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PSII as an in vivo molecular catalyst for the production of energy rich hydroquinones - A new approach in renewable energy

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ABSTRACT

One of the pertinent issues in the field of energy science today is the quest for an abundant source of hydrogen or hydrogen equivalents. In this study, phenyl-p-benzoquinone (pPBQ) has been used to generate a molecular store of hydrogen equivalents (phenyl-p-hydroquinone; $pPBQH_2$) from the*in vivo* splitting of water by photosystem II of the marine cyanobacterium *Synechococcus elongatus* BDU 70542. Using this technique, 10.8 µmol of $pPBQH_2$ per mg chlorophyll *a* can be extracted per minute, an efficiency that is orders of magnitude higher when compared to the techniques present in the current literature. Moreover, the photo-reduction process was stable when tested over longer periods of time. Addition of phenyl-p-benzoquinone on an intermittent basis resulted in the precipitation of phenyl-p-hydroquinone, obviating the need for costly downstream processing units for product recovery. Phenyl-p-hydroquinone so obtained is a molecular store of free energy preserved through the light driven photolysis of water and can be used as a cheap and a renewable source of hydrogen equivalents by employing transition metal catalysts or fuel cells with the concomitant regeneration of phenyl-p-benzoquinone. The cyclic nature of this technique makes it an ideal candidate to be utilized in mankind's transition from fossil fuels to solar fuels.

1. Introduction

Driven by the finite and polluting nature of fossil fuels that drives civilization today, it has been the endeavor of the scientific community to harness the energy from the sun in a sustainable manner. In this regard, many techniques have been employed till date, ranging from the extraction of lipids from plants [1], algae [2] and cyanobacteria [3] and using microbial strains to produce metabolites of high calorific value [4,5]. However, an economically viable process still eludes us. One reason for this is the fact that all the products mentioned above are formed by the respective organism's anabolic pathways. It is a known fact that as metabolism proceeds from catabolism to anabolism, there is a progressive loss of input energy due to the various processes of life taking place inside the organism. As a result, the output (i.e. the lipid, biomass or the metabolite of interest) represents only a fraction of the total input photonic energy. The use of hydrogenases to couple the transmembrane proton gradient formed during the light reactions of photosynthesis with the production of hydrogen harvests the photonic energy much before the commencement of anabolism [6,7]. However, the extreme vulnerability of hydrogenases to molecular oxygen [8], a by-product of photosynthesis, makes this process unsuitable for commercialization using current technology. Hence, a viable process that extracts the input energy at a very early stage of photosynthesis is the need of the hour.

Benzoquinones are a class of compounds that are used by researchers to study the various aspects of photosynthesis. Since they resemble plastoquinone, a molecule that shuttles electrons from photosystem II (PSII) to the cytochrome b_6f complex, they can accept electrons from PSII instead of plastoquinone [9]. These benzoquinones have been used mainly to study the kinetics of PSII operation [10]. However, when a benzoquinone is reduced to its quinol form (more commonly referred to as the hydroquinone form), it also becomes a molecular store of hydrogen equivalents, storing the energy from the photocatalytic splitting of water by PSII in its chemical bonds. These compounds are similar to NADH or NADPH, the predominant molecular stores of reducing power designed by nature. That the quinols are molecular stores of energy is evidenced by the fact that a class of membrane proteins collectively named quinol oxidases tap the free energy of these molecules to create a transmembrane proton gradient [11]. Moreover, there have been studies in which energy from these hydroquinones have been extracted using metal catalysts [12] or fuel/electrochemical cells [13–15]. However, to our knowledge, work on the

Abbreviations: PSII, Photosystem II; pPBQ, phenyl-p-benzoquinone; DMBQ, 2, 6-dimethyl benzoquinone; $pPBQH_2$, phenyl-p-hydroquinone; chl *a*, chlorophyll *a*; *S. elongatus*, *Synechococcus elongatus* BDU 70542; OEC, oxygen evolving complex

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production and extraction of these molecular stores of energy using photosynthesis has not been done till date.

