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Spectrally silent light induced conformation change in photosynthetic reaction centers

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Abstract **Spectrally silent conformation change after photoexcitation of photosynthetic reaction centers isolated from *Rhodobacter sphaeroides* R-26 was observed by the optical heterodyne transient grating technique. The signal showed spectrally silent structural change in photosynthetic reaction centers followed by the primary P+BPh− charge separation and this change remains even after the charge recombination. Without bound quinone to the RC, the conformation change relaxes with about 28 μs lifetime. The presence of quinone at the primary quinone (QA) site may suppress this conformation change. However, a weak relaxation with 30–40 μs lifetime is still observed under the presence of QA, which increases up to 40 μs as a function of the occupancy of the secondary quinone (QB) site.**

Keywords: Transient grating; Heterodyne detection; Reaction center

1. Introduction

There are several types of photosynthetic reaction centers (RCs) in living organisms (PS-I and PS-II of plants and cyanobacteria, RCs of purple and green bacteria; see e.g. [1]) which are developed to convert light energy into chemical potential in a series of (photo)physical and (photo)chemical reactions.

The basic processes of the photosynthetic energy conversion in all types of the RCs include (a) electron excitation of chlorine pigments by light, (b) charge separation and stabilization reactions between redox active cofactors bound to the protein, (c) rearrangement of the dielectric medium and hydrogen bond interactions (including protonation and deprotonation of specific amino acids), and (d) conformational movements within the protein [including transition of (sub)states between dark and light adapted forms].

There are several evidences that RCs are in different conformation states in dark and light, e.g. [2–6]. Kinetic components in transient absorption were observed at specific wavelength [7,8], in capacitive potentiometry [9] and time resolved FTIR spectroscopy [10,11] which are generally assigned to conformational transients related to $Q_A^-Q_B$ to $Q_AQ_B^-$ electron transfer. Recent crystallographic experiments did not show large quinone displacements at the Q_B site on the time scale of the secondary electron transfer [12,13] in agreement with FTIR studies [14,15]. It seems reasonable to conclude that even larger quinone movement is not necessarily accompanied with considerable structural change [13].

Transient grating technique was successfully used for many applications including thermodynamics and kinetics of CO binding of myoglobin [16,17], the photocycle of the photoactive yellow protein (PYP, [18]), determining diffusion coefficient of proteins and DNA [19] and conformational changes of photosensor proteins [20–23]. In a recent publication we have shown that this method can be successfully applied to investigation of light induced charge transfer processes and accompanied protein relaxation movements in bacterial reaction centers [24]. Here, we give further evidences.

1.1. Principle of the OHD-TG measurement

The principle of the optical heterodyne detection (OHD) of the transient grating (TG) signal was described previously [25–28]. After photoexcitation of a sample with a grating pulsed light, a signal field ($E_S(t)$) is created by the diffraction of a probe light. If it is interfered with a local oscillator (LO) field (E_{LO}) the observed light intensity is

$$I(t) = \alpha|E_{LO} + E_s(t)|^2 \quad \sim I_{LO} + \delta n(t)\cos\Delta\phi I_{ex}I_{pr}, \tag{1}$$

where α is an instrumental constant, $\delta n(t)$ is the refractive index change, $\Delta\phi$ phase difference between the local oscillator (LO) and probe light fields and I_{ex}, I_{LO}, and I_{pr} are the intensities of the excitation, LO, and probe light, respectively. Here we assume that the refractive index change is the dominant source of the signal, and the signal field is weak compared to E_{LO} ($|E_{LO}| >> |E_S|$). Since the local oscillator light intensity is a constant, I_{LO} provides constant background. The second term of Eq. (1) represents the OHD-TG signal. The OHD-TG signal intensity depends on the relative phase difference between the LO and signal fields, $\Delta\phi$. The maximum intensity of the thermal grating component of a reference sample is achieved at $\Delta\phi = 0$.

Two main factors contribute to the refractive index change: the thermal effect [thermal grating, $\delta n_{th}(t)$] and a change in

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Abbreviations: BPh, bacteriopheophytin; DEAE, diethylaminoethyl; LDAO, N,N-dimethyldodecylamine-N-oxide; LO, local oscillator; OHD, optical heterodyne detection; P, primary donor; Q_A, primary quinone; Q_B, secondary quinone; *R.*, *Rhodobacter*; RC, photosynthetic reaction center; TG, transient grating; TrL, transient lens; UQ-10, ubiquinone-10

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chemical species by the reaction [species grating, $\delta n_{spe}(t)$]. The species grating component consists of the population and volume terms. Hence, we can monitor the temporal changes of the energy, absorption change, and molecular volume by analyzing the OHD-TG signal.

When the energy relaxation is faster than the diffusion process, the temporal profile of $\delta n_{th}(t)$ is determined by the thermal diffusion, and is given by

$$\delta n_{th}(t) = \delta n_{th}^0 \exp(-D_{th} q^2 t), \quad (2)$$

where D_{th} is the diffusion constant, q is the grating wavenumber.

The kinetics of the species grating signal intensity, $\delta n_{spe}(t)$, is given by the difference of the refractive index changes due to the reactant (δn_r) and product (δn_p). If the back reaction from the product to the reactant is comparable to the diffusion process it is given by

$$\delta n_{spe}(t) = \left\{ \delta n_p^0 - \frac{k}{k + (D_r - D_p)q^2} \delta n_r^0 \right\} \exp\{-(k + D_p q^2 t)\} + \frac{\delta n_r^0 (D_r - D_p) q^2}{k + (D_r - D_p) q^2} \exp\{-D_r q^2 t\}, \quad (3)$$

where D_r and D_p are diffusion coefficients of the reactant and the product, respectively.

