supernatant after centrifugation of the sonicated cells shows that the low molecular weight fraction usually adheres to Sephadex but can be readily chelated by desferrioxamine, EDTA, or transferrin. None of the iron found in the ferritin peak at the void volume is transferred either to chelating agents or to transferrin.

When Chang cells have been "labeled" with [^{59}Fe] transferrin so that radioiron is normally distributed through the cell fractions, subsequent transfer of the culture to a medium containing desferrioxamine results in rapid iron depletion of the cell. The [^{59}Fe] ferritin content falls more rapidly than the low molecular weight ^{59}Fe, but as desferrioxamine has been shown not to chelate ferritin iron directly, mobilization presumably occurs via the low molecular weight pool. Similar mobilization of intracellular iron is observed when the cells are incubated with transferrin, the amount removed being inversely related to the transferrin saturation.[17] Direct estimation of the cytosol iron by atomic absorption spectrometry shows it to amount to about 20 fg/cell, but after incubation for 7 days in a medium containing 170 μmole/liter iron this quantity is increased to about 100 fg/cell.

The incubation of pure cultures of human polymorphonuclear leukocytes, lymphocytes, and monocytes under the same conditions as those used in the Chang cell experiments shows that despite wide differences between different cell types and the variations in disease states iron uptake is related to transferrin saturation in all cases, and "cytosol" iron is present in both ferritin and nonferritin forms.[18] The characteristics of the nonferritin form, however, are not the same in all cases. Gel chromatography of the polymorphonuclear leukocyte cytosol reveals the presence of a sharp peak corresponding to the position of lactoferrin. In studies of small intestinal epithelial cells, though there is a component of low molecular weight, estimated to be less than 3500,[19] a protein iron binder of molecular weight similar to transferrin is also found.[19,20]

While all these cell types differ in their functions and in iron metabolism they all appear to contain a low molecular weight iron complex which is easily available either for uptake by ferritin, heme, transferrin, or chelators. The universality of such an intracellular intermediate cannot automatically be assumed, but the abundant evidence for its existence both in erythrocyte precursors and in other cells, together with the difficulty in explaining intracellular iron metabolism without postulating such an intermediate iron pool, suggests that it is probably present in many tissues.

FUNCTIONS OF THE INTERMEDIATE POOL

The importance of the intracellular iron pool can be seen from its focal position in relation to intracellular iron metabolism (Fig. 1). It provides iron for heme synthesis and other metabolic needs for iron compounds. A wide variety of processes such as DNA synthesis,[21] globin chain synthesis,[22] and tyrosine hydroxylase[23] and proline hydroxylase[24] activities are inhibited by desferrioxamine and may be assumed to depend on the availability of iron in the intracellular intermediate pool. Other substances such as the biogenic amines[25] or enzymes such as those of the pentose phosphate shunt[26] or α-glycerophosphate dehydrogenase[27] appear to depend on an adequate tissue iron status for their normal function, and thus presumably depend on the presence of a metabolically functional intracellular iron pool.

Enlargement of this pool beyond normal metabolic needs results in the stimulation of ferritin synthesis[28,29] and an increased availability for uptake by transferrin. Wills[30] has demonstrated the role of this reactive iron pool in lipid peroxidation and it is probably of major importance in the iron toxicity found in overload states. In this respect its availability for chelation by therapeutic agents such as desferrioxamine is important not only as the route whereby the level of iron stores can be reduced but also as a means of "detoxicating" iron-loaded cells. Inhibition of lipid peroxidation by desferrioxamine in vitro has been demonstrated.[30]

The interaction of this pool with extracellular transferrin and thus similar intermediate pools in other tissues provides a route through which an equilibrium can be established not only between the various metabolic processes in the cell but also between different organs in the body. The equilibrium between the intermediate pool in the small intestinal epithelium and those in the rest of the body probably plays a major role in the regulation of iron absorption.[31]

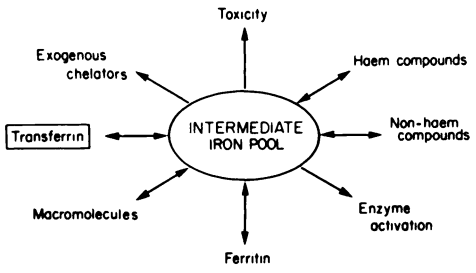

Fig. 1. Relation of intermediate iron pool to intracellular iron metabolism.

