Electrochimica Acta 113 (2013) 603–608

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

The peak effect of the photocurrent on the concentration of electron mediator (*para*-benzoquinone) in thylakoids

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ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 6 September 2013
Accepted 7 September 2013
Available online 7 October 2013

Keywords:
Photocurrent
Photosynthesis
Electron mediator
Benzoquinone

ABSTRACT

This work investigates the photocurrent harvested from the isolated thylakoids. Several tests have been used to verify that the photocurrent measured is indeed from the photosynthesis on the thylakoid membranes. The photocurrent has a linear dependence on light intensity; the photocurrent shares similar frequency dependence as that of absorption spectrum of chlorophyll; the photocurrent decreases or disappears with the application of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea as an inhibitor. The new finding of this report is the observation of a peak in the photocurrent as a function of the concentration C_{BQ} of electron mediator *para*-benzoquinone (p-BQ). It is found that the photocurrent measured increases at small C_{BQ}, and a maximum current is obtained at $C_{BQ} \approx 1.8$–2 mM and decreases with further increase in C_{BQ}. A simplistic model has been proposed to explain the peak. The effect of bias voltage applied between the electrodes on photocurrent is studied as well.

1. Introduction

Nature's most sophisticated and efficient solar energy system is found in photosynthetic organisms, including plants, algae and some bacteria. They utilize photosynthesis to convert solar energy to chemical energy, split water and generate O_2, H^+ and high energy electrons. The energy stored in the high energy electrons is used to synthesize sugars and polysaccharides in oxygenic photosynthetic organisms.

Chloroplasts are the organelles that are responsible for photosynthesis in plant cells and some eukaryotic organisms. Inside the chloroplast are the stroma where starch and sugar are produced and the stacked thylakoid compartments (also called granum). Light-dependent photosynthesis takes place inside the thylakoid membrane. To capture the excited electrons from the thylakoids, the chloroplast membrane needs to be broken down and the stacked thylakoid can then be isolated from the chloroplast.

Inside the thylakoid, the electron transport follows a so-called "Z scheme mode" [1], which can be described briefly as follows. First, photosystem II (PS II) P680 complex absorbs light energy and it is then excited into P680*. The high energy electron from P680* is then transported along the electron transport chain to photosystem I (PS I). These electrons subsequently reduce chlorophylls $P700^+$ in PS I and finally reduce $NADP^+$ to NADPH, making biological energy ATP used by the organisms. During the light reaction of photosynthesis, water molecules are split at the site of oxygen evolving complex. The electrons generated from the water splitting reduce $P680^+$ in PS II and enable the photosynthesis light reaction cycle to be repeated.

Understanding of the charge transfer from photosystems to the transport chain, especially charge extraction to an electron mediator and subsequent transport to an outside circuit is thus paramount to potential applications of the bioelectricity.

Recent studies have successfully shown the feasibility of harvesting the high energy photosynthetic electrons. For example, direct extraction of excited photosynthetic electrons from a single living algal cell could generate pico-ampere bioelectricity [2]. Different approaches have also been studied. The photosynthetic reaction centers (photosystem II or I) could be isolated from the chloroplast of the photosynthetic organisms [3–7]. They can be chemically attached to linker molecules to conductive electrodes [8], such as carbon nanotubes [9–11], a GaAs surface [12], a gold substrate [13] or a nano-porous gold leaf [14]. In other studies, intact chloroplasts [15,16], single algal protoplasts [17–20] or isolated thylakoid membranes [21] have been used in the photosynthetic electrochemical cell with the help of various electron mediators including 2-hydroxy-1,4-naphthoquinone (HNQ) [22], 2,6-dimethyl-1,4-benzoquinone (DMBQ) [23], flavin mononucleotide (FMN) [20] or phenazine methosulfate (PMS) [21].

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0013-4686/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.electacta.2013.09.079

It is thus critical to understand the roles of an electron mediator in the charge extraction, especially the effect of concentration of mediator. In this work, careful studies on the dependence of photocurrent as a function of concentration of electron mediator, *para*-benzoquinone (p-BQ) [24–27], are reported. p-BQ was chosen in the experiment for the relatively small time constant of the photocurrent and being insensitive to illumination in the absence of thylakoids.

2. Experimental

2.1. Thylakoid isolation

The thylakoid pellets were isolated from spinach leaves using a thylakoid isolation protocol [3,28,29]. Small pieces of fresh spinach leaves were initially pulverized in a pH 7.7 grinding buffer consisting of 0.3 M NaCl, 3 mM $MgCl_2$, 30 mM Tricine/NaOH using a kitchen blender [28], and filtered through an 8-layer cheesecloth. The filtrate was then centrifuged at $2500 \times g$ (relative centrifugal force) for 4 min. The dark green pellet was then re-suspended in a pH 7.5 washing buffer consisting of 0.2 M sucrose, 3 mM $MgCl_2$, 10 mM KCl, 20 mM Tricine/NaOH [28], and centrifuged at $1000 \times g$ for 40 s to remove the cell debris. Finally, the supernatant fluid was centrifuged at $5000 \times g$ for 10 min twice. The transparent liquid at the top was discarded. The remaining pellets at the bottom of centrifuge tube were the isolated thylakoid membranes, which could be frozen under −20 °C for future use.

