Journal of Photochemistry & Photobiology, B: Biology 180 (2018) 140–148

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

The effect of light and temperature on the dynamic state of *Rhodobacter sphaeroides* reaction centers proteins determined from changes in tryptophan fluorescence lifetime and $P^+Q_A^-$ recombination kinetics

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ARTICLE INFO

Keywords:
Photosynthetic reaction center
Tryptophan fluorescence
Temperature dependence
Fluorescence decay kinetics
Recombination kinetics

ABSTRACT

The temperature dependencies of the rate of dark recombination of separated charges between the photoactive bacteriochlorophyll and the primary quinone acceptor (Q_A) in photosynthetic reaction centers (RCs) of the purple bacteria *Rhodobacter sphaeroides* (*Rb. sphaeroides*) were investigated. Measurements were performed in water – glycerol and trehalose environments after freezing to −180 °C in the dark and under actinic light with subsequent heating. Simultaneously, the RC tryptophanyl fluorescence lifetime in the spectral range between 323 and 348 nm was measured under these conditions. A correlation was found between the temperature dependencies of the functional and dynamic parameters of RCs in different solvent mixtures.

For the first time, differences in the average fluorescence lifetime of tryptophanyl residues were measured between RCs frozen in the dark and in the actinic light. The obtained results can be explained by the RC transitions between different conformational states and the dynamic processes in the structure of the hydrogen bonds of RCs. We assumed that RCs exist in two main microconformations – "fast" and "slow", which are characterized by different rates of P^+ and Q_A^- recombination reactions. The "fast" conformation is induced in frozen RCs in the dark, while the "slow" conformation of RC occurs when the RC preparation is frozen under actinic light. An explanation of the temperature dependencies of tryptophan fluorescence lifetimes in RC proteins was made under the assumption that temperature changes affect mainly the electron transfer from the indole ring of the tryptophan molecule to the nearest amide or carboxyl groups.

1. Introduction

The pigment–protein complexes of photosynthetic reaction centers (RCs) are photoactive enzymes whose conformational dynamics play a crucial role during all stages of light energy conversion, including the stage of temporary stabilization of electrons in the acceptor part of the RC [1–5]. Effective electrostatic stabilization of electrons in the quinone acceptors in RCs (Q_A) and (Q_B) of the pheophytin-quinone type significantly slows down the process of dark recombination of separated charges associated with displacements of protons in their protein environment. As was shown for the RC of purple bacteria, the binding of H^+ in the presence of semiquinones in the RC is not a diffusely controlled process; it is regulated by changes associated with conformational transitions between "available" and "unavailable" states prior to the actual binding or transfer of H^+ in the RC structure [6]. Our previous comparative study of the kinetics of redox conversions of the primary electron donor (dimer of bacteriochlorophyll P) in the RC structure of the purple bacteria *Rb. sphaeroides* and the final quinone Q_B electron acceptor in their individual absorption bands in the course of a one-electron reversible charge transfer between P and Q_B demonstrated that the kinetics of the dark reduction of P^+ by Q_B^- recorded in the Q_B absorption bands (335 nm and 420–450 nm) was slower than the kinetics recorded in the Q_x-absorption band of P (600 nm) [7]. We explained this phenomenon by the relaxation processes in the Q_B environment associated with electrostatic stabilization of the electron transferred to Q_B due to displacement of protons in hydrogen bonds of the protein environment of the RC. The activation energy (U_a) and the characteristic time (τ_r) of this process were 1.2 kcal/mol (= 0.052 eV) and 0.1 s, respectively, for RCs in the water–buffer environment at physiological temperatures. The relatively small value of activation energy as compared with the thermal energy and rather high (on the molecular scale) characteristic relaxation time indicate a specific

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https://doi.org/10.1016/j.jphotobiol.2018.01.027
Received 8 December 2017; Received in revised form 22 January 2018; Accepted 29 January 2018

mechanism of the relaxation process due to displacements of protons and deformation of hydrogen bonds of the Q_B environment. Important information about the relationship of the electron-transport activity of the RC and its intramolecular dynamics was provided by experiments involving RC freezing to cryogenic temperatures in the dark or in activating light in different solvents followed by a parallel study of the temperature dependencies of the functional and dynamic values of RC preparations during heating. Previously, this type of research allowed us for the first time to confirm our hypotheses about conformational changes in the RC associated with the electron transfer that regulate the basic constants of this transfer, in particular, during changes of temperature [3,8].

The fluorescence of tryptophanyl residues in proteins is an informative internal natural indicator of the conformation of proteins, as well as their dynamics and intra- and intermolecular interactions. It is well known that the fluorescence of indole chromophore is highly sensitive to the state of its surrounding, including hydrogen bonds, and can be affected even by small changes in the environment [9]. If the structure of a protein is known, the changes in tryptophanyls can be interpreted in terms of structural changes in atomic resolution. The fluorescence maximum of tryptophanyl residues in proteins, as determined by the relaxation characteristics of the polar environment of the excited chromophore, has been used for many years as an indicator of the state of the intramolecular dynamics of proteins, including the variation of the temperature [10]. The interpretation of the effects of changes in the environment on the fluorescence lifetime of tryptophanyl residues in proteins is more complicated. However, in recent years, an informative approach that associates the changes in the lifetime of excited tryptophanyls with the alteration of the environment, including the state of hydrogen bonds, has been developed [11].

