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Comparison of nonlinear properties of monomer and dimer of bacterial phytochrome from *Deinococcus radiodurans*

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ABSTRACT

Current medicine might be greatly enhanced by the ability to *in vivo* control and monitor neurons using opsins/phytochromes expressed in neural cells. The fundamental challenge with non-invasive neural cell activity regulation is a high absorption of visible light into biological tissues. This drawback could be mitigated by the photoconversion of phytochromes in spectral ranges with higher tissue transparency. In this study, we first demonstrated two-photon Pr→Pfr conversion of monomeric phytochrome at 1.2 μm wavelength. We did a comparison of linear and nonlinear conversion of truncated DrBphP bacterial phytochromes. This work provides a structured understanding of the optical properties of the dimer and monomer of phytochrome as well as their potential for use in optogenetics.

Keywords: monomer, dimer, phytochrome, tunable ultra-short pulsed laser, two-photon photoconversion, nonlinear conversion, optogenetics, Pr state, Pfr state, iRFP

1. INTRODUCTION

Early diagnostics and treatment of neurological diseases are great challenges for modern societies with the ageing (> 65 yrs.) population soaring in many countries from 6% up to 28% [1]. Therefore, many scientists are looking for advanced methods to ease the burden on National Health Systems. One of the possible solutions is to adopt optogenetic techniques in dementias research, which could offer a significant advancement in medicine. The current challenge for the therapeutic use of optogenetic tools is the delivery of enough power through biological tissues for actuation of target proteins (opsins/phytochromes) expressed in neural cells. In order to overcome these difficulties, long-wavelength phytochromes are particularly remarkable for scientific study because of their ability to be reversibly converted with linear and nonlinear light absorption in spectral ranges with a higher tissue transparency. Phytochromes represent a group of photoreceptors that could make feasible applications of optogenetics in the near-infrared spectrum.

In this study, we first demonstrate the linear and nonlinear photoconversion of wild-type dimeric *Deinococcus radiodurans* (DrBphP) bacterial phytochrome and a comparison of optical properties with its monomerized variant. Phytochromes found in bacteria are usually dimers, and it is still unclear if reversible Pr↔Pfr conversions require photoactivation of both monomeric parts. Determining the optical properties after light absorption into just one dimer's monomer, two times smaller, is therefore an important task for the development of optogenetic tools [2].

2. MATERIALS AND METHODS

For all experiments, dimeric and monomeric variants of bacterial phytochromes DrBphP-PCM (Photosensory Core Module) with a concentration of 60 μM were used. The PCM consists of the PAS, GAF, and PHY domains and the linear tetrapyrrole biliverdin, which is located in the pocket of GAF domain and covalently attached to the N terminus of the PAS domain, as a chromophore. The detailed description of monomer and dimer of DrBphP-PCM bacterial phytochromes are shown in [3, 4].

In our experiments, we measured the absorption spectra of dimeric and monomeric phytochromes after linear and nonlinear Pr↔Pfr conversion. The linear photoconversion was performed using 660 nm and 780 nm LEDs directed to a cuvette with a phytochrome sample.

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The absorption spectra of the monomer and dimer were carried out using a halogen lamp operated in a limited wavelength range of 550-850 nm. The beam was directed to a 1 mm sample cuvette and focused into a spot of 30 μm diameter. The transmitted light then was focused and collected by a collimator and recorded using a high-resolution spectrometer (Ocean Optics HR4000CG-UV-NIR). During the experiments, the cuvette was left open to allow a diffusion of the sample.

For nonlinear Pr→Pfr conversion, we used a Titanium: Sapphire laser pumping an optical parametric amplifier which generated 50 fs laser pulses at 1.2 μm wavelength with a repetition rate of 4 kHz. In our study, we tuned a wavelength from 1180 nm to 1360 nm and average power from 1 to 9 mW. The laser beam was collimated and aligned with the halogen lamp beam, the spot size for the laser beam was 40 μm in diameter. In dark conditions, phytochrome samples were photoconverted to Pr state after light illumination with a wavelength of 780 nm for 2 minutes. Then sample was illuminated by ultra-short pulsed laser operating at 1.1-1.3 μm. After 30 seconds, the halogen beam was unblocked to immediately measure the absorption spectrum. The schematic view of the experimental setup is shown in Figure 1.

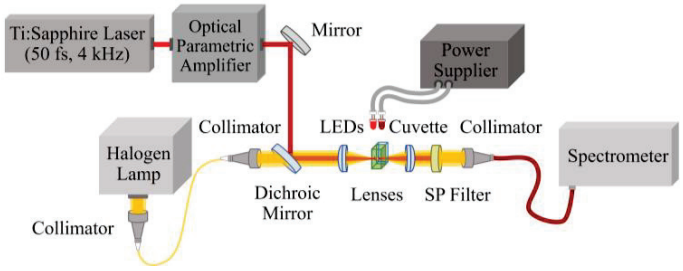

Figure 1. Schematic view of an experimental setup for measuring the absorption spectra of phytochrome samples.

3. RESULTS

The mechanism of linear photoconversion is similar for dimeric and monomeric phytochromes since both have linear tetrapyrrole biliverdin, which changes cis–trans isomerisation due to near-infrared light absorption. The far-red absorbing (Pr) and the red absorbing (Pfr) spectral states are photoreversable. Figures 2a and 2b demonstrate the absorption spectra of monomeric and dimeric DrBphP-PCM bacterial phytochrome, respectively.

