CHEMICAL PROPRIETIES OF THE IRON-QUINONE COMPLEX IN MUTATED REACTION CENTERS OF *Rb.sphaeroides.*

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INTRODUCTION

We investigated photosynthetic bacterial reaction centers of type II, which contain a quinone - iron complex (Q_A –Fe- Q_B) on the acceptor side. The non-heme iron (NHFe) is a very conservative component of type II reaction centers (RCs) [1, 2]. The role of the non-heme iron (NHFe) in the electron transfer between the primary (Q_A) and secondary (Q_B) quinone acceptor is unclear. It was always observed in a reduced state under physiological conditions. However, it can occur in two spin states (high and low spin state), what suggests that it might regulate dynamical properties of the iron – quinone complex and the protonation and deprotonation events in its neighbourhood.

In order to get insight into the function of NHFe, we preformed Mössbauer studies of a wild type and two mutated forms of *Rb. sphaeroides* (two residua near the Q_B binding site, which are expected to participate in proton transfer, were replaced by neutral amino acids and one neutral near the Q_A binding site replaced by a tyrosine). Here, we present how the introduced mutations influence the spin state of NHFe the Q_A –Fe- Q_B complex.

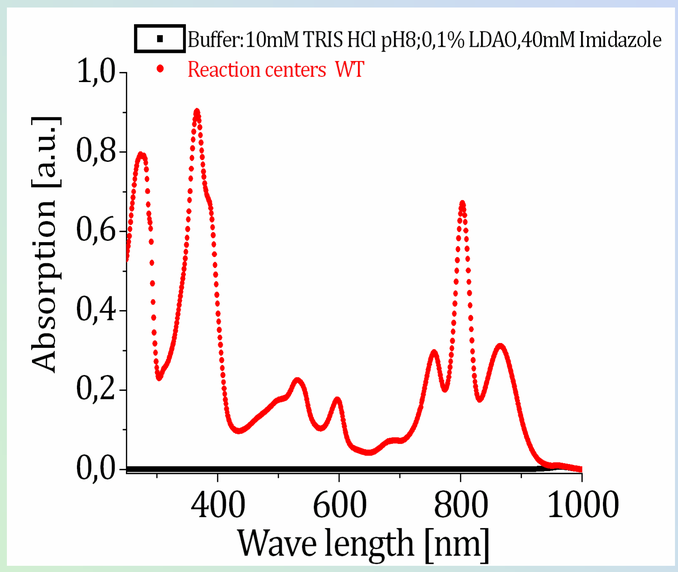

Figure 1. *The absorption spectrum of WT reaction centers isolated from Rb.sphaeroides.*

MATERIAL AND METHODS

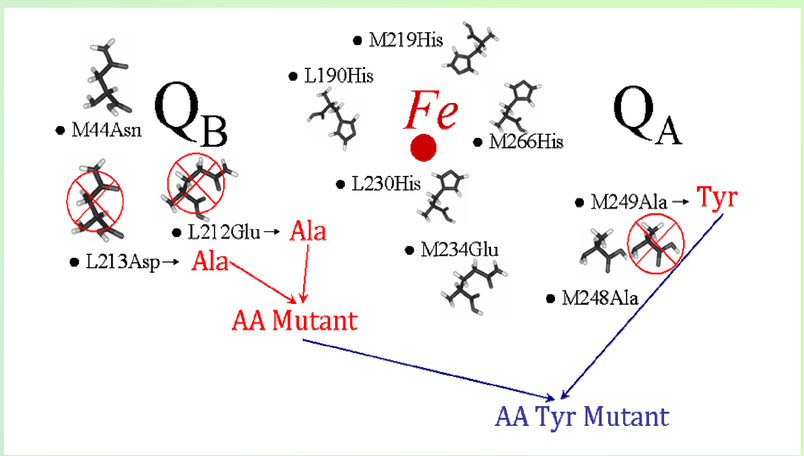

Figure 2. *The arrangement of amino acids from the proteins: M, L and H in the vicinity of the NHFe binding site for Rb.sphaeroides.*

We performed measurements on BRCs isolated from WT and two photosynthetically incompetent mutants of *Rb.sphaeroides* [3]. The double mutant, called 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 binding place of the ubiquinone Q_A (M249Ala->Tyr).

