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Chemical proprieties of the iron-quinone complex in mutated reaction centers of *Rb. sphaeroides*

Agnieszka Hałas · Valerie Derrien · Pierre Sebban ·
Krzysztof Matlak · Józef Korecki · Jerzy Kruk ·
Květoslava Burda

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Abstract We investigated type II bacterial photosynthetic reaction centers, which contain a quinone - iron complex (Q_A -Fe- Q_B) on their acceptor side. Under physiological conditions it was observed mainly in a reduced high spin state but its low spin ferrous states were also observed. Therefore, it was suggested that it might regulate the dynamical properties of the iron-quinone complex and the protonation and deprotonation events in its neighbourhood. In order to get insight into the molecular mechanism of the N_HFe low spin state formation, we performed Mössbauer studies of a wild type of *Rb. sphaeroides* and its two mutated forms. Our Mössbauer measurements show that the hydrophobicity of the Q_A binding site can be crucial for stabilization of the high spin ferrous state of N_HFe.

Keywords Photosynthetic reaction center · Non-heme iron

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A. Hałas · K. Burda

Faculty of Physics and Computer Science, Department of Medical Physics and Biophysics,
AGH University, Kraków, Poland

V. Derrien · P. Sebban

Laboratoire de Chimie Physique, University of Paris XI, Orsay, France

K. Matlak · J. Korecki

Faculty of Physics and Computer Science, Department of Solid State Physics, AGH University,
Kraków, Poland

J. Kruk

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University,
Kraków, Poland

K. Burda (✉)

Faculty of Physics and Computer Science, AGH University, al. Mickiewicza 30,
30-059 Kraków, Poland
e-mail: kvetoslava.burda@fis.agh.edu.pl

Abbreviations

Bacterial reaction center	BRC
<i>Rhodobacter sphaeroides</i>	<i>Rb. sphaeroides</i>
<i>Rhodospirillum rubrum</i>	<i>Rp. rubrum</i>
Quinone-iron complex	Q _A -Fe-Q _B
Non-heme iron	NHFe
Electron transfer	ET
Ubiquinone	UQ
Wild type	WT
Isomer shift	IS
Quadrupole splitting	QS

1 Introduction

The bacterial photosynthetic reaction center (BRC) is a membrane protein complex, which converts light energy into chemical free energy. BRC of *Rhodobacter sphaeroides* (*Rb.sphaeroides*), which belongs to purple bacteria, is considered as a model system to study the dynamical and structural proprieties of the photosynthetic reaction centers of type II. Each BRC is composed of three protein subunits L, M and H. The L and M proteins are connected on the acceptor side by the non-heme iron (NHFe) situated between the primary and secondary ubiquinone acceptor Q_A and Q_B, respectively. The ubiquinone is tightly bound to the Q_A site contrary to the one at the Q_B site. The Q_A ubiquinone, bound to the M subunit, is in a hydrophobic area whereas the Q_B ubiquinone, bound to the L subunit, is surrounded by charged and polar residues. The molecular mechanisms of the electron transfer between Q_A and Q_B ubiquinones have been a subject of challenging studies but the role of the NHFe, which is a very conservative component of RCs of the type II, in this process is still unknown [1, 2]. Especially the role of NHFe in stabilization of the Q_A and Q_B binding sites and in the primary electron transfer (ET) is not clear. A change of the NHFe valence state has never been detected in any system of type II [3]. But mixed spin states (low and high spin state) were observed in BRCs of *Rb. sphaeroides* and of *Rp. rubrum* [4, 5] and an exclusively low spin state in photosystem II of algae PSI⁻ mutant [6, 7]. We focused on studies of the NHFe valence and spin states in BRCs isolated from a wild type (WT) and two mutants of *Rb. sphaeroides* (see Fig. 1) [8, 9]. The double mutant, called later in this paper AA, has two point mutations near the Q_B acidic cluster: (L212Glu/L213Asp -> Ala/Ala). The triple mutant, called AATyr, has additionally a point mutation near the Q_A binding site (M249Ala->Tyr).

