The primary photosynthetic energy conversion in bacterial reaction centers - Stepwise electron transfer and the effect of elevated exposure levels

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Abstract: The primary reaction in photosynthetic reaction centers from Rhodobacter sphaeroides is investigated for different experimental conditions. Agreement with stepwise electron transfer via a reduced bacteriochlorophyll was observed at low excitation rates.

1. The primary reactions of photosynthetic reaction centers

Photosynthetic energy conversion in bacterial reaction centers (RC) can be taken as a prototype reaction not only for other photosynthetic systems but also for artificial solar energy conversion devices. In this context a sound understanding of the primary reaction steps is of major importance. The observation of these processes requires excitation at the appropriate wavelength, weak intensities to avoid overexposure and a very sensitive detection over a wide range of probing wavelengths. In the 1990ies, a simple stepwise reaction model (1), based on non-adiabatic Marcus-type electron transfer (ET), was proposed to explain the molecular processes and to understand optimization of photosynthesis. Here, an electron is transferred from the originally excited special pair P via the accessory bacteriochlorophyll B_A and the bacteriopheophytin H_A to the quinone Q_A (for the spatial arrangement of the chromophores in the RC see [1,2]).

$$P \xrightarrow{h\nu} P^* \xrightarrow{3.5\,ps} P^+B_A^- \xrightarrow{0.9\,ps} P^+H_A^- \xrightarrow{200\,ps} P^+Q_A^- \qquad (1)$$

Detailed simulations using evolutionary optimization have shown that this simple reaction scheme is able to explain the high quantum yield $\eta > 95\%$ and the good energy efficiency of the primary photosynthetic reaction [3]. More recently, new experimental investigations have been published which partially deviate from original publications and suggest alternative reaction principles [4,5]. In this context we present here the results of new experiments recorded with a high signal to noise ratio and use improved data analysis algorithms. We find (i) a pronounced influence of exposure and repetition rate on the dynamics of the RC and that (ii) the data recorded at low exposure levels are in excellent agreement with the stepwise ET presented in equation (1).

2. Techniques

Pump-probe experiments were performed with femtosecond pulses from a Clark-MXR CPA-2001 laser-amplifier system operated at a repetition rate of 1 kHz. The excitation pulse ($t_P \approx 175$ fs , $\lambda_{exc} = 865$ nm) is generated by a non-collinear optical parametric amplifier (NOPA). A white light continuum with smooth spectral and temporal properties throughout the 600 - 1040 nm range was produced in a YAG crystal from pulses supplied by an optical parametric amplifier (OPA) operated at 1122 nm. The excitation rate was adjusted by choppers between 50 Hz and 500 Hz. Stirring combined with rapid transversal motion of the sample cuvette allowed to replace the irradiated volume and to ascertain a defined exposure for the whole sample volume (0.3 ml). A special method was used to identify the relevant signal components in a Singular Value Decomposition (SVD) based procedure and to quantify the components related to the ET reaction.

3. Results

Experiments at low excitation intensities, with optimized sample exchange and low exposure levels (established by the low repetition rate of 50 Hz) yielded data which could be well fitted by a sum of exponential functions with time constants of 1.1 ps, 3.5 ps, 190 ps and a long-lasting component. The SVD-analysis shows that the time traces related to significant singular values (SV) are fitted well by this exponential 4-component model. Conversely, a 3-component model does not fit the significant time traces with sufficient quality. Figures 1-a and 1-b show the time traces of the fourth and fifth SV together with a multi-exponential fit for the 3-component (solid-blue) and the 4-component model (solid-red). The comparison demonstrates that a fourth kinetic of ca. 1 ps is required to describe

the primary ET-reaction. In order to obtain additional molecular information on the related processes we performed different analysis procedure. Spectral signatures for the intermediate states indicated in equation (1) can be computed from the fit amplitudes using the corresponding rate equation model. The rate constants are assumed in the order 3.5 ps, 1.1 ps and 190 ps. On the contrary, if the two first reaction rates are interchanged, difficulties arise to consistently describe the time dependent emission recorded in a previous publication [6]. Finally, a diverse investigation of the spectral signatures can be performed with target analysis [7]. In this case we obtain again nice agreement with the reaction scheme (1). Both procedures clearly show that the intermediate state populated upon the decay of the excited special pair P* contains the reduced bacteriochlorophyll B_A.

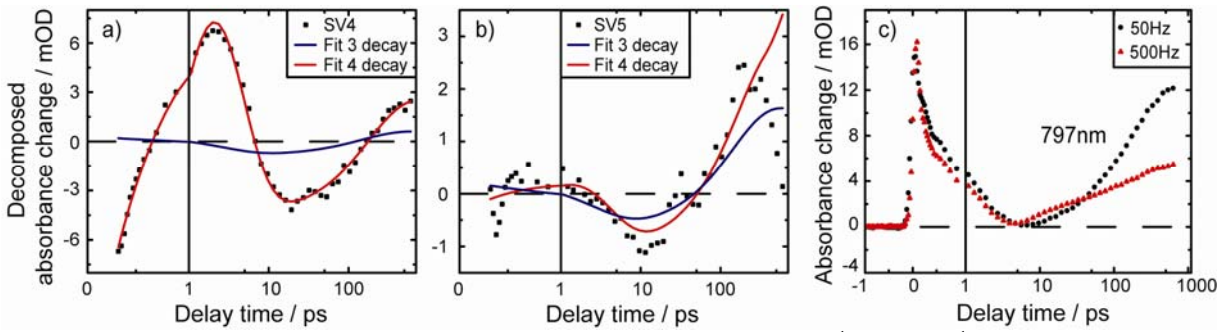

Figure 1: Decomposed transient signal for measurements with 50Hz rep. rate corresponding to the 4th SV (a) and 5th SV (b). Transient absorption signal at 797nm measured with different repetition rates (c). Displayed data has parallel polarization. The time scale is linear until 1ps and logarithmic afterwards.

Experiments performed at higher repetition rates, with reduced sample exchange speed and with chemically treated RC-solutions, show only qualitatively similar results. However the amplitude of the ca. 1 ps component is decreased and an additional kinetic appears with a time constant in the 7 ps range. Moreover there is a pronounced change in dichroism. The discrepancies are evident in the spectral range around 797 nm. When the 50 Hz excitation rate is used (figure 1-c, black points), the absorption features are well fitted by the 4-component model. The transient absorption recorded with a 500 Hz excitation rate (figure 1-c, red points) display pronounced differences. These results indicate that the illumination of the RC at high rates for extended times leads to the accumulation of products, generating a considerable modification in the reaction properties. A most straight forward explanation would be the incorporation of an additional charge in the RC which changes the energy landscape and therefore the ET-kinetics. Apparently new reactions, presumably of non-physiological nature, appear under high exposure conditions.

In conclusion, the photosynthetic reaction centers display primary reactions with a pronounced change in the transfer dynamics, depending on exposure and repetition rate. Nevertheless, data recorded at low exposure levels are in excellent agreement with the stepwise ET model described in the early 1990ies (see equation (1)).

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