CHEMICAL NATURE OF THE INTERMEDIATE POOL

The nature of the intermediate iron pool is unknown; it appears to be a low molecular weight complex of iron, or perhaps a mixture of such complexes. The notion of iron chelates playing a key role in iron metabolism is not new.[32,33] Mazur et al.[34] have demonstrated the transfer of iron from transferrin to ferritin in liver slices with the participation of ascorbic acid and ATP, an iron $(ATP)_2$ complex being the final iron donor. A similar process has been observed in developing erythrocytes.[35] Although Mazur et al. felt it is unlikely that any other substances are required as intermediates, a number of other candidates have been suggested and indeed any naturally occurring intracellular ligand can be considered. Miller and Perkins[36] have found that in the transfer of iron from transferrin to ferritin a reducing agent is essential and has three functions: first, to assist in the removal of iron from transferrin; second, to form an intermediate ferrous chelate; and third, to ensure that iron is available in the Fe^{2+} state for uptake by ferritin. Ascorbic acid appears to fulfill this role effectively, though transfer is enhanced if another chelator is also present. The intermediate chelate should not be so stable as to prevent the uptake of iron by ferritin. Citrate, sugars, amino acids, and nucleotides may all function as intermediate ligands in iron transfer.[36]

One of the problems in identifying low molecular weight intermediate iron compounds within the cell is that of distinguishing artifacts occurring during the homogenization process, either through interaction between cell constituents or with the buffer, from the original iron complex in vivo.

The identification of intermediate iron compounds will not only depend on their chemical characterization but also on their ability to display the appropriate metabolic functions. It is of interest that iron-ATP not only forms ternary chelates with a number of biogenic amines whose activity has been linked with iron metabolism, but that it can also form a chelate with phosphatidyl serine, a membrane component.[37] Barnes et al.[38] have shown that a number of iron compounds are incapable of donating iron to mitochondria, though some of the compounds they have studied, such as transferrin and ferritin, are rather too large to be considered in the present context. Their only successful candidate, desferrioxamine, can hardly be considered physiologic in mammalian systems. There is evidence that both ferric ADP and ferric ATP can donate iron to mitochondria,[39] though Cederbaum and Wainio[40] have shown that ATP inhibits mitochondrial iron uptake. Evidence for the presence of large amounts of iron nucleotides in red cell precursors[41-43] is based on the examination of acid extracts of the cells, which might be considered an unphysiologic procedure. Ferric citrate can be utilized directly by the reticulocyte for heme synthesis,[44,45] and the same is true for a number of nonphysiologic low molecular weight chelates such as nitriloacetate,[44] fusigen, and ferricrocin.[45]

There is no reason why the intermediate iron pool should consist of a single type of complex—though clearly all the component compounds will share a number of properties. It is not impossible that in vivo much of the intermediate iron could be nonspecifically bound to protein. At the very least it seems probable that there is an equilibrium between Fe^{2+} and Fe^{3+} compounds, determined by the redox potential within the cell or possibly at different intracellular

sites. It is likely that the iron incorporated into ferritin is initially in an Fe^{2+} form before its oxidation by the protein and that on its release it undergoes reduction.[28] At the same time ferritin synthesis and iron incorporation are inhibited by desferrioxamine, an Fe^{3+} chelator, which presumably alters the equilibrium within the iron pool. Conversely, there is evidence that mitochondrial iron uptake is from the Fe^{3+} form followed by an interaction with the respiratory chain within the organelle to form Fe^{2+}.[46] Iron uptake for heme synthesis, however, is inhibited by α,α-dipyridyl, an Fe^{2+} chelator.

There is a striking contrast between our detailed knowledge of iron proteins and our ignorance regarding the intermediate pool. Further work on this aspect of cell metabolism should prove particularly rewarding. Clarification of the chemistry within the intermediate iron pool will give a better insight into intracellular iron metabolism and thereby into a number of pathologic mechanisms. It may also uncover relationships between iron metabolism and other metabolic pathways which at the present time are unsuspected.

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