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Mössbauer studies of frataxin role in iron-sulfur cluster assembly and dysfunction-related disease

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Abstract Friedreich ataxia is a disease that is associated with defects in the gene coding for a small protein frataxin. Several different roles have been proposed for the protein, including iron chaperoning and iron storage. Mössbauer spectroscopy was used to probe these hypotheses. Iron accumulation in mutant mitochondria unable to assemble iron sulfur clusters proved to be insensitive to overexpression of frataxin, ruling out its potential involvement as an iron storage protein similar to ferritin. Rather, it was found that frataxin negatively regulates iron sulfur cluster assembly.

Keywords Mössbauer spectroscopy · Frataxin · Iron sulfur cluster · Iron accumulation

1 Introduction

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disease with an occurrence of 1 in 40,000 in the caucasian population. This inherited disorder was

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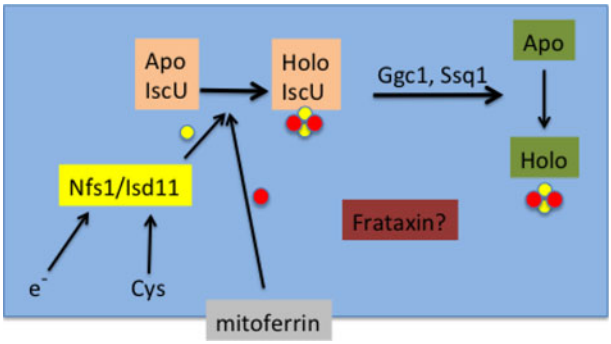

Fig. 1 Partner proteins involved in iron sulfur cluster biogenesis. Iron (●) is imported through mitoferrin, sulfide (●) from cysteine by Nfs1/Isd11

identified in 1843 by Friedreich. The most frequent symptom and disease cause of FRDA is hypertrophic cardiomyopathy that develops ca 25 years after onset of the disease, which occurs generally around age 14 [1]. Genetic studies in the mid-1990s have shown that the disease is associated with a deficiency in a specific protein that was coined frataxin (FXN). FXN is a small (14 kDa) mitochondrial protein highly conserved from bacteria (CyaY) to yeast (Yfh1) to humans (FXN). Its deficiency is associated with abnormalities of iron metabolism: decreased iron–sulfur cluster (ISC) biogenesis, accumulation of iron in mitochondria and depletion in the cytosol, enhanced cellular iron uptake, and impaired heme biosynthesis. It was recently shown that ISC biogenesis is the first defect, while iron accumulation occurs in a later stage of the disease [2]. Figure 1 illustratres the various processes and proteins involved and the proposed potential roles of FXN.

Several structures of FXN of prokaryote as well as eukaryote origins are available both in crystalline and in solution states [3]. They reveal that FXN comprises a β sheet and two α helices, and that one helix possesses a patch of negatively charged aspartate/gutamate residues, possibly involved in iron binding. Indeed it was shown that in excess of iron and oxidative conditions FXN oligomerizes and binds a high number of iron atoms that assemble to form an aggregate, a behavior reminiscent of that of the iron storage protein ferritin, which led some authors to propose that FXN is an iron storage protein involved in mitochondria protection against oxidative stress [4].

Mössbauer spectroscopy, which evidences iron whatever its chemical form and its spin state, is perfectly suited to trace it in cells, and studies of iron distribution in organelles have recently been developed as a tool to follow iron metabolism in native or stress conditions [5]. Mössbauer has also been extensively used to characterize ISC clusters in proteins and enzymes [6] and recently in the scaffold proteins involved in ISC biogenesis [7]. We took advantage of these available background informations to investigate two possible roles of FXN as (i) an iron storage protein or (ii) a regulator of ISC biogenesis.

2 Experimental section

^{57}Fe Mössbauer experiments were operated as already described [8, 9]. Analysis of the data was performed with the program WMOSS (WEB Research, Edina, MN).

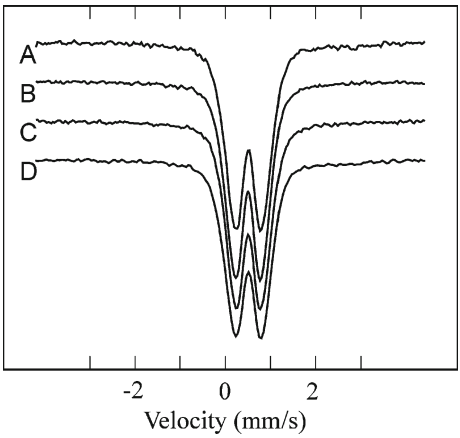

Fig. 2 Mössbauer spectra of mitochondria from different yeast strains: Δyfh1 **a**, Δssq1 **b**, Δggc1 **c**, Δggc1-YFH1 **d**. Spectra recorded at 78 K in zero applied magnetic field

