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Power Scavenging and Optical Absorbance Analysis of Photosynthetically Active Protoplasts

Plants and photosynthetic bacteria hold protein molecular complexes that can efficiently harvest photons. This article presents fundamental studies to harness photochemical activities of photosynthetically active protoplast extracted from Arabidopsis plants. The conversion of photonic energy into electrical energy was characterized in the presence and absence of light. The photoinduced reactions of photosynthesis were measured using a patch clamp measurement system at a constant voltage. The optical characterization was also performed on the extracted protoplast. It showed absorption bands at a number of wavelengths. The current–voltage measurements done on protoplast extracts showed two orders of magnitude increase in current from dark to light conditions. The absorbance measurements showed very large bandwidth for extracted protoplasts. The analysis of the optical data measurements showed that protein complexes obtained from photosynthetic cells overcame the limitation of traditional organic solar cells that cannot absorb light in the visible-near infrared spectrum. The demonstration of electrical power scavenging from the protoplast of the plant can open avenues for bio– inspired and bio-derived power with better quantum electrical efficiency. [DOI: 10.1115/1.4007657]

Keywords: photosynthesis, Arabidopsis, absorption, protoplast, solar energy, power scavenging

1 Introduction

Natural systems have very efficient energy carrying and conversion capabilities. The processes involved in the conversion of photonic energy into electrical energy have thus attracted considerable attention since the 70s [1]. In the past few decades, a lot of work has been focused on various applications of photosynthetic proteins in technology, ranging from light-induced electron transfer, sensing of herbicides, hydrogen production, etc. [2–5]. There are fundamental limits in harnessing photochemical activities that have hampered the use of natural systems in meeting the energy needs of humans. With ever-increasing use of energy, the current sources are becoming overburdened and depleted [6]. The urgent need for more efficient and economical alternatives, notably renewable energy sources, cannot be overemphasized [7]. It is thus important to develop nonconventional fuels. Environmental safety concerns are also often mitigated using alternative energy, in comparison to fossil or nuclear systems. Therefore, the use of organic molecules instead of chemicals is attractive and has gained a lot of attention in research.

The wavelength of solar electromagnetic radiation reaching earth's atmosphere ranges from 10^2 to 10^6 nm and above. Ultraviolet (UV) radiation (100–400 nm) is mostly absorbed by the atmosphere and very little hits the earth's surface [8]. Visible light spans over 400—700 nm whereas infrared (IR) spectrum extends from 700—5×10^5 nm. Visible-near IR (NIR) region (600–1000 nm) corresponds to more than 70% power of solar radiation and the conventional organic solar cells are not capable of absorbing light in this region due to their larger bandgaps [9]. Most organic semiconductors have bandgaps higher than 2 eV that limits the absorbed spectrum to $<$600 nm, consequently these can possibly absorb only 33% of total solar radiation [10].

This article focuses on using a natural resource that has evolved into one of the most efficient energetic systems to capture solar

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radiation, the most abundant form of energy on earth. Solar energy, a carbon-neutral energy source, is the ultimate and reliable key to the energy and environmental challenges [11,12]. Various applications of solar energy have been aggressively pursued for many years now [13]. Various studies have focused on improving energy conversion efficiencies and development of organic solar cells to use light-induced electron transfer [2,10,14,15]. Organic solar cells have quite a few advantages over traditional inorganic solar cells due to much lower production cost, flexibility and large area applications; however, the traditional organic solar cells have the limitation of not being able to absorb light in the visible and NIR region that make up the majority of the power (over 70%) of total solar radiation [16]. Plants and photosynthetic bacteria, on the other hand, contain molecular complexes that can efficiently harvest photons and overcome these limitations by absorbing light in the visible-NIR range [16]. Photosynthetic reaction centers harvest photons with a quantum yield that can exceed 95% [17,18]. However, in all photosynthetic systems, wasteful dissipation of the most of bright sunlight through nonphotochemical quenching is the primary reason for the lower-than-theoretical efficiency and productivity [15]. The work on photosynthetic proteins in light-induced electron transfer, sensing of herbicides, and hydrogen production, [3–5] can directly contribute toward achieving an alternate source of energy in a cleaner and greener fashion. The basic steps for converting solar energy to another form, electricity or biomass, can be described by promotion of electrons to higher energy levels, or excited states when photons are absorbed. The promotion of an electron into an excited state repositions it into new spatial and energy coordinates. In the process, it leaves a positive charge behind. In other words, the electron is reduced and positive charge left behind is chemically oxidized. In case of plants, the sunlight is absorbed by the photosynthetic antenna. Antenna later transfers the excited electrons to the reaction centers. We demonstrate an efficient capture and remarkable photonic efficiency of these photosynthetic protein complexes, extracted from *Arabidopsis* plant.

