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# Characterization of the photolyase-like iron sulfur protein PhrB from *Agrobacterium tumefaciens* by Mössbauer spectroscopy

**T. O. Bauer · D. Graf · T. Lamparter · V. Schünemann**



**Abstract** High field Mössbauer spectroscopy has been used to characterize the $[4Fe\text{-}4S]^{2+}$ cluster of the protein PhrB from *Agrobacterium tumefaciens* which belongs to the cryptochrome/photolyase family (CPF) and which biological function has previously been shown to be DNA repair. Mössbauer spectra taken of the as prepared protein reveal $\delta = 0.42\ \text{mms}^{-1}$, and $\Delta E_Q = 1.26\ \text{mms}^{-1}$ as well as an asymmetry parameter of $\eta = 0.8$. These parameters are characteristic for a ferredoxin-type $[4Fe\text{-}4S]^{2+}$ cluster. In order to investigate whether this cluster is involved in DNA-repair the protein has also been studied in its photoactivated state during DNA binding. The so obtained data sets exhibit essentially the same Mössbauer parameters as those of the non-activated PhrB. This indicates that during DNA repair the $[4Fe\text{-}4S]^{2+}$ cluster of PhrB has no significant amounts of transition states which have conformational changes compared to the resting state of the protein and which have life times of several seconds or longer.

**Keywords** Iron proteins · DNA repair enzymes · Iron sulfur centers

## 1 Introduction

A DNA photolyase is a DNA repair enzyme of 450–550 amino acids and possesses two non-covalently bond chromophore cofactors [1]. One of the cofactors is always

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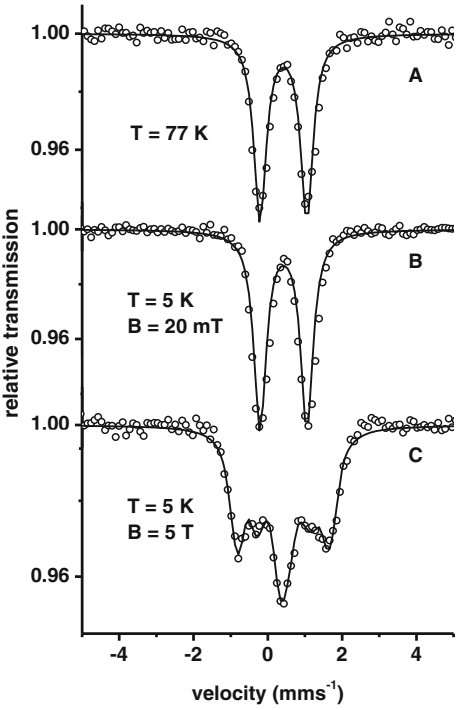


**Fig. 1** Mössbauer spectra of as prepared PhrB (**1**) taken at (**A**) T = 77 K, (**B**) T = 5 K with an external field of 20 mT and (**C**) T = 5 K with an external magnetic field of 5 T. The magnetic fields were applied perpendicular to the $\gamma$-beam. The *solid lines* are simulations with the parameters given in the text

FAD (flavin adenine dinucleotide) as a primary catalytic cofactor, which is photoreducable by blue light [2] and the second one is either MTHF (methenyltetrahydrofolate), 8-HDF (8-hydroy-7,8-didemethyl-5-deazari-boavin) [3] FAD, FMN or 6,7 dimethyl 8 ribityl-lumazin (DMRL). The second chromophore acts always as antenna to maximize light capture [2]. PhrB represents the first prokaryotic (6–4) photolyase and the first member of the cryptochrome/photolyase family (CPF) that contains an iron-sulfur $[4Fe\text{-}4S]^{2+}$ cluster [2] and DMRL as antenna chromophore [4]. Here we report the first Mössbauer spectroscopic study of this protein both in the non-photoactivated and in its photoactivated state.

