Photosynth Res (2015) 126:161–169
DOI 10.1007/s11120-015-0075-3

REGULAR PAPER

The Photosystem II D1-K238E mutation enhances electrical current production using cyanobacterial thylakoid membranes in a bio-photoelectrochemical cell

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Received: 2 June 2014 / Accepted: 2 January 2015 / Published online: 15 January 2015

Abstract The conversion of solar energy (SEC) to storable chemical energy by photosynthesis has been performed by photosynthetic organisms, including oxygenic cyanobacteria for over 3 billion years. We have previously shown that crude thylakoid membranes from the cyanobacterium *Synechocytis* sp. PCC 6803 can reduce the electron transfer (ET) protein cytochrome c even in the presence of the PSII inhibitor DCMU. Mutation of lysine 238 of the Photosystem II D1 protein to glutamic acid increased the cytochrome reduction rates, indicating the possible position of this unknown ET pathway. In this contribution, we show that D1-K238E is rather unique, as other mutations to K238, or to other residues in the same vicinity, are not as successful in cytochrome c reduction. This observation indicates the sensitivity of ET reactions to minor changes. As the next step in obtaining useful SEC from biological material, we describe the use of crude *Synechocystis* membranes in a bio-photovoltaic cell containing an *N*-acetyl cysteine-modified gold electrode. We show the production of significant current for prolonged time durations, in the presence of DCMU. Surprisingly, the presence of cytochrome c was not found to be necessary for ET to the bio-voltaic cell.

Keywords Photosynthesis · Cyanobacteria · Solar energy conversion · Cytochrome c · Electrochemistry

Abbreviations

BPC	Bio-photoelectrochemical cell
Chl	Chlorophyll
Cyt c	Horse heart mitochondrial cytochrome c
CV	Cyclic voltammetry
ChAmp	Chronoamperometry
ET	Electron transfer
D1-K238E	K238E mutant of D1 protein
LHC	Light-harvesting complexe(s)
NAC	*N*-Acetyl cysteine
PS	Photosystem (s)
PSI	Photosystem I
PSII	Photosystem II
RC	Reaction center (s)
RSS	Wild-type D1 protein encoded by *psbA2*
SAM	Self-assembled monolayer
SEC	Solar energy conversion
Syn	*Synechocystis* PCC sp. 6803

Electronic supplementary material The online version of this article (doi:10.1007/s11120-015-0075-3) contains supplementary material, which is available to authorized users.

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Introduction

The light-driven stages of the photosynthetic process in photoautotrophic organisms consist of a series of electron transfer (ET) steps from donors to acceptors (Blankenship 2014). These processes tightly couple energetically favorable steps in order to efficiently produce reduced components and/or a proton gradient for the production of ATP. Forward ET must be tightly controlled to ensure maximal activity without loss of the absorbed energy to competing processes and without damaging the system due to over excitation and production of harmful intermediates. Photosynthetic membranes (thylakoids) contain photosystems (PS) consisting of photochemical reaction centers (RCs) and light-harvesting antennas. It has been estimated that most thylakoids are quite densely packed with PSs, enabling maximal light absorption, ET and product formation (Armond et al. 1977; Staehelin 2003). While each PS is optimized for its environmental niche, (Blankenship and Hartman 1998; Neilson and Durnford 2010), many of the systems are also quite flexible, enabling maximal activity over rather wide range of parameters. Additional components found in photosynthetic systems are the small, soluble redox active proteins that act as mobile electron donors and acceptors, electrically linking the PSs (Bashir et al. 2011). These proteins include c-type cytochromes, plastocyanin, ferredoxin, and others.

Over the past few years, the potential of using SEC by biological photosynthetic systems for society's energy requirements has been explored. Experiments in the use of isolated photosynthetic complexes merged into inorganic/synthetic voltaic devices have been described in the literature (Harrold et al. 2013; Kargul et al. 2012; Kato et al. 2014; Nguyen and Bruce 2014; Toporik et al. 2012; Yehezkeli et al. 2014). Other research groups are actively working to mimic the mechanisms found in biological photosynthesis using completely synthetic components (McConnell et al. 2010; Ronge et al. 2014; Schultz and Yoon 2014). The use of intact cells has also been examined (McCormick et al. 2011; Summers et al. 2010). The question arises whether these attempts could potentially replace or augment other photovoltaic solar cell technologies. Upon isolation from the cell, the photosynthetic components can be driven at their maximal rates; however, in many cases this leads to rapid (within a few minutes) loss of activity since the connection with the D1-protein repair systems is disrupted. A recent description of dried PSI on nanostructured semiconductor electrodes shows superb current and power densities and a very high fill factor (Nguyen and Bruce 2014). Other PS types have also been utilized in a variety of cells, with a variety of chemistries determining the manner of PS/electrode association and electrochemical cell composition.

