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INFLUENCE OF SOLVENT EFFECTS ON Qy TRANSITIONS IN CHLOROPHYLL

J E Smit[a], L C Prinsloo[b], J M Nel[b], A F Grobler[c], R W Sparrow[a]

[a] CSIR Biosciences, PO Box 395, Building 20, Pretoria, 0001, South Africa
[b] Department of Physics, University of Pretoria, Pretoria, South Africa
[c] Unit for Drug Research and Development, North-West University, Potchefstroom Campus, Potchefstroom, South Africa

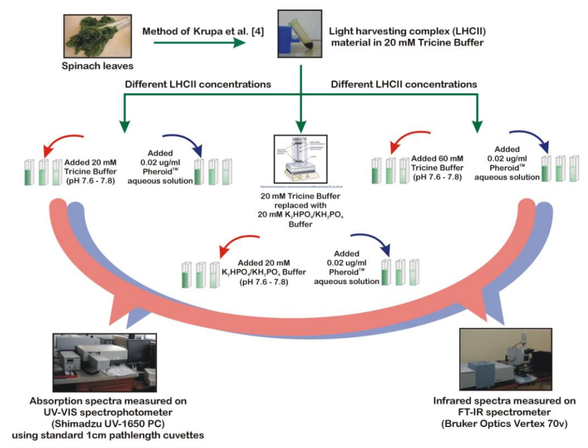
INTRODUCTION

The most abundant and efficient light harvesting, energy transfer and transduction systems are found in nature within the process of photosynthesis. Although the processing sequences of an absorbed solar photon in the photosynthetic apparatus have been deciphered [1–3], the underlying physical basis of photosynthesis is not well understood yet. Our research aims to contribute to this understanding by characterising the level of organisation of the Light Harvesting II complexes (LHCII) and energy transfer systems when incorporated into artificial lipid vesicles called Pheroid™. As a matrix to stabilize the system we are using a micro-emulsion containing 3.8% lipid content consisting mainly of ethyl esters of the fatty acids obtained from soybean oil, rather than conventional phospholipid-based combinations, which enables the production of small, elastic Pheroid™ vesicles. Previous work has shown that photosynthetic light harvesting material can be incorporated into the Pheroid™.

LHCII was extracted from spinach leaves in a 20 mM Tricine buffer to stabilise the proteins. The Qy transitions of chlorophyll in the red (Qy) region of the absorption spectra appeared to red-shift by 3.5 – 5.5 nm; indicating a possible change in organisation of the light harvesting system after incorporation into the Pheroid™. These shifts however could also be interpreted as bathochromic solvent effects due to the Tricine buffer. The objectives of this study were

1. to investigate whether the red-shifts were due to the Tricine buffer and
2. if so, whether the alternative use of a 20 mM K_2HPO_4 / KH_2PO_4 buffer could eliminate the bathochromic solvent effects.

MATERIALS AND METHODS



RESULTS AND DISCUSSION

Figure 2 shows the absorbance spectra at 293 K for samples containing a photosynthetic material (LHCII) concentration of 1.74 mg/ml in either a 20 mM or 60 mM Tricine buffer solution without (**Figure 2(a)**) and with an added 0.02 µg/ml Pheroid™ vesicle solution (**Figure 2(c)**). Spectra were an amalgamation of chlorophyll *a* (Chl a) and *b* (Chl *b*) with main peaks around 435 nm (Chl b) and 670 nm (Chl *a*) respectively. Carotenoids attributed a peak around 470 nm, while shoulders around 590 nm and 620 nm could be attributed to phycoerithrin and Chl *b* respectively (Soret region not shown).

20 mM vs 60 mM Tricine: Spectra at 293 K of 1.74mg/ml LHCII

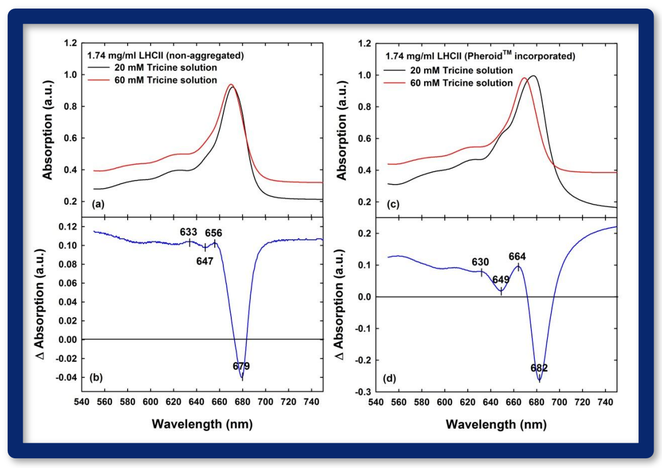


Figure 2: Absorption spectra at 293 K of 1.74 mg/ml LHCII in the Qy region (a) without and (c) with added 0.02 µg/ml Pheroid™ vesicle solutions. Black line: LHCII in 20 mM Tricine buffer. Red line: LHCII in 60 mM Tricine buffer. (b) and (d): Calculated absorption difference spectra 60 mM Tricine minus 20 mM Tricine buffer (blue line) for the Qy regions of (a) and (c) respectively

