Possible Roles of Near-infrared Light on the Photosynthesis in Synechocystis sp. PCC6803 under Solar Simulating Artificial Light

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Solar simulating light (SSL) has been widely used for evaluating the performance of photovoltaic cells and algal photosynthesis. Green plants and algae utilize chlorophylls, thus, the chlorophyll-targeting light components mostly contribute to photosynthesis. In contrast, near infrared (NIR) light hardly energizes photosynthesis. Since SSL spectrum covers a wide range of light from ultraviolet to NIR, we examined the roles of NIR components in SSL during photosynthetic O2 evolution in Synechocystis (sp. PCC6803), by selectively and step-wisely eliminating the NIR using several NIR-cut filters. Here, the effects of intact SSL spectrum and the NIR-cut filtered SSL spectra (lacking NIR light greater than 690, 710, 750, or 810 nm) were examined. We observed that the 750 nm shortpass filter lowered the maximal photosynthetic velocity (\(P_{\text{max}}\)), whereas no significant change was observed with the 810 nm shortpass filter. We concluded that the 750–810 nm band may contain the photosynthesis-stimulating NIR component acting differently from the known phenomenon (Emerson effect). In contrast, Synechocystis unexpectedly regained the photosynthetic performance by eliminating all range of NIR (>710 nm), suggesting that 710–750 nm far-red band corresponding to the absorption band for bacterial phytochrome is possibly inhibitory to photosynthesis.

Keywords: cyanobacteria, hill equation, near infrared, oxygen electrode, photosynthesis, solar simulating light

INTRODUCTION

Solar simulators were designed to emit the light with spectral features mimicking the sunlight by combining the xenon lamp-based light sources and various optical filters. Solar simulating light (SSL) has been frequently employed for evaluating the performance of photovoltaic cells (Senadeera et al., 2003) or photocatalysts (Yu et al., 2009; Sakai et al., 2016). Since the photovoltaic cells and photocatalysts are mostly sensitive to ultraviolet (UV) and visible (VIS) lights regions, most SSL are designed to reproduce the spectra of solar ray in these regions. However, spectral features of most SSL developed to date, failed to reproduce the NIR spectrum found in solar ray as shown in Fig. 1.

For studying the natural photosynthesis, several groups employed SSL in place of natural sun light (Nagasawa et al., 2015). Photosynthesis in green plants/algae utilizes the VIS light, after harvesting photons with antenna pigments such as chlorophylls (Chls). Therefore, the bands of light corresponding to the absorption by chlorophylls (i.e. 665 nm and 430 nm for Chl a and 465 nm and 645 nm for Chl b) mostly contribute to photosynthesis. In contrast, light components not targeting such pigments hardly energize the photosynthesis (while there are recent intensive debate on the roles for green colored light in photosynthesis; Terashima et al., 2009). Although both the spectra of solar ray and SSL ranging between 400 and 700 nm shows broad intensive peaks, the action spectra for photosynthesis suggests that only limited bands reflecting the absorption spectra for Chls are utilized by photosynthesis (Nagasawa et al., 2015). Interestingly, when the plants were exposed to Chl-targeting red light together with the light with wavelength greater than 680 nm (peaking at around 700 nm), higher rate of photosynthesis can be induced (as known as Emerson effect) (Govindjee et al., 1960). In the present study, we examined the roles of NIR components in SSL during photosynthetic O2 evolution in Synechocystis sp. PCC6803, by selectively and step-wisely eliminating the long wavelength light components using several NIR-cut shortpass filters.

MATERIALS AND METHODS

Photosynthetic bacteria

As a simplest model photosynthetic organism, a cyanobacterium would be one of the best materials since the photosynthetic apparatus in this organism is simple but equivalent to the one found in algae and terrestrial plants (Jarvis, 2004).