We have recently embarked upon an attempt to use PSII in its native environment toward a similar goal (Larom et al. 2010). PSII has many intrinsic characteristics that make it an attractive target for SEC, especially its use of water as the electron donor eliminating the need for mobile electrolytes (Brudvig 2008) in a bio-photoelectrochemical cell (BPC). PSII has the strongest redox potential of any biological system (up to 1.8 V vs. SHE)(Barber 2012); however, these same characteristics lead to a relatively short complex lifetime. PSII activity both in vivo and in vitro is typically lost within minutes due to radical-induced damage within the RC. In vivo PSII is continually repaired by a complex system that replaces the D1 protein component and also reattaches all of the necessary pigments and co-factors. It has been shown that isolated PSII from cyanobacterial thylakoid membranes can drive ET at rates of up to 2,500–3,000 μmol O_2 × mg chl^{-1} × hr^{-1} (Shen and Kamiya 2000). This is equivalent to a current of about 300 mA per mg chl. Cyanobacterial cells can be grown quite easily and very cheaply in extremely large quantities, and thus can serve as the source of the SEC raw material.

We have previously shown (Larom et al. 2010) that we can mutagenize PSII of *Synechocystis* sp. PCC 6803 (*Syn*) in a fashion that affords reduction of the ET protein cytochrome c (cyt c) by isolated thylakoid membranes. We believe that in this mutant, where Lys238 of the D1 protein was modified to a glutamic acid (D1-K238E), an alternative ET pathway leading from the primary acceptor Q_A to the stromal surface was revealed. Introduction of this mutation did not significantly affect normal cell growth of the D1-K238E strain or its normal photosynthetic activities. Addition of an inhibitor which blocks forward normal ET to the secondary acceptor, Q_B, significantly increased the amount of reduced cyt c obtained. The activity of the mutated PSII in these isolated membranes was also shown to protect PSII activity, most likely by the prevention of over-excitation of Q_A. Use of thylakoid membranes instead of isolated complexes introduces many complications as to assessing the effects of all the different parameters affecting ET and SEC. On the other hand, isolation of complexes requires many energy-intensive steps, as well as the use of polluting chemicals (such as detergents). We propose that use of crude photosynthetic membranes could in the future be developed into a relatively environmentally clean material for a SEC device.

In this paper, we show that the membranes of the D1-K238E strain are superior to other D1 protein variants in their ability to reduce cyt c. We also show that crude cyanobacterial membranes can indeed drive light-induced current in a BPC.

Materials and methods

Preparation of a thylakoid suspension from Synechocystis

A late log phase culture ($OD_{730} = 1$) was pelleted and resuspended in 20 mM Tris pH8, 400 mM NaCl, and 15 mM $MgCl_2$ (Buffer A). Cells were then passed twice through a microfludizer (Microfluidics Corporation) under pressure of 30 psi. The unbroken cells were pelleted at 2,000 rpm, and the supernatant containing the thylakoid membranes was centrifuged for 20 min at 15,000 rpm. The thylakoids were suspended in 50 mM MES/NaOH pH 6, 15 mM NaCl, and 5 mM $MgCl_2$ (Buffer B). The final concentration of Chlorophyll *a* was determined in 80 % (v/v) acetone according to Arnon (Arnon 1949).

Spectroscopic analysis of cytochrome c reduction in solution

The equivalent of 5 μg chl of isolated thylakoids was incubated with 30 μM horse heart mitochondrial cyt c (Sigma-Aldrich) in buffer B and then illuminated for 3 min with an incandescent light providing photosynthetically active illumination at an intensity of 1,000 μE m^{-2} s^{-1} or ~220 Wm^{-2}. The concentration of reduced cyt c was calculated using the equation: $\Delta\varepsilon 550–542 = 21$ mM^{-1} cm^{-1} obtained by a standard calibration curve.