Difference spectra between the LHCII in the 60 mM and 20 mM Tricine buffer solutions were calculated. **Figures 2(b) and (d)** depict these spectra without added Pheroid™, and LHCII material incorporated into the Pheroid™ vesicles respectively. To facilitate comparisons between spectra, absorption spectra were normalised at 405 nm (**Figures 2(a) and (c)**). Difference spectra in the red (Qy) region show a strong transition around 679 nm (**Figure 2(b)**) and 682 nm (**Figure 2(d)**) indicating protein aggregation (compare with [5]) of the incorporated material in the 20 mM Tricine buffer, but not in the 60 mM Tricine buffer. Hence, it does not appear that the Tricine buffer causes any bathochromic solvent effects, since a larger red shift would be expected with the use of the 60 mM Tricine buffer.

As an alternative to the 20 mM Tricine buffer to stabilise the proteins, samples were dialysed in a 20 mM K_2HPO_4 / KH_2PO_4 buffer. **Figure 3** shows the 20 mM Tricine versus 20 mM K_2HPO_4 / KH_2PO_4 buffer absorption and difference spectra at 293 K for samples similar to **Figure 2**. Results for the potassium phosphate buffer were similar to those of the 60 mM Tricine buffer.

20 mM Tricine vs 20 mM K_2HPO_4 / KH_2PO_4: Spectra at 293 K of 1.74mg/ml LHCII

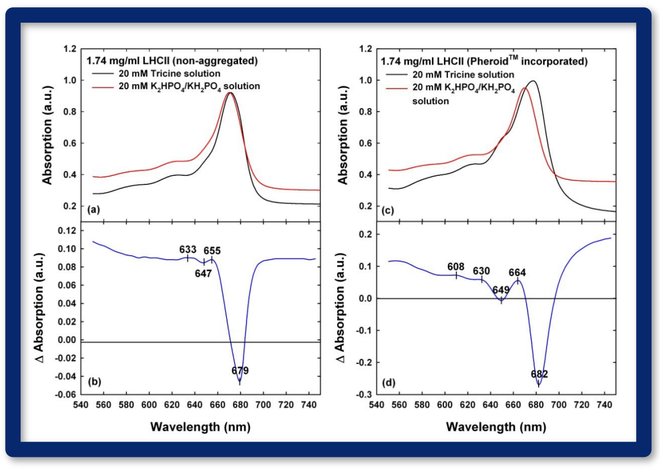


Figure 3: Absorption spectra at 293 K of 1.74 mg/ml LHCII in the Qy region (a) without and (c) with added 0.02 µg/ml Pheroid™ vesicle solutions. Black line: LHCII in 20 mM Tricine buffer. Red line: LHCII in 20 mM K_2HPO_4 / KH_2PO_4 buffer. (b) and (d): Calculated absorption difference spectra 20 mM K_2HPO_4 / KH_2PO_4 minus 20 mM Tricine buffer (blue line) for the Qy regions of (a) and (c) respectively

Although the Tricine and potassium phosphate buffered results appeared to be similar, Fourier Transform Infra-red (FTIR) spectra of the samples indicated a shift in the Amide I and II peaks to around 1630 cm^{-1} and 1551 cm^{-1} (Tricine buffer) compared to 1650 cm^{-1} and 1543 cm^{-1} (potassium phosphate buffer) (**Figure 4**). Thus the α-helix conformation of the 25 and 27 kDa apoproteins in the LHCII have been changed to β-sheet conformation [6]. This conformational change may influence the apoproteins' ability to aggregate further, hence favouring the use of the potassium phosphate buffer to stabilise the proteins.

