Spin states of non-heme iron and protein local motions in reaction centers from purple photosynthetic bacterium *Rhodospirillum rubrum* modified by Cd^{2+}

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INTRODUCTION

We show that NHFe in RC isolated from *Rhodospirillum* (*Rs.*) *rubrum* occurs almost exclusively in a low spin state, while in RC from *Rhodobacter sphaeroides* it was observed in a high spin state [1]. These intriguing observations prompted us to investigate in detail NHFe in *Rs. rubrum*, in particular, how its low spin state is affected by fluctuations of protein matrix and by Cd2+ ions, which are known to bind in the vicinity of the iron-quinone complex [2, 3]. To this end, Mössbauer spectroscopy was applied to compare the valence and spin states of Fe atoms in native RC from *Rs. rubrum* and in RC treated with Cd2+ salt. Further, nuclear inelastic scattering (NIS) was used to monitor the collective motions in the NHFe-binding sites in both the native and the Cd2+-treated RCs.

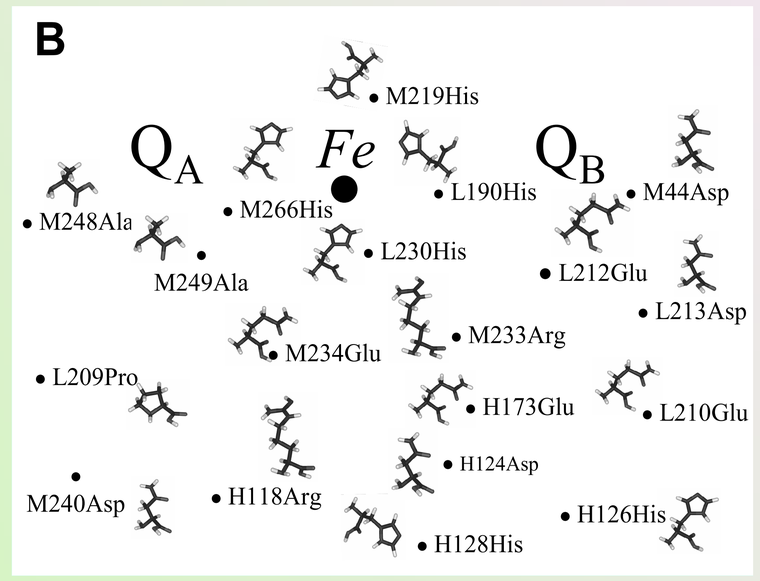

Figure 1. ***The arrangement of aminoacids from the proteins: M, L and H in the vicinity of the NHFe binding site for Rs. rubrum [4]. The aminoacids of the H protein are taken from Rb. sphaeroides.***

METHODS

Appliying *Mössbauer spectroscopy* , we studied valence and spin states of the iron atoms. The Mössbauer 57Fe spectra were recorded in a home made cryostat using 50 mCi 57Co/Rh as a source of 14.4 keV g radiation and a proportional counter to detect the radiation. The temperature stabilization was within 0.1 K. The isomer shifts are given vs. metallic Fe at room temperature. The recorded spectra were fitted using a Recoil program.

Nuclear inelastic scattering of synchrotron radiation experiments were performed at the Nuclear Resonance beamline ID18 at the European Synchrotron Radiation Facility in Grenoble, France. The storage ring was run in hybrid mode, providing 24 groups of 8 radiation pulses with the period of 88 ns. X rays were monochromatized to the energy bandwidth of 0.5 meV. The energy of incident radiation was tuned around the 14.4 keV energy of 57Fe nuclear transition within the range from -40 meV to 100 meV for 60 K and from -80 meV to 100 meV for 240 K.