Working electrode fabrication

Standard microscopy glass slides (25 mm × 76 mm × 0.70 mm) were cleaned with absolute acetone, isopropanol, methanol, and ethanol before use. A 1,000Å-thick gold film was deposited on the glass slide primed with 150Å titanium with an E-beam evaporator (AIRCO TEMESCAL FC-1800)(Pierson 1999). The electrodes prepared in this manner were incubated overnight in a 0.1 M *N*-acetyl cysteine (NAC, $C_5H_9NO_3S$) solution at 4 °C.

Electrochemical measurements

Electrochemical experiments with commercial cyt c (Sigma) were performed using a Zennium electrochemical workstation (Zahner Electrik, Germany). All other measurements were carried out in a single-compartment in-house manufactured cell (Fig. S1) using either two-electrode mode (for chronoamperometric (ChAmp) measurements) or three-electrode mode (for cyclic voltammetry (CV) or ChAmp measurements). In the 3-electrode configuration cell, the NAC-modified gold was used as a working electrode, a platinum electrode as counter electrode, and an Ag/AgCl/3 M NaCl as a reference electrode. For CV, current potentials were scanned between 200 and 600 mV at a scan rate of 50 mV/sec. In this cell, the anode compartment is composed of a NAC-modified gold electrode immersed in a photoactive electrolyte solution containing thylakoid membranes and cyt c with finite volume determined by a silicone o-ring (Fig. S1). The anode was illuminated from above. The light source used for thylakoid illumination was solar simulator (Oriel Sol3A, Newport Inc.). Thylakoid membranes were not stirred during the measurements. To assess current production, photo-ChAmp was performed. During these measurements, a constant potential of 0.75 V was applied to the working electrode, which was typically exposed to alternate 200 s light/dark cycles and the resulting current was measured over time.

Hydrogen detection

A two-compartment cell in 3-electrode setup was fabricated in the following fashion: a NAC-modified gold electrode was immersed in buffer B and DCMU in the anode compartment and a Pt wire was immersed in 1 M HCl in the cathode compartment. The D1-K238E membranes were placed on the anode (50 μg chlorophyll *a*), and 0.5 V was applied using a Zennium electrochemical workstation (Zahner Electrik, Germany). The anode was illuminated from above using a solar simulator (Oriel Sol3A, Newport Inc.). A sample of headspace gas was removed from the cathode compartment by a gas syringe and was injected into the gas chromatograph to determine the concentration of H_2. The system consisted of an Agilent 7890. A gas chromatograph was equipped with HP-Plot-Q column (30 m, 0.53 mm, 40 μm) and a thermal conductivity detector . Nitrogen was used as the carrier gas.

Results and discussion

Reduction of cyt c in other mutated strains

The engineering of a docking site for cyt c by replacing K238 with glutamic acid was based on the analysis of the electrostatic interactions between the positively charged surface of cyt c and a negatively charged site on the surface of PSII. We anticipated that the interaction would be strong enough to enable specific and correctly positioned attraction of the cyt c without resulting in binding of a magnitude that would inhibit cyt c turnover (Adir et al. 1996). This is a similar situation as found in bacterial photosynthesis (Axelrod et al. 2002). In order to explore the site further, we analyzed the interaction between cyt c with other variants of PSII (Fig. 1). The *Syn* genome contains a gene family of three *psbA* genes encoding the D1 protein, called