Among cyanobacteria, Synechocystis sp. PCC6803 was chosen since it is one of the most widely studied strains capable of growth under both autotrophic and...
heterotrophic conditions (Ohkawa et al., 2000). In addition, this strain was used as a model for studying the evolutionary emergence of photosynthetic eukaryotes through symbiosis development between cyanobacteria and protzoa (Ohkawa et al., 2011). Cells of PCC6803 were precultured in BG-11 medium (at 23 ± 1°C) under a continuous fluorescent natural-white light at ca. 3000 lux (which is equivalent to 29.6 μmol m⁻² s⁻¹). Culture was renewed at 2-week intervals.

Prior to experiments, cells were harvested by centrifugation at 3,000 rpm for 10 min, then re-suspended in fresh medium and the optical density (OD) of the cell suspension was monitored at 680 nm by a spectrophotometer UV-1800 (Shimadzu Co., Kyoto, Japan). OD was adjusted to be 1.0, at which cellular density can be prepared to be approximately at 10⁸ cells/ml. The cell suspension was then kept in darkness for 30 min.

Irradiation by SSL

The light source used was a solar simulator (Cell Tester YSS-50, Yamashita Denso, Tokyo). The spectrum of the light source was measured using a portable NIR field spectro-radiometer (FieldSpec HandHeld 2, ASD Inc., Atlanta, GA, USA). Spectral pattern found in SSL emitted by the above equipment was highly similar to the shape of the spectrum of sunlight especially in the UV-VIS regions while NIR spectra largely differed between the solar light and SSL (Fig. 1b). Four NIR-cut shortpass filters removing greater wavelength range of light (>690, 710, 750, or 810 nm) (Asahi Spectra Co., Ltd., Japan) were used and their performance was confirmed with a spectral irradiance meter (KONICA MINOLTA, Tokyo, Japan).

The intensity of the light was scored as photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹). The light intensity for illuminating the algal cells was adjusted by inserting the the layers of black-colored plastic meshes (pore size, 1 mm) between the light source and the algal samples.

Monitoring of dissolved O₂ concentration in the cyanobacterium culture

For monitoring the evolution of O₂ from the cyanobacterium culture, a Clerk-type oxygen electrode (Oxygraph Plus System, Hansatech Instruments Ltd., Norfolk, United Kingdom) was used with some modification of the oxygen electrode chamber as previously described (Nagasawa et al., 2015.). The electrode chamber was covered with black cardboard with an opening (optical window; diameter, 10 mm). Instead of a plastic lid of the electrode chamber, a transparent acrylic plug was used as the optical window, so that SSL radiation directly reaches the level of algal cell suspension through the optical window. For each measurement of O₂ evolution by living cells, except for dark control, the cell suspension was subjected to a dark cycle of 300 s followed by a light period of 600 s.

Kinetic handling of light response curves (PI-curves)

Platt and Jasby (1976), British marine ecologists, have selected an equation similar to Michaelis-Menten equation (MME), as the most reliable photosynthetic equation, after examining a variety of equations. MME was originally designed for analysis of the velocity (V) of enzyme reaction as a function of substrate concentration ([S]) (Michaelis et al., 1913). In MME, two key factors, namely, maximal V (Vmax) and Michaelis constant (Km) are emphasized. By analogy, Platt-Jasby equation (PJE) is consisted of MME-like formalism as shown below (eq. 1). In fact, most PI-kinetic models are derivatives of MME. Today, MME-derived PJE remains the standards for generation of PI-curves in various algae and phytoplankton (Marra et al., 1985; Nagasawa et al., 2015), and also in higher plant communities (Okamoto et al., 2016).
where $P$ is the rate of gross photosynthesis, $P_{\text{max}}$ is maximal photosynthetic rate, $J$ is the intensity of light, and $K_j$ is the Michaelis constant for light intensity.

