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Molecules in focus

Intracellular labile iron

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Abstract

Cells maintain organellar pools of "labile iron" (LI), despite its propensity for catalyzing the formation of reactive oxygen species. These pools are identifiable by iron-chelating probes and accessible to pharmacological agents. Cytosolic LI has been assumed to have a dual function: providing a rapidly adjustable source of iron for immediate metabolic utilization, and for sensing by iron-regulatory proteins (IRPs) that regulate iron uptake and compartmentalization via transferrin receptors and ferritin. However, it now appears that IRPs may respond both to fluctuations in LI *per se* and to secondary signals associated with redox-active species. Recent information also indicates that iron can be delivered to mitochondria via pathways that circumvent cytosolic LI, suggesting possible alternative mechanisms of cell iron mobilization and trafficking. We discuss the changing views of intracellular LI pools in relation to iron homeostasis and cellular distribution in physiological and pathological states.

Keywords: Iron; Fluorescence; Chelators; Sensors

1. Introduction

The existence of a cellular pool of metabolically available, labile iron (LI), has remained controversial. Firstly, its inherently transient nature, susceptibility to exchange between chemical ligands and to environment-sensitive redox conversions, have made it difficult to chemically quantify or even characterize. Secondly, the actual concept of cellular pools of LI might be considered counterintuitive, as it would entail the heavy cost of coping with continual iron-catalyzed generation of toxic reactive oxygen species (ROS). The development of iron-sensitive fluorescent probes has provided much evidence in favor of intracellular LI pools. Cytosolic LI is still portrayed as a key player in the cell iron-sensing machinery and hub of iron homeostasis (reviewed in Kakhlon & Cabantchik, 2002; Kruszewski, 2003). Yet, there remains considerable uncertainty about the mechanisms by which it affects iron-handling proteins and is in turn influenced by them. The notion of diffusible LI that is randomly bound to cellular ligands of variable affinities does not exclude the possibilities of escorted iron delivery to organelles and ensuing compartmentalization. The purpose of this review is to critically examine these evolving concepts and open questions, and point out new directions for investigating cellular LI.

Abbreviations: LI, labile iron; CAL-G, calcein green or fluorescein–bis(methyliminodiacetic acid); DMT-1, divalent metal transporter; Phen-Green, fluorescein–phenanthroline; ROS, reactive oxygen species; SIH, salicylaldehyde isonicotinoyl hydrazone; IRP, iron-regulatory protein

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2. Probing intracellular labile iron

Probes for LI are comprised of a fluorescent reporter group coupled to a high-affinity iron(II or III) chelator. The fluorophore responds to metal binding by undergoing a change in fluorescence, ideally in a stoichiometric manner. The most commonly used fluorophores include fluorescein and rhodamine derivatives attached to either desferrioxamine, phenanthroline, bipyridyl, ethylene-diamine tetraacetate, ferrichromes (reviewed in Esposito, Epsztejn, Breuer, & Cabantchik, 2002) or pyridinones (Ma, DeGroot, Liu, Hider, & Petrat, 2006).

As LI is might be in dynamic equilibrium with various cell components and switch between redox states as a function of cell metabolism, its chemical probing should reliably reflect the level of LI in a particular redox condition. Probes that can fulfill such requirement ought to carry intermediate- to high-affinity chelating moieties and display relatively fast reaction rates. Calcein green (CAL-G) is a prototypic probe that partially satisfies this requirement (Esposito et al., 2002), while minimally affecting cell viability or inhibiting cell growth (Glickstein and Cabantchik, unpublished). A complementary approach for estimating cell LI is based on metal-driven catalysis of ROS formation. As the rate of ROS generation is proportional to LI concentration, the relative contribution of LI to total cell-generated ROS can be estimated from chelator-mediated inhibition (Glickstein, Ben-El, Shvartsman, & Cabantchik, 2005). The advantages of this method are high sensitivity and selectivity for redox-active LI.

3. Functions and properties of intracellular LI

3.1. Cellular LI as a chelator-accessible source of reactive oxygen species

The generation of reactive oxygen species (ROS) via the Fenton reaction is virtually unavoidable in cellular systems that contain both redox-active LI and generate reactive oxygen intermediates such as superoxide and/or H_2O_2. Redox activity requires the cycling of iron between the di- and tri-valent states, which may be expected within cells due to the reducing intracellular environment and activity of ferric reductases. It has been shown that $\geq$80% of the $\sim$0.4 μM LI in erythroleukemia K562 cells is accessible to iron(II)-selective chelators (Breuer, Epsztejn, & Cabantchik, 1995). Also, $\sim$5.8 μM LI in the cytosol of hepatocytes was detected using an iron(II)-specific probe (Petrat, de Groot, Sustmann, & Rauen, 2002). Generally, iron(II) is the form involved in cellular processes, including incorporation into iron-requiring enzymes and ferritin, transport across membranes, or release upon degradation of heme (Wallander, Leibold, & Eisenstein, 2006).

Chelators such as desferrioxamine, deferiprone and deferasirox (Glickstein et al., 2005, 2006) or the pyridylcarboxaldehyde isonicotinoyl hydrazones (Whitnall & Richardson, 2006), prevent redox-cycling of iron and substantially lower both cellular LI and ROS production. Various pro-oxidant stresses elevate LI, possibly by releasing iron from sensitive sites, such as iron sulfur clusters (Kruszewski, 2003; Rouault, 2006), thus exacerbating the damage. Stress stimuli for which this has been observed include peroxides, nitrofurantoin, UV light, nitric oxide and hypothermia injury. Both the LI release and associated damage can be largely prevented by pre- or co-treatment with iron chelators. The iron-storage protein ferritin is remarkably effective at scavenging labile iron(II) and preventing ROS generation. Similarly to exogenous chelators, ferritin overexpression in cells lowers both cytosolic LI and protects cells from ROS-mediated damage (Kakhlon & Cabantchik, 2002).

3.2. Regulation of cellular LI

The accepted model of steady-state LI levels is based on a continuously self-adjusting system that maintains an even balance between cells' iron requirements and supply (Fig. 1). Iron delivery via transferrin receptors 1 and 2 in conjunction with DMT-1 is coordinated with that of the LI regulator ferritin and with the demand of the principal consumers, the mitochondria and various iron-requiring proteins. The control occurs at the mRNA level, mainly via the IRPs (Rouault, 2006; Wallander et al., 2006), although their mode of sensing cell iron levels is still controversial. Candidate signals of iron-repletion to IRP2, leading to its proteosomal degradation, include: (i) heme, (ii) an iron and oxygen requiring dioxygenase (iii) nitric oxide, (iv) iron-independent phosphorylation, as well as (v) cytosolic LI bound to cysteines near the N terminus (Wallander et al., 2006).

It has been suggested that all of the identified IRP-2 regulatory mechanisms may be relevant, but to different degrees in different cells or situations (Rouault, 2006; Wallander et al., 2006). Cytosolic LI may rise and become the predominant signal following: (i) inhibition of iron extrusion resulting from hepcidin-mediated down-regulation of ferroportin, in iron repletion or inflammation (De Domenico et al., 2006); (ii) exposure to nitric oxide and other pro-oxidants; (iii) chronic influx of non-transferrin bound iron (Glickstein et al., 2006).