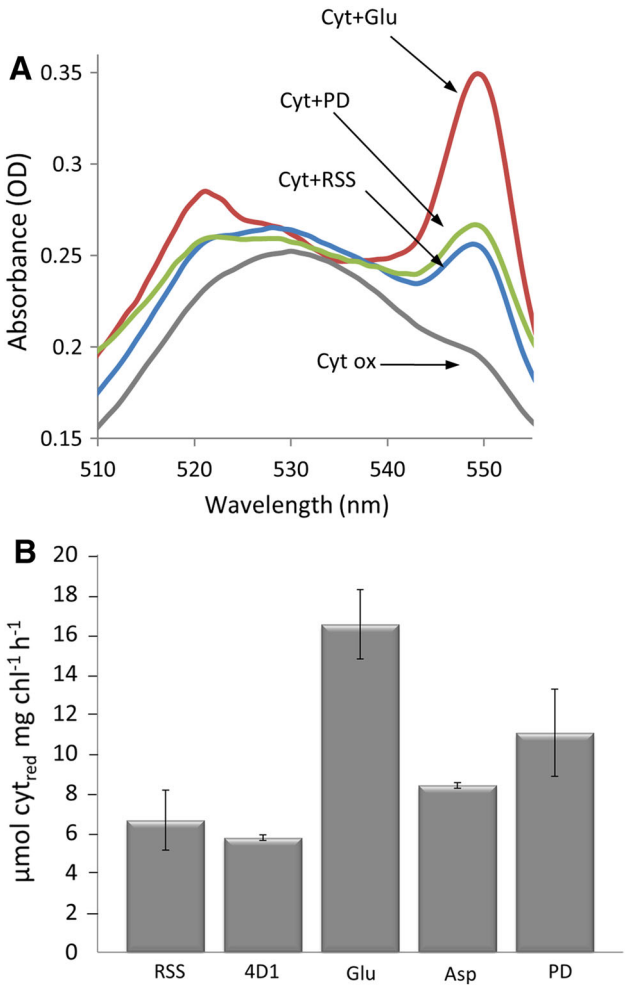

Fig. 1 Cytochrome c is photoreduced more efficiently by D1-K238E strain membranes. **a** Thylakoid membranes of different forms of the D1 protein of PSII were illuminated for 3 min in the presence of oxidized cyt c and DCMU and the light–dark difference absorption spectrum was monitored. Cyt c ox: Spectra of oxidized cyt c is shown for reference. **b** Cyt c reduction rates of thylakoids obtained from the modified strains were analyzed in the presence of DCMU at 550 nm. Error bars were calculated from at least three independent experiments. RSS, D1 protein encoded by the *psbA3* gene. PD, D1 protein lacking the 225–239 PEST-like sequence loop, D1-K238E, D1 protein with K238E mutation in the *psbA3* gene. 4D1, D1 protein encoded by the *psbA2* gene. D1-K238D, D1 protein with K238D mutation in the *psbA3* gene

psbA1-3 (Kulkarni and Golden 1995; Sugiura and Boussac 2014; Vinyard et al. 2013).

In order to ascertain whether the D1-K238E phenotype resulted from expression from the *psbA3* gene site, we analyzed the reduction of cyt c by the 4D1 strain, carrying only wild-type *psbA2* gene (Debus et al. 1988). This strain was compared to the RSS strain carrying only the *psbA3* gene (Figs. 1a, b). We did not expect any major differences since the amino acid sequences of the two paralogs are identical. Indeed, use of thylakoid membranes of the 4D1 strain revealed similar rates of cyt c reduction rate to RSS membranes (Fig. 1b). We also engineered a mutation in the D1 protein where K238 was replaced by an aspartic acid (D1-K238D). This mutation should introduce a negative charge similar to D1-K238E at the stromal surface of PSII; however, due to the lack of a single methylene group, the potential charge would be embedded deeper within PSII. Surprisingly, although quite similar to the D1-K238E strain, membranes isolated from the D1-K238D strain displayed limited photoreduction rate of cyt c compared to the D1-K238E strain (Fig. 1b). This difference in cyt c reduction between the D1-K238E and D1-K238D strains indicates that the docking site for cyt c is very sensitive to small differences in the structure and position of the involved amino acid residues. An additional modified D1 protein containing strain that we analyzed was the PD strain (Mulo et al. 1998; 1997). In this strain, amino acids 225–239 have been omitted by deleting the PEST-like domain, resulting in extensive changes to the vicinity of the putative cyt c docking site. Incubation of cyt c with membranes isolated from this strain resulted in about twice the cyt c reduction rate as compared to the RSS strain but not as high as the D1-K238E strain (Figs. 1a, b). These results indicate that the nature of amino acids forming the cyt c docking site which in concert produce the electrostatic and surface area is critical in determining how cyt c binds, is reduced, and at what rate it can dissociate from the PSII complex. Even the very small difference between D1-K238E and D1-K238D significantly changed the ability of cyt c to abstract electrons.

