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SHORT COMMUNICATION

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Influence of osmolytes on the stability of thylakoid-based dye-sensitized solar cells

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Summary

In recent years, there has been considerable interest in incorporating naturally occurring components of the photosynthetic apparatus into man-made solar cells, because of the high quantum efficiency of photosynthetic reaction centers. One hurdle to overcome regarding the use of native membranes in these devices is their limited lifespans. In this study, we used stabilizers to increase the long-term viability of biomolecules in vitro, thereby alleviating this challenge. In this regard, it is known that osmolytes, such as glycine betaine (GB) and sucrose, preserve photosynthetic activity in isolated photosystems. Upon investigation of the thermal protection properties of GB and sucrose in thylakoid-based dye-sensitized solar cells, we report that the addition of GB and sucrose to the thylakoid photosensitizer maintains nonzero photocurrent in the thylakoid-based solar cell upon heating to 50°C. At 50°C, the GB-containing cell displayed about a fourfold increase in photocurrent than the control cell, in which the photocurrent was decreased to nearly zero. The addition of 0.5M and 1M sucrose has respectively caused nearly 40% and

Abbreviation: DSSC, dye-sensitized solar cell; GB, glycine betaine; ITO, indium tin oxide; OEC, oxygen evolving complex; PSI, photosystem I; PSII, photosystem II

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70% increases in photoinduced electron transfer activity over the control at 35°C. Similarly, though to a lesser extent, 1M GB caused an approximate 40% increase in electron transfer activity as well. Moving forward, this approach will be extended to alternative membrane protein isolation strategies, allowing for an accurate comparison with traditional detergent-isolated complexes, with the ultimate goal of developing a cost-effective and sustainable solar cell.

KEYWORDS
glycine betaine, solar cell, stabilization, sucrose, thylakoid membrane

1 | INTRODUCTION

The photosynthetic apparatus of plants and bacteria is a highly evolved nanostructure enabling the high efficiency conversion of light energy to chemical bond energy.[1-3] Its elegance, high efficiency, and adaptability is the result of millions of years of evolution.[1,4,5] This interminable period of refinement has led to the quantum yield of charge separation in both photosystem 1 (PSI) and photosystem 2 (PSII) reaction centers to approach unity.[6] In addition, the highly abundant pigment-protein complexes provide a cost-effective, renewable, and environmentally benign source of photoactive material. Because of these properties, components of the photosynthetic apparatus have earned considerable interest for light-to-electricity conversion by artificial or biohybrid systems.[7-13]

One of the first investigations into the application of the photosynthetic apparatus for electricity generation was published by M.J. Allen in 1977.[14] In that work, a platinum electrode covered with thylakoid membrane preparations was characterized by a potentiometric technique. In spite of apparent nonzero photocurrent, the authors noted that thylakoid membranes were not efficient as photosensitizers because of their relatively short lifespan.[14] Nevertheless, since this pioneering work, the direct conversion of light energy to electricity by means of biological pigment-protein complexes has been the focus of many investigations to follow.

At present, there are two main problems regarding the design and development of bio-based solar cells. The efficiency of these cells remains very low, and pigment-protein complexes are very susceptible to degradation over time in vitro, yielding a short lifespan of such solar devices.[15] In the former case, lower efficiency of bio-based solar cells can be attributed to the narrow absorption bands of photosynthetic pigments and small light-absorption cross sections of photosystem complexes.[15,16] In the latter case, deterioration of biological structures during the bio-based preparation is also expected to lead to the low efficiencies currently observed in these solar cells. When incorporated into devices, these proteins or lipid-protein membranes are exposed to elevated temperatures, high light intensities, and high levels of atmospheric oxygen, irreversibly damaging the macromolecules. Thus, stability of the extracted and immobilized pigment-protein complexes remains a significant challenge in bio-based solar cell development. Previously published data suggest that the specialized galactolipid environment of the thylakoid membrane stabilizes the PSII and PSI complexes.[17-19] From this viewpoint, it can be proposed that retention of the native membrane environment may preserve photosystem proteins after membrane extraction.[10,20] However, the bilayer structure of the thylakoid membrane is also sensitive to high temperatures, which can cause phase transitions of the surrounding lipids and subsequent cessation of electron transport.[21,22] Nevertheless, investigations conducted over the past 20 years demonstrate the potential for the use of protein containing thylakoid membranes in bio-based solar cells.[15,23-25] To solve the technical problems regarding stability of macromolecules immobilized on inorganic electrodes, several stabilizing molecules such as surfactant peptides, organic self-assembled monolayers, and redox-active polymers have been used.[9,26-28] These stabilizers all have inherent advantages and disadvantages; however, their incorporation is largely hindered because of limited abundance and/or high cost, revealing the need to find more cost-effective, nonresource-limited alternatives.