The study focused on understanding the photosynthetic reduction of phenyl-p-benzoquinone (pPBQ) by a marine cyanobacterium *Synechococcus elongatus* BDU 70542 (*S. elongatus*), obtained from National Facility for Marine Cyanobacteria, Bharathidasan University, Tamil Nadu, India. The choice of pPBQ as the electron acceptor was made due to its higher efficiency in electron capture from PSII [9]. The photosynthetic reduction of pPBQ can be estimated by measuring a drop in the A_{374} of a suspension containing *S. elongatus* and pPBQpost light treatment, since phenyl-p-hydroquinone ($pPBQH_2$; the reduced form of pPBQ) does not absorb at this wavelength [16]. However, on the absorption of light at specific wavelengths, benzoquinones are subject to molecular excitation, with a subset of the species entering the triplet state. These species react with water to form a photohydrate that subsequently cleaves into the corresponding hydroquinone and hydroxyquinone [17]. In order to quantify the photosynthetic reduction of pPBQ, one needs to remove the 'noise' due to its autocatalytic activity in the presence of light.

It is a well-known fact that mild heat treatment of acyclic photosynthetic systems inactivates the oxygen evolving complex (OEC) of PSII [18,19]. The inactivation of the OEC results in the cessation of photosynthetic activity and hence no reduction of pPBQ by photosynthesis is possible in these cells. Hence measuring the drop in A_{374} of a mixture of pPBQ in normal cyanobacterial culture and comparing it with the drop in A_{374} of its heat treated counterpart post light treatment would give an accurate view of the photosynthetic reduction of pPBQ by *S. elongatus*. Moreover, the extent of the drop in A_{374} in the solution containing heat treated *S. elongatus is* a direct measure of its autocatalytic activity.

2. Materials and Methods

2.1. Growth of S. elongatus

S. elongatus was grown in ASN III medium in a small scale glass bioreactor (perforated silicon membrane sparging system; air sparging rate: 0.4 lpm). A 16:8 light: dark diurnal cycle was followed with a light intensity of 20 $\mu E\, m^{-2}\, s^{-1}$. Post growth, the cells were harvested by centrifuging at 6000 $\times g$ for 5 min. The pellet was resuspended in fresh ASN III medium and left overnight in the above mentioned light regime. The culture used in all experiments was dark adapted for 5 min unless otherwise stated. All photoreduction experiments were performed just after the dark cycle. ASN III Media composition is as follows: NaCl: 25 g, $MgSO_4{\cdot}7H_2O$: 3.5 g, $MgCl_2$: 0.95 g, $CaCl_2{\cdot}2H_2O$: 0.5 g, KCl: 0.5 g, Citric acid: 3 mg, Ferric ammonium citrate: 3 mg, EDTA: 0.5 mg, Trace Metals Mix A5: 1 ml, $NaNO_3$: 0.75 g, K_2HPO_4: 0.69 g, Na_2CO_3: 20 mg, Vitamin B_{12}: 10 μg. These chemicals were dissolved in distilled water and made up to 1 l. Trace Metals Mix A5 contains the following composition – H_3BO_3: 2.86 g, $MnCl_2{\cdot}4H_2O$: 1.81 g, $ZnSO_4{\cdot}7H_2O$: 0.22 g, $Na_2MoO_4{\cdot}2H_2O$: 0.39 g, $CuSO_4{\cdot}7H_2O$: 0.079 g, $Co(NO_3)_2{\cdot}6H_2O$: 0.049 g. These chemicals were dissolved in distilled water and made up to 1 l.