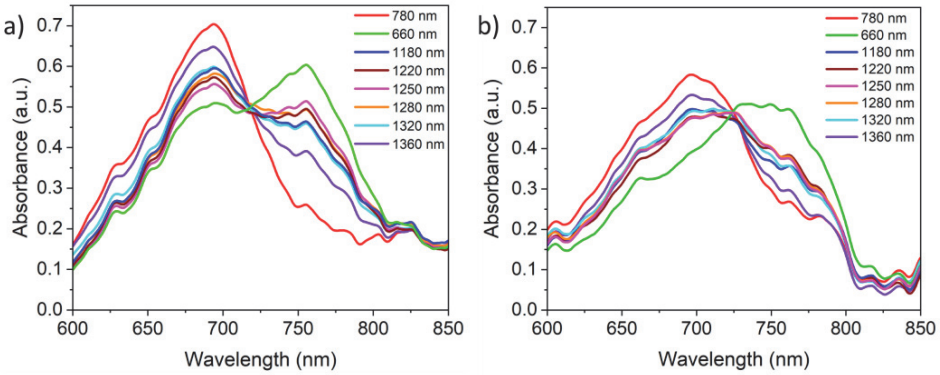

Figure 2. Linear Pr↔Pfr conversion and two-photon conversion from Pr to Pfr state after absorbing of different wavelengths: (a) monomeric and (b) dimeric DrBphP-PCM bacterial phytochromes.

The absorption spectra of the Pr state (red line) were measured after 2 minutes illumination of the sample by 780 nm LED. For both monomeric (Fig. 2a) and dimeric (Fig. 2b) variants of DrBphP, the absorption peaks are at a wavelength of 700 nm. The absorption spectra for monomer and dimer samples after two minutes of irradiation with a 660 nm LED (Pr→Pfr conversion) are shown by the green line in Figures 2a and 2b, respectively. Both monomeric and dimeric phytochromes have a maximum absorption peak at 750 nm wavelength.

Our experimental results showed that the dimeric and monomeric variants of DrBphP bacterial phytochrome have similar optical properties regarding the linear absorption of light. Both samples demonstrated reversible photoconversion between Pr and Pfr states and fluorescence at the wavelength of 720 nm by absorbing light at 450 nm or 630 nm.

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Therefore, even when a phytochrome is truncated preventing the monomer from its dimerization it keeps its photoreversibility.

For nonlinear Pr→Pfr conversion, we used an ultra-short pulsed laser with a tunable wavelength from 1180 to 1360 nm and average power of 4 mW (fluence of 283 mJ/cm^2). The absorption spectra after two-photon conversion using different wavelengths for monomer and dimer are shown in Figures 2a and 2b, respectably. Spectra of both samples show a decrease in the 700 nm peak and an increase in absorbance at a wavelength of 750 nm. These changes prove that two-photon absorption stimulates isomerisation to trans-configuration. For the dimeric phytochrome, absorption at 1360 nm (violet line in Fig. 2b) results in a 20% Pr→Pfr conversion based on comparison with the absorbance spectra of DrBphP after a linear conversion of the Pr and Pfr state (red and green lines). The best result for nonlinear conversion (54%) was demonstrated by the use of a 1220 nm wavelength laser source (dark red line in Fig. 2b). For monomeric phytochrome, the highest percent of Pr→Pfr conversion (62%) was shown after absorption of 1250 nm wavelength light (magenta line in Fig. 2a). We also demonstrated that the Pfr yield can be increased to 72% for monomeric and 55% for dimeric variants with the laser fluence of 636 mJ/cm^2, however, it would not be further improved by increasing the irradiation time or intensity.

Our experiments have shown that even the fluence of 78 mJ/cm^2 and 6.3 mJ/cm^2 are enough to start the process of transferring the monomeric and dimeric DrBphP-PCM phytochrome from Pr to Pfr states. Similar results were obtained in another study of dimer *Deinococcus deserti* (DdPCM) bacterial phytochrome [5], where two-photon conversion of phytochrome was examined using a femtosecond pulsed laser (fluence of 18 mJ/cm^2) operating at 1190 nm wavelength. They showed that the dimer variant could be potentially two-photon converted under an fluency of 1 mJ/cm^2. Therefore, dimeric phytochromes can be more easily transferred to the Pfr state, since the two monomers in the dimeric variant, help each other during the transition from one state to another by two-photon absorption. However, all the energy is shared between these monomeric parts, and when the absorbed energy reaches a level sufficient to initiate an independent non-linear Pr→Pfr conversion of one monomer, the monomeric DrBphP demonstrates a higher percentage of conversion to Pfr yield than dimeric phytochrome with the same concentration. These results prove the potential benefits of using monomeric phytochromes for optogenetics.

4. CONCLUSION

The two-photon photoconversion of monomeric and dimeric variants of a DrBphP bacterial phytochrome was demonstrated for the first time. This study shows a comparison of the efficiency of different wavelengths for the nonlinear Pr→Pfr conversion of phytochromes, as well as threshold intensities for triggering two-photon photoconversion and achieving maximum Pfr yields for both variants. We also investigated optical properties such as linear reversible conversion and fluorescence of samples, analyzed and discussed the uniqueness of monomeric and dimeric phytochromes.

Nonlinear conversion could significantly increase the spatial resolution for precise optogenetic stimulation, since the 1.1-1.3 μm wavelengths utilised for two-photon absorption provide better penetration into skin, skull and brain. The ability of monomer to be nonlinearly converted by II near-infrared laser sources facilitates the development of new *in vivo* optogenetic applications.

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