Optimization and Stabilization of Phycobilisomes for use in Applied Phototechnology

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ABSTRACT

Photovoltaic cells are remarkably useful devices for producing power by converting the energy provided by the sun to usable electricity. Annually, the earth receives $4 \times 10^{24}$ J of energy from the sun, but the diffuse nature of this energy makes it difficult to harness. Unfortunately, current photovoltaic devices have a limited cost effectiveness because of the diffuse nature of solar radiation, the high cost of the materials used in these cells, and a limited efficiency. An effective solar concentrator used in tandem with a photovoltaic cell increases the efficiency and cost effectiveness of these devices. In nature, cyanobacteria have been using solar power for ~ 3.5 billion years and have dealt with the problem of diffuse solar radiation by manufacturing light antennae, phycobilisomes, that contain ~ 1,500 pigments each. These natural solar concentrators absorb high energy blue-green light and funnel this light energy to the red absorptive chlorophyll A which drives photosynthesis. Phycobilisomes are naturally engineered light concentrators, and have shown promise for use in solar concentrators for photovoltaic cells. Phycobilisomes from 4 cyanobacteria, Synechococcus sp. WH8102, Synechococcus sp. WH7803, Synechocystis sp. PCC6803, and Thermosynechococcus elongatus, were evaluated for use in solar concentrators for photovoltaic cells. Intact phycobilisomes are very labile in aqueous solution; to combat this problem phycobilisomes were covalently stabilized by cross linking with formaldehyde. The structure and function of these stabilized and non-stabilized phycobilisomes were evaluated using fluorometric analysis, analytical ultracentrifugation, circular dichroism and SDS-PAGE. Phycobilisomes isolated from Synechococcus sp. WH8102 show the most optimal properties for use in the current application. Phycobilisomes isolated from this organism gave an effective absorption over 240 nm of the visible spectrum (430 nm to 670 nm). Moreover, they also showed the largest shift between absorption and emission of the phycobilisomes investigated, which may maximize the transmittance of captured light to a photovoltaic device.
INTRODUCTION

Organic photovoltaic cells are a promising source of inexpensive, renewable energy; however, their low efficiency keeps them from being a practical source of energy. The problem with traditional silicon based solar cells is that they must absorb photons and undergo charge separation. Thick films optimize the former function while the latter function is optimized by thin layers of silicon, as shown in panel C of figure 1. Separating the optical and electrical duties of the device by incorporating an antenna to absorb light and transfer it to a conventional photocell to produce electricity can alleviate this problem. Panel C of figure 1 suggests that the combination of an antenna and an organic photocell has the potential to double the performance of organic solar cells. Photosynthetic organisms harvest and use solar energy in much the same way. Light is primarily harvested by an antenna structure and transferred to a reaction center where the solar energy is converted to a usable form. In panel A of figure 1, traditional organic photocells, photosynthesis, and the proposed photocell and antenna combination are compared. Cyanobacteria have optimized their solar antenna complexes, phycobilisomes, for harvesting light for photosynthesis. The same properties that make them ideal in nature also gives them optimal properties for use in the proposed antenna/PV combination.