2.2. Measurement of Chl a Concentration

1 ml of the culture was taken and centrifuged at 10,500 $\times g$ for 10 min. The supernatant was discarded. 1 ml of cold 100% methanol solution (4 °C) was added to the pellet and sonicated for 5 s to re-suspend it. The suspension was incubated at 4 °C for 30 min. After the time elapse, the suspension was centrifuged at 10,500 $\times g$ for 10 min. The A_{665} and A_{720} of the supernatant were recorded. The chl *a* concentration of the culture was arrived at as follows [20]:

$$\text{Chl } a\ (\mu g/ml) = 13.42*(A_{665} - A_{720})$$

2.3. Estimation of the Autocatalytic Activity of pPBQ in Culture

S. elongatus was taken in a 50 ml falcon tube and heat treated at 70 °C for 5 min and subsequently cooled at room temperature. A stock solution of pPBQ (10 mg/ml) in 100% acetone was prepared. 1 ml of the heat treated culture at a given chl *a* mass containing different concentrations of pPBQ were taken in separate 2 ml centrifuge tubes and placed at a light source from an 85 W CFL lamp for 5 min at an intensity of 20 $\mu E\, m^{-2}\, s^{-1}$. The lamp was mounted parallel to the plane of the centrifuge tubes. Concurrently, an identical set of tubes were dark incubated for 5 min. Post this time period, the tubes were centrifuged at 2200 $\times g$ for 30 min. The A_{374} of the supernatant was recorded. Similar experiments were performed in plain buffer (without heat treated *S. elongatus*).

2.4. Estimationof pPBQ Reduction by S. elongatus

2.4.1. A Factor for the Partitioning of pPBQ in S. elongatus

Since some amount of pPBQ partitions into the pellet during centrifugation, a factor to account for this phenomenon was calculated as follows: 1 ml of heat treated *S. elongatus* containing different concentrations of pPBQ were taken in separate 2 ml centrifuge tubes in the dark, mixed thoroughly and centrifuged at 2200 $\times g$ for 30 min and the A_{374} of the supernatant was recorded. Using this data, a standard curve was constructed for all chl *a* concentrations used in the experiments. The slope of this curve was used to estimate the amount of pPBQ reduced.

2.4.2. Estimation of pPBQ Reduction (Illumination Duration Upto 5 min)

1 ml of *S. elongatus*at a given chl *a* mass containing different concentrations of pPBQ were taken in separate 2 ml centrifuge tubes. These tubes were illuminated at an intensity of 20 $\mu E\, m^{-2}\, s^{-1}$ fora specified time duration. Post this time period, the tubes were centrifuged at 2200 $\times g$ for 30 min. The A_{374} of the supernatant was recorded. The amount of pPBQ reduced is estimated as:

$$\mu\text{mol pPBQ reduced/mg chl } a/\text{min} = W/(\text{chl } a \text{ mass} * \text{time duration})$$

$$W = \text{Initial pPBQ concentration} - \left[\frac{A_{374}}{\text{Slope of the line corresponding to the partitioning experiment}}\right]$$

2.4.3. Estimation of pPBQ Reduction over a Longer Time Frame

A given initial concentration of pPBQ in *S. elongatus* suspension is illuminated at an intensity of 20 $\mu E\, m^{-2}\, s^{-1}$ for 5 min. After this, a fixed amount of pPBQ is introduced into the solution every 5 min; the cycle is repeated eleven times (total time period: 1 h). Post this time period, the tubes were centrifuged at 2200 $\times g$ for 30 min. The amount of pPBQ reduced is estimated as

$$\mu\text{mol pPBQ reduced/mg chl } a/\text{hour} = W/(\text{chl } a \text{ mass})$$

$$W = \text{Total pPBQ added} - \left[\frac{A_{374}}{\text{Slope of the line corresponding to the partitioning experiment}}\right]$$

The rationale for adding pPBQ on an intermittent basis is based on the solubility limit of pPBQ in culture media (~600 μM).

2.5. Recovery and Characterization of $pPBQH_2$ post Photoreduction

During the photoreduction process, black particles started to precipitate from the suspension and were separated through sedimentation by placing the tubes upright in a stand for 3 h. The supernatant was then pipetted out. The resulting precipitate was washed and centrifuged five times with Milli Q water (centrifugation speed: 10,500 $\times g$ for