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Report:

Background of the studies

Several types of metal centers, such as Mg, Fe and Mn, are intrinsically involved in photosynthesis, a fundamental biological process. For instance, redox active transition metal Fe serves as an electron carrier but its functioning undergoes strong modulation by the immediate environment. Iron ions can act either as an electron/group transfer center or a coordination center. The former activity is found in complexes which contain heme iron (HFe) as a cofactor what is observed for cytochrome b559 in photosystem II (PSII) occurring in thylakoids of higher plants or cyanobacteria (1). The latter one involves e.g. non-heme iron (NHFe), a conservative component of Q type photosynthetic reaction centers (RCs) in which its role is still open to discussion (2). Photosynthetic reaction centers from purple bacteria belong evolutionary to the same photosystems as PSII because these reaction centers on their acceptor side have an iron quinone complex: Q_A-Fe-Q_B (where Q_A and Q_B are primary and secondary quinone acceptors). The arrangement of cofactors on the donor and acceptor sides of PSII resembles that one in bacterial RCs. The core of PSII RC is formed by two main proteins D1 and D2, which consists of five membrane helices and structurally correspond to the L and M subunits of the reaction centers from purple bacteria (3, 4). Due to sequence homologies and similarities in folding of the core proteins the electron transfer (ET) cofactors in the Q-type photosystems are arranged symmetrically in two branches, A and B, with the NHFe localized between the two core peptides and bound to the quinone Q_A (on M or D2) and Q_B (on L or D1). However, there are some differences. Additionally, PSII contains two redox active tyrosines (TyrZ and TyrD) and calcium-manganese complex on the donor side. In the case of purple bacteria RCs cytochrome c_2 donates electrons to the oxidized bacteriochlorophyll special pair (P870) whereas in the case of PSII the electrons came form the 4Mn-Ca complex which is able to oxidized water. Besides some other differences in small protein contents between the Q-type photosystems, the presence of cytochrome b559 being a transmembrane hem-protein in PSII seems to be the most important evolutionar change between bacterial RCs and PSII.

The aim of our project was to give the evidence for the existence of a mechanism coupling the electron transfer on the donor and acceptor side of photosynthetic reaction center PSII isolated from a wild type and mutated tobacco, having a single point mutation on cytochrome b559. However, we started our investigations from more stable and simple systems , i.e. bacterial reaction centers. We found out that the non-heme iron (NHFe) form the iron-quinone complex in the RCs isolated from *Rhodobacter* (*Rb.*) *sphaeroides* exists in two different spin states, in a low spin state (LS) and a high spin state (HS), whereas in the case of *Rhodospirillum* (*Rsp.*) *rubrum* it is observed mainly in the low spin state. So far it was believed

that NHFe can be present in native Q-type photosystems only in the reduced high spin state. The exception was PSII from algae PSI- mutant (5). These intriguing observations prompted us to investigate in detail the properties of NHFe in RCs from these two different types of purple photosynthetic bacteria. Especially, that the temperature dependence of electron transfer (ET) steps and the role of NHFe in photosynthetic charge separation are among the most challenging issues (6,7). Intriguingly, Q_A in RCs from various organisms remains fully active at cryogenic temperatures whereas ET from reduced Q_A to Q_B slows down at temperatures below 200±20 K (13, 31-36, 38- 44). This phenomenon, predicted also theoretically, suggests that perhaps some intrinsic flexibility in the protein matrix in the vicinity of the Q_A–Fe–Q_B complex is required for efficient ET to the acceptor side. This is in line with the fact that thermal fluctuations of proteins among different conformational arrangements, with the time constants of relaxations from picoseconds to seconds, strongly affect rates of ET in donor-acceptor pairs. Furthermore, both fast and slow collective motions of the protein matrix in RCs, activated at T > 180 K, seem to be crucial for the protonation and deprotonation events accompanying ET within the Q_A–Fe–Q_B complex (8). To this end, it is not clear whether NHFe plays any structural role in stabilization of the Q_A and Q_B binding sites and/or it is actively involved in the primary ET. The phonon-assisted Mössbauer effect with synchrotron radiation, i.e Nuclear Inelastic Scattering (NIS) allowed us to provide new evidence for the importance of NHFe in the Q-type photosystems. We performed measurements at two different temperatures 60 K and 240 K for native bacterial reaction centers and treated with Cu^{2+} or Cd^{2+} cations. The heavy metal ions were shown to interact in the vicinity of the non-heme iron. In addition cadmium and copper cations were shown to change the ferrous state of NHFe from its high spin state into the low spin one (9-11). These data suggest that one should also expect influence of theses metal ions on the dynamic properties of the NHFe.

Results and Discussion

We applied Mössbauer spectroscopy to compare the valence, spin states and dynamic properties of Fe atoms in the two RCs and the nuclear inelastic absorption of synchrotron radiation (NIS) technique to monitor the modes of the NHFe collective motions in the Q_A – Fe – Q_B complex.