It has previously been described that specific secondary metabolites in cells, also referred to as cosolutes, offer protection to macromolecules from aggressive environmental factors such as freezing and elevated temperature, pressure, and salinity. The following cosolutes have been reported to stabilize extracted macromolecules in vitro: some amino acids and anionic derivatives, polyols, sugars, methylamines, and methylsulfonium compounds.[29] More specifically, the most studied cosolutes for photosynthetic proteins are glycine betaine (GB), sucrose, trehalose, proline, and glycerol.[29] Therefore, we

decided to take a closer look at these cosolutes to determine if their incorporation could increase the stability of macromolecules immobilized in a solar cell. In this work, we investigate the thermal stability of thylakoid membranes incorporated into a solar cell alongside the cosolutes GB and sucrose. GB and sucrose are ideal candidates for this application because of their low cost and reported stabilizing effect on extracted photosystems.[21,30-32]

GB or *N*,*N*,*N*-trimethylglycine is an amphoteric osmolyte that has been observed to accumulate in a variety of plant species in response to various environmental stresses. It has also been shown that GB provides a stabilizing effect on enzyme activity following protein isolation.[31,33,34] Further, it has been reported that GB accumulation was more effective in chloroplasts than in the cytosol.[35] Regarding its structure, presented on Figure 1 A, GB possesses both amphoteric and amphiphilic properties and as such can simultaneously interact with both hydrophilic and hydrophobic domains of a membrane protein.[33] It has been presumed that in addition to being a nontoxic, cellular osmolyte, GB is able to stabilize the structure of protein complexes (thereby preserving activity) and might also maintain the integrity of membranes against the damaging effects induced by thermal transitions.[36] Several studies have described the mechanism of this GB stabilizing effect on proteins by two prevailing models: (*a*) GB has been selectively excluded from binding to the protein surface in such way that a tightly associated hydration shell has formed around the hydrophilic domains of the protein, stabilizing its native structure[32,37,38]; (*b*) the methyl-rich, hydrophobic portion of GB has bound directly to the hydrophobic domains of the protein to solvate these hydrophobic domains.[32,39] It is possible that both of these processes may occur simultaneously during interaction with membrane proteins. Similarly, numerous studies have shown that sucrose can stabilize the photosynthetic apparatus by at least two mechanisms, unrelated to those proposed for GB. First, sucrose is proposed to replace/expel water molecules from the protein surface, driving the proteins to attain a conformation that minimizes their solvent-exposed surfaces.[29] The second mechanism proposes an altered macroorganization of the lipid-protein interface, inducing nonbilayer lipid phase separation.[21,40]

To date, an investigation of the stabilization of photocurrent in bio-based solar cells by the addition of GB or sucrose has not been conducted. There are several environmental factors that affect the activity of pigment-protein complexes in solution and the efficiency of solar cells composed of these complexes, including light intensity, spectral quality, temperature, and humidity. For example, elevated temperature and high light intensity cause the deterioration of macromolecules. We hypothesized that the GB and/or sucrose can protect macromolecules from destruction by high temperature. In a real-world scenario, protein containing solar cells would experience prolonged high light irradiance. Therefore, in order to combat the elevated temperature that results from this setting, optimizing the thermostability of these devices is of the utmost importance.[23]

The main goals of this research are to compare the effects of temperature (with and without osmolytes) on photosynthetic activity in isolated thylakoid membranes and to determine the overall photocurrents of biohybrid solar cells incorporating these membrane preparations.

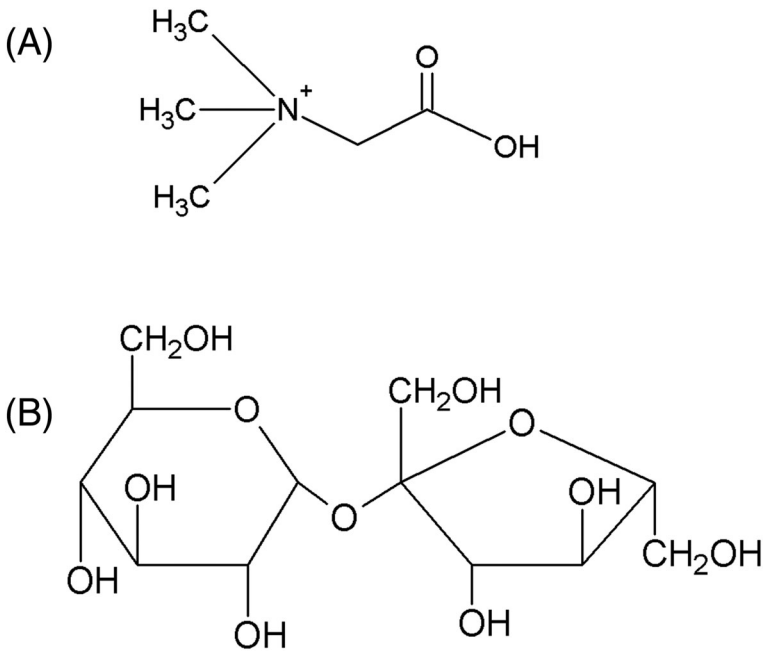

FIGURE 1 Structures of glycine betaine A, and sucrose B,

The results of this study will allow us to better understand the stabilizing effect of chemically diverse osmolytes and evaluate their validity for use in biohybrid solar devices. The solar cells in this work are modeled after the dye-sensitized solar cell (DSSC), with thylakoid membrane serving as the sensitizer.[41] The simplified DSSC has a sandwich-like design that is composed of two glass electrodes coated with a layer of transparent conductive oxide. The conductive side of the anode is covered by a film of mesoporous, nanostructured TiO_2. Thylakoid membrane fragments are immobilized on the TiO_2 semiconductor surface. After sensitization, the electrodes are attached to each other, and cavities between them (owing to the nonplanar geometry of TiO_2) are filled by electrolyte solution.[42] In order to investigate the protective effect of GB and sucrose against elevated temperatures for these cells, photosynthetic activity was characterized by measuring the oxygen evolution rate from the PSII oxygen evolving complex (OEC) and PSII maximum quantum yield.