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. The cultures were grown in darkness at 30°C. Wild-type and mutated BRCs containing ^{57}Fe at the acceptor side were purified according to [4, 5].

RESULTS

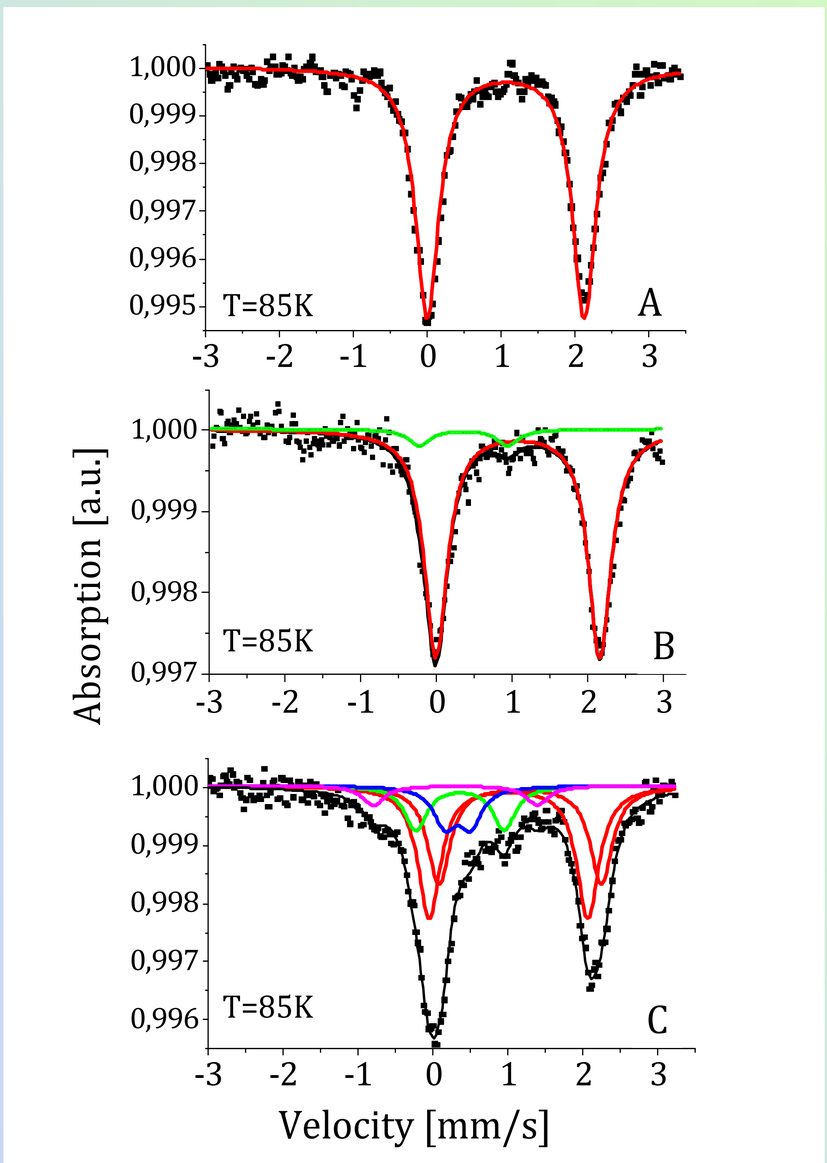

Figure 2. *The ^{57}Fe Mössbauer spectra of reaction centers isolated from Rb.sphaeroides. A:WT ; B:AA mutant ; C: AATyr mutant. The lines represent fits assuming symmetrical lorentzian doublets.*

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

Table1. *Hyperfine parameters obtained from the theoretical evaluations of the Mössbauer spectra : Isomeric shift (IS); Quadrupole splitting (QS); Contribution (%) for WT and mutated reaction centers.*

bRC	UQ_B/RC
WT	0,24
AA	0,77
AATyr	0

Table2. *The ratio of ubiquinone Q_B per reaction center in WT, AA and AATyr BRCs, respectively.*

CONCLUSION

The occupation of the Q_B site UQ is not directly related to the state of NHFe.

NHFe is not sensitive to the presence of the ubiquionone 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.

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