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Mössbauer spectroscopy of the chloroplast-targeted DnaJ-like proteins CDJ3 and CDJ4

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Abstract The heat shock protein 70 (HSP70) in the chloroplast of *Chlamydomonas reinhardtii*, termed HSP70B, interacts with chloroplast-targeted DnaJ-like proteins (CDJs). In this work we focus on two CDJ co-chaperones (CDJ3 and CDJ4) of HSP70B which contain a redox-active Fe-S cluster (Dorn et al. Biochem. J. **427**, 205 2010). We have performed Mössbauer spectroscopy on ^{57}Fe enriched CDJ3 an) CDJ4. Our results indicate that both proteins have unusual $[4Fe4S]^{2+}$ clusters showing structural inhomogeneity of the two $[Fe^{2.5+}\text{-}Fe^{2.5+}]$ pairs. The spectra have been analyzed by means of two components with δ-values characteristic for $Fe^{2.5+}$ centers, but the differences in ΔE_Q indicate variations in their tetrahedral coordination spheres.

Keywords Iron sulfur cluster · Mössbauer spectroscopy · Chloroplast chaperones · Chloroplast DnaJ-like proteins

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1 Introduction

Photosynthesis is one of the most important biological processes on earth. The conversion of light energy from the sun into the energy of chemical bonds (carbohydrates) occurs in chloroplasts. Knowledge about chloroplast proteostasis is important to understand these processes. Molecular chaperones (HSP70 proteins) are found in all living organisms, except for some archaea [1]. HSP70s participate in a large number of cellular functions like protein folding and refolding after stress or protein quality control [2, 3]. In this work we focus on the major HSP70 in the chloroplast of *Chlamydomonas reinhardtii*, termed HSP70B, which interacts with chloroplast-targeted DnaJ-like proteins termed CDJ1-6 [4]. CDJ3-5 contain Fe-S clusters that are located between a N-terminal J domain and a C-terminal domain [1]. The exact function of these proteins has not yet been elucidated to date. In order to obtain a better structural and functional characterization of the Fe-S clusters of CDJ3 and CDJ4, we present here a field-dependent Mössbauer spectroscopic study.

2 Materials and methods

Recombinant CDJ3 and CDJ4 proteins were expressed in *E. coli* ER2566 cells carrying plasmids pMS336 and pMS337, respectively [1]. Cells were grown in minimal medium (M9) supplemented with ^{57}Fe to label the Fe-S clusters of CDJ3 and CDJ4. To remove undesired ^{56}Fe, all glassware and centrifugation tubes used were washed with 6 N HCl and rinsed thoroughly with deionized water. Moreover, the M9 salt solution was passed through a column packed with 10 g of Chelex 100 resin (Biorad) to remove polyvalent metal ions. ^{57}Fe was dissolved in a small volume of 12 N HCl and the acid was evaporated by exposing the solution to nitrogen gas. Resulting $FeCl_2 \bullet 4H_2O$ was added to the medium at a final concentration of 50 μ M. Bacteria were grown in 4 l M9 medium at 37 °C to an OD600 of 0.3 and IPTG was added to a final concentration of 0.7 mM. The expression of recombinant protein was allowed to proceed for 4 hours at 25 °C, then cells were collected by centrifugation at 5000 rpm for 5 min and pellets stored at -20 °C. Cell pellets were resuspended in 10 ml of ice-cold lysis buffer (20 mM HEPES-KOH pH 7.6, 0.5 M NaCl, 1 mM EDTA, 0.25× protease inhibitor cocktail (Roche), 0.1% Triton X-100) per l of medium and sonicated on ice. Lysed cells were centrifuged for 30 min at 13,000 rpm at 4 °C and the supernatant was applied twice at 4 °C to a column packed with 6 ml chitin beads (New England BioLabs). Subsequently the column was washed with 100 ml of lysis buffer, 10 ml of KMH-buffer (20 mM HEPES-KOH pH 7.6, 80 mM KCl, 2.5 mM $MgCl_2$, 5 mM ATP), and 20 ml lysis buffer lacking Triton. The column was then flushed with 10 ml cleavage buffer (20 mM Tris-HCl pH 9.0, 0.5 M NaCl, 1 mM EDTA, 50 mM DTT) and transferred to 25 °C for 16 h. Mature CDJ3 and CDJ4 proteins were eluted with 10 ml lysis buffer lacking Triton, dialysed against 3 l of 5 mM PBS, concentrated in Amicon Ultra-4 tubes (Millipore) and stored at -80 °C.