One of the challenges of the measurements described here (and below) is the complexity of the crude membrane system. These membranes contain the known PSII heterogeneities found in all photosynthetic organisms, due to the D1 turnover process. In addition, the accessibility of different membrane layers in the preparation as well as ongoing changes that occur to PSII during oxygen evolution and ET leads to increased variance in activity. The observation that the different mutants (including the RSS strain) have reduction rates that are on the same order, would appear to indicate that a major difference is in the attraction (binding) of the cyt c to the correct site. In the presence of DCMU, ET to cyt c reaches about 80 % of the rate for small molecule lipophilic acceptors (Larom et al. 2010) used in the absence of DCMU. The rate of recombination from the $S_2Q_A^-$ state is about 20-fold faster than from the $S_2Q_B^-$ state (Rappaport and Lavergne 2009), so we can safely assume that forward ET to cyt c is on the same order as the reduction of Q_B or other exogenous Q_B site-binding acceptors. Since oxygen evolution rates sustained by cyt c are about 30 % greater than the rate of cyt c reduction obtained spectroscopically, we can assume that this proportion of cyt c is reoxidized either by the presence

of O_2 (which is unavoidable in the presence of active PSII) and/or other membrane-bound components.

Design of the bio-photoelectrochemical cell

The merging of biological materials with inorganic electrochemical systems has been widely achieved by different chemical linking techniques (Yehezkeli et al. 2013; 2012; 2010). In most reports, isolated proteins (such as glucose oxidase) or complexes (such as PSII or PSI) were either adsorbed to particulate materials or chemically bonded to metallic electrodes (Pt, Au, etc.) or to semi-conductors (crystalline Si, TiO_2, etc.) (Bartlett and Whitaker 1987; Lovley 2012; Willner et al. 2006; Willner and Willner 2001). Affinity-tagged proteins can be produced via heterologous expression, yielding large quantities of protein that can be then purified by affinity chromatography techniques. In order to utilize membrane-bound complexes, they must be isolated from their native environment, via the use of surfactants that solubilize the lipid membrane while conserving activity and preventing precipitation. Following solubilization, the complex must also go through complex series of isolation steps prior to use. In most cases where ET proteins or PSI have been used, the presence of chemical electron donors is required to establish current. These donors must either be replenished periodically by their addition to the solution or their re-reduction must occur on the anode.

In the development of the system we describe here, we decided to use a different approach in the design of the BPC. Using PSII, we avoid the need for the addition of external reducing agents that must be replenished as described above. PSII activity will produce only H_3O^+ ions and molecular oxygen. The second major modification is the direct use of the photosynthetic membranes. Once released from the enclosing cell wall and cytoplasmic membrane, the D1-K238E mutation should allow PSII ET to cyt c without the need for surfactants. The use of PSII has typically been seen as problematic due to damage caused by the production of radical oxygen species, a process that appears to be an inherent characteristic of the complex, at all light intensities. However, our previous study showed that the D1-K238E mutation provides some protection against damage (Larom et al. 2010). While we foresee that the eventual loss of PSII activity is unavoidable, since the membranes are not chemically attached to the electrode, the damaged material can be quickly replaced without harming the BPC.

The proposed ET path in the planned photocell is depicted in Fig. 2. The organization of the ET cofactors at PSII reaction center are denoted as determined by X-ray crystallography (PDB 3ARC)(Umena et al. 2011). Following charge separation (P^*_{680}) and formation of $P_{680}{}^+$/

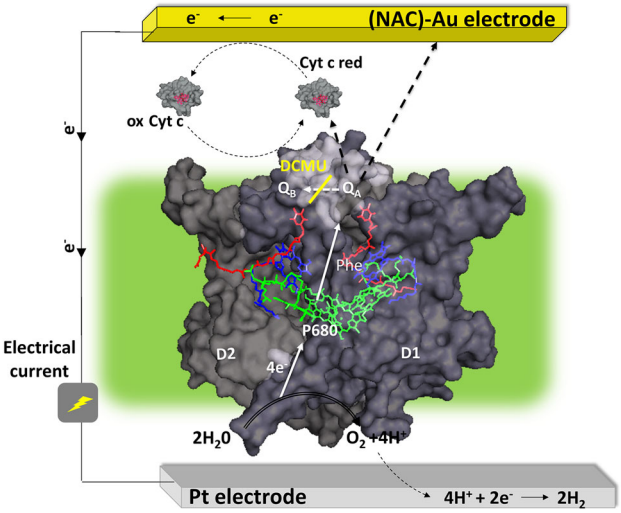

Fig. 2 The experimental system. **a** Schematic presentation of the modified thylakoids in a photoelectrochemical cell. The structure of cyanobacterial core of PSII (D1 and D2 proteins) is presented according to the 3 ARC structure. The structure of the cyt c is depicted according to PDB code 1HRC (Bushnell et al. 1990). Chlorophyll pigments are colored in *green*, pheophytins are in *blue*, and quinones in *red*. The PSII reaction center is embedded in the thylakoid membranes (*green rectangle*). The heme of cytochrome c is colored in magenta. Electron transfer inside the PS II is marked with white arrows. Electron transfer from Q_A to either cyt c or to the modified gold electrode is noted with thick *dashed black arrows*. The inhibition point of DCMU between Q_A and Q_B is indicated with a *yellow line*

