Potential sustainable energy source: Pheroid™ with incorporated light harvesting materials

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INTRODUCTION

The primary source of energy for industrial and domestic use is currently based on fossil fuels. The supplies of these fuels are limited and are becoming depleted. Thus there is a search for alternative and more sustainable energy sources. One such source is solar energy, which has many advantages over fossil fuels. Solar energy is:

- Abundant
- Universally available and not restricted to specific geographical locations
- Environmentally friendly (non-polluting)
- A more dependable supply and less prone to global political and economic fluctuations
- More assured long term sustainability.

Thus research into harvesting, transferring, and converting light energy is of great significance. The most abundant and efficient light harvesting, energy transfer and transduction systems are found in nature with the process of photosynthesis. In the photosynthetic system light energy is absorbed by antenna chlorophylls and this energy is then passed onto a reaction centre chlorophyll molecule where charge separation occurs [1] in less than 100 ps and at about 95% efficiency [2]. It has been shown that organised connective light harvesting complexes are required for long range energy transfer [3]. As a matrix to stabilise the system, we are using a combination of fatty acids and nitrous oxide, rather than conventional phospholipid-based combinations, which enables the production of small, elastic artificial vesicles, called Pheroid™. Previous work has shown that photosynthetic light harvesting material can be incorporated into the Pheroid™ (**Figure 1**).

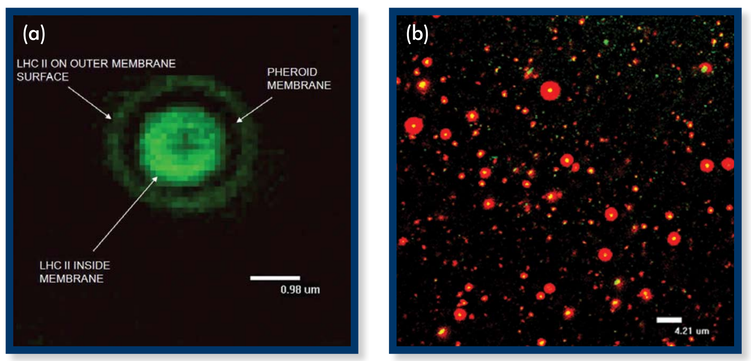

Figure 1: Examples of photosynthetic material incorporated into the Pheroid™ vesicles. (a) 2.8:2 LHCII 5µl and (b) 2.8:1 LHCII 20 µl. Confocal microscopy data collected in collaboration with North-West University (Potchefstroom campus)

In this study we are characterising the level of organisation through protein aggregation on the incorporated light harvesting systems using absorption spectroscopy.

MATERIALS AND METHODS

Photosynthetic materials were extracted from spinach leaves using the method of Krupa *et al.* [4]. Samples consisted of different concentrations of photosynthetic light harvesting material added to either a buffer solution or an 0.02 mg/ml Pheroid™ aqueous solution. The buffer was 20 mM Tricine pH 7.6 – 7.8. Samples were then stored in the dark individually at room temperature to determine the incorporation ratio of the photosynthetic material into the Pheroid™. Absorbance spectra were measured with a UV-VIS spectrophotometer (Shimazdu UV-1650 PC) using standard 1 cm pathlength cuvettes.

RESULTS AND DISCUSSION

Figure 2 shows the absorbance spectra at 293 K for samples containing increasing amounts of photosynthetic material (LHCII) added to 0.02 mg/ml Pheroid™ vesicle solutions. Spectra were an amalgamation of chlorophyll a (Chl a) and b (Chl b). Being the main pigments, Chl a and b attributed main peaks around 435 nm (Chl b) and 680 nm (Chl a) respectively, as well as shoulders around 590 nm and 620 nm. Other pigments were also present, with carotenoids possibly attributing a peak around 470 nm.

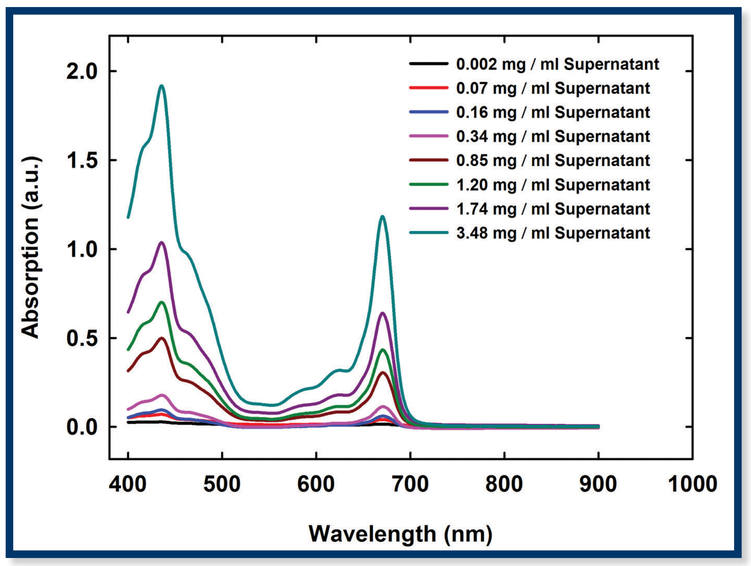

Figure 2: Absorption spectra of different amounts of LHCII material incorporated into 0.02 mg/ml Pheroid™ vesicle aqueous solutions