The nuclear inelastic scattering of synchrotron radiation experiment was performed at the Nuclear Resonance beamline ID 18 at the European Synchrotron Radiation Facility in Grenoble, France. The storage ring was run in hybrid mode, providing 24 groups of 8 radiation pulses every 88 ns. The energy radiation was tuned around a transition energy of ^{57}Fe (14.412 keV) within a range from -40 meV to 100 meV for a temperature of 60 K and from -80 meV to 100 meV for a temperature of 240 K. More details on the experimental method and setup are described elsewhere (12). A statistically meaningful spectrum of iron vibration modes in bacterial RC was obtained after 10-12 hours of data collection, depending on the ^{57}Fe iron concentration in the sample.

In Fig. 1 the scattering data collected at 60 K for the two bacterial reaction centers characterized by different occupation of the high and low spin states of NHFe are shown.

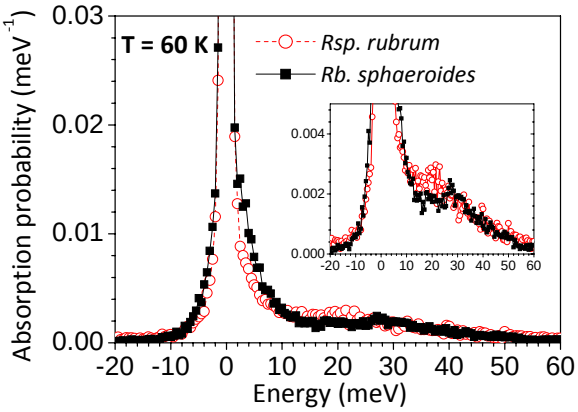

Fig.1. Normalized spectra of nuclear inelastic absorption of synchrotron radiation in the reaction centers from *Rba. sphaeroides* and *Rsp. rubrum*, measured at 60 K. In the inset the absorption probability scale is expended to show fine differences between the spectra.

The vibrational DOS spectra derived from the experimental data for these two RCs, observed at 60 K, show striking differences (Fig. 2A). For an interpretation of the measured vibrational spectra it is essential that both HFe (Cyt c) and NHFe (in the Q_A-Fe-Q_B complex) contribute to the DOS. It is noteworthy that in the present data, most of the spectral intensity is observed between 10 meV and 40 meV, in addition to the

"heme-like" features at low and high energies (13). The heme-iron, regardless of whether it is bound to protein or not, contributes significantly to the DOS spectrum only at energies higher than 30 meV (in Cyt c even higher than 35 meV). In the case of *Rba. sphaeroides*, the DOS has a characteristic shape with some pronounced and relatively sharp peaks (e.g at 28±2 meV or 17±1 meV), while that for *Rsp. rubrum* is rather featureless. These results reveal a clear correlation between the vibrational and spin states of NHFe.

The differences in the DOS for the RCs from *Rba. sphaeroides* and *Rsp. rubrum* can be better visualized in terms of DOS/E (Fig. 2B).

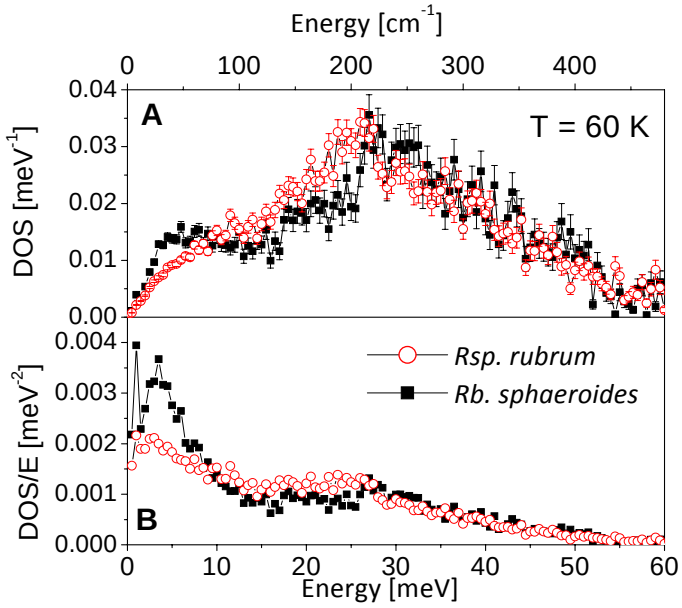

Fig. 2. Density of vibrational states DOS (A) and the density states normalized to their corresponding energies DOS/E (B) in the reaction centers from *Rba. sphaeroides* and *Rsp. rubrum*, measured at 60 K.

For *Rsp. rubrum*, a drastic reduction in the spectral density at low energy is accompanied by an enhancement of modes between 15 and 30 meV. The reduction of the low energy modes means effectively a weaker coupling between NHFe and the protein that correlates well with the expected properties of Fe in a low spin state, the one dominating in RC from *Rsp. rubrum*, as shown by the Mössbauer technique (see above). The low frequency modes originate predominantly from protein vibrations (acoustic) transferred to the resonant ^{57}Fe bound in the Q-Fe complex because only this atom is directly bound to the protein matrix. When the force constants of the iron bonds are comparable to those for the protein matrix, the protein vibrations are transferred effectively to the binding site of NHFe in the HS state. Therefore, in the RC from *Rba. sphaeroides* an enhancement of NHFe fluctuation modes at low energy is seen. The DOS modes of heme-iron from cytochrome show only a very low contribution in this energy range of vibrational frequencies (13). It is important to mention that the increase of temperature from 60 K to 240 K caused an incerase of the contribution of some modes of fluctuations at E < 20 meV in both cases (data not shown).

