Spectroscopic Studies into the self-assembly of the Photosynthetic Pigment, Lutein

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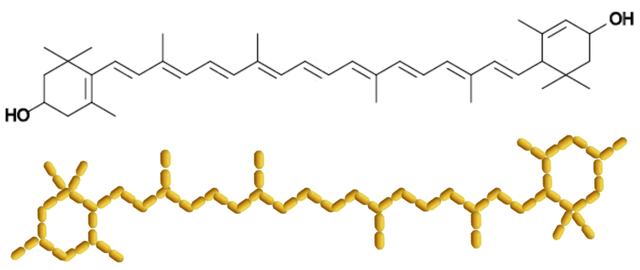
1. Introduction

1.1 Structure of lutein

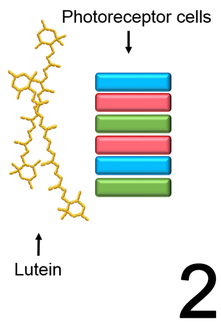
Lutein is a biological pigment which consists of a hydrophobic polyene chain chromophore, capped by a hydroxylated ionone ring at either end. Lutein strongly absorbs blue light making it appear yellow (Britton, 1995).



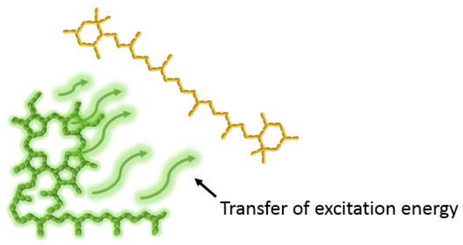
1.2 Lutein in nature

Lutein plays a primarily photoprotective role in nature. In plants, it quenches the chlorophyll triplet state; a cytotoxic species which is formed at high light intensities (Deming-Adams B. et al., 1992) . In humans, it filters out harmful, high energy blue light before it reaches photoreceptor cells in the macula (Krinsky et al., 2003).

In humans

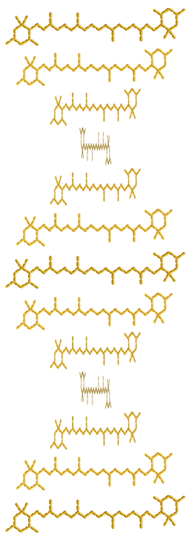


In plants



1.3 Self-assembly

In aqueous environments, lutein assembles into aggregates. This is coupled to a shift of the absorbance from blue to ultraviolet light. Theoretical studies have shown that the formation of helical aggregates cause this shift.

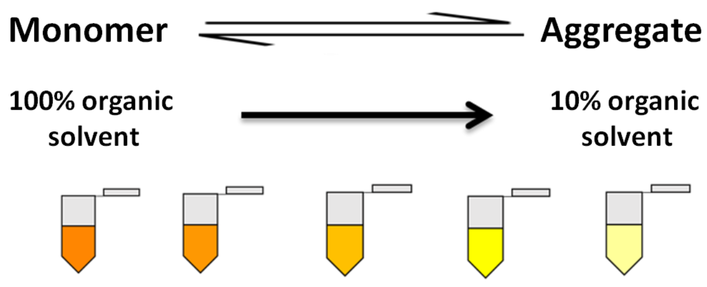


1.4 Aims of this work

The aim of this work was to gain mechanistic insights into the self-assembly process by identifying thermodynamically stable states along the pathway to helical aggregate formation.

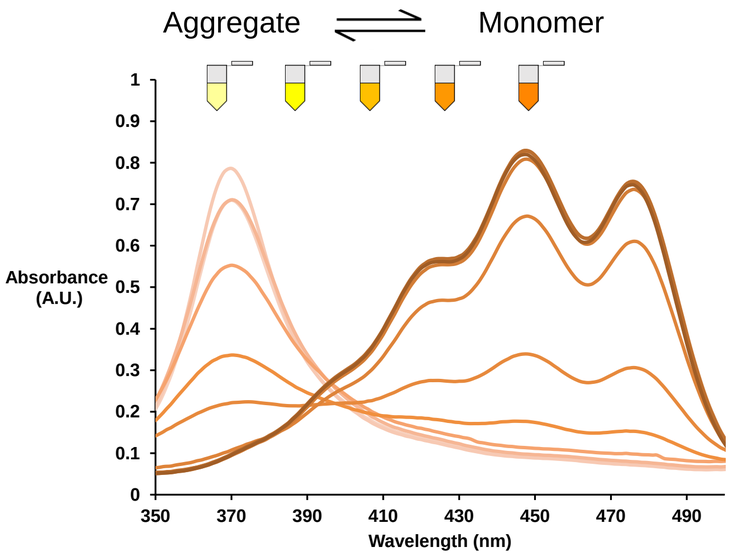
2. Methodology

Samples were made up from 100% to 10% organic solvent in water. The absorbance spectra were recorded and ratios of the absorbances were calculated for each sample.