2 | MATERIALS AND METHODS

2.1 | Materials

Thylakoid membranes were harvested from store-bought spinach. Sodium ascorbate, GB, and sucrose were sourced from Sigma-Aldrich, USA. Polished float glass plates coated with indium tin oxide (ITO) (Delta Technologies, USA) served as electrodes. Resistance of the conductive side of the glass was 9 to 15 Ω; dimensions were 25 × 25 × 0.7 mm. TiO_2 paste Ti-Nanoxide D/SP (Solaronix, Switzerland) was used for the preparation of the mesoporous semiconductor layer. I/I3- electrolyte (HI-30, Solaronix, Switzerland) was used as primary electron donor for the photosynthetic protein complexes in the solar cell. Electrically conductive silver-based adhesive (Contaktol, Russia) served to connect the wires to the glass electrodes. Buffer for oxygen evolution measurements contained MES monohydrate and NaCl (Sigma-Aldrich). Potassium ferricyanide (Sigma-Aldrich) served as electron acceptor in OEC activity assay. Sodium dithionite (Sigma-Aldrich) was used for calibration of the Clark-type electrode.

2.2 | Methods

2.2.1 | Sample preparation

Thylakoid membranes were extracted from fresh spinach leaves according to known procedures.[43] Briefly, leaves lacking ribs were blended in Tris-HCl buffer (20mM, pH 7.8) containing 1 g/L sodium ascorbate. The resulting mixture was centrifuged at 2000*g* for 5 minutes to separate insoluble plant material. The supernatant was further centrifuged at 5200*g* for 30 minutes, to pellet the thylakoid membrane fragments. The thylakoid membrane suspension, with a chlorophyll concentration of 5.01 mg/mL and 20% (v/v) glycerol, was stored at −70°C prior to use.[43] On the day of solar cell preparation, thylakoid membranes were thawed and diluted twofold with buffer containing 25mM MES and 10mM NaCl at pH 6.5. Solutions of GB and sucrose were then introduced to the thylakoid membrane suspension at this point. In the control cell, the photosensitizer suspension did not contain any cosolutes. The amount of thylakoid membrane added to the electrode surface was standardized to chlorophyll concentration. The resulting suspension contained 1.2 ± 0.2 mg chlorophyll/mL as determined by absorbance spectroscopy with a Cary 8454 spectrophotometer (Agilent Technologies, USA).

2.2.2 | Cell preparation

Construction of the solar cell has been described previously.[23] In short, DSSCs incorporate two glass plates coated with conductive transparent layer of ITO.[44] One ITO-glass electrode (the anode) was covered with a paste of nanostructured TiO_2 by a doctor-blade method, followed by high temperature sintering. The electrodes were sintered in the following temperature profile: 100°C for 10 minutes, 200°C for 10 minutes, and 300°C for 20 minutes, then cooled for 15 minutes. The area of electrode covered with TiO_2 was approximately 1.5 cm^2. After the glass was cooled to room temperature, the thylakoid membrane suspension was drop casted onto the TiO_2 surface, until fully covered. The electrode was then incubated overnight (approximately 15 h) at 4°C to achieve complete absorption of the photosensitizer. Next, unbound thylakoid membrane fragments were removed by gentle rinsing with MES buffer. The anode and the cathode (untreated ITO-glass electrode) were then connected to each other using double-sided adhesive tape. The electrodes were slightly offset relative to each other, allowing space for attachment of wires on each electrode. Small wires were connected to both electrodes by electrically conductive adhesive, providing electrical contacts. The space between the two electrodes was filled with iodine/triiodide (I/I3-) electrolyte solution (approximately 10 μL), penetrating into the pores of the mesoporous TiO_2 layer by capillary action.

In this study, three solar cells were compared. The first cell contained thylakoid membrane preparations without any additive, referred to as the control cell. The photosensitizer suspension for the second and third solar cells contained 0.5M GB and 0.5M sucrose, respectively. We also prepared solar cells with thylakoid suspensions containing 1M GB and sucrose, but these suspensions were not successfully immobilized, and all sensitizer was completely washed away from the TiO_2 matrix during the rinsing following incubation.