The goal of this study was to conduct a comparative investigation of the temperature dependencies of the electron transport activity of RCs of the purple bacteria *Rhodobacter sphaeroides* (*Rb. sphaeroides*) - the dark recombination of the P^+ and Q_A^- separated charges measured in the individual absorption bands of the electron donor and acceptor and fluorescence lifetimes of tryptophan in RC samples frozen to cryogenic temperatures in the dark or in the activating light in various solvents during their subsequent heating.

2. Materials and Methods

The study was carried out in RC preparations of the purple bacteria *Rhodobacter sphaeroides*. Bacterial cells were disrupted using an ultrasonicator. Chromatophores were separated by centrifugation and incubated for 30 min at 4 °C in 0.01 M sodium phosphate buffer (pH 7.0) containing 0.5% zwitterionic detergent lauryldimethylamine oxide. The chromatophores were then centrifuged at 144,000*g* for 90 min at 4 °C. The RC fraction in the supernatant was separated by chromatography on hydroxyapatite column as described in [12]. The concentration of the obtained RC suspended in 0.01 M sodium phosphate buffer at pH 7.0 containing 0.05% lauryldimethylamine oxide was approximately 50 μM.

Photoinduced reactions were investigated using a differential single-beam spectrophotometer with double monochromatization of the measuring light designed at the Department of Biophysics, Faculty of Biology of the Moscow State University. An Nd-YAG LS-2131M laser (532 nm, 8 ns, 5 mJ, LOTIS-TII, Belarus) was used as a source of the excitation light. Photoinduced absorption changes were recorded at 600 nm (Q_x absorption band P) and in the bands that reflect the redox conversion of ubiquinones of RCs at 335 nm and at 420–450 nm. The kinetics of the absorption changes at 335 nm and 420–450 nm were practically the same. However, since the amplitude of the signal at 450 nm was the highest, redox transformation of ubiquinones of RCs was described according to the results of measurements at this wavelength. The accumulation and averaging of 8–10 single signals were performed for the improvement of the signal/noise ratio using an Octopus CS 8327 analog-to-digital converter (GaGe, USA). The obtained kinetic curves were approximated using the QRIGIN software package. In experiments that involved freezing in the light, the sample (in the presence of 10^{-2} M *o*-phenanthroline blocking electron transfer from Q_A to Q_B) at room temperature was illuminated with white light using an incandescent lamp of the KGM type (350 W). When the light was on, the temperature decreased to −180 °C within 10 min. The light was then switched off and laser-induced absorption changes were measured. The temperature of the sample was then increased in the dark at increments of 10 °C per min and photoinduced changes in the absorbance were recorded.

Measurements of the protein-fluorescence spectra of the RC samples (λ_{excit} = 280 nm) in the temperature range from −180 °C to +35 °C were carried out using a Hitachi 850 (Japan) computerized spectrofluorometer equipped with a cryostat constructed in the laboratory.

The measurement of the fluorescence lifetime of the RC protein was conducted using a Simple Tau 140 device operating in the photon counting mode (Becker & Hickl, Germany). The sample was placed in a liquid nitrogen-cooled cuvette whose temperature was measured using a thermocouple. In this case, the time of cooling to −180 °C was approximately 10 min and the heating rate was 5–7 °C/min. Upon cooling, the RC was activated by constant light in the visible range with an intensity of approximately 1 W/cm^2. At −180 °C, the activating light was switched off. The fluorescence of the sample was excited at 280 nm using an EPLED 280 ps semiconductor laser (Edinburg Photonics, Ireland); the pulse duration was 870 ps; the spectral width, 10 nm; the pulse repetition rate, 1 MHz. The fluorescence was recorded using a K5900 16-channel multianode photomultiplier (Hamamatsu, Japan), in front of which a grating polychromator (600 grooves/mm) was located. The width of the spectrum at the output of the polychromator was 200 nm, which corresponded to 12.5 nm per channel. The integration time was 30 s. Thus, we obtained the three-dimensional (λ; *t*; *I*, intensity) picture of the fluorescence of tryptophanyls and could get the kinetics of the fluorescence decay at any point of the spectrum within 200 nm. Kinetic fluorescence curves were processed using the SPCImage program (Becker & Hickl, Germany); the best approximation of the model curve with the experimental results was achieved by the two-component approach. The average lifetime (τ_{av}) was calculated as the sum of the products of the normalized amplitudes and times of the corresponding kinetic components.

3. Results and Discussion

During the cooling of RCs in the dark electron transfer from Q_A to Q_B was almost completely inhibited even at −30–40 °C. The rate (25–30 ms)$^{-1}$ of charge recombination between Q_A and P^+ was measured in samples frozen to −180 °C in the dark. At room temperature, the time of this reaction was substantially higher – approximately 100 ms. At room temperature and at −180 °C, the kinetics of the recombination can be well approximated by a single exponential. However, when samples frozen to −180 °C in the dark were then heated, the recombination kinetics became essentially non-exponential between the cryogenic and room temperatures. A different pattern was observed for measurements of the recombination kinetics in RC samples cooled in the activating light. Under these conditions, the time of dark charge recombination between the P^+ and Q_A^- remained close to that observed at room temperature (i.e., approximately 100 ms). Upon subsequent defrosting to −110 °C, a slight decrease in this time was observed, while further heating increased the time to values typical for room temperature. These results are explained by light-induced transitions of RCs between different conformational substates, modulated by the temperature factor [5,13]. In our experiments, we did not observe any differences in the $P^+Q_A^-$ recombination rate constants obtained by kinetic measurements at 450 and 600 nm in RCs containing 70% glycerol during heating of RCs frozen in the dark in the entire range of temperatures. Therefore, in the presence of 70% glycerol in the