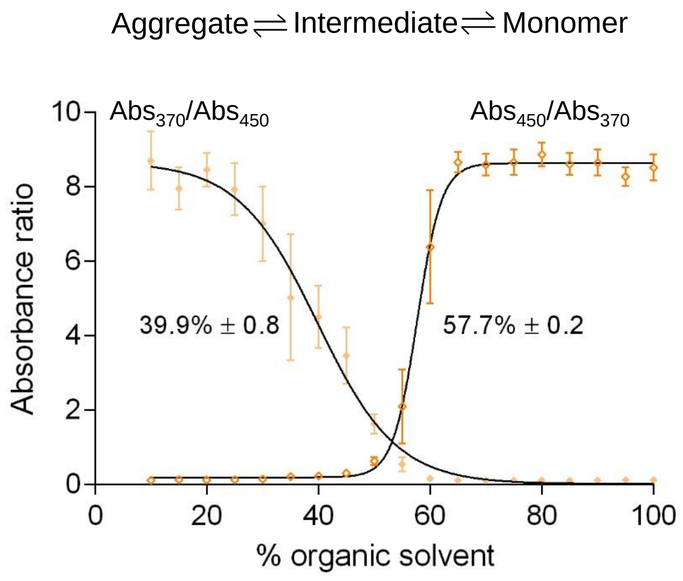
3. Results

3.1 Monomer and Aggregate state



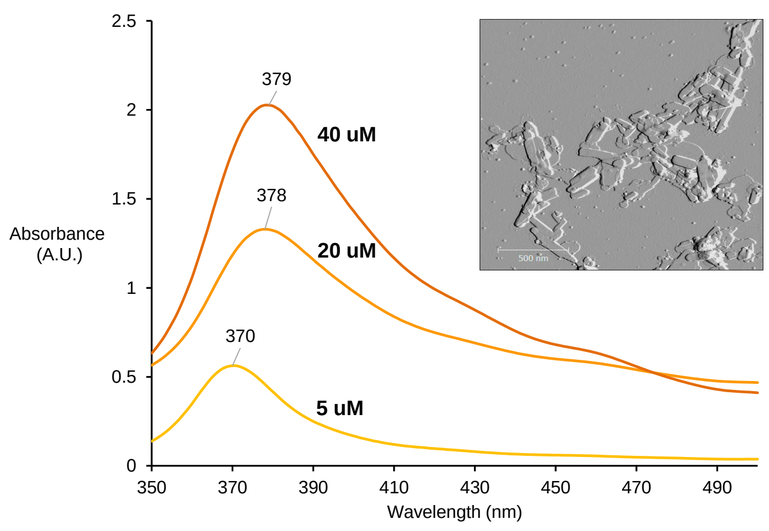
The monomer has a peak absorbance at 450 nm and the aggregate at 370 nm. The aggregate dominates in the most aqueous mixtures, whereas the monomer dominates at high ethanol concentrations. The 450 nm peak is well established as being due to monomer, the 370 nm peak has been previously fitted to a helical aggregate model (Spano, 2009).

3.2 Intermediate state



Ratios of monomer absorbance/aggregate absorbance and the inverse were plotted against % of organic solvent. The curves were fitted to a two-state model giving midpoints for each transition. There is a difference of almost 20% suggesting that there is a thermodynamically stable intermediate state.

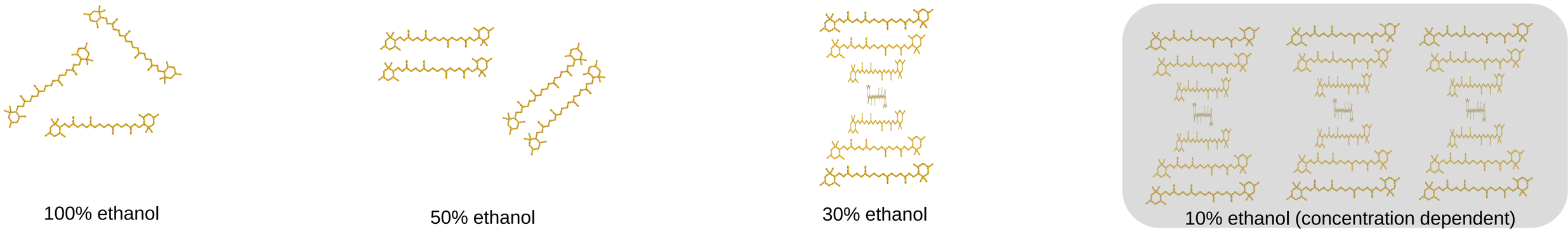
3.3 Crystalline state



At increasing concentrations of lutein (10% ethanol), the aggregate peak shifts to higher wavelengths. AFM images show the presence of crystalline structures in samples at above 100 uM lutein. Hence the absorbance shift may be caused by lateral stacking of the helical strands during the formation of these nanocrystals.

4. Model for self-assembly

Monomer ⇌ Intermediate ⇌ Helical aggregate ⇌ Crystalline aggregate



100% ethanol — 50% ethanol — 30% ethanol — 10% ethanol (concentration dependent)

5. Discussion and future work

A model for the self-assembly mechanism based on spectroscopic data is presented above. The transition from monomer to intermediate results in loss of the 450 nm peak which is likely to be caused by association of lutein molecules into dimer or oligomer assemblies. This buries hydrophobic surface area on the molecule making these structures more favourable in the presence of water. The oligomer species associate into helical aggregates indicated by the appearance of a peak at 370 nm (Spano, 2009). This buries more hydrophobic surface area and potentially results in favourable hydrogen bonding interactions between the hydroxyl groups. At higher concentrations, the helical assemblies associate laterally forming crystalline structures as observed in AFM images. Future work will explore the relative sizes of each species along the pathway using DLS and further characterise the concentration dependence of the equilibria.

References

Britton G. (1995) *Structure and properties of carotenoids in relation to function*. FASEB J. 9(15): 1551-1558

Deming-Adams B. and Adams III W. W. (1992) *Photoprotection and other responses of plants to high light stress.* Annu. Rev. Plant. Physiol. Plant Mol. Biol. 43: 599-626

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Spano F. C. (2009) *Analysis of the UV/VIS and spectral line shapes of Carotneoid assemblies: Spectral signatures of Chiral H-aggregates.* J. Am. Chem. Soc. 131: 4267-4278