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Hyperspectral Analysis on the Photosynthetic PS-II system: Study on *Chlamydomonas reinhardtii*

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Abstract

This paper presents a new approach to studying the nano-sac or nano-site of photosystem components in photosynthesis. Instead of analyzing bulk colonies of cells or using complicated biological pigment isolation procedures, hyperspectral characterization is used to investigate individual algal cells. This method eliminates the need for bulk characterization and allows for a more precise analysis of the PS-II nano-sac/nano-sites. The results of the hyperspectral studies using Cytoviva™ are presented in this paper.

Introduction

Photosynthesis is a natural organic process seen in all plants and green algae. Photosynthesis is a complex biochemical process which involves fixing atmospheric carbon dioxide as a sugar complex with the evolution of oxygen back to the atmosphere. The solar energy spectrum catalysis this photosynthesis process through their Photosynthetically Usable Radiation (PUR). The simplified natural photosynthesis can be expressed as equation (1).

$$\underset{[Carbondi\ Oxide]}{6CO_2} + \underset{water}{6H_2O} \xrightarrow{light} \underset{sugar\ complex}{C_6H_{12}O_6} + \underset{Oxygen}{6O_2\uparrow} \quad (1)$$

Although equation (1) represent the photosynthesis reaction, the underlying mechanism are complex with several intermediatory energy transfer stages. Photosynthesis is a complex process involving reaction centers (RC) in chlorophyll pigments. These small sites capture light energy, which raises the photosynthetic pigment to a higher energy level. The energy is then transferred to lower energy levels, leading to redox chemical reactions that reduce NADP to NADPH and release oxygen molecules. This is a crucial process for plants and other photosynthetic organisms, allowing them to convert light energy into usable chemical energy. Scientists have been studying the mechanisms of photosynthesis for decades to understand this fundamental process better.

At this juncture, the photosynthesis is resolved into two photosystems (PS), PS-I and PS-II, extracting energy at two different wavelengths. Usually, PS-II harvests a higher energy level at a lower spectrum wavelength, and PS-I harvests a lower one at a higher wavelength. Initially, understanding of the PS includes the presents of only one machinery extracting energy at a predominant wavelength; Several works on chlorophyll in higher order plants and algae concluded the presence of PS-I as postulated a bulk dispersed chemical complex. But with the understanding of the z-scheme of energy flow in photosynthesis, the presence of nano-sacs or nano-sites of PS-I is established. The presence of the PS-I in lower-order photosynthesis of algae and plants is still debated. The author believes the evolution of the PS-II system marks an addition to the fine line between the lower-order and higher-order plants. In addition to the presence of the PS systems, there is also the presence of Light Harvesting (LH) antennas which improve the photosynthesis efficiency with respect to the available wavelength spectrum. The characterization of these LH systems is species-dependent, and the nutrient availability during the growth [1]–[7]. This paper is concerned with characterizing the PS-II system using hyperspectral imaging of freshwater photosynthetic algae. The algae species of Chlamydomonas Reinhartii is used for this study due to their versatility of growth in the laboratory and the better distinction of the PS-II system in their chlorophyll.

Hyperspectral imaging is a technique that uses light to visualize the chemical and mechanical properties of nanoparticles and sites. Unlike other imaging techniques, such as SEM and FTIR, hyperspectral imaging allows for the simultaneous visualization of these properties. Particles can be characterized on a pixel-by-pixel basis [1], [8], [9] by using a light source of a specific wavelength. This technique requires expertise, but it allows for individual cellular study rather than only studying the bulk response of colonies. Hyperspectral imaging can also study live cells without complex biological pigment isolation procedures, providing a more accurate representation of in-cell pigment performance.

Methods And Procedures

The following procedure and protocols are performed at Optical Bio-Microsystems Laboratory at Concordia University to grow and characterize the freshwater algae (*Chlamydomonas Reinhartii*).

1.1 Algal Growth and harvest

The algal strain CC-125 is grown for harvest at laboratory-controlled conditions for three weeks. Due to the strain's superior growth factor, three weeks of growth yield favourable colony density. These harvested strains are suspended in pH-neutral TAP (Tris-acetate-phosphate medium) nutrient media for three days to reach the exponential growth phase of multiplication before the commencement of the study [10]. The algal strains are monitored at 24-hour intervals for any contamination and abnormalities. Upon successful contamination-free growth in the exponential phase (UV-Vis absorption >1; three days of TAP suspension), the algae are subjected to hyperspectral imaging.

