(GNMT). GNMT is an abundant enzyme in liver that catalyzes the methylation of glycine by using S-adenosylmethionine (AdoMet). GNMT KO mice spontaneously develop steatohepatitis and fibrosis. It has been shown that eight months old MAT1A KO mice developed spontaneous NASH and the majority of them developed HCC at the age of eighteen months. Consequently, these KOs mice have been chosen as model for the study of the metabolic pathways implicated in the development of NASH.

Phosphorylation is a key regulation event in cell signalling and in consequence, in the function of biological systems. The use of phosphoprotein enrichment procedure is a method to simplify the proteome of KO and WT liver mice. Phosphoprotein enrichment was performed using Qiagen kit for phosphoprotein purification. The phosphoproteins were loaded into a 2D SDS-PAGE, visualized with Sypro Ruby and further analyzed by PDQuest software. In parallel, the phosphoproteins were digested by trypsin and titanium oxide and IMAC enrichment was performed on the tryptic peptides. Analysis of the phosphopeptides recovered for this second enrichment step at the peptide level was performed by LC-MS/MS using a nanoAcquity-UPLC system (Waters) coupled to a QToF Premier (Waters). The characterization of the proteins was carried out using Mascot Database Searching.

Differences in phosphorylation have been observed by Sypro Ruby staining of 2D gels for the phospho-proteomes of KOs and WTs mice. The biological analysis of these changes in phosphorylation levels of phosphoproteins between KO and WT liver homogenate mice it will provide valuable information about the role of phosphorylation in the development of the disease.

Major targets of iron-induced protein oxidative damage in frataxin-deficient yeasts are magnesium-binding proteins

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Introduction

Iron accumulation has been associated with several pathological conditions such as Friedreich ataxia (FRDA). This human disorder is caused by decreased expression of frataxin (Roy & Andrews, 2001). Oxidative stress due to iron-overload promoted selective damage to proteins. Such damage can be evaluated by analyzing protein- carbonyl content (Tamarit et al, 1998, Cabiscol et al 2002, Schacter et al 1994). In yeast cells lacking the frataxin ortholog *YFH1*, we have identified a set of 14 carbonylated proteins which include mitochondrial ATP synthase, phosphoglycerate kinase, pyruvate kinase and molecular chaperones. The fact that most of the target proteins are magnesium and/or nucleotide-binding proteins, leads us to postulate that when iron accumulates, it replaces magnesium at the corresponding metal-binding site, promoting selective damage to these proteins.

Materials and methods

- *Western blot Analysis and carbonyl content quantitation*.- Oxidative damage to proteins was evaluated by carbonyl-group derivatization with 2,4-dinitrophenyl hydrazine (DNPH). Antibodies against DNPH allow the immunodetection of this compound bound to proteins by classic western-blot techniques. Crude extracts were separated in one- or two-dimensional gels (Irazusta et al, 2006). In both cases, antibodies against DNPH (Dako) were used at 1:5,000 dilution. Images were acquired in a ChemiDoc XRS System (Bio-Rad) and analyzed with PDQuest or Quantity One software (Bio-Rad). Proteins were identified by peptide mass fingerprinting after tryptic digestion and MALDI-TOF analysis.

Results

- *Selective protein oxidation in Δyfh1 mutants*.- To analyze oxidative damage to specific proteins, crude extracts from wild type and Δ*yfh1* cells grown in YPG where prepared and analyzed by western blots from two-dimensional electrophoresis. Most of the carbonylated proteins in Δ*yfh1* were mitochondrial ones: Ssc1, Hsp78, Cta1, Atp1, Atp2 and Ilv5, highlighting the relevance of oxidative stress in this organelle in Δ*yfh1* cells. These results also showed that iron can promote selective oxidation of proteins containing ATP/magnesium binding sites. Pure preparations of such proteins incubated with iron/ascorbate as oxidant system showed increased carbonylation when ATP was present in the assay.

Conclusions

Our work uncovers a mechanism for understanding the specificity of protein oxidative damage. These results confirm that iron-induced oxidative damage plays a relevant role in FRDA disease and, as a consequence, it is conceivable that this would apply to all the pathologies in which iron accumulation is involved.

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