## 2 Materials and methods

PhrB was expressed in *E. coli ER2556* as described in [4]. The *E. coli* cells were grown in iron-free medium and $^{57}FeCl_2\ 4H_2O$ was added up to a final concentration of 50 μM. The protein purification was performed according to the procedure described in [4]. The final concentration of the protein solution before transfer to the Mössbauer sample holder was 1.0 mM (sample **1**). The interaction of as prepared PhrB with DNA was studied by adding single stranded DNA ("t-repair1", AGGTTGGC) in equimolar amounts for 20 min and subsequent freezing using dry ice (sample **2**). For studying the interaction of PhrB with damaged DNA a further sample was prepared by adding equimolar amounts of 6–4 photodamaged t-repair1 oligonucleotides (after HPLC purification [4]) to protein which was photoreduced in the presence of 10 mM DTT using blue light ($\lambda$ = 470 nm,

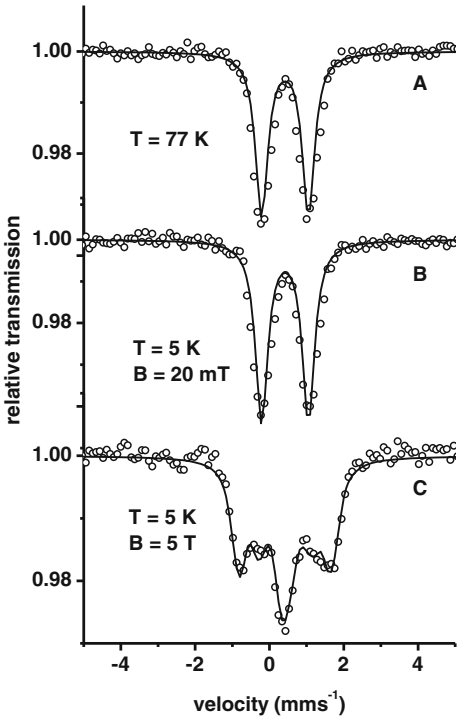


**Fig. 2** Mössbauer spectra of PhrB plus DNA **(2)** taken at (**A**) T = 77 K, (**B**) T = 5 K with an external field of 20 mT and (**C**) T = 5 K with an external magnetic field of 5 T. The magnetic fields were applied perpendicular to the $\gamma$-beam. The solid lines are simulations with the same parameters used in Fig. 1 and are given in the text

55 μmol $s^{-1}$ $m^{-2}$). After mixing, the sample was again irradiated with blue light ($\lambda$ = 470 nm, 55 μmol $s^{-1}$ $m^{-2}$) for 10 min to induce photorepair. The sample was then frozen with dry ice and further stored in liquid nitrogen (sample **3**).

Mössbauer spectra were recorded using a spectrometer in the constant-acceleration mode and a closed cycle cryostat equipped with a superconducting magnet as described earlier [5]. All spectrometer parts were purchased from WissEl GmbH. The Mössbauer drive MA260 was connected to the corresponding drive unit MR 260A. A laser sealed source ($^{57}$Co in Rh matrix, manufactured by Cyclotron Co. Ltd. with an activity of ∼20 mC was used. Detection of the transmitted radiation was performed with a proportional counter also from WissEL GmbH (LND-45431). The counter signal was amplified by a PEA 6A and stored by means of a multi channel analyzer module (CMCA-550). As a function generator served a DFG 1000. The spectrometer was run in constant acceleration mode with 512 channels and a maximum velocity of ∼12 $mms^{-1}$. This was done in order to check for unspecifically bound $^{57}$Fe the spectral signature of which often occurs as a very broad magnetic feature in the background. Velocity calibration was performed with an $\alpha$-Fe foil at room temperature. Folding of the experimental data and spectral analysis was performed with the VINDA Add On for Excel 2003 [6]. Experimental errors with respect to the reported isomer shifts and quadrupole splittings are ∼ ±0.03 $mms^{-1}$. Isomer shifts are given relative to $\alpha$-Fe at room temperature. The spectra were analyzed by least-square fits using Lorentzian line shapes. Mössbauer spectra obtained under high magnetic fields were simulated by means of the spin Hamiltonian Formalism which enables full diagonalization of the nuclear Hamiltonian and subsequent powder averaging [7].