RESULTS

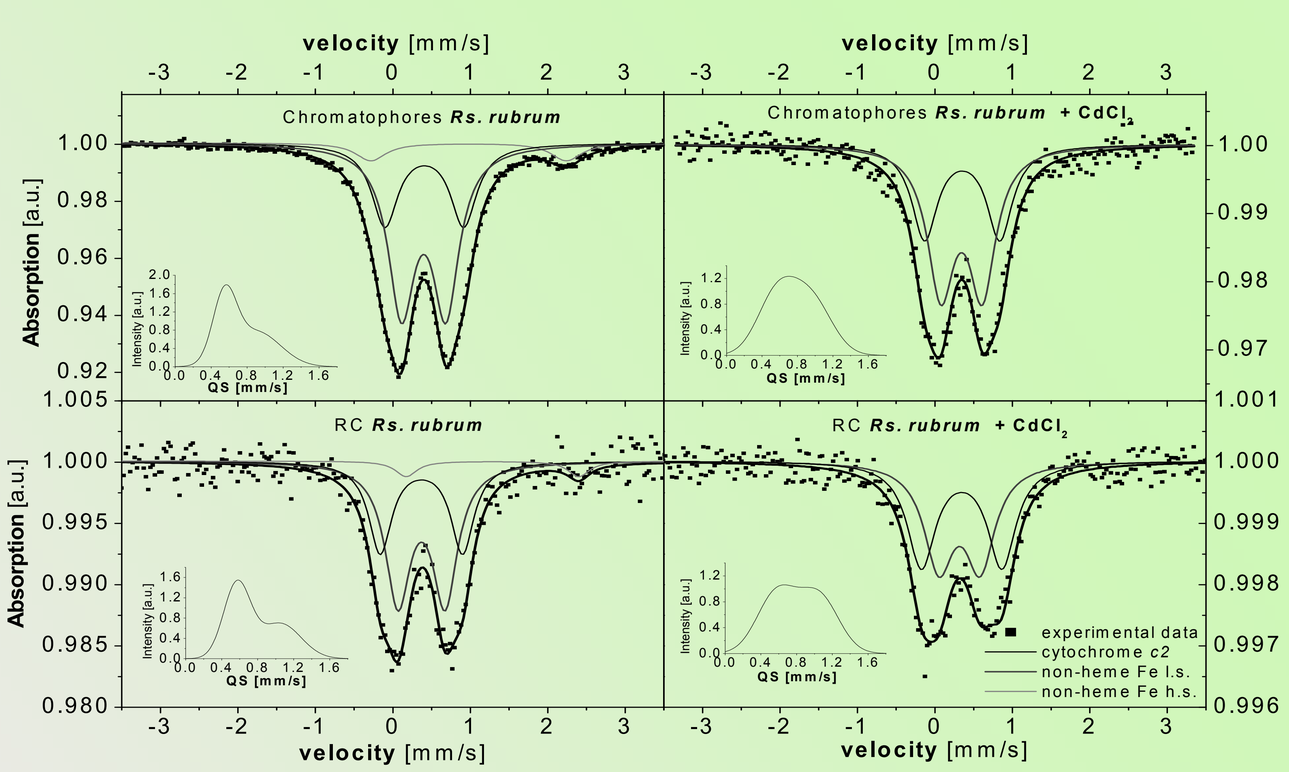

Figure 2. *The ^{57}Fe Mössbauer spectra of chromatophores and reaction centers isolated from Rs. rubrum, left panel - control and right panel - treated with $CdCl_2$. The spectra were measured at 85 K. The lines represent fits assuming symmetrical doublets. Subspectra correspond to NHFe and HFe in cytochrome c_2 (marked in the figure). From the distributions of the quadrupole splitting (QS) (see insets) we found that the central doublet detected for chromatophores and RCs can be approximated by two doublets with the line-width of 0.21 ± 0.02 mm/s and 0.19 ± 0.02 mm/s, respectively.*