2.2.3 | Measurement of oxygen evolution

Oxygen evolution of PSII in thylakoid membrane suspension was measured using a Clark-type electrode (Hansatech, UK) at various temperatures. Fixed temperature was provided by a circulating water bath (Huber KISS K6, Germany). The analysis was carried out in 950 μL of buffer solution containing 25mM MES (pH 6.5), 10mM NaCl, and 5mM ferricyanide followed by addition of 50 μL of thylakoid membrane suspension. Chlorophyll concentration of resulting mixture was approximately 30 μg/mL. Light intensity was 1000 μmol photons m-2 s-1. The system was calibrated at each temperature by the addition of sodium dithionite to 1 mL of distilled water. The samples were dark adapted for 5 minutes before being irradiated with full-spectrum white light. The suspension was stirred with a magnetic stirrer during the measurement.

2.2.4 | Fluorescence measurement

PSII maximum quantum efficiency was measured using MINI version of the IMAGING-PAM (WALZ, Germany). Three drops of sample were deposited on a Peltier module (thermoelectric cooler) and covered with glass in the active area of the IMAG-PAM camera. The Peltier module was controlled with a regulated power source (YIZHAN, China). Heat from the backside of the cooler was removed with cold tap water. Each experiment was repeated at least five times ($n = 5$). Frequency of the probe light source was 1 Hz with an intensity of 20 μmol photons m-2 s-1. Intensity of the saturating pulse was about 6000 μmol photons m-2 s-1. The sources of both lights were blue LEDs with emission maxima at 460 nm. The samples were dark adapted for 5 minutes before measurements. At the beginning of the measurement, the samples were exposed to probe light for 4 seconds, after which the saturation pulse was turned on.

2.2.5 | Investigation of solar cells

Photocurrent of the solar cells under varying light and temperature conditions was analyzed using a previously described custom measurement system.[23] An analog-to-digital converter E14-440 (L-card, Russia) measured photocurrent and exported data to the computer in real time. A Peltier element kept temperature constant during each measurement, ranging from 5°C to 50°C. The regulated power supply allowed for temperature variation through a programmed ramping of the supply voltage or photocurrent variation. A thermocouple connected to the temperature regulator module (OVEN, Russia) measured the working temperature.

Chronoamperometric measurements at various temperatures were carried out on solar cells previously dark adapted for 5 minutes, to determine temperature dependence of photocurrent generation under each solar cell treatment. During these measurements, temperature and light intensity had fixed user-defined values. Photocurrent values measured at 500th second (±1 s) after light irradiation were compared. A chronoamperometry curve depicting photocurrent approaching and following this time point can be found in Figure 2. In most cases, the curve reached a plateau before 500th second, but not in all experiments. The measurements of photocurrent dependence on temperature were performed at white light intensity equal to approximately 100 ± 5 μmol photons m-2 s-1.

3 | RESULTS

3.1 | Oxygen evolution rate of PSII

OEC activity was measured across temperatures ranging from 5°C to 55°C, and the curves obtained are shown in Figure 3. The temperature that yields maximum oxygen evolution is about 25°C for all samples. One can see that the suspension with 1M sucrose lies above the other curves for all temperatures. The curves for the control and 0.5M GB-containing suspensions have no significant differences. Samples with 1M GB and 0.5M sucrose demonstrate distinctly higher oxygen evolution rates in comparison with the control suspension at 35°C. Thus, we can say that the addition of 1M sucrose increases the activity of the OEC most significantly over a wide temperature range. At the same time, addition of the cosolutes does not cause notable expansion of the temperature range of OEC activity. For all samples tested, oxygen evolution ceases at approximately 55°C. One can conclude from these results that all samples succumb to highly elevated temperature in the same

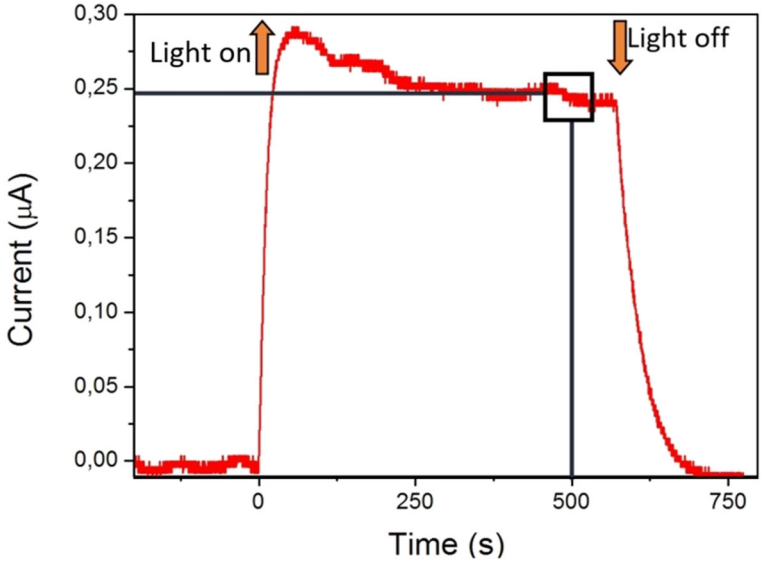

FIGURE 2 Photocurrent versus time of dark-adapted solar cell. Photocurrent values at 500th second after light turned on were taken for comparison of various solar cell designs [Colour figure can be viewed at wileyonlinelibrary.com]