Pheo, the electron is transferred within 200 μs to Q_A and from there to Q_B. For every two absorbed photons, Q_B undergoes two reduction and protonation reactions to plastoquinol (Q_BH_2), which then dissociates from PSII into the thylakoid membrane. Addition of DCMU inhibits ET from Q_A to Q_B typically resulting in charge recombination. In the Glu mutant previously described (and to a lesser extent in WT PSII), illumination of isolated thylakoid membranes drives cyt c reduction (Larom et al. 2010) and the addition of DCMU diverts more electrons toward cyt c reduction.

Cytochrome c is reversibly oxidized in the bio-photoelectrochemical cell

It has previously been shown that different reduced ET proteins can be oxidized by electrodes within an electrochemical cell (Amdursky et al. 2014; Silveira and Almeida 2013). Gold electrodes are efficient and stable electrodes; however, proteins that associate with gold typically denature or adhere irreversibly. Therefore, in order to allow a reversible binding of cyt c to the electrode and to promote a rapid heterogeneous ET, a chemical modification of the metal surface is imposed (Fedurco 2000). In this work, we

used NAC, an acetylated derivative of cysteine that was previously shown to form a self-assembled monolayer (SAM) on gold surface. This sulfur-containing SAM is negatively charged at neutral pH and known to promote electron exchange between cyt c and gold electrode in a reversible manner. CV of cyt c in buffer phosphate at pH 7.0, at potentials between −0.2 V and 0.5 V (vs. a reference Ag/AgCl/3 M NaCl electrode), indicated neither oxidation nor reduction of cyt c when measured on a bare gold electrode at a scan rate of 50 m Vs^{-1} (Fig. S2, blue lines). However, the electrochemistry of cyt c on a NAC-modified gold electrode displayed both cathodic and anodic peaks indicating that the reaction is reversible, in agreement with previous results (Fig. S2, red lines) (Digleria et al. 1986).

Generation of electrical current in the BPC

In order to detect the electric current produced by thylakoid membranes, the BPCs were constructed as described in the Methods section (Fig. S1). We assume protons are being reduced to hydrogen in the cathode compartment, since no other electron-accepting compounds are present, and preliminary measurements using gas chromatography show the presence of dissolved hydrogen gas following the reaction (Fig. S3). CV measurements were performed in 3-electrode mode with an Ag/AgCl/3 M NaCl reference electrode. ChAmp measurements were performed using either a two-electrode (in which the working electrode was connected to the anode and both counter and reference electrodes connected to the cathode) or three-electrode mode as described above. A gas-tight BPC is being developed to enable quantitative real-time measurement of hydrogen production.

The electrochemical response of the BPC system was monitored under constant applied potential (bias) of 0.75 V using alternate dark–light cycles. Photo-induced currents were measured and current densities (μA cm^{-2}) were calculated. CV measurements of the BPC system showed minimal light response without the presence of thylakoids (Fig. S4A). When thylakoid membranes were added to the BPC and CV performed, significant light-dependent currents were obtained (Fig. 3); however, the peak oxidation position was shifted to a potential of 50 mV higher than that measured with pure cyt c. In addition, increasing the concentration of cyt c lowered the apparent current density (Fig. 3). CV measurements using the RSS membranes showed essentially the same pattern, with significantly lower signals. We then measured the current density by ChAmp using the D1-K238E membranes in the presence of DCMU with different concentrations of cyt c. In agreement with the CV measurements, we found that the maximal current density (>35 μA cm^{-2}) was obtained in the absence of cyt c (Fig. 4). Addition of cyt c inhibited ET in a concentration-dependent manner (Fig. 4, insert). Since no other mobile and soluble redox component was added to the system, this would suggest that the thylakoid membranes contain a redox component (either membrane bound or released from the membrane upon application to the electrode, but not found in the soluble fraction) that is reduced by PSII before Q_B, diffuses out of the membrane

