DIAMAGNETIC STATE OF NON-HEME IRON IN BACTERIAL REACTION CENTERS TREATED WITH Cu^{2+}

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INTRODUCTION

The non-heme iron (NHFe) is a very conservative component of type II reaction centers (RCs) but its role in photosynthetic charge separation and the temperature activation of electron transfer (ET) between the two terminal quinone acceptors remains one of the most challenging issues [1, 2]. Here, we present our studies of the influence of copper ions, Cu^{2+}, on the NHFe binding site in the bacterial RCs isolated from the purple bacterium *Rhodobacter (Rb.) sphaeroides*. Previously, it has been found that Cu^{2+} preserves the low spin ferrous state of this iron center in PSII BBY from a *Chlamydomonas reinhardtii* mutant deficient in photosystem I and that it enhances the covalence of bonds to NHFe. [3, 4]. Recently, we have observed that NHFe in the bacterial RCs, which contained contaminations of cytochrome c2, exist in two forms, in the high and low spin ferrous states [5], and that interactions with Cu2+ ions cause its transition to a diamagnetic state (unpublished data). In this investigations we applied Mössbauer spectroscopy to monitor the local electronic and structural properties of NHFe in highly purified bRCs and to gain direct information about its diamagnetic states, unavailable by other methods.

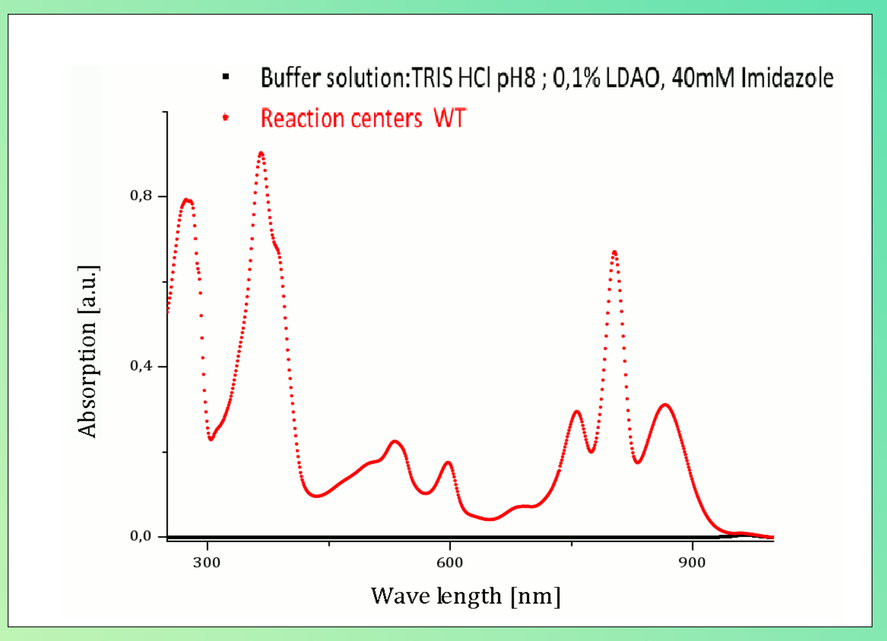

Figure 1. The absorption spectrum of WT reaction centers isolated from Rb. sphaeroides.

METHODS

Rb. sphaeroides cells were grown in a malate-yeast medium supplemented with kanamycin (20µg/mL) and tetracyclin (1,25µg/mL). The medium was enriched with an iron isotope, ^{57}Fe, a Mössbauer probe. The cultures were grown in darkness at 30°C. Wild-type bRCs, containing ^{57}Fe at the acceptor side, were purified from *Rb. sphaeroides* and concentrated using (Vivaspin, MWCO 30 kDa) according to [6, 7].
A sample of bRC (0.3 mM) was incubated 15 min at room temperature with a 800-fold molar excess of CuCl2 under illumination with white light and continuous stirring. Then the unbound Cu^{2+} ions were removed by rinsing the sample several times with the suspension buffer (free of Cu^{2+}), containing EDTA in the first step. Each time, the bRC sample was stirred for 10 min in the buffer at ambient temperature and then pelleted by ultracentrifugation. This procedure was repeated 3 times before the sample was centrifuged and stored at -80°C. We estimated, using AAS, that the ratio of Fe : Cu was about 1 : 0.6 in the final preparation.