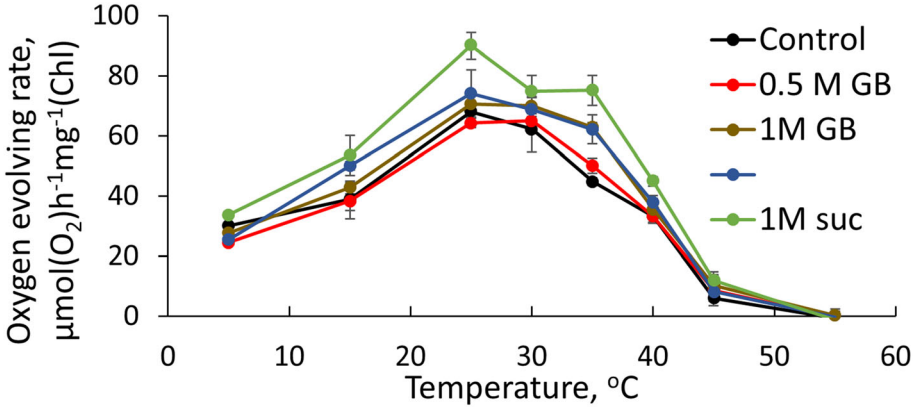

FIGURE 3 Dependence of oxygen evolution rate on the temperature expressed in absolute values. Error bars represent the standard deviation between samples (n = 3). GB, glycine betaine [Colour figure can be viewed at wileyonlinelibrary.com]

manner. Further, these data show that 0.5M to 1M sucrose and 1M GB samples are more thermostable than the control at 25°C to 35°C.

The results of a *t*-test this test, shown in Table 1 for 25°C, 35°C, and 40°C, suggest there are significant differences between O_2 evolving rates for 1M sucrose-containing thylakoid preparations compared with the control. At higher temperatures (but still below 40°C), differences between 0.5M sucrose and control preparations are also significant. The number of

TABLE 1 Oxygen evolution activity of osmolyte-treated thylakoid preparations compared with no osmolyte treatment at various temperatures

	Effect of Osmolyte Addition (% Change Relative to No Osmolyte Control)			
Temperature, °C	**0.5M GB**	**0.5M Sucrose**	**1.0M GB**	**1.0M Sucrose**
25	94.6% ± 7.9%	108.9% ± 24.4%	103.6% ± 12.4%	132.4% ± 14.8%**
35	111.7% ± 8.6%	138.8% ± 14.9%*	140.3% ± 6.1%**	167.8% ± 15.6%**
40	99.8% ± 6.2%	113.8% ± 4.3%*	106.9% ± 14.0%	134.9% ± 5.0%**

Abbreviation: GB, glycine betaine.

**$P < .01$.

*$P < .05$.

repetitions is 3 (n = 3) for all measurements; values are considered significantly different if $P < .05$. The most profound effect of osmolyte addition was observed at 35°C (Table 1). The addition of 0.5 GB did not impact the oxygen evolving rate at room and higher temperatures. Moreover, it is possible that photosynthetic activity is depressed by 0.5M GB and 0.5M sucrose at low temperature (Table 1). Lastly, 1M sucrose shows significant impact over the 25°C to 40°C temperature range.

3.2 | Maximum quantum yield of PSII

Figure 4 demonstrates the temperature dependence of PSII via measurement of maximum quantum yield. Fv/Fm gradually decreases as temperature increases from 5°C to 50°C for all samples. At 55°C, all except sucrose-containing samples demonstrate significantly lower values of Fv/Fm and large measurement errors. At temperatures below 50°C, no significant difference can be seen in the yield between the control and samples containing stabilizers. However, at elevated temperature, sucrose-containing samples demonstrate clear thermostability over other sample conditions. Thylakoid membrane preparations containing 0.5M GB show higher susceptibility to elevated temperature compared with the sucrose stabilized solar cells, which is evident when we compare results at 50°C. The control, 0.5M GB, and 1M GB-containing samples demonstrate very similar results over the entirety of the temperature range. At 55°C, the sample containing 1M sucrose retains 66% of its maximum activity in terms of PSII maximum quantum yield, and 0.5M sucrose retains about 62% of this maximal activity (measured at 5°C, $P < .01$).

3.3 | Photocurrent dependence on temperature

Thylakoid membranes with 1M and 5M GB, as well as 1M sucrose, were unable to be immobilized onto the TiO_2 semiconductor surface. Thus, we do not have results to report for cells with these sensitizer/cosolute concentrations. Figure 5 shows the dependence of photocurrent on temperature at fixed light conditions. All three cells present photocurrent maxima above the lowest temperature tested (5°C), at approximately 10°C, 20°C, and 35°C for the control, 0.5M GB, and 0.5M sucrose, respectively (Figure 5). At temperatures approaching 40°C, the photocurrent for the control cell falls by approximately 90% relative to its maximum value, whereas the GB-containing cell loses approximately 56% and the sucrose-containing cell loses only approximately 30% of its maximum photocurrent. Lastly, the optimal temperature at which photocurrent is greatest was higher for the sucrose-containing solar cells compared with GB-containing and control cells.

