

POSTER PRESENTATION - SHORT COMMUNICATION

Characterization of mitochondrial iron uptake in HepG2 cells

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ABSTRACT

There is increasing evidence that accumulation of redox-active iron in mitochondria leads to oxidative damage and contributes to various neurodegenerative diseases, such as Friedreich's ataxia and Parkinson's disease. In this work, we examined the existence of regulatory mechanisms for mitochondrial iron uptake and storage. To that end, we used rhodamine B-[(1,10-phenanthroline-5-yl)amino carbonyl] benzyl ester, a new fluorescent iron-sensitive probe that is targeted specifically to the mitochondrion. We found that extracellular iron was incorporated readily into mitochondria in an apparently saturable process. Moreover, the rate of iron incorporation responded to the Fe status of the cell, an indication that the mitochondrion actively regulates its iron content.

Key terms: mitochondria, iron homeostasis, RPA, HepG2 cells

INTRODUCTION

The mitochondrion is the main generator of endogenous reactive oxygen species (ROS) and at the same time, it must ensure an appropriate iron supply for the synthesis of heme and Fe-S clusters. There is increasing evidence that accumulation of redox-active iron in mitochondria can lead to oxidative damage and contributes to various human diseases, such as Friedreich's ataxia and Parkinson's disease (Zecca et al., 2004). The use of fluorescent probes that chelate Fe and are targeted to specific organelles allows the determination of compartmentalized pools of labile (reactive) iron, (LIP) (Espósito et al., 2002; Petrat et al., 2002). In this study, we examined the existence of regulatory

mechanisms for mitochondrial iron uptake and storage. To that end, we studied the changes in the mitochondrial labile iron pool, mLIP in HepG2 cells and the generation of ROS upon long- and short-term exposure to iron.

RESULTS AND DISCUSSION

Cells were loaded with the mitochondrial, iron-responsive probe rhodamine B-[(1,10-phenanthroline-5-yl)amino carbonyl] benzyl ester (RPA) (Petrat et al., 2002), and fluorescence was recorded with a fluorescence microplate reader. After a basal reading, iron, as ferrous ammonium sulfate (FAS), was offered to cells. Fe was rapidly taken up by mitochondria, as demonstrated by RPA quenching (Fig. 1).

RPA quenching was apparently saturable at concentrations of FAS above 10 μM . These results suggest that mitochondria have a relatively fast and saturable iron uptake system.

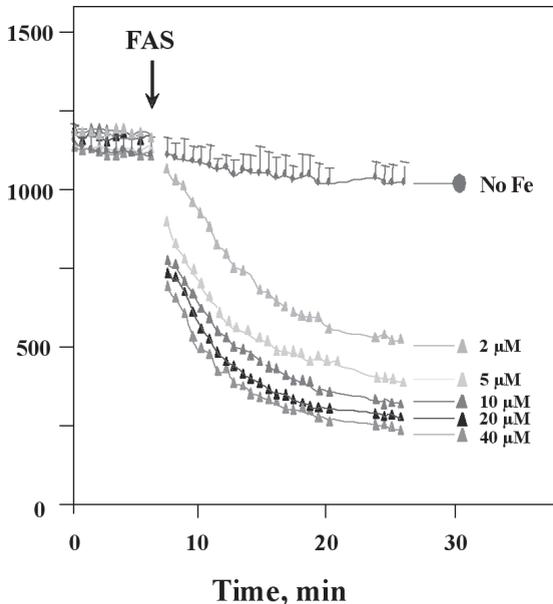


Figure 1. Changes in mitochondrial iron in response to changes in extracellular iron. HepG2 cells grown in microplate wells were incubated with RPA and fluorescence was followed in a microplate fluorescence reader after the addition of 0, 2, 5, 10, 20 or 40 μM ferrous ammonium sulfate (FAS) to the culture medium. Entry of Fe was fast and apparently saturable.

We determined levels of mitochondrial iron in long-term cultures of HepG2 cells. RPA fluorescence in cells grown for 5 days in iron-deficient medium was stronger than fluorescence in cells grown in control (5 μM Fe) medium, whereas cells cultured in 20 μM Fe presented the lowest RPA fluorescence values. No further significant decreases in RPA fluorescence were found in the 20-100 μM Fe range (data not shown). These results suggest that mitochondrial iron levels reflect the iron supply to the cells. We have seen similar relationships between extracellular iron and cytosolic LIP determined by calcein quenching (Núñez et al., 2001).

We then measured the generation of reactive oxygen species (ROS) associated with Fe uptake, by incubating cells previously exposed for 20 min to 5, 10 and 20 μM FAS with dihydrodichlorofluorescein diacetate (DHDCF-DA) and recording the appearance of green fluorescence due to oxidation of the probe (Núñez et al., 2001). The rate of ROS production increased by 42, 48 and 90% of the control value with the addition of 5, 10 and 20 μM FAS. Similarly, compared to control medium containing 5 μM Fe, cells grown for 5 days in 10 μM Fe presented increased levels of ROS, whereas no further increases in ROS were found in cells grown in the 10-100 μM Fe range. Similarly, no loss in cell viability was found with up to 100 μM Fe in the culture medium. Thus, HepG2 cells, unlike neuronal cells (Aguirre et al., 2005a, 2005b), have very effective mechanisms to control iron-induced oxidative stress.

In summary, the results presented here show that extracellular iron is readily incorporated into mitochondria, in an apparently saturable process. Moreover, the rate of iron incorporation responds to the Fe status of the cell, an indication that the mitochondria actively regulate their iron content. Although iron uptake was accompanied by ROS production, HepG2 cells effectively regulated ROS generation. These results suggest that HepG2 cells tightly regulate their mitochondrial iron levels.

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