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Report:

Rhodobacter sphaeroides (Rb.sphaeroides), a non-sulphur purple bacterium, is a model system to study the dynamical and structural proprieties of type II photosynthetic reaction centers (RCs). The core of bacterial RC consists of three protein subunits L, M and H. The subunits L and M are connected via the non-heme iron (NHFe) symmetrically situated between the primary and secondary ubiquinone acceptors Q_A and Q_B, respectively. The ubiquinones are separated about 18 Å from each other. NHFe is a very conservative component of type II RCs. Together with the two ubiquinones it forms a quinone-iron complex (Q_A-Fe-Q_B). The NHFe role in photosynthetic charge separation and in the temperature activation of electron transfer (ET) between Q_A and Q_B ubiquinones remains unclear. No change of the NHFe valence state was observed in native type II RCs, which usually occurs in a high spin ferrous state. However, in native photosynthetic systems isolated from algae and purple bacteria, different spin states of the reduced NHFe were detected.

The aim of this project was to investigate long range regulatory mechanisms controlling the activity of the photosynthetic bacterial reaction centers (BRC) of type II, and to understand the NHFe role in these processes. The mechanisms are related to the flexibility of the BRC core proteins which contain cofactors participating in electron transport (ET) and residues involved in forming hydrogen network and in tuning the redox potential of these cofactors. Some of the residues, conserved during the evolution process of photosynthetic systems of type II, are important for proton transfer (PT) to the quinones Q_A and Q_B bound within the iron-quinone complex on the acceptor side of the reaction centers. The knowledge of how mutations of these residues change a partial photon density of NHFe states in comparison to a wild type of BRC is crucial for understanding of the influence of a local protein flexibility on distal redox active sites. In particular, we concentrated on the studies of bacterial reaction centers isolated form a wild type and mutant strains of *Rb. sphaeroides* with the modified Q_A (M249Ala → M249Tyr) and Q_B binding sites (L212Glu → L212Ala and L213Asp →L213Ala) (Fig. 1). It was shown that the double mutation L212Ala and L213Ala results in reduction of proton transfer to Q_B by 3 orders of magnitude and an additional mutation M249Tyr restored the stochiometry of the proton uptake but not the kinetics of the process. Moreover, the neutron scattering experiments showed that these double and triple point mutations also lead to an increase of the flexibility of the whole BRC protein system in comparison to the wild type at temperatures above the characteristic transition temperature (~230 K) when fast and slow collective motions occur.

We performed studies of the NHFe motions in the mutated BRCs to learn about intermolecular interactions responsible for functioning of photosynthetic systems of type II. The phonon-assisted Mössbauer effect with synchrotron radiation, i.e. Nuclear Inelastic Scattering (NIS) is an excellent tool to achieve this goal. Our investigations of the His-tagged RCs allowed us to obtain, for the first time, a pure spectrum of vibrational state density originating from the slow collective motions of the protein matrix in the vicinity of NHFe. Moreover, we showed that the double and triple mutations in the vicinity of the Q_A and Q_B sites influence these collective vibrations (Fig. 2A and B), what suggests that the modification of the hydrogen network due to the exchange of the following residua L212Glu, L213Asp and M249Ala can be crucial for the activity of the acceptor side in BRCs of type II. These mutations results additionally in modification of the NHFe spin state [1].

The obtained results were partially presented during the conference ICAME 2011 in Kobe (Japan) and some of them are included in a paper submitted to BBA Bioenergetics [2].

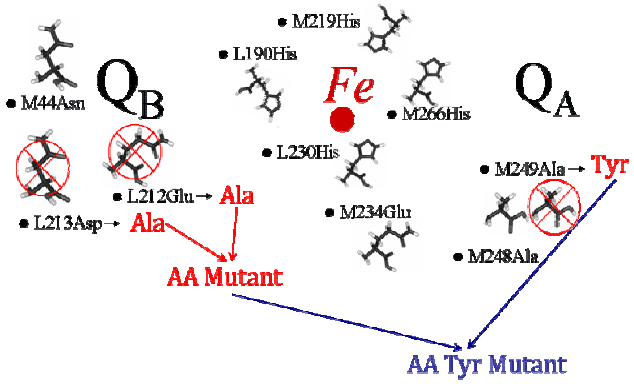
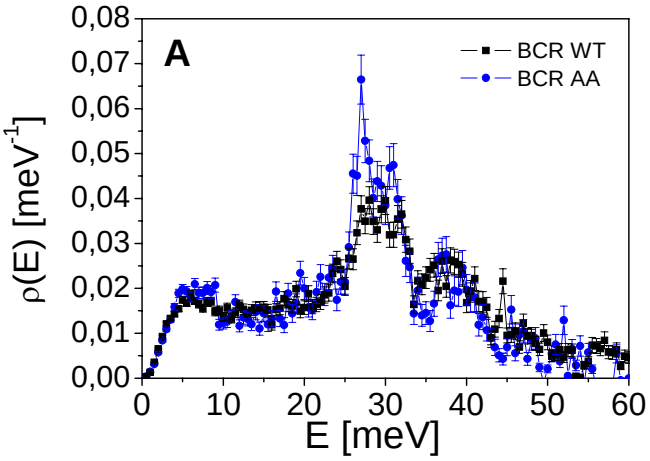


Fig. 1. The quinone-iron complex of the bacterial reaction center (BRC) of *Rb.sphaeroides.*



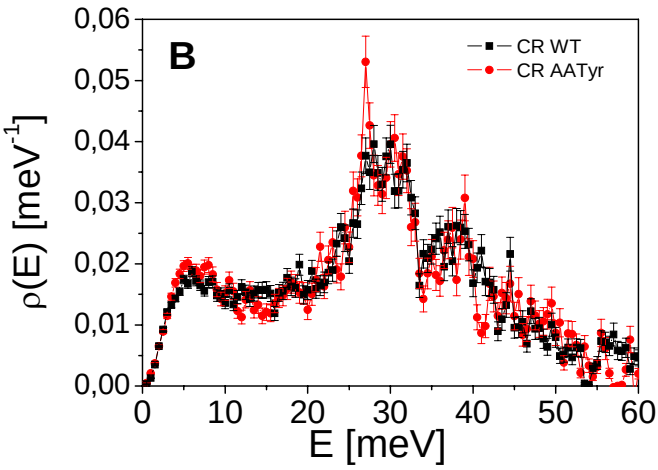


Fig. 2. Density of vibrational states ρ(E) obtained from NIS experiments (T = 60 K) for (A) WT and AA (a double mutant; L212Ala and L213Ala); (B) WT and AATyr (a triple mutant; L212Ala and L213Ala and MB249Tyr RCs.

[1] A. Hałas, V.Derrien, P.Sebban, K. Matlak, J.Korecki, J. Kruk, K. Burda, *"Chemical properties of the iron-quinone complex in mutated reaction centers of Rb. sphaeroides"* Hyperfine Interactions (2011) DOI 10.1007/s10751-011-0451-0

[2] A. Hałas, P. Sebban, K.Matlak, J.Korecki, A. Orzechowska, J. Fiedor, A.Chumakov, M.Zając, T.Ślęzak, K.Strzałka, L.Fiedor and K. Burda, (2011) *Dynamical properties of the non-heme iron in bacterial reaction centers from Rb.sphaeroides.* submitted to BBA – Bioenergetics