T-test results shown in Table 2 reveal a significant difference between control and sucrose-containing solar cells appears only for temperatures exceeding 30°C. At temperature above 40°C, both sucrose- and GB-containing solar cells generate significantly higher photocurrent than their respective control cells ($P < .05$, n = 3).

4 | DISCUSSION

The remarkable efficiency of the photosynthetic apparatus makes it an attractive prospect for solar energy harvesting device integration to produce electrical energy. To this end, it is necessary to stabilize these protein

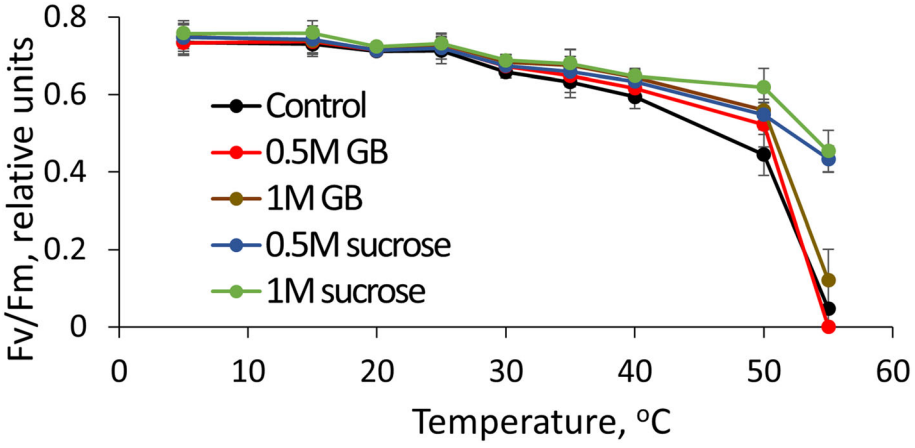

FIGURE 4 Dependence of PSII maximum quantum yield on the temperature expressed in absolute values. Error bars represent the standard deviation (n = 5). GB, glycine betaine; PSII, photosystem 2 [Colour figure can be viewed at wileyonlinelibrary.com]

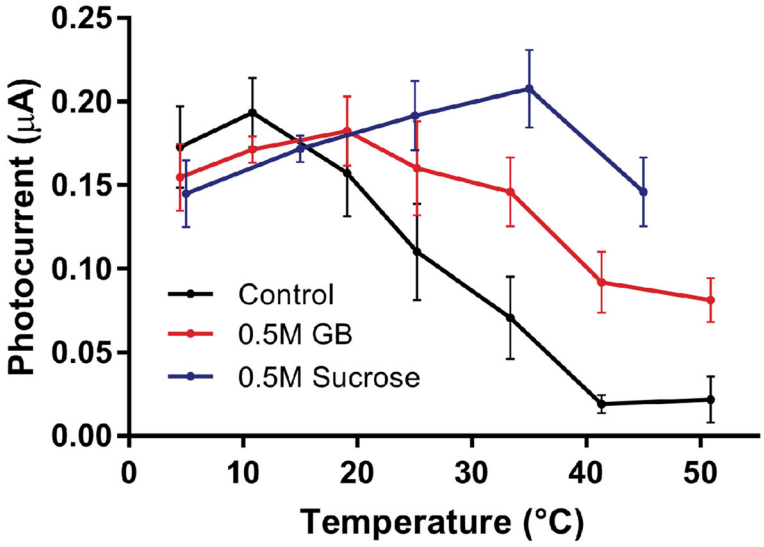

FIGURE 5 Effect of temperature on photocurrent generation. Light intensity = 100 ± 5 μmol photons m-2 s-1. Error bars represent the standard deviation between samples (n = 3). The difference between the photocurrent values was not statistically significant at temperatures below 30°C. GB, glycine betaine [Colour figure can be viewed at wileyonlinelibrary.com]

complexes in an in vitro (commonly inorganic) setting for the most effective deployment of this technology to meet our growing energy needs. In nature, the ability of photosynthetic protein complexes to convert photonic energy to a charge separated state and subsequent electron transfer is facilitated via a complex system of photochemical and redox reactions, which occur simultaneously and in close proximity. Bound in the thylakoid membrane, photosynthetic complexes comprise both more stable (eg, reaction centers) and more susceptible (eg, OEC) components. This complexity makes the determination of overall in vitro activity in the presence of osmolytic cosolutes difficult.[21,32,45] Upon immobilization onto the inorganic matrix, efficiency of the photosynthetic reaction center protein complexes is clearly altered, potentially because of proposed conformational changes imparted onto these protein complexes through this process.[23] We suggest that cosolutes shown to stabilize the photosynthetic apparatus in suspension also will carry this effect when immobilized on the TiO_2 matrix. In addition, comparing the impact of cosolutes on the activity of photosynthetic reaction centers in suspension, to incident photon to electron conversion efficiency in the thylakoid-based solar cell, may elucidate which components of the photosynthetic apparatus are critical to energy conversion in the device.