In our studies we applied Mössbauer spectroscopy to investigate the valence and spin state of the probing atom i.e. the isotope ⁵⁷Fe.

2 Materials and methods

The HIS tagged cells of *Rb.sphaeroides* (native and harbouring mutations) were grown in the medium deprived of natural iron and enriched with an iron isotope ⁵⁷Fe. The growing conditions and the BRC purification have previously been described

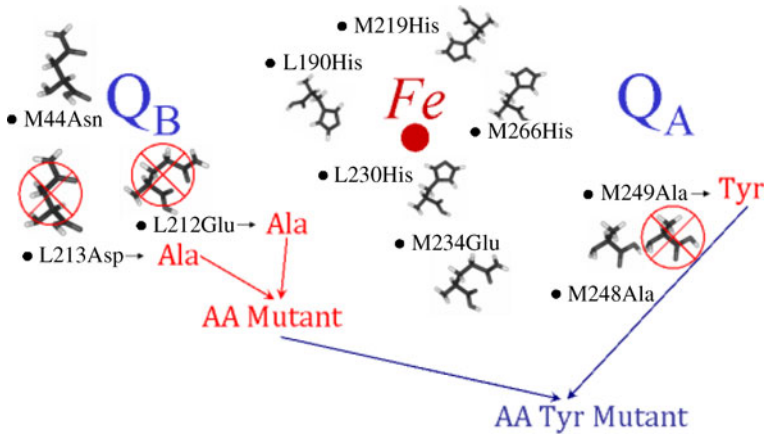
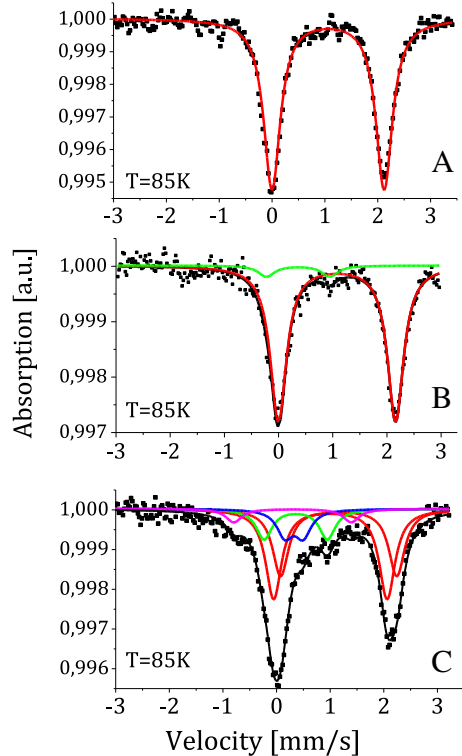


Fig. 1 The arrangement of amino acids at the proximity of the iron-quinone complex (Q_A-Fe-Q_B) in *Rb. sphaeroides*

Fig. 2 Mössbauer spectra of WT (a), AA (b) and AATyr (c) BRC isolated from *Rb. sphaeroides*. The solid lines represent fits assuming symmetrical lorentzians. Different components are indicated by various colors. The spectra were measured at 85 K



in [9]. We used 900 nmol, 800 nmol and 1400 nmol in the WT, AA and AATyr BRCs, respectively. No differences were observed in the absorption spectra of the WT BRCs and its two mutated types (data not presented). The Mössbauer spectra were recorded in a home made cryostat using 50 mCi ⁵⁷Co/Rh and a proportional

Table 1 The hyperfine parameters of NHFe fitted to the Mössbauer spectra of WT, AA and AATyr BRCs