Determination of coefficients

We have recently performed eco-toxicological simulations of the lethal impacts of contaminating chemicals based on limited data size or data points from model experiments using the cells of green paramecia (Takaichi and Kawano, 2016). In this approach, the problems with apparently incomplete curves for toxicity response due to limited experimental data points was overcome by using practically arranged Hill-type equation assisted by curve fitting with graphical elucidation of Gauss-Newton algorithm (GEGNA), enabling the determination of LC$_{50}$ values and Hill coefficient (Takaichi and Kawano, 2016). By definition, Gauss-Newton algorithm iteratively finds the value of the variables which minimizes the residual sum of squares (RSS) as often applied to determination of variables (V$_{\text{max}}$ and K$_{m}$) for MME (Björck, 1996). RSS is the sum of the
squares of the difference between the recorded and the simulated values as shown below (eq. 2).

\[ RSS = \sum_{i=1}^{n}(y_i - f(x_i))^2 \]  

(2)

By analogy, PJE was converted to Hill-type equation as below (eq. 3)

\[ \frac{P_{\text{max}}}{K_j} \cdot \frac{J^x}{J^*} \]

(3)

where \( \alpha \) is Hill coefficient and other variables are identical with PJE. Here, modified PJE (eq. 3) assisted by GEGNA was applied to analyze the photosynthetic performance of PCC6803 cells.

RESULTS AND DISCUSSION

Effect of light intensity on \( O_2 \) evolution

Typical results for measurements of light intensity-dependent \( O_2 \) evolution under SSL are shown (Fig. 2). Here, the effects of intact SSL spectrum and the NIR-cut SSL spectra (lacking NIR light greater than 810, 750, 710, or 690 nm) were compared (Fig. 3). Then, key parameters (\( P_{\text{max}} \) and \( K_j \)) for photosynthetic curves expressed by eq. 3 were compared (Fig. 4). Notably, we observed that the application of the 750 nm shortpass filter resulted in lowered \( P_{\text{max}} \) and \( K_j \) whereas no significant change was observed with the 810 nm shortpass filter (Fig. 4). We concluded that the 750–810 nm band may contain the photosynthesis-stimulating NIR component acting differently from the Emerson effect.

The \( K_j \) value employed here is analogous to Michaelis constant in the enzymatic Michaelis-Menten’s equation. By definition, alteration of this value may reflect the change in sensitivity to light intensity. Therefore one may expect that the lowered \( K_j \) in the presence of NIR component (\( \geq 750 \)) is reflecting the sensitization to lower light, however, the obtained curves with and without removal of the NIR component (\( \leq 750 \)) are almost overlapping in the lowrange of light intensity (up to ca. 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \); Fig. 3c). It is conclusive that lowered \( K_j \) could be simply worked out as \( P_{\text{max}} \) is significantly lowered in the presence of NIR component (\( \geq 750 \)). Therefore, we would like to propound that the \( P_{\text{max}} \) value is the key target of the inhibitory action of the 750–810 nm NIR light component.

In the present study, we focused on the impact of specific band of NIR light on the Michaelis-Menten-like kinetics (Platt-Iasby kinetics) for PI-curve, especially the changes in \( P_{\text{max}} \) and \( K_j \). On the other hand, as far as we know, the experimental demonstration of Emerson effect do not focus on the maximal photosynthetic values (such as \( P_{\text{max}} \) or \( K_j \)). The so-called red-drop phenomenon could be less obvious as the intensity of the light increases approaching the \( P_{\text{max}} \) level. Usually, demonstrations of Emerson effect are made under the fixed intensity of the pared light sources (Govindjee et al., 1960). Therefore, our experimental design is not suitable for performing or confirming the Emerson effect.

In contrast to the negative effect of 750 nm-shortpass filter describe above (Fig. 3c) performed in the step-wise series of NIR removal, Synechocystis cells regained the photosynthetic performance by eliminating the most NIR (wavelengths, \( \geq 710 \) nm; Fig. 3b), suggesting that 710–750 nm band may include NIR components inhibitory to photosynthesis possibly corresponding to the absorption band for bacterial phytochrome (Igamberdiev et al., 2014).

At present, preliminary data were obtained as shown above. We need to further examine the phenomena in the future research.

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