Phycobilisomes (PBS) function as light harvesting devices in cyanobacteria and red algae. These large pigment-protein complexes are capable of absorbing light over a broad range of the visible spectrum and efficiently delivering this captured energy to the photosynthetic reaction center (1,2). PBS exist as large macromolecular complexes attached to the thylakoid membrane and transfer absorbed energy to Photosystem II (or under certain circumstances to Photosystem I) (3). Optimized over the last ~3.5 billion years, the PBS are able to transfer absorbed energy with near 100 percent efficiency (4). The main components of PBS are the phycobiliproteins (PBP) which contain linear tetrapyrrole pigments, called bilins (5). The total number of bilins per PBS is
highly variable between different species (4, 6, 7) and even within a species under different
growth conditions (8–12). Although no high resolution structure exists for any intact PBS, low
resolution structures based on EM and AFM, have shown a common structural organization, where
the PBS is organized with a series of attached hexameric disks organized into several non-parallel
rods, emanating from a core structure that contains two or more parallel cylinders, with a
triangular arrangement being the most common(1,13). The PBS core contains allophycocyanin
(APC), and the rods contain phycocyanin (PC) that absorbs higher energy light, and some
organisms may also contain phycoerythrin (PE) at the distal ends of the rods (14). In general, non-
marine cyanobacteria have PBS tuned to absorb more red light (i.e. PBS lacking PE) while marine
cyanobacteria must absorb available blue-shifted light found deeper in the water column; this is
accomplished by incorporating the blue absorbing PBP phycoerythrin-I, the bilin content of which
is composed mostly of phycoerythrobin, or a further blue-shifted absorbing PBP phycoerythrin-II,
the bilin content of which is composed mostly of phycourobilin, into the distal ends of the PBS
rods (15). The PBPs have their bilins arranged in such a way that high energy radiation is captured
at the outermost portion of the PBS and conducted through the PBS to the core (16). PE, with an
absorption maxima near 570 nm, forms the outermost portion of the rod, while PC, with an
absorption maxima near 625 nm, forms the portion of rod adjacent to the core (4). The PBS core
itself, is formed almost entirely of APC which has an absorption maxima near 650 nm. This layout
forms a complex in which absorbed energy is rapidly conducted downhill from PE->PC->APC->
Chl where energy from absorbed photons is transduced to chemical energy through
photosynthesis.

Linker proteins contribute 10-15% of the mass to PBS and are necessary for the
arrangement of PBPs into the PBS complex (8,17,18). In assembling the PBPs into the PBS, linkers
serve to direct efficient energy migration throughout the PBS by tuning the properties of the PBPs
Four major types of linkers exist: the core membrane linker (L_{CM}) which anchors the core of the PBS to the thylakoid membrane and is the major terminal energy transmitter to PSII, small core linkers (L_{c}) which are associated with the periphery of the APC core, rod-core linkers (L_{rc}) which attach the rods to the core, and rod linkers (L_{R}) that associate the PBPs into rods (3,4,16).

*In vivo*, organisms use several mechanisms to regulate the assembly, number, location, and even PBP composition of PBS (4,10,19,20). These mechanisms are necessary to adapt to a changing environment and regulate the large biosynthetic commitment that the PBS represent. Under high light conditions or during nitrogen starvation, PBS may be remodeled and/or consumed by the organism as necessary (19,21). It has been shown that linker proteins (of PCC6803 at least others?) are dephosphorylated under these conditions, which appears to be instrumental in degradation of the PBS *in vivo* and contributes to their complete dissociation *in vitro* (19). Methylation of the PBPs also appears to contribute to stabilization of the PBS complex and significantly contributes to the efficient energy transfer of the PBS (20).

PBPs have been used for many years as fluorescent tags in cell labeling, fluorescence activated cell sorting, flow cytometry, immunoassay and histochemistry (22,23). The properties of the PBPs that make them useful in these applications (large stokes shift, shielded location of bilins within PBP, large range of absorption, rapid and efficient energy transfer) is magnified when the PBPs are organized together in the PBS complex. *In vitro*, the high efficiency of energy transfer within the PBS has only been observed in high phosphate buffer (0.7M-1.0M). Although the individual PBPs themselves appear to be stable in aqueous solution, the quaternary organization of the PBS complex itself is labile. Upon dilution of the PBS into low ionic solutions, there is a uncoupling of energy transfer from PE-PC-APC, this uncoupling is the result of structural changes that lead to complex disassembly of the rods from the core complex.
Unfortunately, phycobilisomes are labile unless kept in high phosphate buffer, so while investigating them for use in the proposed application we must simultaneously investigate ways in which to keep them functional when removed from high phosphate buffer and later in polymeric films. Here, crosslinking with formaldehyde is investigated as a possible treatment for transferring stability to intact phycobilisomes. Phycobilisomes from 4 cyanobacteria, Synechococcus sp. WH8102, Synechococcus sp. WH7803, Synechocystis sp. PCC6803, and Thermosynechococcus elongatus were evaluated for use in a solar concentrator before and after crosslinking using fluorometric analysis, analytical ultracentrifugation, circular dichroism and SDS-PAGE.