Difference spectra between the LHCII incorporated into the Pheroid™ vesicles (from **Figure 2**) and non-aggregated LHCII in the Tricine buffer were calculated. **Figures 3(b)** and **(d)** depicts these spectra for an LHCII concentration of 1.74 mg/ml in the Soret and Qy regions respectively. To facilitate comparisons between difference spectra with increasing LHCII concentrations, absorption spectra were normalised at 405 nm (**Figures 3(a)** and **(c)**). Difference spectra in the Soret region (**Figure 3(a)**) show a decrease in the transition amplitudes at 419 nm and 435 nm (Chl a), as well as a broad band appearing around 494 nm. This indicates protein aggregation of the incorporated material [5]. Unlike the results from [5] which were measured at 77 K, the amplitude of the 475 nm (Chl b) and 454 nm (xanthophyll) transitions appear increased. In the red (Qy) region, a strong transition occurs around 685 nm (Chl a), with increased absorption at 624 nm and 649 nm (Chl b).

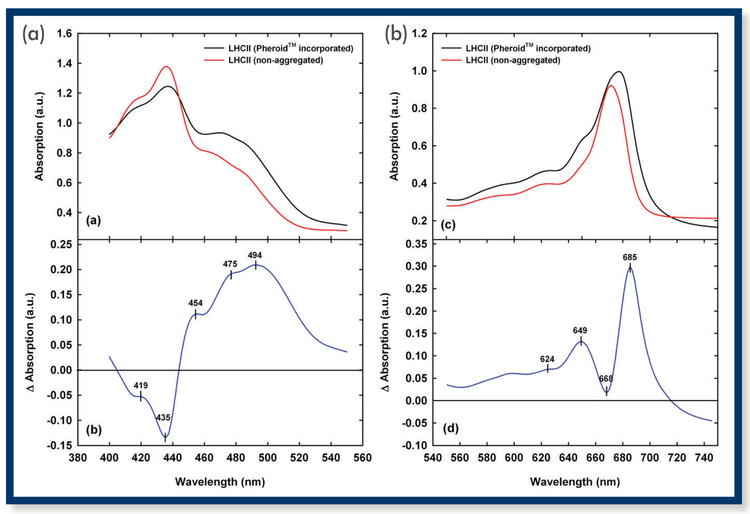

Figure 3: Absorption spectra at 293 K of 1.74 mg / ml LHCII in the (a) Soret and (c) Qy regions. Black line: LHCII incorporated into Pheroid™. Red line: Non-aggregated LHCII in Tricine buffer. Calculated absorption difference spectra incorporated-minus-non-aggregated (blue line) for the (b) Soret and (d) Qy regions.

The Qy transitions of chlorophyll in the red (Qy) region appears to red-shift by 3.5 – 5.5 nm (**Figure 3(c)**); an indication of aggregation of the LCHII material [5, 6]. **Figures 4(a)** and **(b)** shows the change in peak shifts of the maximum peaks in the Soret and Qy regions respectively. Peak shifts for incorporated LHCII of almost all concentrations considered confirms the observed red-shifts of the difference spectra.

Visibly inspected dark-stored samples showed an incorporation ratio of LHCII material: Pheroid™ of 13.33: 1 (i.e. all concentrations less than or equal to 0.34 mg/ml were completely incorporated into the Pheroid™ vesicles). Blue-shifting of the maximum Soret region absorption for added LHCII/Pheroid™ ratios less than 13.33 µl/µl (**Figure 4(a)**) compares well with these observations and possibly indicates a separation of the Chl a protein complexes.

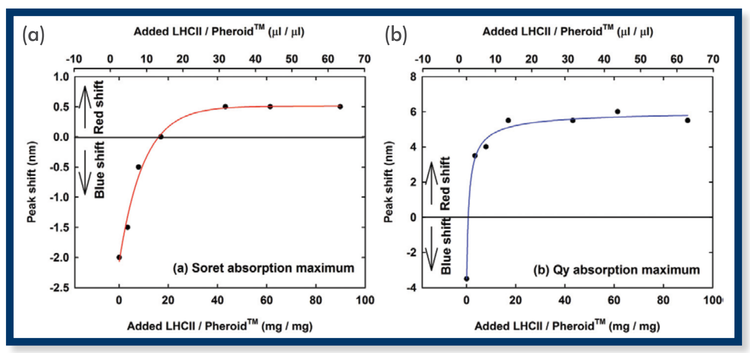

Figure 4: Peak shifts in the Soret and Qy absorption maxima of LHCII after incorporation into Pheroid™ vesicles.