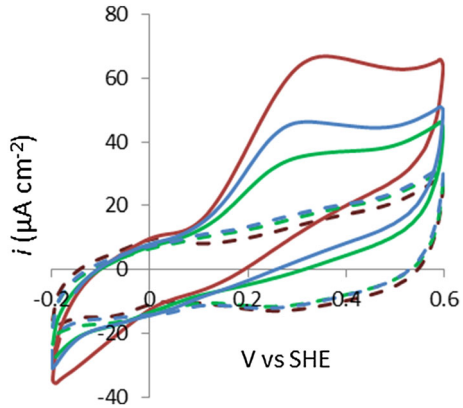

Fig. 3 CV measurements of D1-K238E thylakoid membranes in the presence of different concentrations of cyt c. CV measurements were performed on D1-K238E membranes (0.05 mg chl *a*) in the presence of DCMU with the addition of 0 (red), 0.9 (blue), or 1.8 mg ml^{-1} cyt c (green). Measurements were performed in three-electrode mode in the dark (*dashed lines*) or under standard solar irradiation of 100 mW/cm^2 (AM1.5; *solid lines*). In all measurements, the scan rate was 50 mV/sec

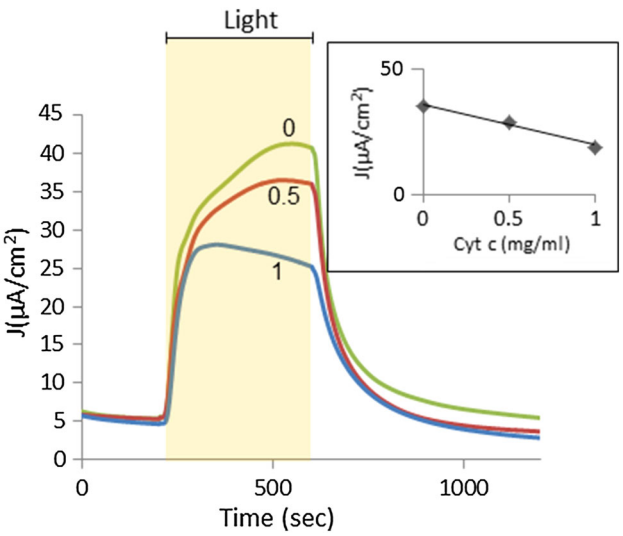

Fig. 4 Cyt c impairs photocurrent generation. A. ChAmp measurements were performed on D1-K238E membranes (0.05 mg chl *a*) in the presence of DCMU with the addition of 0, 0.5, or 1 mg/ml cytochrome c. The insert shows that the photocurrent decreases linearly with cyt c concentration. The measurements were all made in 2-electrode mode at 0.75 V bias under standard solar irradiation of 100 mW/cm^2 (AM1.5). The light period is indicated in *yellow*

and is oxidized on the anode. Alternatively, the membranes may transfer electrons directly to the NAC-modified gold anode. The current was measured following gravitational settling of the membranes onto the anode; however, they could be removed by simple washing, indicating that there was no strong adsorption onto the anode as a result of the NAC modification.

When CV measurements were performed in the BPC in the presence of wild-type (RSS) or D1-K238E thylakoid membranes in the absence of cyt c (Fig. 5 and Fig. S4), the D1-K238E thylakoids (magenta lines) provided significantly greater current as compared to the RSS thylakoids (red lines). Thus it is quite clear that irrespective of the actual mechanism of ET, the D1-K238E thylakoids are superior in their ability to obtain light-dependent current in the BPC. A typical ChAmp measurement of D1-K238E versus RSS thylakoids (in the presence of DCMU) is shown in Fig. 6. Illumination of the RSS membranes resulted in a photocurrent of 9 ± 2 μA cm^{-2} (average of three independent experiments, measuring the first illumination period). When the same measurement was performed on thylakoid membranes from the D1-K238E strain, current densities were between 16 ± 2 μA cm^2 (average of three independent experiments, measuring the first illumination period). As previously seen, the D1-K238E mutation imparts significantly more efficient alternative ET that is enhanced by the presence of DCMU. Current could be obtained from the thylakoid membranes for more than 30 min (Fig. S5), much longer than observed when measuring typical PSII activity in vitro (such as oxygen evolution), indicating that ET to the BPC has a protective effect. This is similar to the protective effect of cyt c previously reported (Larom et al. 2010). Following termination of illumination, the current drops slowly, indicating a capacitor effect of the BPC. Further experiments will be required to elucidate the entire ET mechanism in our BPC. The present BPC design could not enable the quantitative measurement of the formation of hydrogen gas at the cathode during illumination. Hydrogen formation was measured by removing an aliquot of the BPC solution following illumination and gas chromatographic analysis (Fig. S3). The formation of hydrogen requires the addition of significant external bias (0.5 V).