MATERIALS AND METHODS

Organism and Culture Conditions. Synechocystis sp. PCC6803 was grown until late log phase in BG-11 at 30°F under a light intensity of 70μE in an airlift fermenter (Bethesda Research Labs, Bethesda MD) bubbled with 0.2 μm filtered air and no additional CO₂ was added. Thermosynechococcus elongatus was grown in the airlift fermenter under similar light conditions.
with a temperature of 56°C in modified media D (24). *Synechococcus* sp. WH7803 was grown in A+ media (25) under ~20 μE at 20°C without bubbling in 2L culture bottles with occasional stirring. *Synechococcus* sp. WH8102 was grown in AN media (26, 27) under ~20 μE at 20°C in 2.8L Fernbach flasks with occasional stirring.

**Phycobilisome Isolation** - Phycobilisomes from *Synechocystis* sp. PCC6803, *Synechococcus* sp. WH7803, *Synechococcus* sp. WH8102, and *Thermosynechococcus* elongatus were isolated as described respectively (4,11). Cells were collected at 5000 x g for 5 min, and resuspended at 0.12 g wet weight/ml in 750 mM NaKPO₄ (350mM NaPO₄, 350mM KPO₄, pH 8.0), and lysis was achieved by passing through the French Press at 20,000 psi twice. *T. elongatus* samples were resuspended in 750 mM NaKPO₄ (pH 8.0), 500 mM Sorbitol, and 1% lysozyme and incubated for 2 hours at 42°C in the dark before French Pressing. The lysate was incubated with 1-5% Triton-X100 with stirring for 45 min at room temperature, and phase separation was achieved by centrifugation at 31,000 x g for 30 min (for WH7803 and WH8102, 7,000 x g, and for *T. elongatus* 40,000 x g), the middle phase containing phycobilisomes was carefully collected and loaded on a step gradient of 2 M, 1 M, 0.75 M, 0.5 M and 0.25 M sucrose in 750 mM NaKPO₄ (pH 8.0), 98,000 x g 18°C for 16 hr; (for WH7803 and WH8102, a 2 M, 0.75 M, 0.62 M, 0.5 M, 0.37 M and 0.25 M sucrose gradient was used and 40,000 x g 18°C for 16 hr). Intact phycobilisomes were then collected and refrigerated in the dark until use. Upon use, phycobilisomes were diluted to desired concentration with either 750 mM NaKPO₄ (pH 8.0) or ddH₂O, incubated in dark at room temperature for 2 hrs.

**SDS-PAGE** - Samples of isolated phycobilisomes were loaded at two concentrations on a continuous tris-tricine gel (28) with 4% stacking, 10% spacer, and 16.5% seperating gel. Gels were run at constant voltage (105 V for 16 hr) at room temperature. Gels were stained with Coomassie Brilliant Blue.
Absorbance Spectra- Absorbance spectra of isolated phycobilisomes were obtained using a UV-Vis UV-160 spectrophotometer (Shimadzu).

Fluorescence Spectroscopy- Fluorescence emission was performed in a dual emission fluorometer (PTI, New Jersey). Emission spectra were obtained of crosslinked and non-crosslinked phycobilisomes in 750 mM NaKPO$_4$ as well as in 50 mM NaKPO$_4$ to compare stability. *Synechocystis sp. PCC6803* and *T. elongatus* samples were excited at 555 nm and emission was monitored from 560 nm to 800 nm, *Synechococcus sp. WH7803* samples were excited at 480 nm and emission was monitored from 486 nm to 800 nm, and *Synechococcus sp. WH8102* samples were excited at 450 and emission was monitored from 455 nm to 800 nm.

Circular Dichroism- A Circular dichroism (CD) spectrometer (Model 202, Instruments Inc.) was used, sample concentration was monitored to 0.6mg protein/ml solvent. CD spectra were taken of both crosslinked and non-crosslinked samples suspended in both 750 mM NaKPO$_4$ (pH 8) and in ddH$_2$O.