Hyperfine parameters	Component 1	Component 2	Component 3	Component 4	Component 5
WT					
IS [mm/s]	1,06 ± 0,01				
QS [mm/s]	2,12 ± 0,01				
A [%]	100 ± 2				
AA					
IS [mm/s]	1,08 ± 0,01		0,36 ± 0,10		
QS [mm/s]	2,16 ± 0,01		1,17 ± 0,20		
A [%]	93 ± 2		7 ± 1		
AA Tyr					
IS [mm/s]	1,15 ± 0,03	0,99 ± 0,03	0,25 ± 0,04	0,13 ± 0,05	0,17 ± 0,10
QS [mm/s]	2,12 ± 0,02	2,10 ± 0,03	0,98 ± 0,10	1,9 ± 0,1	0,44 ± 0,10
A [%]	38 ± 7	33 ± 6	10 ± 2	9 ± 1	10 ± 2

IS: isomeric shift, QS: quadrupole splitting, A: contribution in the spectrum. The line width in all cases was 0.18 ± 0.01 mm/s

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 [10].

3 Results and discussion

In Fig. 2 we present Mössbauer spectra for BRCs of high purity obtained from wild type, AA and AATyr mutant of *Rb. sphaeroides*.

The hyperfine parameters of NHFe obtained from theoretical fits are given in Table 1.

The spectrum of WT BRCs is composed of a single doublet with hyperfine parameters characteristic for a high spin Fe^{2+} state [4, 11]. In the case of the AA mutant, the spectrum contains component of the high spin reduced state of the iron atom and a small contribution of a component, whose hyperfine parameters are more similar to those observed usually in the case of the reduced low spin state of the heme-iron in cytochromes [4, 12]. The AATyr spectrum is very different from those detected for WT and AA mutant BRCs. It requires five components to get a fit of good quality. The two components having isomer shift (IS) of about 1 mm/s are typical for a high spin ferrous state. Our estimation of this spectrum with a distribution of a quadrupole splitting (QS) showed that we can deconvolute this part of the spectrum using two sub-spectra. Component with IS = 0.13 mm/s and QS = 1.9 mm/s is more typical for iron in octahedral coordination, when one of the perpendicular ligands has a different character than the others. It is very often observed for heme - iron ligated with O_2 [13] but in our BRCs we have six-coordinated non-heme iron, what means that at least one of its ligands had to be modified in comparison to the state when NHFe occurs in a high Fe^{2+} state. The sub-spectrum with QS ≈ 1.0 mm/s is most probably related to the same NHFe state which is observed in the AA mutant but having a higher symmetry of the binding site. The sub-spectrum with the lowest quadrupole is related to the low spin ferrous state

of NHFe. Its hyperfine parameters do not change with the increasing temperature. A similar NHFe state was already observed in other photosynthetic systems of type II [4–7]. To understand the complexity of the AATyr spectra we performed measurements of the amount of ubiquinone binding at the Q_B site per reaction center using HPLC [14]. The ratio of ubiquinone Q_B per reaction center is 0.24, 0.77 for WT and AA, respectively, and no ubiquinone at this site was detected for AATyr BRCs. From those results, it turns out that the occupation of the Q_B site by UQ is not directly related to the state of NHFe. It was already shown in the reconstitution experiments, when independently of the amount of bound UQ at the Q_B site, only high spin ferrous state of NHFe was observed [11]. Therefore, we suggest that the presence of various states of NHFe in the case of AATyr BRCs is caused by the modification of the hydrophobicity in the vicinity of the Q_A binding site, whose differences between the double AA and triple AATyr mutant are confirmed (see Fig. 1).

4 Conclusion

The presented results suggest that the spin state of NHFe is not sensitive to the presence of the ubiquinone at the Q_B site but it is easily affected by the hydrogen network in the vicinity of the Q_A binding site. Especially, the hydrophobic character of the Q_A binding site is very important for the stabilization of the high spin ferrous state of NHFe, having been proved by the obtained Mössbauer spectra for the AATyr mutant in which M249Ala was substituted into a hydrophilic amino acid, tyrosine. The planned temperature dependent measurements will show if this modification of the acceptor site near the Q_A binding site may also influence the local flexibility of NHFe.

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