2 Materials and Methods

Arabidopsis thaliana, an angiosperm, is one of the most widely used plant for the study of a broad range of problems in development, metabolism, genetics, environmental adaptation, and pathogen interactions [19]. *Arabidopsis* belongs to the mustard family *Brassicaceae,* also known as *Cruciferae*, which is an extensively distributed family of approximately 340 genera and approximately 3350 species [15]. Although of no inherent economic significance, *Arabidopsis* with its small size, rapid life cycle, small and simple genome, prolific seed production and the accessibility of numerous mutant plant lines puts forward various advantages for rapid genetic and molecular analysis. This weed is generally self-pollinated and takes only 5–6 weeks from seed germination to the production of a new generation of seeds. It has thus, turned out to be the focus of the first plant genome project to realize the biology of a flowering plant at the molecular level [15]. In our work, we isolated *Arabidopsis* protoplast, cells without the cell walls, to study the photonic to electronic conversion efficiency of the photosynthetic protein complexes and as a model system to prove the concept that organic cells can be modified with photosynthetic complexes for higher efficiency than that for traditional inorganic cells.

2.1 Growing *Arabidopsis*. A soil mixture consisting of Redi-earth plug and seedling mix (Sun Gro, Bullevue, WA), fine vermiculite, and perlite in 1:1:1 (v:v:v) was prepared in controlled environment at 22 °C, 60±5% relative humidity, and a 12 h photoperiod under light intensity of 100 μmol/m^{2}s to grow the plants. For all experiments, four to six week old plants were used.

Specifically, pots were filled with the soil mixture, and soaked for 48 h in water. Soaked soil mixture was covered with a layer of dry vermiculite and finally covered with a screen mesh. Seeds were suspended in 0.1% agarose solution and sown in five spots of the pot. The pots were labeled and kept at 4 °C for two days.

Fig. 1 Growth of *Arabidopsis* after sowing (starting from left, weeks 1, 2, and 3)

After two days, the pots were moved to a growth chamber (Percival) and kept at the environmental conditions specified above. Figure 1 show the growth of *Arabidopsis* in 3 weeks after sowing.

2.2 Isolation of Protoplast. Protoplast was extracted from the leaves following a protocol described before, with slight modifications [20]. Briefly, approximately 50 young, fully expanded leaves were plucked carefully and blended in 100 ml of de-ionized water for 5 min. Then the blended solution was filtered using a 100 μm nylon mesh. The residues which consisted of epidermal and mesophyll cells were transferred to a flask that contained a mixture of 500 μl 0.7% cellulysin, 50 μl of 0.01% poly-vinyl-pyrrolidone, 500 μl of 0.25% bovine serum albumin (BSA), and 2.75 ml of 55% basic medium (0.5 mm $CaCl_2$, 0.5 mm $MgCl_2$, 5 mm MES, and 500 mm D-sorbitol). Then the mixture was incubated in dark for 3 h at 25 °C, at 0.78 × g (using Labnet orbit 1000) and filtered again through 100 μm nylon mesh. The residue was collected in a flask containing a mixture of 500 μl 1.5% cellulose, 500 μl of 0.03% Pectolyase, 500 μl of 0.25% BSA, and 2.95 ml of basic medium. This mixture was incubated in dark for 2 h at 18 °C at a speed of 0.38 × g. After incubation the mixture was filtered through four 100 μm nylon meshes and the filtrate was collected in a centrifuge tube. The filtrate was centrifuged at 1000 × g for 5 min. This process was repeated twice to isolate the protoplast. The extracted protoplast was stored in dark at 4–10 °C. The protoplast consisted of live cells and these easily died if exposed to too much light or heat (Fig. 2). Before the experiments, protoplasts were inspected under the microscope to make sure that the cells were alive as indicated by the integrity of the cells and organelles. The samples were used when more than 90% of the cells were alive.