1.2 Microscope algal fixing

Simplified live algal cell fixation is used in this study. 6.5 µl of suspended algae are placed in a microscopic slide and covered with a 25x25mm slide cover to fix the algae to the microscopic sides. A simple chemical-free fixing method is preferred to eliminate any additional spectral signal due to the fixing agent. The cover is sealed with nail polish along the corners for archiving.

1.3 Hyperspectral imaging

The hyperspectral imaging is performed using Cytoviva™ at 60x and 100x magnification. A halogen lamp of a broad visible spectrum is used for this study. Maximum light intensity is allowed across the sample at an exposure of 300ms. The focus point is maintained at high intensity possible (maximum aperture size) with low exposure time. The alternative method of minimum aperture size with high exposure time (>2s) failed to provide clear output signals. The hyperspectral image is analyzed using the ENVI software package.

Results And Discussion

Figure 1 represents the hyperspectral images of Chlamydomonas Reinhartii samples at day 3 growth. The inserted red circles represent the clear light harvesting spot apparatus.

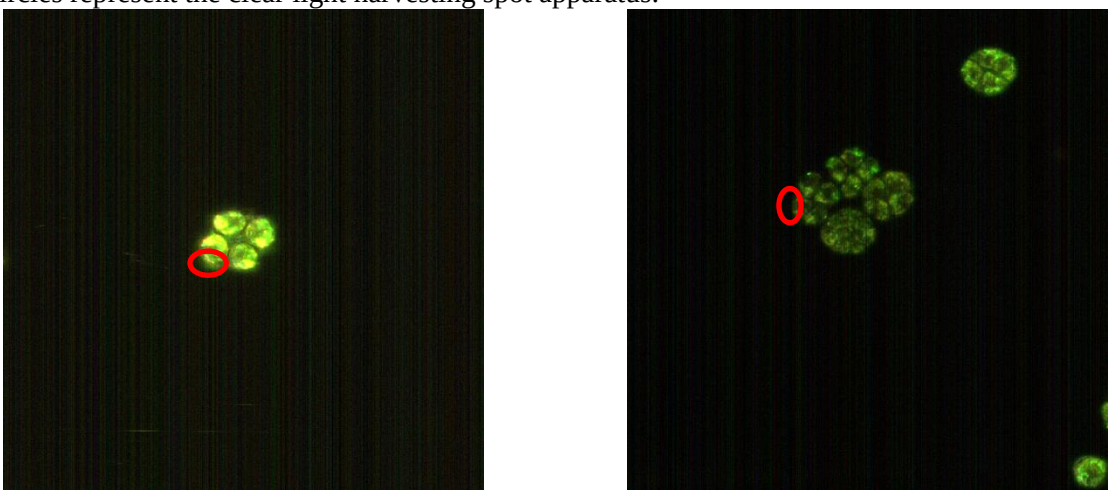
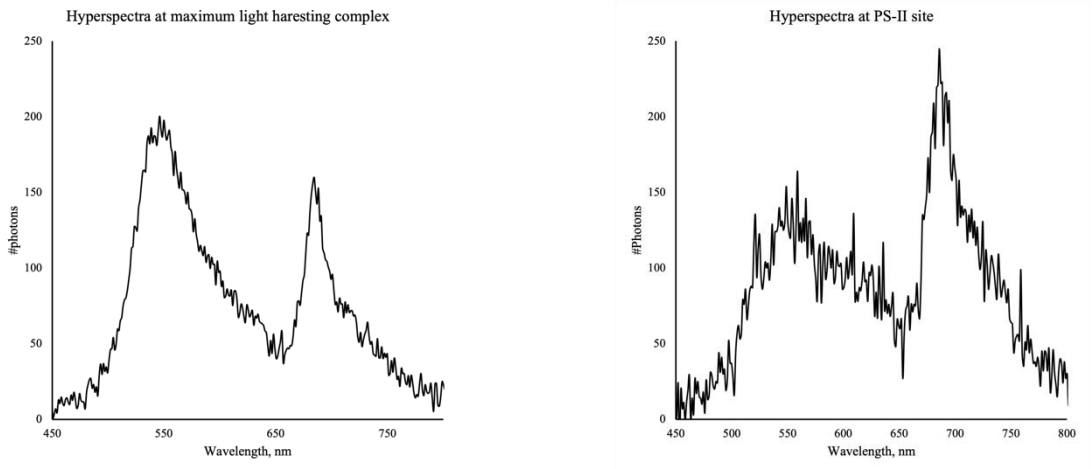


Figure 1: Hyperspectral image of Chlamydomonas Reinhartii at exponential growth phase: Day 3 of growth, exposure time 300ms at maximum photon count >200/pixel

The spectral response of the light-sensitive photosystems in live Chlamydomonas Reinhartii is shown in Figure 2.