Samples of mitochondria strains were prepared as described [10]. Sample preparation for kinetic analysis through Mössbauer spectroscopy was done under strict anaerobic conditions (within an inert atmosphere box with an oxygen content <2 ppm) by mixing the scaffold protein IscU, the desulfurase IscS, the reducing agent DTT, a $^{57}Fe^{2+}$ salt and cysteine in the ratio 1 mM/10 μM/5 mM/1.2 mM/5 mM. Two parallel experiments were performed, one in the absence and one in the presence of 50 mM of bacterial frataxin CyaY. The reaction was initiated by addition of cysteine and the samples were frozen at different times: 0, 5, 15, 30, 60 or 120 min. The expriments were run in triplicate with overlapping time domains to assess the reproducibility of the observations. In addition, control experiments were performed on mixtures lacking a component, and no Fe-S cluster formation was observed in any of these controls. All samples and controls for Mössbauer analyses were frozen in liquid nitrogen within the box and kept at that temperature until measurement.

3 Results

3.1 Iron accumulation in mitochodria

Iron accumulation is a regular symptom observed in FRDA patients. It is associated with a defect in frataxin and can be observed in mutant strains in which the protein is lacking (Δyfh1 strain). However it can be observed also in strains that possess FXN and assemble ISC but lack one of the ISC transporter chaperones (Δssq1 and Δggc1 strains), and are therefore unable to transfer ISC to their target proteins. Previous Mössbauer studies of yeast mitochodria Δyfh1 strain [10] had identified the aggregate form as polydisperse nanoparticles of iron phosphate. To assess whether iron accumulates always in the same chemical form we compared Δyfh1 to Δssq1 and Δggc1 strains. In addition, we studied Δggc1-YFH1, a particular strain lacking Ggc1 but overexpressing FXN, to evaluate if FXN can act as an iron storage protein.

Figure 2 illustrates the Mössbauer spectra of the strains under consideration in the present study. Consistent with previous observations [10], the 4.2 K Mössbauer spectrum of Δyfh1 mitochondria (Fig. 1a) displayed one slightly asymmetric quadrupole