RESULTS

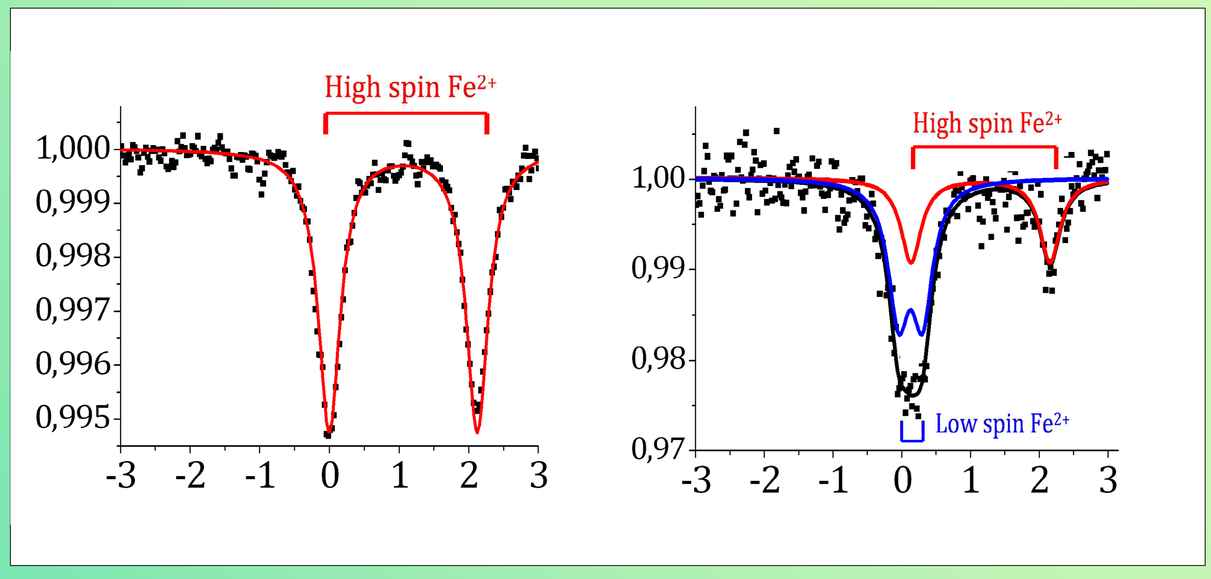

Figure 2. The ^{57}Fe Mössbauer spectra of reaction centers isolated from Rb.sphaeroides.

Left panel – control bRCs ; right panel – bRCs treated with CuCl2 at the concentration: 800 Cu2+ ions per RC. The spectra were measured at 85 .

The lines represent fits assuming symmetrical lorentzian doublets.

	IS high spin Fe	QS high spin Fe	IS low spin Fe	QS low spin Fe [mm/s]	C high spin	C low spin
WT	1,06 ±0,005	2,12 ±0,01	—	—	100%	
WT+Cu^{2+}	1,17± 0,02	1,95 ±0,05	0,13 ±0,02	0,38 ± 0.03	42%	58%

Table 1. Hyperfine parameters: Isomeric shift (IS), quadrupole splitting (QS) and contribution (C) for native reaction center and treated with copper.

CONCLUSION

- **Our Mössbauer experiments show that copper at low concentrations causes transition of the high spin ferrous state of the non-heme iron into the low spin one(diamagnetic). This suggests that Cu^{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 diamagnetic state of NHFe results in a magnetic decoupling of the metallic center from the primary quinone acceptor Q_A.**

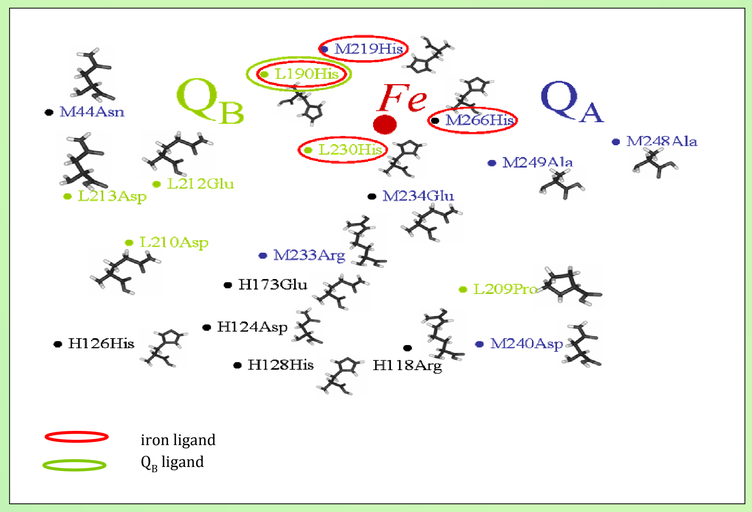

Figure 3. The arrangement of amino acids from the proteins: M, L and H in the vicinity of the NHFe binding site for Rb. sphaeroides.

REFERENCES

[1] D. Kleinfeld, M.Y. Okamura, G. Feher, Biochemistry 23 (1984) 5780-5786.
[2] K. Burda, Cell Biochem. Biophys. 47 (2007) 271-284.
[3] K. Burda, J. Kruk, J. Borgstadt, J. Stanek, K. Strzałka, G.H. Schmidt, O. Kruse, FEBS Lett. 535 (2003) 159-165.
[4] K. Burda, J. Kruk, J. Stanek, K. Strzałka, G.H. Schmidt, O. Kruse, Acta Phys. Polon. A 109 (2006) 237-247.
[5] A. Orzechowska et al. Biochimica et Biophysica Acta 1797 (2010) 1696–1704
[6] Gerenscer, L. Taly, A. Baciou, L.Maroti, P. & **P. Sebban (2002)** ***Biochemistry*** 41, 9132-8.
[7] Paddock wdvn ML, Graige MS, Feher G and Okamura MY 1999 *Proc. Natl. Acad. Sci. USA* 96 6183

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