TABLE 2 Photocurrent output of devices with and without osmolyte at various temperatures

	Effect of Osmolyte Addition (% Change Relative to No Osmolyte Control)	
Temperature, °C	**0.5M GB**	**0.5M Sucrose**
5	89.5% ± 0.09%	83.7% ± 0.09%
10-20	115.9% ± 0.10%	109.2% ± 0.08%
25	145.4% ± 0.12%	174.2% ± 0.11%
32-36	206.2% ± 0.09%	293.3% ± 0.10%*
40-50	484.2 ± 0.06%*	767.7 ± 0.06%*

Abbreviation: GB, glycine betaine.

*$P < .05$.

Results reported in this work are aimed at determining the effect of temperature on the primary processes of photosynthesis and the activity of solar cells containing thylakoid membranes as the sensitizer. More accurately, the goal of this study is to compare the effect of known stabilizers on the activity of light energy conversion, occurring in thylakoid membranes in suspension and immobilized in solar cells. Here, we show that sucrose and to a lesser extent GB increase oxygen evolving activity in thylakoid suspensions at concentrations around 1M in the presence of 5mM potassium ferricyanide (Figure 3). This effect is in agreement with previously published studies.[32,45] We have also shown that the addition of these cosolutes does not expand the overall temperature range of OEC activity. This expands on the work from Allakhverdiev et al from 1996, which showed that GB and sucrose can preserve PSII from irreversible temperature-induced destruction of OEC. In their experiments, BBY-particle suspensions were incubated at elevated temperature for 5 minutes followed by oxygen evolving rate measurements, conducted at 20°C.[32] Incubation at 55°C degraded OEC activity by approximately 55% in the presence of 1M GB or 1M sucrose, while the control was completely inactive after exposure to this temperature.[32] Thus, we have concluded that the presence of these cosolutes increases the activity of the

OEC, potentially through the inhibition of structural changes at elevated temperatures. The strongest evidence supporting this hypothesis is the observation that activity of the OEC with GB or sucrose can be restored after incubation at 55°C. However, even in the presence of GB or sucrose, oxygen evolution ceases at this temperature.

This increase of OEC activity in the presence of the cosolutes may be attributed to a more native protein conformation (as previously stated) and/or the retention of native boundary lipids and their interaction with the protein complexes. Further, the reversibility of OEC activity could be attributed to reversible lipid phase transitions, which have also been shown to be affected by the presence of cosolutes.[21] However, it should be stated that this hypothesis remains controversial, and further investigation is needed to reach a conclusion in this area.[32] At the same time, in this experiment, we were able to measure the rate of oxygen evolution by thylakoid membrane preparations in the presence of exogenous electron acceptors catch electrons from PSI only. From this vantage, the value of O_2 evolved is a measure not only of OEC activity but also of electron transport activity on the scale of the whole electron transport chain from water to the PSI acceptor.

Charge separation in PSII of the dark-adapted thylakoid membranes can occur at 55°C in the presence of 0.5M to 1M sucrose. This is shown in the results from the pulse modulated fluorescence measurement shown in Figure 4. At the same time, PSII maximum quantum yield across the majority of the temperature range (approximately 5°C-40°C) is independent of cosolutes, which is in agreement with a recently reported result by Kotakis et al in 2018.[21] With regard to quantum efficiency of PSII, the stabilizing effect of GB was not significant compared with the control. This may be because Fv/Fm is less susceptible to elevated temperature compared with OEC activity. We reason that this protection may be due to the placement of the reaction center deeper within the protein complex than the peripherally associated and exposed OEC. Also, the oxygen evolving rate measured in the presence of potassium ferricyanide is more properly suited to observe overall activity of the photosynthetic apparatus compared with Fv/Fm, which characterizes the activity of PSII alone. For both OEC activity and Fv/Fm measurements, the figures for GB and sucrose are different. These data suggest that mechanisms of interaction between these osmolyte and thylakoid membranes are distinct.

We were unable to prepare solar cells at 1M concentrations of GB and sucrose, previously tested in solution. It is possible that cosolute molecules if present at high concentrations hinder the absorption of protein containing, thylakoid membrane fragments to TiO_2. This would explain why we were able to observe the effect of 0.5M sucrose and GB on activity of the solar cell (Figure 5). All solar cells in this work demonstrate a maximum in photocurrent with regard to increasing temperature. Such temperature dependence agrees with previously described results for DSSCs incorporating nonbiological sensitizers.[46] In this study by Peng and Berberoglu, the performance of these solar cells using acetonitrile-based redox mediator was observed from 5°C to 50°C. These areas are separated by the temperature that allows for the maximum production of photocurrent. At lower temperature, photocurrent of these devices is primarily diffusion limited. This rate of diffusion increases with increasing temperature, and the faster this diffusion occurs, the higher the resulting photocurrent. In the elevated temperature trials, photocurrent is limited by electron and hole recombination reactions. The rates of these undesirable processes, also referred to as reverse reactions, increase with increasing temperature, as these reverse reactions become more detrimental to overall photocurrent production than ionic diffusion at elevated temperatures.[46] Both the control cell and GB-containing cell produced maximum photocurrent in the temperature range from 10°C to 20°C (Figure 5). The sucrose-containing solar cell demonstrated a photocurrent maximum around 35°C. In addition, the maximum photocurrent generated by the sucrose-containing solar cell was higher than that of the control and GB-containing cells. Temperature dependence of photocurrent can therefore be more appropriately explained by the previously reported properties of the DSSC, rather than the dependence of OEC activity. In other words, advantages of sucrose-containing cells at elevated temperature can be attributed to the preservation of the chlorophyll antennae within PSII and its ability to funnel photonic energy to the reaction center (shown by Fv/Fm), rather than OEC preservation. Overall, our data suggest that OEC activity is not critical to the overall performance of thylakoid-based solar cells. However, this hypothesis requires further confirmation by testing OEC-deficient thylakoid membranes as the sensitizer.