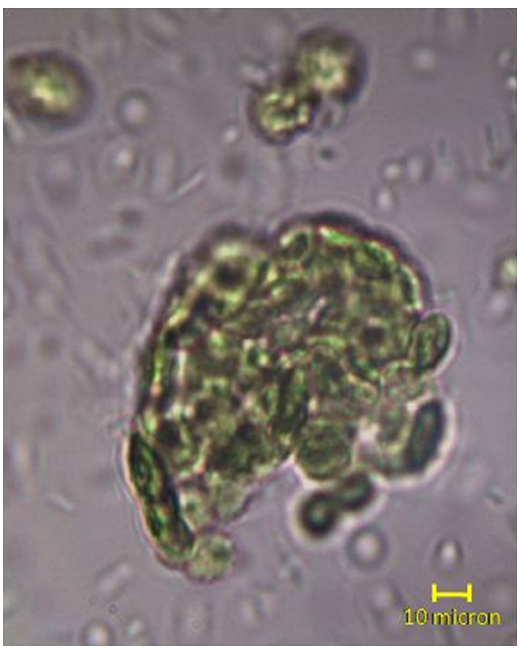

Fig. 2 Optical photomicrograph of intact and viable extracted protoplast

2.3 Optical Characterization of Extracted Protoplast. The protoplast extract of *Arabidopsis* was characterized for optical absorbance from 300 to 1000 nm. The absorbance through the protoplast suspension and the buffer solution was measured with a UV/VIS/NIR spectrometer (Lambda 9, Perkin Elmer, MA). UV WINLAB software was used to control the spectrometer. Three different measurements (protoplast suspension, buffer solution, and empty cuvette) were processed under the same conditions. The data of the empty cuvette and the plain buffer solution were used for background subtraction. The experimental measurements were done in terms of transmittance (T) which is the measure of light intensity after it passes through a solution. The absorbance of light in the protoplast extract solution is a complex function of the scattering and absorption coefficients. The relationship between absorbance (A) and transmittance (T) in % is defined by well-known Beer–Lambert law

$$A = -\log\left[\frac{I}{I_o}\right] = -\log\left[\frac{\%T}{100}\right] \quad (1)$$

where I is the intensity of the transmitted beam and I_o is the intensity of the incident light beam. An increase of a unit of 1.0 in A means transmittance is reduced by a factor of 10%.

2.4 Optical–Electrical Measurements. The *Arabidopsis* protoplast was used for optical to current conversion measurements. Current–voltage (I–V) measurements through protoplast solution were taken in the dark and under light. All the samples were tested 14 times under the same conditions. A LABVIEW program was developed interfacing with a digital multimeter for measurements. The current was measured at fixed voltage as well as by ramping the applied voltage from 0 to 75 mV. Figure 3 shows a block diagram of the measurement setup. Ag/AgCl electrodes (0.8 mm in diameter) were used to measure electrical data. The buffer solution was used as control. The control measurements consisted of I–V data through the buffer solution under the exact same conditions as the protoplast extract suspended in the same buffer solution. The measurement system was housed in an electrically and magnetically shielded enclosure placed on vibration-isolation table.