2.2. Experimental setup

To measure the picoampere photocurrent, a picoamp booster (CHI 200, CH instruments) and a Faraday cage were employed. The medium containing the electron mediator was delivered to a petri dish. Two electrodes (a platinum electrode, diameter 0.5 mm, manufactured by Alfa Aesar, Inc., MA, USA, working as a cathode and a customized carbon fiber electrode, tip diameter 5 μm [30], working as an anode) were placed in the medium. Isolated thylakoid pellets were added into the medium (shown in Fig. 1). The petri dish and the electrodes were placed inside the Faraday cage to minimize background noise. The light source (microscope external light, Titan tool supply, NY, USA) was placed outside the Faraday cage, and the light intensity could be adjusted by a dial setting. The reading of the dial was verified to correspond linearly with the light intensity from a separated flux meter calibration. The maximum intensity at the dial setting of 13 was calibrated to be approximately 300 μW/(cm^2 nm) at 615 nm. An optical fiber was used to guide the light into the cage. An electrochemical analyzer (CHI 660C, CH Instruments) was used to monitor the current signal between the electrodes in the system. Amperometry was applied to monitor the current flow through the circuit at a constant bias voltage, and the scan sample interval was typically set to 0.1 s. A red pass filter (No. 24 semi-band-pass Wratten gelatin filter, Eastman Kodak Company), which transmits light with wavelength above 600 nm and a green filter (No. 61 band-pass Wratten gelatin filter, Eastman Kodak Company), which transmits light with wavelength in between 500 and 570 nm were used to check the frequency dependence in the experiment. A single color blue laser pointer with wavelength of 440 nm (Z-Bolt, Taiwan) was also used as the light source for the blue region. *para*-Benzoquinone (p-BQ) was purchased from Sigma–Aldrich, reagent grade (≥98%).

3. Results and discussion

To extract the high energy electrons from the photosynthetic electron transport chain, electron mediators are needed to siphon the excited electrons from the thylakoids in competition with the natural electron carriers, plastoquinol and plastocyanin [1].

$$BQ + 2e^- + 2H^+ \leftrightarrow QH_2 \quad (1)$$

In Eq. (1), p-BQ (abbreviated as BQ in the text) can be reduced to p-QH_2 (*para*-hydroquinone, abbreviated as QH_2 thereafter) when the reduction potential is more negative than −0.63 V (vs SCE) [27]. The excited electrons from the PS II can thus be siphoned out and then used to reduce BQ to QH_2.

Shown in Fig. 2 is an overlay of photocurrent measured in the presence of 1 mM BQ mediator. In the absence of thylakoid, the BQ molecules in the medium showed no detectable photocurrent (data in red). When the isolated thylakoid membranes were added to the solution, the light-dependent photocurrent could be easily detected. The experiment was performed by measuring electric

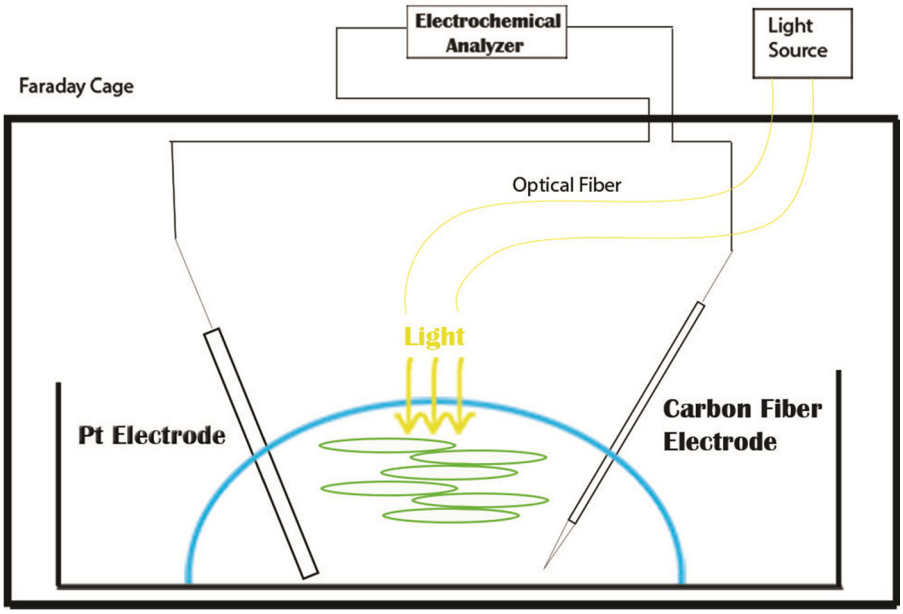

Fig. 1. Schematic drawing of the experimental setup.