For better visualization how heavy metals can modify vibrational states of NHFe we present the density of vibrational states normalized to their corresponding energies for *Rsp. rubrum* treated with Cd^{2+} ions and for *Rb. sphaeroides* tretaed with Cu^{2+} ions in Fig. 3 and 4, respectively. In the case of *Rsp. rubrum* we show the experimental data collected at 240 K because at this temperature the influence of cadmium cations on the collective fluctuations of NHFe is more pronaunced than at 60K. Besides DOS changes at energies from 15-28 meV, a decrase of the contribition of the density of vibrational states at energies lower than 12 meV is observed. Having in mind that Cd^{2+} causes a transfer of NHFe into a new ferrous low spin state, which hyperfine parameters indicate an increased rigidity of the iron bonds (11), this effect can be explained by the same same phenomenon as we discussed above comparing the DOS spectra between the two types of bacterial RCs. The same effect is observed in *Rb. spaheroides* RCs incubated with Cu^{2+}. Copper ions change either the high or low spin state of ferrous NHFe into a new low spin state in all Q-type photosystems (9, 10). Because interaction of Cu^{2+} with the iron-quinone complex is much more specific than of Cd^{2+} and *Rb. spheroids* RCs contained in their native state almost 50% of NHFe in the ferrous high spin state the DOS spectra of this bacterial RCs exhibit significant changes within the whole range of energies < 30 meV already at 60 K.

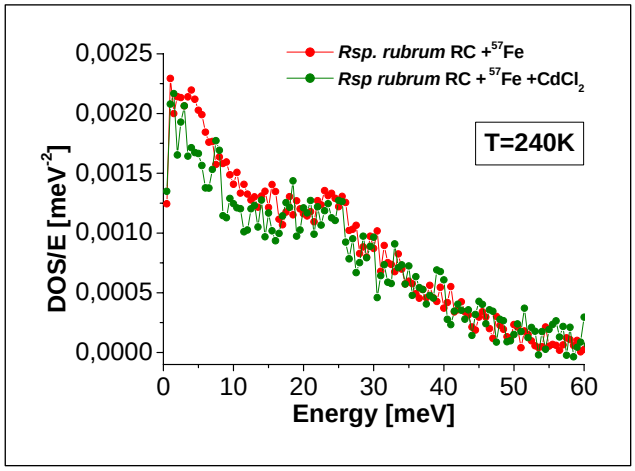

Fig. 3 The density states DOS normalized to their corresponding energies for native and treated with $CdCl_2$ *Rsp. rubrum* measured at 240 K.

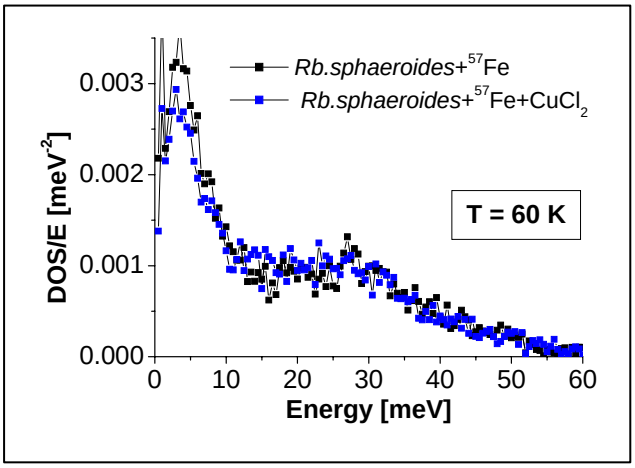

Fig. 4 The density states DOS normalized to their corresponding energies for native and treated with $CuCl_2$ *Rb. sphaeroides* measured at 60 K.

Summarizing, we show that the relationships between Fe spin states, the stability of the Q_A–Fe–Q_B complex, and the efficiency of charge recombination in Q-type reaction centers [14]. From thermoluminescence (TL) and absorption spectroscopy we learnt that the low spin state of NHFe results in decrease in the rate of electron transfer between the two quinone acceptors Q_A and Q_B. Studies of the temperature dependence of the chosen phonon states related with the intramolecular vibrations as well as intermolecular interactions allow us to propose a simple mechanism in which the spin state of non-heme iron directly determines the strength of coupling between the two quinone acceptors and thus controls the rates and stabilization of charge separation between the quinones. The goal could be achieved only by applying the nuclear inelastic absorption of synchrotron radiation (NIS) technique. This knowledge and experience that we gathered during realization of the project will allow us to plan studies of more complex and sensitive biosystems, as for example photosystem II.

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