3 Results

The two samples, one with buffer solution only and the other with protoplast in the same buffer, were studied with I–V measurements. The electrical response of the plain buffer solution under light and in darkness is shown in Fig. 4. The data shows that when the voltage bias were applied through the buffer in dark, the current increased linearly as a function of voltage. It was ~10 nA at 75 mV. When the same experiment was repeated under the light, it showed a slightly higher current but within the same order of magnitude. On the other hand, the experiments on protoplast extract showed two-order increase in current (from dark to light) under the same bias conditions (Fig. 5). Compared with the photocurrent data from the plain buffer solution, the current was indeed primarily generated by the protoplast extracted from *Arabidopsis* when exposed to light, without minimal contribution from the buffer solution. The behavior of protoplast when exposed to light clearly shows that at 75 mV applied bias, a current of 338 nA is observed, which was around ~10 nA in case of dark conditions. If there was no green structure observed, the cells were dead and there was no change in current between dark and light conditions. Figure 5 shows electrical behavior of protoplast suspension in buffer solution in presence and absence of light.

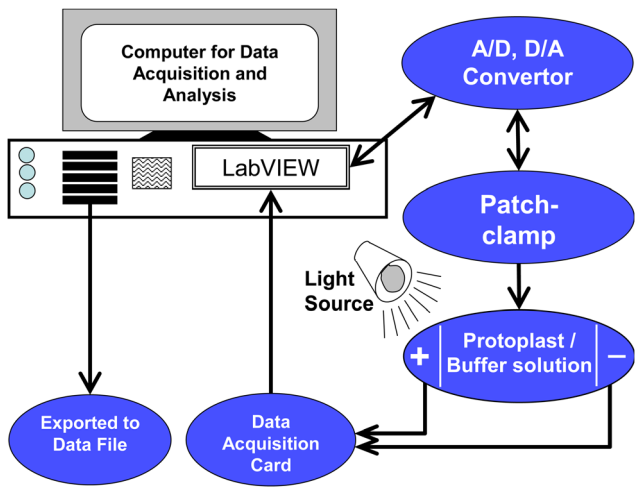

Fig. 3 Block diagram of the system designed for electrical measurements

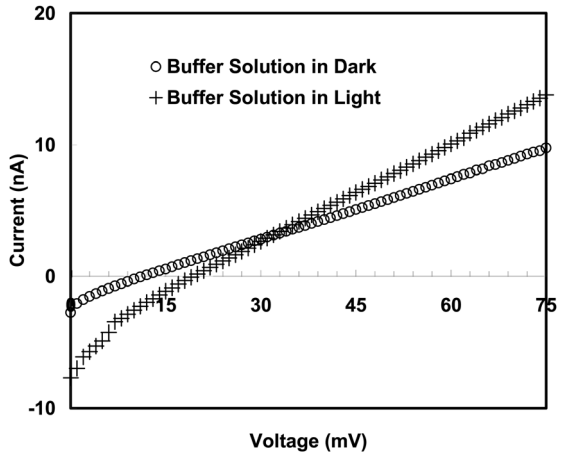

Fig. 4 *I–V* plot showing conductivity through buffer solution in the light and darkness

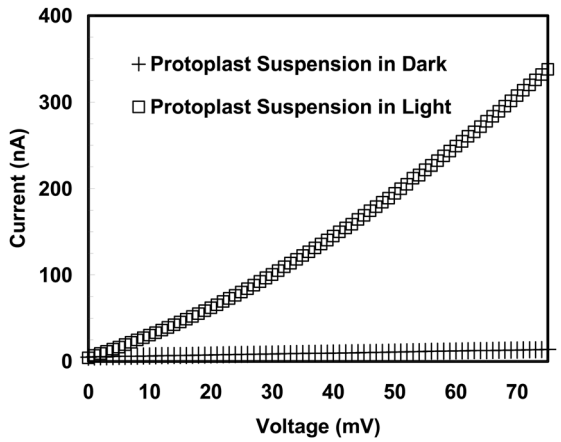

Fig. 5 *I–V* data showing behavior of the extracted protoplast suspended in buffer solution under ramping voltage in the dark and light conditions

For all the experiments ($n = 14$), data were analyzed for mean ± standard deviation. The data indicated that the standard deviation for buffer solution were higher than that of protoplast suspension. In the dark, buffer solution had much smaller standard deviation than that in the presence of light. The average of current values and standard deviations are shown in Table 1.

