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Mini-review

Iron metabolism: the low-molecular-mass iron pool

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Summary. This review examines various aspects of iron metabolism in mammalian and bacterial cells which support the hypothesis of the existence and the biological significance of an intracellular pool of low-molecular-mass iron complexes.

Key words: Intracellular iron metabolism – Low-molecular-mass iron pool – Fenton reaction

Introduction

The control of internal iron concentration is a biological event of considerable importance. In contrast to its biologically beneficial effects (storage and activation of molecular oxygen, reduction of ribonucleotides and dinitrogen, electron transport, etc.), iron may play also a deleterious role through the generation of toxic free radicals (Halliwell and Gutteridge 1989). The great majority of iron is located within polypeptide chains. However, a low-molecular-mass iron pool seems to be a necessity at least in four major biological processes: (a) intracellular iron transport, (b) regulation of the biosynthesis of transferrin receptors and ferritin, (c) control of the activity of iron-containing proteins, (d) catalysis of the Fenton reaction. Although it has been the matter of extensive work and debate, it is still poorly defined.

The intracellular iron transport

The major vehicle for iron delivery to mammalian cells is the serum glycoprotein transferrin, which tightly binds two ferric ions/molecule (Aisen 1989; Crichton and Charloteaux-Wauters 1987). At least two mechanisms for cellular uptake of iron from transferrin have been proposed (Thirstensen and Romslo 1990). The first one involves a receptor-mediated endocytosis. After binding to a specific receptor on the cell surface, the transferrin-iron complex is internalized into an acidic non-lysosomal vesicle. The low pH facilitates iron mobilization from transferrin. This mechanism does not apply to all cell types. In hepatocytes, it is proposed that binding to the receptor results in reduction of iron by a specific membrane diferric-transferrin reductase (Sun et al. 1987). An Fe(II) transport system then participates in the translocation of iron across the membrane to the interior of cell. Within the cytosol, iron is incorporated into ferritin, the iron-storage protein, probably as Fe(II) which becomes oxidized within the protein core. One molecule of ferritin can store as many as 4500 iron atoms, largely in the form of inorganic oxyhydroxide, together with some phosphate (Harrisson and Lilley 1989). Utilization of ferritin iron by the cell requires that it can be reduced and mobilized as Fe(II). However, the physiological reducing system has not been identified. Finally, Fe(II) has to be transported to specific cell compartments, such as mitochondria, where it is incorporated into protein cofactors (protoporphyrin IX) or newly synthesized apoenzymes.

The above-mentioned systems, transferrin, ferritin and mitochondrial proteins, do not interact directly with each other, since they are located within separated individual compartments. Thus the concept has arisen that a low-molecular-mass iron pool, containing small and soluble complexes, was the actual form of iron transport inside the cell from one iron-binding protein to another. It is sometimes called the transit iron pool (Jacob 1977).

Regulation of transferrin receptors and ferritin

Uptake and storage of iron are regulated in a concerted manner and are responsive to an iron signal. When iron is in excess, ferritin synthesis and iron storage are increased while synthesis of transferrin receptors and iron uptake decline. The opposite is observed when iron levels are low. The coregulation occurs at the level

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of mRNA translation/degradation and is due to the presence of the same nucleotide sequence, called IRE (iron responsive element) in both receptor and ferritin mRNA (Theil 1990). The details of this regulation must await characterization of regulatory proteins as well as the identification of the iron signal. Very recently, an IRE-binding protein has been cloned and found to contain cysteine and histidine clusters suitable for binding iron ions (Rouault et al. 1990). On the other hand, even though the molecular form of the iron signal is not known, it is likely that it resides in a small but critical intracellular low-molecular-mass iron pool, which provides iron to regulatory IRE-binding proteins.

This system may be compared to the regulation of iron metabolism in *Escherichia coli* or *Salmonella typhymurium*. Such microorganisms synthesize very efficient low-molecular-mass Fe(III) chelators, named siderophores, for iron solubilization and uptake (Winkelman et al. 1987). Synthesis of aerobactin or enterobactin is responsive to iron concentrations. This is mediated by Fur, the product of the *fur* (ferric uptake regulation) gene, a negative regulator of the siderophore operon. It has been shown that Fur binds Fe(II) but not Fe(III) at high levels of intracellular iron and becomes activated for the inhibition of siderophore transcription (Bagg and Neilands 1987). When iron levels decline, Fe(II) is removed from Fur and biosynthesis of the siderophore is derepressed. Such a model also points to a pivotal role of free or loosely coordinated iron for regulation of the bacterial iron uptake machinery.

Regulation of the activity of iron-binding proteins

A large number of iron-containing proteins belongs to the category of iron-binding proteins according to Williams (Williams 1982). In contrast to true iron-proteins with high binding strength and very little dissociation of iron, such as heme-dependent enzymes and iron-sulfur proteins, these iron-binding proteins readily lose their metallic ions, in particular on isolation. Their activity is directly dependent on the concentration of iron in a form which makes it available to the protein, more likely as free Fe(II). Note that the Fur protein appears to fall into this category. This thus gives the low-molecular-mass iron pool a pivotal function in the regulation of dissociable iron enzymes.

Catalysis of Fenton reaction

Superoxide radicals and hydrogen peroxide are continuously formed under non-pathological conditions in aerobic cells. But their concentrations are extremely low and their deleterious effects are controlled by a variety of effective protective mechanisms. This vital control has to be selective since oxygen activated species may have beneficial effects, for example during phagocytic and inflammatory processes. The most favored theory of oxygen toxicity is based on its conversion to the highly reactive hydroxyl radical. This is achieved through the one-electron reduction of hydrogen peroxide by ferrous iron, i.e. the Fenton reaction (Halliwell and Gutteridge 1989).

Superoxide is also an important mediator of oxygen toxicity. That is why superoxide dismutase plays a vital role in the protection of aerobic cells. Its toxicity may be related to its ability to reduce iron. In particular, a recent but controversial model for superoxide toxicity suggests a reductive mobilization of ferritin iron by superoxide and thus an increased concentration of toxic free iron (Biemond et al. 1988; Bolann and Ulvik 1990).

As far as the Fenton reaction is concerned, it is important to identify the iron catalyst, in vivo. The suggestion that low-molecular mass iron complexes are available for catalyzing this reaction came from the observation that iron complexes with EDTA, nucleotide or citrate were excellent catalysts and that, in general, protein-bound iron, metalloenzymes, and iron transport proteins do not catalyze the formation of free hydroxyl radicals during Fenton-type reactions.

Conclusion

Despite the putative biological importance of low-molecular-mass intracellular iron complexes, it is remarkable that this iron pool has not yet been identified. Crichton referred to it as 'a mysterious intermediate pool whose nature is about as well characterized as that of the Loch Ness monster' (Crichton 1984). Recently, ATP-Fe complexes have been suggested to be a key constituent of this pool (Weaver and Pollack 1989; Weaver et al. 1990). However, the weakness of most of the published data is that there is not a single iron complex, isolated from a biological medium, that has been completely characterized in terms of coordination chemistry. In fact, attempts to assess the nature and magnitude of this iron pool are fraught with difficulty. On the one hand, iron contamination of samples and extraction of protein-bound iron is almost impossible to prevent. On the other hand, we think that the major part of 'free' iron, in vivo, is ferrous iron. Any attempt to isolate it will result in its over oxidation and over-precipitation.

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