Iron Accumulated in Mitochondria of a YFH1 Mutant of the Yeast Saccharomyces cerevisiae Corresponds to Inorganic Ferric Phosphate

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The YFH1 gene is the yeast homologue of the human FRDA gene encoding a protein named frataxin. Mutations of the frataxin gene lead to a decreased frataxin expression causing Friedreich’s ataxia, the most common autosomal recessive neurodegenerative disease of Caucasians [1,2]. A defect in the yeast frataxin homologue leads to several S. cerevisiae phenotypes. Iron uptake is considerably higher compared to wild-type cells, with most of the iron being found in the mitochondria [3]. These cells exhibit defective respiration [3,5,7,8], unstable mitochondrial DNA and hypersensitivity to oxidative stress [3-5]. The assembly of Fe-S centers is impaired and the cells display drastically decreased cytochrome concentrations [4]. One major goal of our project was to uncover the role of yfh1 in mitochondrial iron metabolism of yeast and in particular, to identify the major mitochondrial iron components. For this purpose we analysed mitochondria of wild-type Saccharomyces and of a Δyfh1 mutant strain by means of in situ Mössbauer spectroscopy [9], EXAFS and biochemical methods.

Table 1: Mössbauer parameters of Δyfh1 mitochondria.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Δ [mm/s]</th>
<th>ΔEQ [mm/s]</th>
<th>Γ [mm/s]</th>
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<tbody>
<tr>
<td>4.3 K</td>
<td>0.53(4)</td>
<td>0.63(1)</td>
<td>0.57(1)</td>
</tr>
<tr>
<td>1.9 K(comp.1, 58%)</td>
<td>0.53(4)</td>
<td>0.64(1)</td>
<td>0.73(1)</td>
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No resonance absorption is detectable in Mössbauer spectra of mitochondria purified from a wild-type (YPH499) recorded at 4.3 K in a small perpendicular field of 20mT. This indicates an iron concentration therein below the detection limit of approx. 300µM. In contrast, the mitochondria of the Δyfh1 mutant displayed a well-resolved quadrupole doublet at 4.3 K (see Table 1) These Mössbauer parameters are typical of a high-spin ferric iron bound to oxygen/nitrogen in an octahedral arrangement. No ferrous iron was observed. Very similar parameters were found for various bacterioferritins at this temperature [10-12]. A Γ-value of 0.57 mm/s indicates a line-width broadening which can be associated with relaxation or superparamagnetic phenomena. Indeed, further broadening of the Mössbauer lines occurred at 1.9 K (Table 1). Moreover, the formation of a second unstructured component (42% of absorption area) was observed. In contrast to what was found in bacterioferritins, no indication for a distinct magnetic hyperfine field or a narrow ranged field distribution was visible. This and the features of a high field spectrum (7T, not shown) are neither consistent with a superparamagnetic transition as observed in bacterioferritins, nor with a magnetic transition of antiferromagnetically µ-oxo-coupled systems. The featureless broadening is best explained by a broad distribution of individual hyperfine fields originating from many magnetically non-equivalent ferric ions. Thus, our data are consistent with the presence of small and very amorphous nanoparticles of iron in Δyfh1 mitochondria.

Various attempts to visualize these particles on PAGE failed. The material remained in the pockets as seen by Fridovich-staining. There were only very little amounts - if any – of protein associated with these particles (0.1µg protein/µg iron), which could represent unspecific adsorption. Phosphate and iron determination resulted in a Fe/P ratio of 1/2.9(8).
EXAFS data gave best fits for the first shell with 6 oxygen atoms at a distance of 1.98(1) Å. (see Fig. 1). The second shell could be fitted either with two phosphorus and 3 iron atoms or with 4 phosphorus atoms. Taking into account the biochemical data and literature values the second shell environment of iron is explained best with phosphorus. We conclude, therefore, that iron is essentially present in Δyfh1 mitochondria of *Saccharomyces cerevisiae* as nano-particles of ferric phosphate. It is tempting to assume a very similar process in human mitochondria of individuals suffering from Friedreich’s ataxia.

**Figure 1**: EXAFS of Δyfh1 mitochondria.

**References**