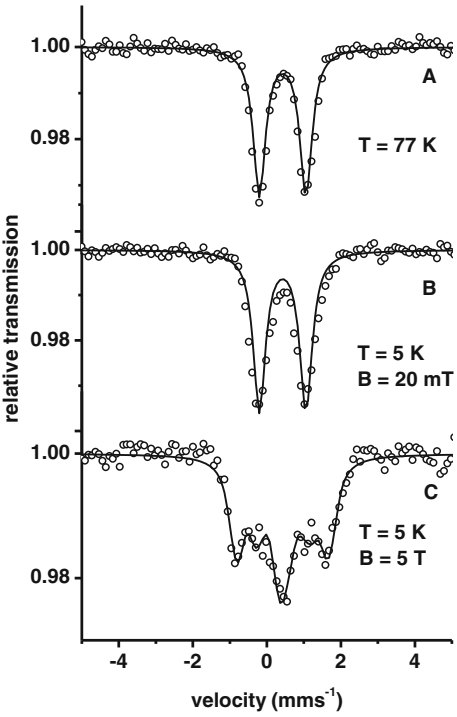


**Fig. 3** Mössbauer spectra of PhrB quenched during DNA repair **(3)** taken at (**A**) T = 77 K, (**B**) T = 5 K,with an external field of 20 mT and (**C**) T = 5 K with an external magnetic field of 5 T. The magnetic field was applied perpendicular to the $\gamma$-beam. The *solid lines* are simulations with the same parameters used in Fig. 1 and are given in the text

## 3 Results and discussion

Figure 1A shows the Mössbauer spectrum of $^{57}$Fe-enriched PhrB taken at T = 77 K. The spectrum has been analyzed by a Lorentz doublet, which exhibits an isomer-shift of $\delta = 0.42$ mms$^{-1}$, a quadrupole splitting of $\Delta E_Q = 1.26$ mms$^{-1}$ and a line width of $\Gamma = 0.40$ mms$^{-1}$. Figure 1B shows the Mössbauer spectrum of PhrB taken at T = 5 K and an external magnetic field of B = 5 T perpendicular to the $\gamma$-beam. The solid line represents the result of a best fit spin Hamiltonian analysis assuming a diamagnetic S = 0 ground state and yields in addition to the parameters mentioned above an asymmetry parameter of $\eta = 0.8 \pm 0.2$. This parameter set is characteristic for a diamagnetic $[4Fe\text{-}4S]^{2+}$ cluster [7, 8]. Such a cluster possesses two mixed-valence iron pairs with two excess electrons each one delocalized over one pair ($Fe^{2.5+}$-$Fe^{2.5+}$). The spins of the two $Fe^{2.5+}$-$Fe^{2.5+}$ pairs $S_{12} = 9/2$ and $S_{34} = 9/2$ are antiparallel coupled, yielding a total cluster spin $S_{tot} = 0$ [7] consistent with the diamagnetic Mössbauer signature shown in Fig. 1C.

In order to check whether photoactivation of PhrB is accompanied by a conformational change of its 4Fe-4S center Mössbauer spectra of PhrB have been collected for samples which were quenched in the Mössbauer sample holder during the reaction with intact DNA (**2**) and damaged DNA after irradiating the protein solution with blue light (**3**).

The so obtained data sets are displayed in Figs. 2 and 3 and they exhibit exactly the same Mössbauer parameters as those of the as prepared PhrB (1) (see above and Fig. 1). Thus both, upon DNA binding and during DNA repair, the 4Fe-4S center of PhrB displays no significant amounts (> 5 %) of transition states which have conformational changes

compared to the resting state of the protein and which have life times of several seconds or longer. This time scale is determined by the protein freezing process prior Mössbauer spectroscopic measurements in our experiments.

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