Crosslinking Optimization- In the crosslinking optimization experiment, intact phycobilisomes isolated from *Synechococcus sp. WH7803* were incubated with 8 concentrations of formaldehyde (4%, 1.7%, 0.722%, 0.3068%, 0.1304%, 0.0554%, 0.0235%, 0.01%) and quenched with .1M glycine at 11 different time points (4 hr, 2 hr, 1 hr, 45 min, 30 min, 20 min, 15 min, 10 min, 5 min, 2 min, 30 sec) in a black 96-well plate. Crosslinked samples were then diluted to a final NaKPO$_4$ concentration of 50 mM and 680 nm emission was measured with an excitation wavelength of 530 nm in a plate reader (Polarstar Optima) to measure the maintenance of intraphycobilisome energy transfer upon dilution from high phosphate buffer. In all other experiments, crosslinked phycobilisome samples were incubated with 1 percent formaldehyde for 1 hour in the dark to induce crosslinking. The crosslinking reaction was quenched with 1mM glycine.
**Analytical Ultracentrifugation** - Phycobilisomes were analyzed before and after cross linking in ddH$_2$O and 750 mm NaKPO$_4$ by centrifugation at 25,000 rpm at 25°C in a Beckman-Coulter XL1 analytical ultracentrifuge, monitoring sedimentation by absorbance optics at three wavelengths: 490 nm, 570 nm, 630 nm. Crosslinked samples were passed over a PD-10 desalting column before analysis to remove excess formaldehyde and glycine and the column buffer was used as the reference buffer.
RESULTS

Figure 2. Stained Tris-tricine gel of isolated phycobilisomes. Lane 1 contains Fisher EZ-Run Rec protein ladder, lanes 2 and 6 contain phycobilisome sample isolated from Synechocystis sp. PCC6803, lanes 3 and 7 contain phycobilisome sample isolated from T. elongatus, lanes 4 and 8 contain phycobilisome sample isolated from Synechococcus sp. WH7803, lanes 5 and 9 contain phycobilisome sample isolated from Synechococcus sp. WH8102, lanes 10 and 11 contain samples from an unrelated project, and lane 12 contains Bio-Rad polypeptide molecular weight standards. The current protocol for T. elongatus phycobilisome isolation is in need of optimization as evidenced by the impurity of samples in lanes 3 and 7. No protocol for T. elongatus phycobilisome isolation has been published; optimization of this protocol is beyond the scope of this project.
Figure 3. Tris-Tricine Gel of Isolated Phycobilisomes. Photograph of gel in Fig. 1 before staining.

The covalently attached chromophores from each phycobiliprotein are clearly visible prior to staining.
Figure 4. Absorbance Spectra of isolated phycobilisomes. A-D represent the normalized absorption spectra of phycobilisomes isolated from *Synechocystis* sp. PCC6803, *Thermosynechococcus elongatus*, *Synechococcus* sp. WH7803, and *Synechococcus* sp. WH8102 respectively. In A and B peaks corresponding to the absorbance of phycocyanin and allophycocyanin are present. In C, a small absorbance corresponding with phycoerythrin II, a large absorbance corresponding with phycoerythrin I, and small absorbances corresponding with phycocyanin and possibly allophycocyanin are present. In D a large absorbance corresponding with phycoerythrin II, a smaller absorbance corresponding with phycoerythrin I, and little to no absorbance corresponding with phycocyanin or allophycocyanin are present.
Emission Spectra

Figure 5. Emission Spectra of Crosslinked and Non-Crosslinked Isolated Phycobilisomes. A-D represent emission spectra of isolated phycobilisome samples from Synechocystis sp. PCC6803, T. elongatus, Synechococcus sp. WH7803, Synechococcus sp. WH8102 respectively. In all cases, crosslinked samples showed a significant improvement in maintaining intracomplex energy transfer when removed from high phosphate buffer in comparison with non-crosslinked samples.
Circular Dichroism