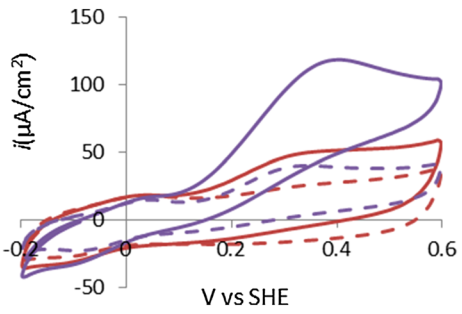

Fig. 5 CV measurements of thylakoid membranes in the BPC. A typical CV measurement performed at a voltage scan rate of 50 mV/sec. 0.05 mg chl *a* of either RSS (*red*) or D1-K238E (*purple*) membranes was allowed to settle onto the NAC-modified gold electrode prior to measurements in the dark (*dashed line*) or in the light (*full line*) in the presence of DCMU

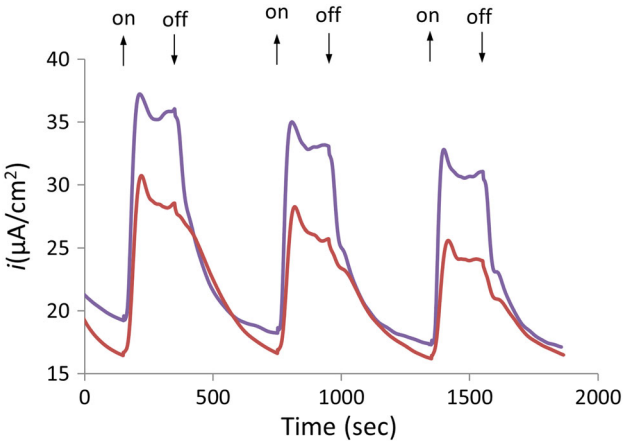

Fig.. 6 The photocurrent response of the D1-K238E-containing cell is higher than the RSS-containing cell. ChAmp response of RSS (*red*) or D1-K238E (*purple*) membranes (0.05 mg chl *a*) in the presence of DCMU was performed in three-electrode mode at 0.75 V bias under chopped standard solar irradiation of 100 mW/cm^2 (AM1.5). The light periods are indicated in *yellow*. The thin *black scale bar* shows boundaries of current measurement

We have previously suggested (Larom et al. 2010) that the main role of DCMU is blocking ET from Q_A to Q_B. On the other hand, thylakoid membranes only contain about 9 plastoquinone (PQ) molecules for every PSII. Thus one could expect that the PQ pool would be rapidly reduced, and in effect this would block any further ET from Q_A. Since the photocurrent continues for many minutes upon illumination, one might expect that this situation would be identical to that of addition of DCMU, yet the behavior of both WT and the D1-K238E membranes in the absence or presence of DCMU is not identical. We can thus suggest that DCMU may have additional roles in our system. The presence of DCMU may prevent rebinding of PQH_2, which could contribute to the known damage that occurs to PSII in vitro. Binding of DCMU may also have subtle structural effects on the stromal surface of PSII in the vicinity of the D1-K238E mutation, as well as changing the redox potential of Q_A (Krieger-Liszkay and Rutherford 1998; Takano et al. 2008).

In conclusion, we show that a crude preparation of thylakoids of cyanobacteria can be used in a BPC to produce a significant amount of electrical current for an extended period of time. Moreover, a modification of an amino acid at the vicinity of the Q_A, that has been

demonstrated to significantly increase the ET from PSII to cyt c in solution, displayed a similar increase in the amount of the electricity produced, however without the requirement of cyt c as a mediator.

Acknowledgments This work has been supported by the US-Israel Binational Science Foundation (2011556), the Israel Ministry of Economy Kamin program, the DIP program of the Deutsche Forschungsgemeinschaft (800027), the Nancy and Stephen Grand Technion Energy Program (GTEP), the I-CORE Program of the Planning and Budgeting Committee and the Technion VPR Gurwin Research Fund. We thank Dr. Pula Mulo for providing us with the PD strain. We thank Yael Talman and Roy Ben Harosh, Technion for assistance in electrode manufacture.

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