2. Materials and methods

2.1. Sample preparations

Rhodobacter sphaeroides R-26 cells were grown photoheterotrophically under anaerobic conditions. RCs were prepared by detergent (LDAO, N,N-dimethyldodecylamine-N-oxide) solubilization followed by ammonium sulfate precipitation and diethylaminoethyl (DEAE) Sephacell anion exchange chromatography [29]. The primary (Q_A) and the secondary (Q_B) quinones were extracted out according to Okamura et al. [30]. RCs with different amount of bound quinones were prepared by addition of ubiquinone-10 (UQ-10) to the solution. The quinone/RC ratio was checked by kinetic absorption change measurement as described by Tandori et al. [29].

2.2. Transient grating measurements

An excitation laser beam (from the second harmonics of a Nd:YAG laser, λ = 532 nm, τ = 10 ns) and a cw IR beam (YAG, 1064 nm) were split by a transmission grating (optical mask) and the first order diffracted beams were combined again on the sample using a concave mirror. In order to avoid overexcitation of the sample, the energy of the excitation beam was reduced by neutral density filters (typically below 100 μJ). One of the cw IR beams was used for the probe beam of the TG signal. The other beam intensity was attenuated about 1/100 by a neutral density filter for the use of the local LO light and of the OHD-TG signal. The filter was slightly adjusted tilting by a computer and used for a fine adjustment of the phase of the LO field to the probe field by changing the optical path length inside. The LO light intensity was detected by a photodiode. The LO light intensity could be changed not only by the interference between the signal and the LO light, but also by the transient lens (TrL) signal created by the pump beam [31]. The OHD-TG signal was obtained by calculating the difference between minimum and maximum LO light intensities matching two opposite phases after filter adjustments.

The spacing of the grating fringe, equivalently, the grating wavenumber, was measured by the decay rate constant of the thermal grating signal from a calorimetric standard sample (bromocresol purple). The signal was averaged and stored by a digital oscilloscope (Tektronics, TDS-520) [17]. In some experiments, absorption change was monitored by a He–Ne laser (590 nm) after the flash excitation. The experimental set up was described in our earlier work [24].

3. Results and discussion

After photoexcitation of the sample solutions, the detected probe light intensity increased abruptly then decreased as a function of time. The two components in the signal (TG and TrL) can be separated based on the phase sensitive nature of the OHD-TG signal. In contrast to TrL, the amplitude and the sign of the OHD-TG signal depends on the phase difference between the LO and the signal (see $\Delta\phi$ in Eq. (1)). The OHD-TG signal changes the sign by phase difference of 180°. Hence, subtraction of the $\Delta\phi$ = 180° signal from the $\Delta\phi$ = 0° signal provides OHD-TG without TrL contribution. The curves TG+ and TG− in Fig. 1 show the probe light intensities measured at $\Delta\phi$ = 180° and $\Delta\phi$ = 0°, respectively in Q_B

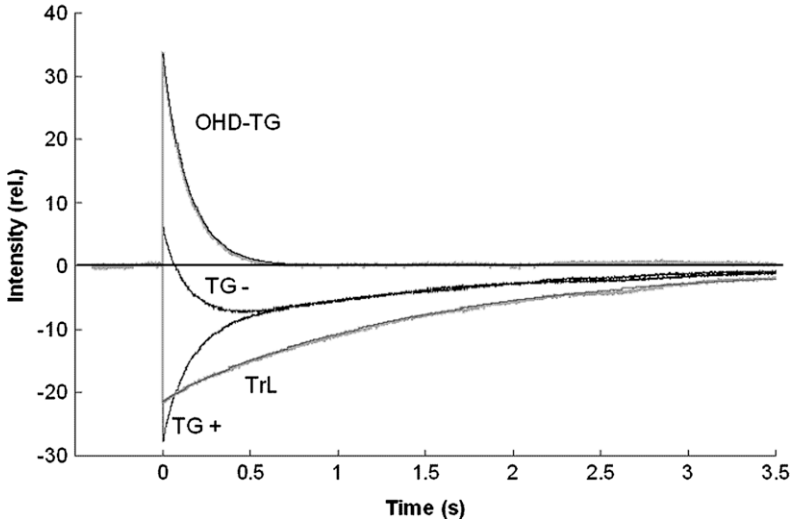

Fig. 1. The intensity of different components of the measured grating signal at different LO light conditions for Q_B reconstituted RCs. TG+ and TG− indicate the original data measured with two opposite phases of the LO light ($\Delta\phi$ = 180° and $\Delta\phi$ = 0°, respectively). TrL and OHD-TG indicate the transient lens and grating components (gray curves), respectively, calculated from TG+ and TG− as described in the Section 1.1. The best fitted curves are shown by the black lines. The best fitting parameters for the OHD-TG and TrL components are $I = 0.97\exp(-t/0.15\ \text{s}) + 0.03\exp(-t/0.055\ \text{s})$ for the OHD-TG component and $I = -0.97\exp(-t/1.5\ \text{s}) - 0.03\exp(-t/0.11\ \text{s})$ for the TrL component. Here the amplitudes are normalized to 1 for better comparison.