Figure 6. CD Spectra of Crosslinked and non-crosslinked isolated phycobilisomes. Panels A-D represent CD spectra of crosslinked (CL) and non-crosslinked (non-CL) phycobilisomes isolated from Synechocystis sp. PCC6803, Thermosynechococcus elongatus, Synechococcus sp. WH7803, and Synechococcus sp. WH8102 respectively.
Analytical Ultracentrifugation

Figure 7. Analytical Ultracentrifugation Data of Crosslinked Phycobilisomes isolated from *Synechococcus WH7803*. Strongest signal was obtained from absorbance at 490 nm which corresponds with PEII; a representative SV profile of the uncrosslinked phycobilisomes in high phosphate is shown in the top panel. Sedimentation velocity data were analyzed using the program SEDFIT to perform direct boundary modeling using the continuous c(s) distribution model (29). The corresponding sedimentation coefficient distribution profiles are shown for each condition: uncrosslinked in phosphate, pink trace; uncrosslinked in water, black trace; crosslinked in water, purple trace. Phycoerythrin containing phycobilisomes regularly sediment within days at 1g. Therefore, it is difficult to conclusively interpret sedimentation velocity data, but the purple trace indicates that the dissociation event is prevented by formaldehyde crosslinking.
Figure 8. Data from Crosslinking Optimization of Intact Phycobilisomes Isolated from Synechococcus WH7803. In a 96-well plate, intact phycobilisomes isolated from Synechococcus sp. WH7803 were incubated with 8 concentrations of formaldehyde and quenched with .1M glycine at 11 different time points. In a fluorescence plate reader (Polarstar Optima), 680 nm emission was measured with an excitation wavelength of 530 nm. The results are graphed as a function of time crosslinked in panel A, as a function of formaldehyde concentration in panel B, and three dimensionally in panel C. Both cross linking at low concentrations of formaldehyde for a long period of time, or at high concentrations of formaldehyde for a shorter period of time appear to be effective. At high concentrations of formaldehyde, the non-specific crosslinker caused the phycobilisomes to form intercomplex crosslinks, forming large aggregates which caused precipitation at the longer time points. This caused the 680 nm emission of these samples to be diminished. In comparison with the positive control (phycobilisomes in 750 mM NaKPO₄), the crosslinked samples with the highest 680 nm emission were only equal to about half (~14,000 counts/sec) the emission of the positive control (~28,000 counts/sec) (data not shown).
DISCUSSION

While initially characterizing the isolated phycobilisomes from all four organisms it became apparent that the phycobilisomes isolated from Synechococcus sp. WH8102 did not contain the APC core. First, looking at the absorbance spectra in Figure 4D the isolated phycobilisomes show nearly no absorbance corresponding with APC at 650 nm. Second, looking at the emission spectra in Figure 5D only one peak is apparent near 650 nm which likely corresponds to phycocyanin. When compared to Figure 5C, the emission spectra of phycobilisomes isolated from Synechococcus sp. WH7803, it is easy to see that there are two peaks near 650 nm corresponding to phycocyanin and allophycocyanin. The phycobilisomes isolated from Synechococcus sp. WH8102 are missing this second peak in the emission spectra and absorbance spectra and also showed no activity when excited at 650 nm, the excitation wavelength for allophycocyanin. From this data it was concluded that the phycobilisomes isolated from Synechococcus sp. WH8102 likely did not contain the allophycocyanin core. The reason for this was not determined.

Organisms are known to digest their phycobilisomes under conditions of nitrogen starvation, but this normally occurs from the distal end of the rod toward the core (30). In the case of Synechococcus sp. WH8102 one would imagine that the organism would begin digesting the phycobilisomes at the PEII disks and gradually work its way toward the core so that the phycobilisomes remain functional for as long as possible. In conditions of high light and white light some Synechococcus strains have been shown to decrease their amount of PEII and even PEI because they are simply not needed to harvest enough usable light from the environment (9). Once again, these changes occur from the outside in so it does not make much sense for the organism to cut off the core and leave the rods because the core is necessary to transfer the captured energy to either PSII or PSI. If the organism did not remove the core in vivo, then the
change must have occurred in vitro. At some point during the isolation procedure the core must have been lost, but it is entirely unclear where that would have occurred. A published procedure for isolating phycobilisomes from *Synechococcus sp. WH8102* was followed (27). In the end, it was not clear where, when, or how the cores of the phycobilisomes were lost.