The density of protoplast per millimeter was calculated using hemocytometer. The average density was $\sim 1.96 \times 10^6$ #/ml (Table 2).

Figure 6 show the optical absorbance of the buffer solution and protoplast suspension at wavelength range of 300–1000 nm. The absorbance spectrum of protoplast demonstrated that the extracted protein complexes had very broad absorption band which enabled

Table 1 Mean ± standard deviation of current from 14 experiments performed independently

Voltage (mV)	Buffer solution in dark (nA)	Buffer solution in light (nA)	Protoplast suspension in dark (nA)	Protoplast suspension in light (nA)
0	−11.93 ± 18.40	−10.76 ± 9.01	4.74 ± 0.30	4.08 ± 0.27
5	−9.14 ± 16.12	−8.50 ± 10.13	5.72 ± 0.30	16.60 ± 0.27
10	−7.11 ± 13.86	−6.66 ± 11.05	6.31 ± 0.30	30.40 ± 0.27
15	−5.15 ± 11.47	−5.26 ± 11.00	6.89 ± 0.52	45.60 ± 0.27
20	−3.16 ± 9.08	−3.77 ± 10.40	7.25 ± 0.48	62.00 ± 0.27
25	−1.20 ± 6.68	−2.23 ± 9.48	7.83 ± 0.48	80.44 ± 0.30
30	0.60 ± 4.60	−0.57 ± 8.36	8.38 ± 0.41	100.75 ± 0.30
35	2.77 ± 1.74	1.16 ± 7.06	8.96 ± 0.30	122.35 ± 0.30
40	4.75 ± 0.72	2.89 ± 5.72	9.63 ± 0.30	145.05 ± 0.30
45	6.70 ± 3.09	4.61 ± 4.36	10.17 ± 0.30	169.05 ± 0.30
50	8.36 ± 5.00	6.48 ± 2.59	10.77 ± 0.30	194.55 ± 0.30
55	9.928 ± 6.85	8.37 ± 0.95	11.37 ± 0.30	221.65 ± 0.30
60	11.50 ± 8.80	10.21 ± 1.11	11.97 ± 0.30	248.95 ± 0.30
65	13.27 ± 10.86	12.25 ± 2.98	12.57 ± 0.30	277.82 ± 0.45
70	15.12 ± 12.96	14.17 ± 4.81	13.17 ± 0.30	307.73 ± 0.46
75	16.98 ± 15.07	16.15 ± 6.80	13.87 ± 0.30	338.59 ± 0.60

Table 2 Densities of protoplast suspended in buffer solution for experiments performed independently

Experiments #	Protoplast density (#/ml)	Experiment #	Protoplast density (#/ml)
1	1.66×10^6	8	2.01×10^6
2	1.86×10^6	9	2.11×10^6
3	1.89×10^6	10	1.96×10^6
4	1.98×10^6	11	1.95×10^6
5	1.87×10^6	12	2.20×10^6
6	1.84×10^6	13	1.85×10^6
7	1.99×10^6	14	1.83×10^6

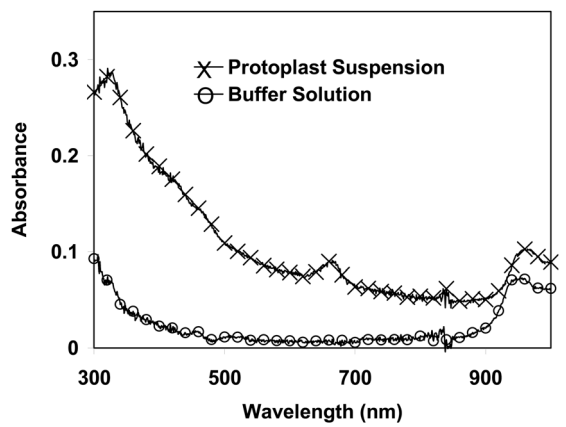

Fig. 6 Optical absorbance spectrum of buffer solution and protoplast suspension at wavelength from 300 to 1000 nm. The jump observed at ~840 nm is an artifact that occurred when the light source in the spectrometer changed.