CONCLUSIONS

Characterisation of the level of organisation and incorporation ratio of photosynthetic light harvesting material : Pheroid™ has been investigated. Peak shifts and difference spectra in the Soret and Qy regions indicated a possible change in organisation of the light harvesting system after incorporation into the Pheroid™. The next stage will be to further investigate the light harvesting organisation before and after incorporation in more detail using steady state optical techniques such as fluorescence and CD (Circular Dichroism) measurements, as well as multiphoton multimodal (MPF, SHG, THG) microscopy. SDS-PAGE (Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis) will also be used for identification of the proteins in order to confirm which light photosynthetic complexes are present. Future work will include assessing the dynamics of the potential energy transfer capabilities using ultra-fast pump-probe transient absorption spectroscopy.

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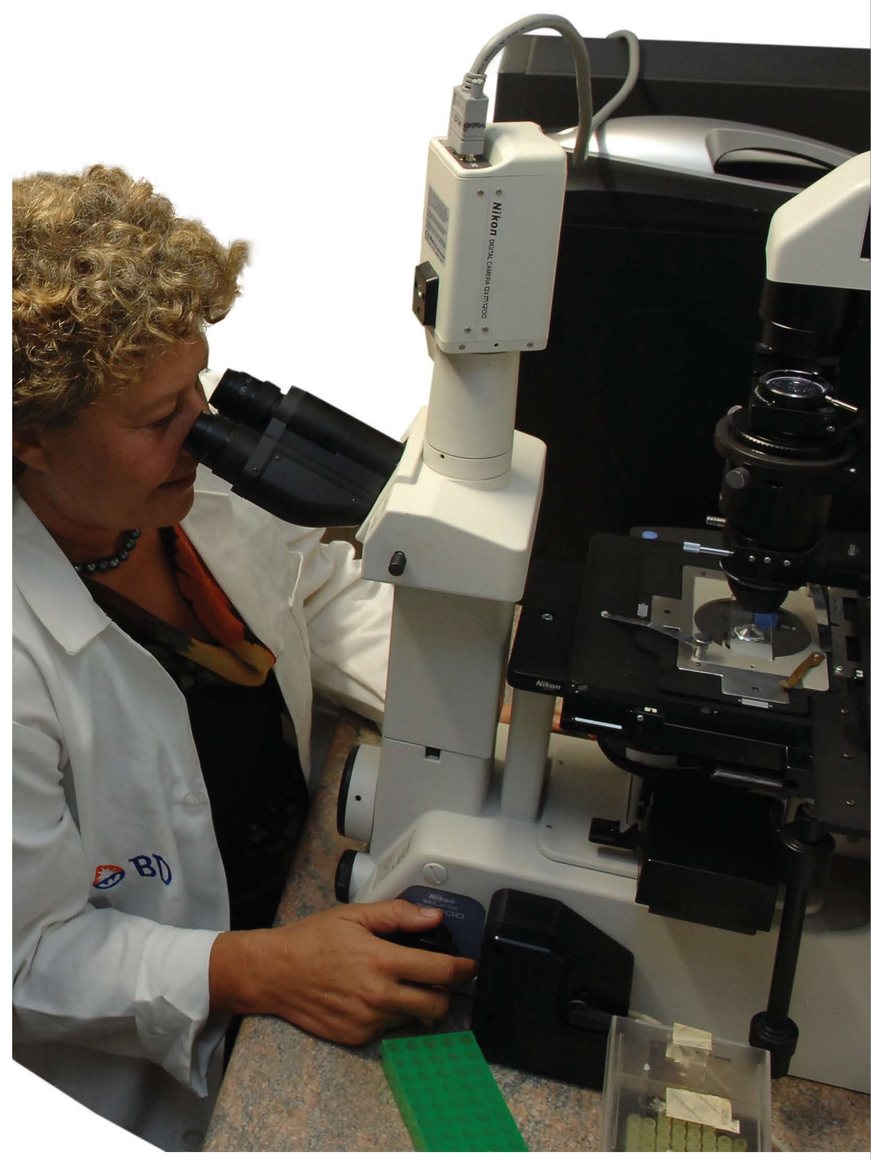
The most efficient light harvesting and energy transfer systems are found in nature as part of the photosynthesis process. By extracting these system fragments and maximising their organisational structure, researchers are developing similar artificial systems for use as potential sustainable energy sources.



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