(a) (b)

Figure 2: Spectral responses (a) at the maximum light-harvesting pigments (shown in red circles on Figure 1) and (b) at the PS-II pigment

The significance of PS systems in photosynthesis is highlighted by the results shown in Figure 1 and Figure 2. The spectral response indicates that the maximum absorption correlates to a peak wavelength of around 550nm, which is critical as it is the starting point of the energy flow chain in photosynthesis. This short wavelength (550nm) provides the energy required to create the special pair (Qa) in the photosynthesis chain within the PS-II system. The occurrence of a strong peak at 550nm and another at 680nm is observed repeatedly across various samples. We analyzed two separate sample growths and validated the spectra with their peaks for this study. These findings demonstrate the vitality of hyperspectral imaging in the analysis of photosynthesis.

Conclusion

Based on our research using hyperspectral analysis of live algal cells, we have found evidence supporting the existence of PS-II systems at approximately 680nm. This method of examining photosynthesis pigments provides a more streamlined

approach than traditional biological methods. However, further research is necessary to distinguish the PS-I peak at wavelengths exceeding 700nm. Our findings indicate that Cytoviva™ could be an invaluable tool for investigating photosynthetic organisms in academic research.

Reference

[1] K. Kuruvinashetti, S. Pakkiriswami, and M. Packirisamy, "Gold Nanoparticle Interaction in Algae Enhancing Quantum Efficiency and Power Generation in Microphotosynthetic Power Cells," *Adv. Energy Sustain. Res.*, vol. n/a, no. n/a, p. 2100135, doi: 10.1002/aesr.202100135.

[2] K. Kuruvinashetti, S. Pakkiriswami, and M. Packirisamy, "Algal Extracellular Vesicles for Therapeutic Applications," in *2020 IEEE 20th International Conference on Nanotechnology (IEEE-NANO)*, Jul. 2020, pp. 354–357. doi: 10.1109/NANO47656.2020.9183452.

[3] M. D. Archer and J. Barber, *Molecular To Global Photosynthesis*. World Scientific, 2004.

[4] I. Pottosin and S. Shabala, "Transport Across Chloroplast Membranes: Optimizing Photosynthesis for Adverse Environmental Conditions," *Mol. Plant*, vol. 9, no. 3, pp. 356–370, Mar. 2016, doi: 10.1016/j.molp.2015.10.006.

[5] L. Nikkanen, D. Solymosi, M. Jokel, and Y. Allahverdiyeva, "Regulatory electron transport pathways of photosynthesis in cyanobacteria and microalgae: recent advances and biotechnological prospects," *Physiol. Plant.*, vol. n/a, no. n/a, doi: https://doi.org/10.1111/ppl.13404.

[6] P. Darvehei, P. A. Bahri, and N. R. Moheimani, "Model development for the growth of microalgae: A review," *Renew. Sustain. Energy Rev.*, vol. 97, pp. 233–258, Dec. 2018, doi: 10.1016/j.rser.2018.08.027.

[7] J. S. Guest, M. C. M. van Loosdrecht, S. J. Skerlos, and N. G. Love, "Lumped Pathway Metabolic Model of Organic Carbon Accumulation and Mobilization by the Alga Chlamydomonas reinhardtii," *Environ. Sci. Technol.*, vol. 47, no. 7, pp. 3258–3267, Apr. 2013, doi: 10.1021/es304980y.

[8] A. S. Schwartz-Duval *et al.*, "Intratumoral generation of photothermal gold nanoparticles through a vectorized biomineralization of ionic gold," *Nat. Commun.*, vol. 11, no. 1, Art. no. 1, Sep. 2020, doi: 10.1038/s41467-020-17595-6.

[9] G. Elmasry, M. Kamruzzaman, D.-W. Sun, and P. Allen, "Principles and Applications of Hyperspectral Imaging in Quality Evaluation of Agro-Food Products: A Review," *Crit. Rev. Food Sci. Nutr.*, vol. 52, no. 11, pp. 999–1023, Nov. 2012, doi: 10.1080/10408398.2010.543495.

[10] K. Kuruvinashetti, S. Rahimi, S. Pakkiriswami, and M. Packirisamy, "Simple, Economical Methods for the Culture of Green Algae for Energy Harvesting from Photosynthesis in a Microfluidic Environment," *Curr. Protoc.*, vol. 1, no. 12, p. e322, 2021, doi: 10.1002/cpz1.322.