It is important to note, however, that the phycobilisomes isolated from *Synechococcus sp. WH8102*, due to their high phycourobilin content, show absorbance over the largest portion of the visible spectrum and have the largest shift between absorbance and emission of the phycobilisomes investigated. This will allow these phycobilisomes to capture more light to transfer to a photovoltaic device and their large shift between absorption and emission will minimize the chance for self absorption by the phycobilisomes themselves. Also, the phycobilisomes isolated from *Synechococcus sp. WH8102* show the highest absorption of high energy light which further makes them optimal for use in the proposed application. Had the phycobilisomes been isolated with their allophycocyanin cores, their properties would have been even more ideal.

**Crosslinking**

Based on the work of Cubicciotti, all of the formaldehyde crosslinking for the emission spectra, circular dichroism, and analytical ultracentrifugation was done at a formaldehyde concentration of one percent incubated for one hour (31). Looking at the emission spectra, the crosslinked phycobilisomes of *Synechocystis sp. PCC6803* and *Thermosynechococcus elongatus* (figure 5 A & B) out of high phosphate buffer showed identical spectra when compared to the non-crosslinked phycobilisomes in high phosphate buffer. The crosslinked phycobilisomes of *Synechococcus sp. WH7803* and *Synechococcus sp. WH8102* (figure 5 C & D) showed a marked improvement in stability out of high phosphate buffer in comparison to the non-crosslinked phycobilisomes, but did not show identical behavior as the non-crosslinked samples in high phosphate buffer. This could be due to a variety of reasons.
First, the phycobilisomes of *Synechocystis* sp. *PCC6803* and *Thermosynechococcus elongatus* contain only allophycocyanin and phycocyanin, so there are less phycobiliproteins to link together to stabilize them. The phycobilisomes of *Synechococcus* sp. *WH7803* and *Synechococcus WH8102* also contain allophycocyanin and phycocyanin, as well as phycoerythrin I and II. This could make them more difficult to stabilize through crosslinking.

It has also been suggested that there could be several populations of phycobilisomes after crosslinking: those that are crosslinked and stabilized properly, those that are not crosslinked at all, those that are crosslinked to other phycobilisome complexes, etc. If fifty percent of the phycobilisomes crosslinked as desired (the phycobiliproteins linked to each other to stabilize the complex) and fifty percent were not stabilized properly this could cause the in-between result seen in figure 5 C & D of the crosslinked samples in low phosphate buffer. After crosslinking, the samples could be loaded and run over a sucrose gradient again to separate the aforementioned populations. While another sucrose gradient step would be simple to perform, it would further dilute the sample and substantially decrease yield, which would be quite undesirable. Unfortunately, it may be near impossible to create a uniformly crosslinked population without some sort of further purification step after crosslinking.

*After determining that the crosslinking of the phycobilisomes isolated from* *Synechococcus sp. WH7803* and *Synechococcus sp. WH8102* was less than ideal, an experiment was designed to quickly assay several incubation times and concentrations of formaldehyde for stabilizing the phycobilisomes of *Synechococcus sp. WH7803*. Concentrations and times showing the highest 680 nm emission were still only equal to about half the control which agrees with the data seen in the original emission spectra after crosslinking with one percent formaldehyde for one hour. It appears that crosslinking with higher concentrations of formaldehyde for a short period of time or crosslinking with a low concentration of formaldehyde for longer periods of time are ideal.
Formaldehyde is a non-specific crosslinker, so using less formaldehyde for a longer time yields more useful samples than the higher concentrations of formaldehyde for shorter time periods; at high concentrations of formaldehyde enough inter-complex crosslinks were formed to cause precipitation of the large phycobilisome aggregates minimizing 680 nm emission. While the crosslinking conditions used for the other experiments may not have been the most ideal, crosslinking with one percent formaldehyde for one hour does transfer a significant amount of stability to the phycobilisomes investigated. Further investigation is necessary to determine the optimal crosslinking conditions. It is possible that a combination of crosslinkers may be ideal for stabilizing the phycobilisomes; a combination of a short crosslinker like formaldehyde with a longer crosslinker may offer stability that a short crosslinker alone cannot.