FTIR spectra of LHCII material: 20 mM Tricine vs 60 mM Tricine vs 20 mM K_2HPO_4 / KH_2PO_4

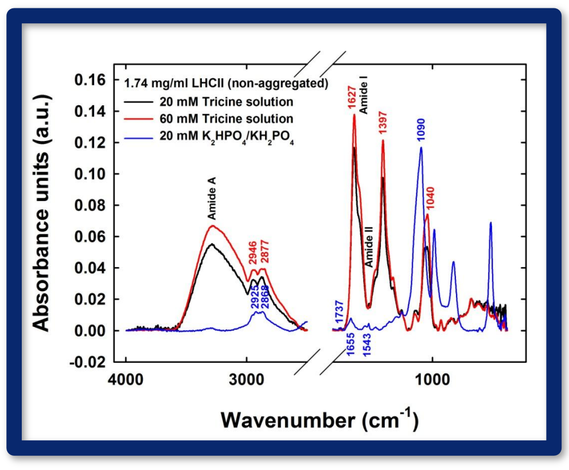


Figure 4: FTIR spectra of LHCII showing the Amide I, Amide II and C=O stretching regions for 20 mM Tricine buffer (black line), 60 mM Tricine buffer (red line) and 20 mM K_2HPO_4 / KH_2PO_4 buffer (blue line). Spectra for LHCII material incorporated into Pheroid™ were similar to these results and are not shown

Spectra for the 20 mM and 60 mM Tricine buffers (without Pheroid™) were similar (**Figure 4**), with the main change a decrease in the carbonyl ester peak (C=O stretching group) around 1737 cm^{-1}. Since this ester group is involved in the aggregation of the LHCII [7], it may account for the lack of red-shifting in the 670 nm peak in **Figure 2(a)**.

The 20 mM Tricine buffer had no observable influence on the Pheroid™ vesicles, but the carbonyl ester peak around 1737 cm^{-1} disappeared with the 60 mM Tricine buffer (**Figure 5**). This may explain the difference in red-shifting of the Qy peak (677 nm compared to 670 nm) in **Figure 2(c)**. FTIR spectra for incorporated LHCII material were similar to those in **Figure 4** and are not shown.

FTIR spectra of 0.02 µg/ml Pheroid™: 20 mM Tricine vs 60 mM Tricine vs 20 mM K_2HPO_4 / KH_2PO_4

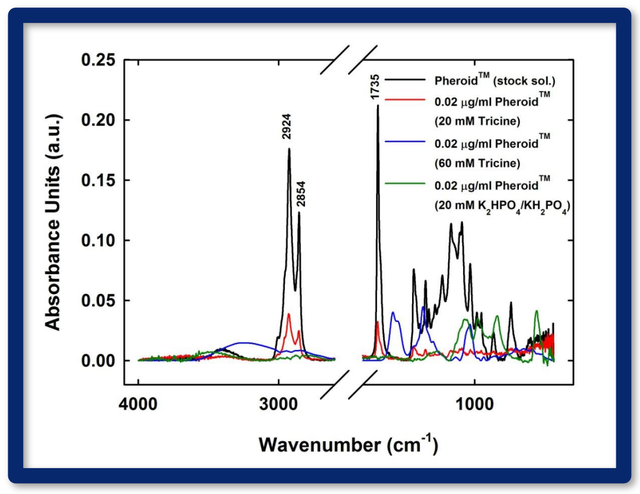


Figure 5: FTIR spectra of 0.02 µg/ml Pheroid™ in 20 mM Tricine buffer (red line), 60 mM Tricine buffer (blue line) and 20 mM K_2HPO_4 / KH_2PO_4 buffer (green line) solutions. Pheroid™ stock solution (black line) added as reference

The influence of the potassium phosphate buffer on the Pheroid™ was more pronounced than with the Tricine buffer. The C-H stretch modes around 2950 – 2880 cm^{-1} were reduced by about 90%, as was the carbonyl ester around 1735 cm^{-1} (**Figure 5**). The latter was also shifted to 1770 cm^{-1}. Hence spectra for the incorporated LHCII material were almost identical to spectra without added Pheroid™ (not shown). This suggested that although the α-helix secondary structure of the apoproteins will result in the correct quaternary structure formation of the LHCII aggregates as found in PSII, the additional carbonyl esters in the Pheroid™ may play a significant role in aiding this aggregation process.

CONCLUSIONS

The absence of a larger red shift with the use of the 60 mM Tricine buffer indicated the observable absence of a bathochromic effect due to the Tricine buffer. Even though results for the 60 mM Tricine and 20 mM K_2HPO_4 / KH_2PO_4 buffers were similar, FTIR spectra indicated a difference in the LHCII's underlying structure with the use of the different buffers. It appears that the use of the potassium phosphate buffer favours retainment of the α-helix conformation of the LHCII proteins compared to the Tricine buffer. It interacts, however with the Pheroid™ vesicles, inhibiting further aggregation of the incorporated light-harvesting material.

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ACKNOWLEDGEMENTS

This work was supported by a CSIR Young Researchers Establishment Fund Grant.