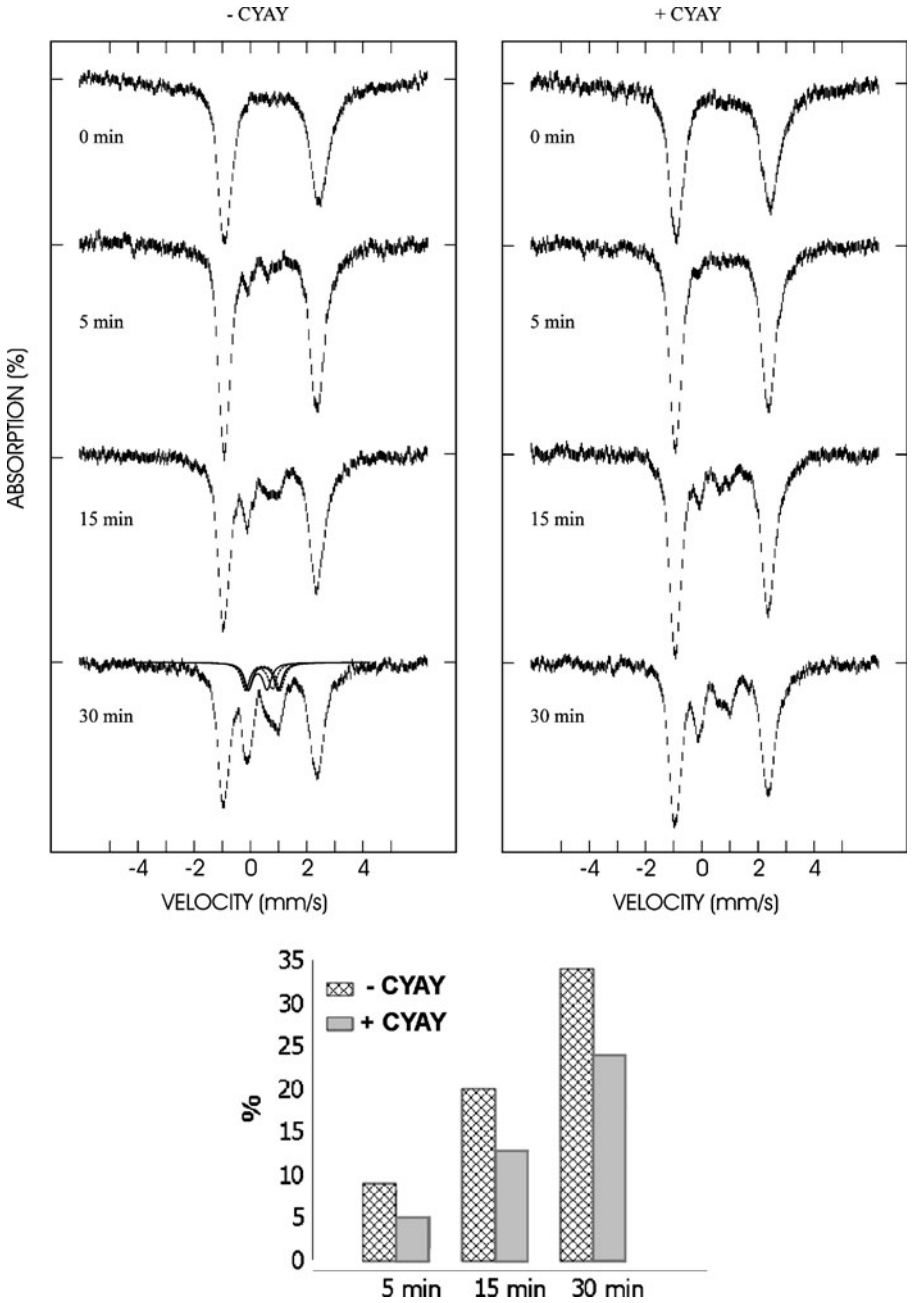

Fig. 3 (*top*) Monitoring of the FeS cluster assembly reaction through Mössbauer spectroscopy. The spectra were recorded in the absence (*left panel*) or in the presence (*right panel*) of CyaY. The spectra were recorded at 4.2 K and with a magnetic field of 60 mT applied parallel to the γ rays. The deconvolution into the two FeS clusters is shown on the spectrum recorded in absence of CyaY at 30 min. (*bottom*) Time dependence of the total cluster formation in absence and presence of CyaY

doublet with $\delta = 0.52(1)$ mm/s, $\Delta EQ = 0.63(2)$ mm/s and $\Gamma = 0.52/0.50(2)$ mm/s, typical of a high-spin ferric iron bound to oxygen/nitrogen in an octahedral arrangement. Spectra recorded in applied magnetic fields up to 7 T reflected a distribution of hyperfine fields, similar to those observed by Lindahl et al. in their study of mitochondria from Yah1p- and Atm1p-depleted cells [10]. The spectra of the strains Δssq1 and Δggc1 (Fig. 2b and c) are identical to that of Δyfh1 mitochondria indicating that iron aggregates are the same whatever the process leading to the aggregation. It is of interest that these aggregates differ strongly from those formed in the well known iron storage protein ferritin [11, 12]. Comparison of the two strains Δggc1 and Δggc1-YFH1 (Fig. 2c and d) again reveals no difference in the aggregates. This is an important observation with respect to the potential role of FXN as an iron storage protein. Indeed, its overexpression in conditions of potential iron availability does not bring any noticeable change in the iron distribution and thus does not support this role.

3.2 Iron sulfur cluster biogenesis

Very recent results have evidenced that ISC formation occurs within a complex involving the scaffold protein IscU, the cysteine desulfurase assembly IscS and Isd11 and FXN [2]. Biochemical and optical studies of ISC formation in a bacterial system have suggested that FXN could be a negative regulator of ISC synthesis [13] but an inverse effect was reported in a eukaryot [14].

In order to verify the effect of FXN on bacterial ISC formation and in particular its molecular aspects we used Mössbauer spectroscopy to monitor the respective formation of the [2Fe2S] and [4Fe4S] clusters upon cysteine addition to a mixture of a $^{57}Fe^{2+}$ salt, dithiothreitol, the scaffold protein IscU and the cysteine desulfurase IscS in the presence and in the absence of bacterial frataxin CyaY. The reaction mixture was prepared anaerobically and frozen at fixed time points after cysteine addition. Figure 3 top illustrates the respective Mössbauer spectra recorded at four reaction times: 0, 5, 15 and 30 min. The initial spectra (t = 0 min) consist of a broad doublet assigned to a tetrahedral ferrous thiolate. As the reaction proceeds, new components appear and grow in, which correspond to IscU bound $[2Fe2S]^{2+}$ and $[4Fe4S]^{2+}$ clusters [7], as shown by the deconvolution of the spectrum recorded after 30 min in absence of CyaY. Comparison of the spectra in absence vs presence of CyaY reveals two interesting features: (i) a higher amount of clusters is formed in absence than in presence of CyaY (Fig. 3 bottom), as previously reported [13], and (ii) the presence of CyaY does not bring any significant difference in the respective proportions of the two clusters. These observations thus (i) confirm that bacterial frataxin CyaY down-regulates ISC synthesis, and (ii) indicate that this regulation is a global effect and does not affect the reductive transformation of the $[2Fe2S]^{2+}$ clusters into $[4Fe4S]^{2+}$.

4 Conclusion

These studies illustrate the high potential of Mössbauer spectroscopy to monitor the transformation of iron species during biological processes. More specifically, in the present case, they support the potential role of frataxin as a regulator of

ISC biogenesis and disfavor its involvement as an iron storage protein. Work is in progress to precise the molecular aspects of frataxin regulatory activity.

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