Circular Dichroism
The circular dichroism results shown in figure 6 are far from ideal results. During the latter runs the lamp on the machine overheated several times and the machine may or may not have been working properly. Further, the samples were read at a dilute concentration which did not give good data. The only close to intelligible data in figure 6B show a difference between crosslinked phycobilisomes in water and non-crosslinked phycobilisomes in water, but the crosslinked sample in water does not closely resemble the samples in the presence high phosphate. This seems to suggest that crosslinking is not preserving the structure of the phycobilisomes seen in the presence of high phosphate buffer once the phycobilisomes are removed from this buffer. I am reluctant to make any conclusions from this data though, as these experiments need to be redone with a higher protein concentration and possibly a new lamp.

Analytical Ultracentrifugation
Interpreting the results of the analytical ultracentrifugation, it appears that there were not any intact phycobilisomes in the sample. It is believed that the large size of the phycobilisome complex
(~15MDa) causes the complex to sediment in the AUC before a reading begins. Therefore, the peaks shown in Figure 7 correspond with phycobilisome components left in solution. The pink trace has a peak corresponding with the size of PE disks at a sedimentation coefficient near 5 and a peak corresponding with disks arranged in a rod structure at a sedimentation coefficient near 14. From this data, it appears that PEII disks and rods are in equilibrium with disks dissociating from the rods and being reattached constantly while in high phosphate buffer. Once treated with formaldehyde the disks appear to be locked in place on the rod, as there appears to be no peak corresponding with single disks in the crosslinked sample. Due to the large size of the phycobilisome complex, dynamic light scattering may be a better technique than AUC for studying phycobilisomes.

CONCLUSIONS

In conclusion, phycobilisomes isolated from Synechococcus sp. WH8102 show optimal absorbance and emission for use in a solar concentrator for organic photovoltaic devices of the four organisms investigated. Second, covalent crosslinking of phycobilisomes with formaldehyde applies significant stability and maintenance of optical properties when phycobilisomes are removed from high phosphate buffer, and crosslinking with lower concentrations of formaldehyde for longer time yields more stable, more soluble phycobilisomes with less inter-complex crosslinks. Also, crosslinking with a combination of a short crosslinker like formaldehyde with a longer crosslinker may provide more stability and energy transfer of phycobilisomes out of high phosphate buffer. Finally, it appears that phycobilisomes are too large to be studied by analytical ultracentrifugation and other methods such as dynamic light scattering should be investigated for the study of phycobilisomes.
FUTURE DIRECTIONS

For the progression of this work I propose the following directions for the future. First, the growth conditions of *Synechococcus* sp. WH8102 and the phycobilisome isolation from *Synechococcus* sp. WH8102 must be optimized to ensure recovery of intact phycobilisomes and a higher phycobilisome yield. Then, the investigation of phycobilisomes isolated from *Synechococcus* sp. WH8102 may be expanded to include the incorporation of the phycobilisomes from *Synechococcus* sp. WH8102 into a transparent polymer to test energy transfer of fixed phycobilisomes to a photovoltaic device. Also, the crosslinking optimization experiment should be expanded to include more timepoints, more crosslinker concentrations, and alternative crosslinkers alone and in combination. Further, crosslinked samples should be purified further to ensure the recovery of intact, stabilized phycobilisomes only. Finally, dynamic light scattering should be attempted with phycobilisomes samples to determine if better data can be retrieved than what was seen by analytical ultracentrifugation.

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