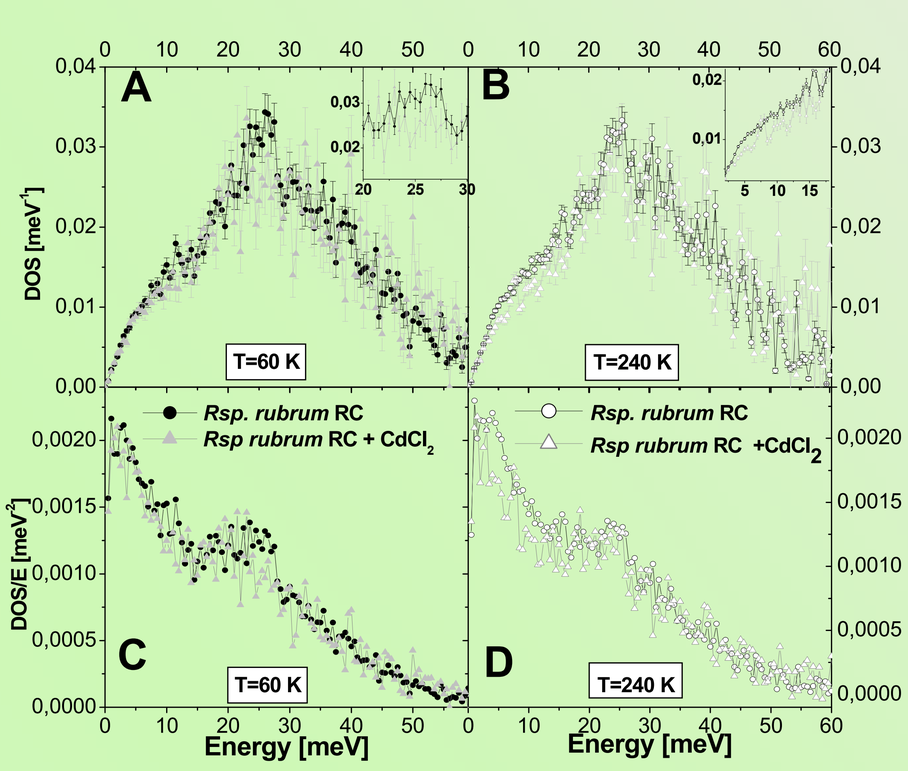

Figure 3. *The iron-partial density of vibrational states, DOS, of the native and treated with $CdCl_2$ RCs isolated from Rs. rubrum measured at 60 K (A) and at 240 K (B). The reduced DOS of the native and treated with $CdCl_2$ RCs measured at 60 K (C) and at 240 K (D). The error bars along the horizontal line (A and B) evaluate the statistical reliability of the data. The insets show the mentioned in the text differences in the DOS spectra. The way of evaluation of the data is presented in [1, 5]*

CONCLUSIONS

-Exposure of RCs to Cd^{2+} ions causes transfer of either the high or low spin state into a new low spin state. This indicates that Cd^{2+} binds in the vicinity of the iron-quinone complex and it does not remove NHFe from this complex (at least at the applied concentrations).

-The NIS experiments shows that Cd^{2+} ions bound in the vicinity of the quinone-Fe complex influence the low energy protein fluctuations causing their damping, especially at 240 K.

-Because electron transfer (ET) between the two acceptor quinones Q_A and Q_B is down regulated by Cd^{2+} and is initiated only at temperatures above 200 K, we suggest that the collective fluctuations damped by cadmium cations at about 3-16 meV are responsible for a proper activation of the coupling between Q_A and Q_B and efficient ET. This means that a certain flexibility of the RC core is required for the efficient action of the Q^- type photosystem. This process can additionally be accompanied/regulated by protonation and deprotonation of residues located near NHFe as well as near the Q_A and Q_B ubiquinones binding sites (see Fig. 1). This, in turn, may not only affect the strength of coordination bonds to NHFe but also regulate its redox potential.

References

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ACKNOWLEDGEMENTS:

This work was supported partially by grant No N N302 195035 (2008-2011) from Polish Ministry of Science and Higher Education. The groups cooperate within the BIONAN project. Project operated within the Foundation for Polish Science MPD Programme co-financed by the EU European Regional Development Fund. (A.H)