Mössbauer spectra were recorded in the horizontal transmission geometry using a constant acceleration spectrometer operated in conjunction with a 512-channel analyzer in the time-scale mode. The detector consisted of a proportional counter filled with argon-krypton-xenon. For measurements at 77 K, samples were placed in a continuous flow cryostat (OptistatDN, Oxford Instruments). Field-dependent conventional Mössbauer spectra at low temperatures were recorded with a closed-cycle cryostat from CRYO Industries of America, Inc. equipped with a superconducting magnet as described earlier [5]. Spectral data were transferred from the multi-channel analyzer to a PC for further analysis using the

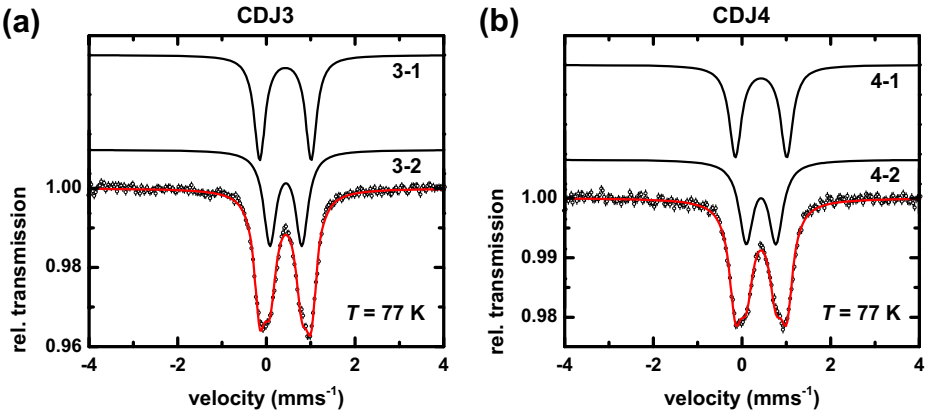

Fig. 1 Mössbauer spectra of CDJ3 (**a**) and CDJ4 (**b**) taken at $T = 77$ K. The black solid lines are the result of least-squares fits using Lorentzian line shapes. All resulting parameters are given in Table 1

Table 1 Mössbauer parameters obtained from the least-square fits using Lorentzian line shapes (black solid lines) shown in Fig. 1

	δ (mms^{-1})	ΔE_Q (mms^{-1})	Γ (mms^{-1})	Area (%)
CDJ3				
component 3-1	0.43 ± 0.02	1.16 ± 0.03	0.30 ± 0.02	50 ± 2
component 3-2	0.44 ± 0.02	0.72 ± 0.03	0.34 ± 0.02	50 ± 2
CDJ4				
component 4-1	0.43 ± 0.02	1.16 ± 0.04	0.33 ± 0.02	50 ± 2
component 4-2	0.43 ± 0.02	0.67 ± 0.04	0.38 ± 0.03	50 ± 2

public domain program Vinda running on an Excel 2003® platform [6]. The spectra were simulated on the basis of Lorentzian line shape or in case of magnetically-split spectra by the spin Hamiltonian approximation [7]. Isomer shifts are given relative to α-iron at room temperature. All values are rounded to the last given digit.

3 Results and discussion

Figure 1 shows the Mössbauer spectra of CDJ3 and CDJ4 taken at $T = 77$ K. Both spectra have been analyzed by means of two components with a spectral ratio of 1:1. Component 3-1 in Fig. 1a has an isomer shift of $\delta_{3-1} = 0.43$ mms^{-1} and a quadrupole splitting of $\Delta E_{Q3-1} = 1.16$ mms^{-1}. Component 3-2 shows $\delta_{3-2} = 0.44$ mms^{-1} and $\Delta E_{Q3-2} = 0.72$ mms^{-1}. The δ-values of both components are characteristic of tetrahedrally sulfur coordinated $Fe^{2.5+}$ centers of mixed-valence iron pairs with a delocalized excess electron, which is typical for $[4Fe4S]^{2+}$ clusters in iron sulfur proteins [8]. The differences in ΔE_Q of the components 3-1 and 3-2 indicate variations in their tetrahedral coordination spheres. The experimental data of CDJ4 are quite similar to those of CDJ3 (see Table 1). The isomer