Taking these results into account, we can make an inference as to how these osmolytes offer stability to the thylakoid membrane sensitizer in the solar cell. Thylakoids membrane fragments are absorbed into the porous TiO_2 and are ultimately exposed to the electrolyte. We propose that the portions of membrane exposed to the liquid electrolyte are damaged to a greater extent from elevated temperature as compared with the TiO_2-associated part of the membranous sensitizer. Osmolyte molecules can therefore stabilize the exposed parts of the membranes by excluding the bulk of the iodine/triiodide mediator solution (Figure 6).

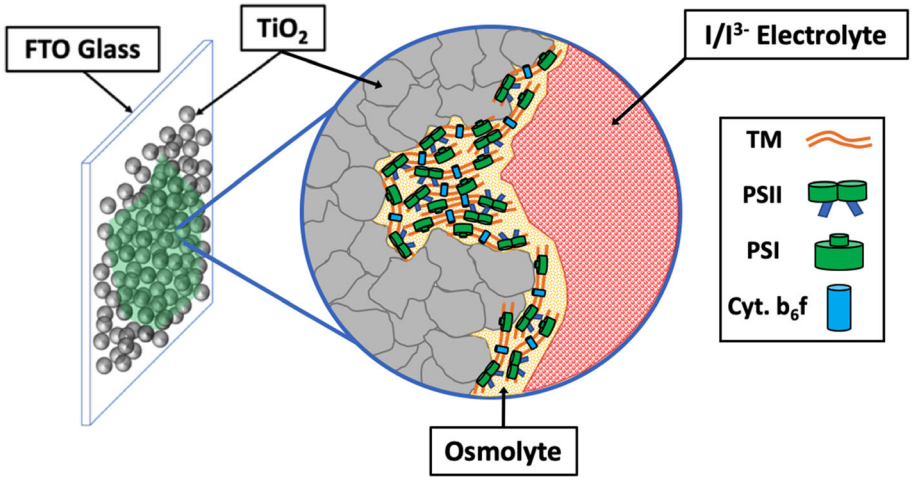

FIGURE 6 General scheme of biohybrid solar cell with osmolyte. Magnified cross section of thylakoid membrane fragments packed into TiO_2 pore is shown. The yellow dotted region represents high concentration of osmolyte molecules. The red region signifies the bulk iodine/triiodide redox mediator solution. PSI, photosystem 1; PSII, photosystem 2 [Colour figure can be viewed at wileyonlinelibrary.com]

5 | CONCLUSIONS

In solution, we observe that GB and sucrose protect the OEC from the temperatures approaching 55°C; however, they do not enable the OEC to operate above this temperature. With regard to PSII photochemistry, sucrose stabilizes the reaction center at 55°C, showing significantly increased quantum yield compared with the control cell, which is fully degraded at this temperature. From the perspective of solar cell design, sucrose and GB at high concentration (≥1M) hinder absorption of thylakoid membranes to TiO_2 functionalized electrodes. Further, at 0.5M sucrose and GB, photocurrent of the thylakoid-based solar cells is increased at elevated temperatures; this effect is more pronounced for sucrose than GB. Taken together, these data suggest that the OEC, apparently, does not participate in the charge transfer of the thylakoid-based solar cell. Therefore, it is possible that it may impede desirable processes, yet this hypothesis requires further testing.

The nature of the interaction between thylakoid membranes and these cosolutes remains unknown and will require further investigation. However, we can state several findings on the basis of our results presented here:

1. Sucrose is more effective than GB in increasing the oxygen evolving rate in thylakoid membranes in the presence of the PSI acceptor.
2. The effect of osmolyte is most noticeable at temperature values about 35°C.
3. The effect of 0.5M sucrose is comparable with effect of 1M GB; effect of 1M sucrose is most dramatic (67% increasing at 35°C); 0.5M GB shows no significant effect.
4. Maximal quantum yield of PSII does not depend on the presence of osmolytes in a wide temperature range.
5. At temperatures exceeding 40°C, the effect of sucrose appears, and at 55°C, this becomes significant. GB is unable to preserve PSII activity at such temperatures.
6. It is very likely that the chemical action of GB differs from that of sucrose in causing these stabilizing effects.
7. The addition of exogenous sucrose and GB (to a lesser extent) at 0.5M does increase the efficiency of thylakoid-based solar cells at elevated temperatures
8. The OEC does not appear to participate in the charge transfer of the thylakoid-based solar cell.
9. The addition of exogenous sucrose and GB (to a lesser extent) does increase the stability and efficiency of thylakoid-based solar cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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