it to absorb light even in the visible-NIR region (600–1000 nm). The absorbance peaks of the protoplast sample show that UV light was being absorbed at a greater quantity in the sample of *Arabidopsis* than in the buffer or cuvette. These peaks indicated higher absorbance at certain wavelengths.

4 Discussion

The results from *I–V* measurements indicated that the change in conductance of the buffer solution from dark to light was almost negligible compared to the change in the conductance of protoplast from darkness to light. When protoplast extract was exposed to light, photons were absorbed by protein complexes generating bound electron-hole pair (excitons) rather than free charges. These excitons which carried energy but no net charge diffused through the solution and were dissociated into electrons and holes possibly when these reached at the Ag/AgCl electrodes. These charges were collected at the respective electrodes—holes at anode and electrons at cathode, and were injected into the external circuit, resulting in an increase of output current [21]. Ideally all photogenerated excitons should reach a dissociation site in order to have maximum quantum efficiency. Thus their diffusion length should be large enough to reach the dissociation site otherwise they would recombine without contributing to the output current and thus photons would be wasted. Exciton diffusion ranges in polymers and pigments are usually around 10 nm [22]. Therefore, excitons generated close to electrodes contributed more to the total output current [21]. Second, excitons and charge transport usually need to hop from molecule to molecule in an organic material. Therefore, closely packed molecules provide rapid exciton transport channels due to more intermolecular overlap and the dense packing of molecules also promotes the absorption coefficient [23]. Hence, as shown in Figs. 4 and 5, we can conclude that light triggered a rapid sequence of events that included absorption of photons, generation and diffusion of excitons, charge separation and collection; resulting into a significant increase in the output current. Thus, the photosynthesis process can be efficiently used with our simple system to convert photo energy to electrical energy. These experiments were repeated multiple times, to confirm this phenomenon, with similar results.

The absorption of light by the chloroplast present in protoplasts promoted the electrons to the higher energy level. The charge left behind was chemically oxidized, once the electron was reduced [24]. In any photosynthesis process, chloroplast plays a key role in capturing the photons and converting them to electrical energy.

The absorbance peaks of the protoplast suspension showed that UV light was being absorbed at a greater quantity in the sample of *Arabidopsis* than the controls. The maximum absorbance represented by a peak of 340 nm showed that cryptochromes were the primary UV absorbers. The absorption by phytochromes can be represented by the peak of 669 nm. Also, it was predicted that the buffer solution should have behaved differently at the beginning because the ionic charges in the solution required time to stabilize before these started flowing in response to the voltage supplied. Statistical analysis confirmed this prediction for buffer solution, there were larger deviations in the buffer solution current as compared with protoplast suspension. The protoplast suspension showed very small deviation, almost negligible, in absence of light and even smaller deviation in the presence of light.

5 Conclusion

The results clearly indicate that plant protoplasts can be efficient, environmental friendly and cheaper alternates for energy needs. Though there are certain limitations such as integration of biomolecular complexes in solid state electronic devices and low quantum efficiency, yet low band gap (less than 1.1 eV) makes plants and photosynthetic bacteria viable options for better harvesting of sunlight that can lead to enhanced efficiency of organic solar cells. It is safe to conclude that exposure of protoplast to light cause a series of events including absorption of photons, exciton generation, exciton dissociation, and hence a collection of charges that results in an increase in the net current output.

Utilizing protoplasts as a natural and instant energy source can greatly impact our rapidly growing world, by providing natural energy. As protoplasts absorb light especially in UV region, it is chemically converted into energy; this energy can be harnessed and used for many electrical applications. Protoplasts can be integrated in new class of solar panels.

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