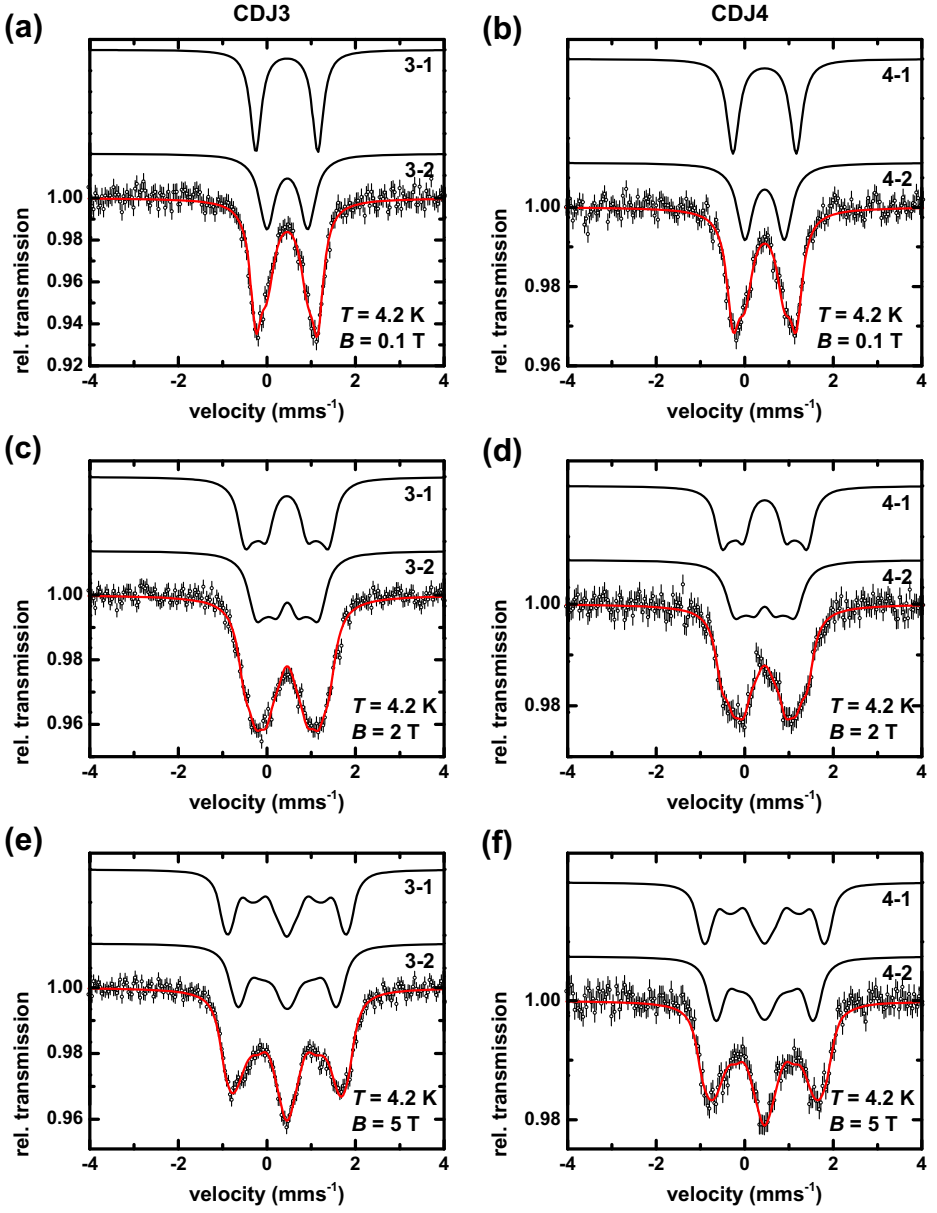

Fig. 2 Field dependent Mössbauer spectra taken at $T = 4.2$ K with an external magnetic field of 0.1 T applied parallel to the γ-beam of CDJ3 **(a)** and CDJ4 **(b)**, with $B = 2$ T for CDJ3 **(c)** and CDJ4 **(d)** and with $B = 5$ T for CDJ3 **(e)** and CDJ4 **(f)**. The solid lines in **(a)** and **(b)** are the result of least-squares fits using Lorentzian line shapes and in **(c) – (f)** of a spin-Hamilton analysis assuming a diamagnetic ground state. All resulting parameters are given in Table 2

shifts and quadrupole splittings of component 4-1 and 4-2 are equal within the experimental errors to component 3-1 and 3-2. Only the quadrupole splitting of component 4-2 differs ($\Delta E_{Q4-2} = 0.67$ mms^{-1}).

Table 2 Mössbauer parameters obtained from the least-squares fits using Lorentzian line shapes and spin-Hamilton analysis (black solid lines) shown in Fig. 2. All simulations shown in Fig. 2 have been analyzed with one consistent data set assuming a total spin of $S = 0$, except some changes in linewidths Γ given in the corresponding footnote

	δ (mms^{-1})	ΔE_Q(mms^{-1})	Γ* (mms^{-1})	η	Area (%)
CDJ3					
component 3-1	0.45 ± 0.02	1.40 ± 0.04	0.33 ± 0.02	1.0 ± 0.3	50 ± 2
component 3-2	0.46 ± 0.02	0.92 ± 0.04	0.37 ± 0.02	1.0 ± 0.4	50 ± 2
CDJ4					
component 4-1	0.45 ± 0.02	1.43 ± 0.04	0.30 ± 0.02	1.0 ± 0.3	50 ± 2
component 4-2	0.45 ± 0.02	0.89 ± 0.04	0.34 ± 0.02	1.0 ± 0.4	50 ± 2

*Γ at 0.1 T for comp. 3-1: 0.30 ± 0.02 mms^{-1}; for comp. 3-2: 0.42 ± 0.02 mms^{-1}; Γ at 2 T for comp. 3-1: 0.35 ± 0.04 mms^{-1}; for comp. 3-2: 0.40 ± 0.04 mms^{-1}; Γ at 0.1 T for comp. 4-1: 0.33 ± 0.02 mms^{-1}; for comp. 4-2: 0.42 ± 0.02 mms^{-1}; Γ at 2 T for comp. 4-1: 0.32 ± 0.02 mms^{-1}; for comp. 4-2: 0.42 ± 0.04 mms^{-1}

The Mössbauer spectra of CDJ3 and CDJ4 taken at $T = 4.2$ K and different magnetic fields at 0.1 T, 2 T and 5 T are shown in Fig. 2. The spectra exhibit for both proteins a symmetric doublet at $B = 0.1$ T (Fig. 2a and b) and at higher fields of 2 T and 5 T a magnetic splitting which is only due to the external magnetic field (Fig. 2c–f). This aspect leads to the conclusion that both, CDJ3 and CDJ4, have a diamagnetic ground state. At low temperatures Component 3-1 has been found to have the following parameters: $\delta_{3-1} = 0.45$ mms^{-1} and $\Delta E_{Q3-1} = 1.40$ mms^{-1}. Component 3-2 has $\delta_{3-2} = 0.46$ mms^{-1} and $\Delta E_{Q3-2} = 0.92$ mms^{-1}. Both components have been simulated with an asymmetry parameter $\eta = 1.0$. In this case the sign of the quadrupole splitting is not defined. It should be noted that simulations for component 3-2 with $\eta = 0.6$ also lead to an acceptable fit of the high-field data shown in Fig. 2c and e. In this case the quadrupole splitting would be negative ($\Delta E_{Q3-2} = -0.92$ mms^{-1}). On the basis of the data presented we cannot discriminate these two scenarios and have given the corresponding simulations with $\eta = 0.6$ in Fig. S1 of the Supporting Information.

The spectra for CDJ4 were fit assuming the same two species with very similar Mössbauer parameters as for CDJ3 at low temperatures. Only the quadrupole splittings of component 4-1 and 4-2 differs ($\Delta E_{Q4-1} = 1.43$ mms^{-1} and $\Delta EQ_{4-2} = 0.89$ mms^{-1}). All experimentally determined parameters at low temperatures and different magnetic fields are given in Table 2.

It should be mentioned that a second parameter set with $\delta_{3-1++} = 0.35$ mms^{-1}, $\Delta E_{Q3-1++} = 1.22$ mms^{-1} (50%) and $\delta_{3-2++} = 0.57$ mms^{-1}, $\Delta E_{Q3-2++} = 1.20$ mms^{-1} (50%) also fits the reported data (see Fig. S2 and Table S2). However, the values for $\delta_{3-1++}$and δ_{3-2++} correspond not to the isomer shift range observed for $Fe^{2.5+}$-$Fe^{2.5+}$ pairs in diamagnetic 4Fe-4S clusters which ranges to our experience from 0.42 – 0.46 mms^{-1}. Therefore, we consider the Mössbauer parameters given in Table 2 as reliable. We have also found a second parameter set for CDJ4 with similar values (see Supporting Information Fig. S3 and Table S3).

The quadrupole splitting of both components of CDJ3 and CDJ4 shows a significant temperature dependent increase (0.20 – 0.27 mms^{-1}). The same behavior was observed by C.E. Johnson and coworkers who have performed Mössbauer measurements at different temperatures on *C. pasteurianum* ferredoxin (2x $[4Fe4S]^{2+}$ clusters) and obtained an increase of

the quadrupole splitting of 0.17 mms^{-1} between $T = 77$ K and 4.2 K [9]. E. Münck and coworkers also report Mössbauer studies of a synthetic analogue for the 4Fe4S Cluster with an increase of ΔE_Q of 0.14 – 0.20 mms^{-1} between $T = 100$ K and 4.2 K [10]. They have pointed out that the orientation of the S-C_β-Cystein bond, which connects the iron sulfur cluster to the protein environment, influences the tetrahedral coordination sphere of the iron atom and is hence responsible for this behavior.

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