TESTING CTAB REVERSE MICELLES TO STUDY LATE EMBRYOGENESIS ABUNDANT PROTEINS

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Introduction

Intrinsically disordered proteins (IDPs) are proteins that do not have a specific three-dimensional structure under aqueous conditions. Many of these proteins gain a defined structure under stressed conditions, such as dehydration or change in pH. Late embryogenesis abundant (LEA) proteins are a class of IDPs found in plants and other organisms. As the name suggests, they are present during embryogenesis of seeds and are thought to aid in allowing the seeds to remain viable after being dormant in extreme conditions.

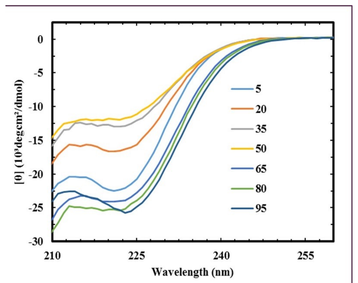

Fig 1. CD wavelength scans of G3LEA in $\omega_0 = 8$ reverse micelle solution collected at various temperatures as indicated in the legend (in °C).

In a previous study of a specific Group 3 LEA (G3LEA) peptide found in soybeans, Circular Dichroism (CD) spectroscopy was used to find that the peptide has a helical structure at low temperatures, and as temperature increases, it loses its helicity, as expected. However, at about 55ºC, it gains helical structure (Fig 1). We suspected the proteins dimerize to form coiled-coils.

Background

Forster resonance energy transfer, or FRET, is a method where energy from one molecule is transferred another molecule. The amount of energy transferred is dependent on the separation between them (Fig 2). In a previous experiment, the amino acids cyanophenylalanine (PheCN) and tryptophan (Trp) were used as chromophore molecules since their emission and absorption spectra overlap. G3LEA peptides were tagged with these amino acids. The excitation of PheCN is at 240 nm and emission is at 280 nm. The excitation of Trp is at 280 nm and emission is at 350 nm. AOT reverse micelles were used to simulate conditions of low peptide hydration.

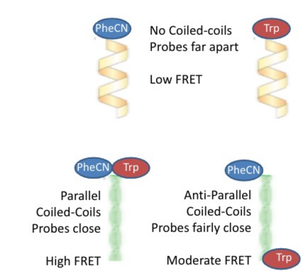

Fig. 2. Levels of FRET under different conditions of separation between PheCN and Trp

Figures 3 and 4 show the results of the temperature dependent FRET scans performed on PheCN-G3 LEA alone in reverse micelles and on PheCN-G3 LEA and Trp-G3 LEA both in reverse micelles, respectively. If FRET was happening, a large decrease in PheCN-G3 LEA fluorescence was expected along with a large increase in Trp-G3 LEA fluorescence. An unexpected large, broad band is seen in both graphs.

Background

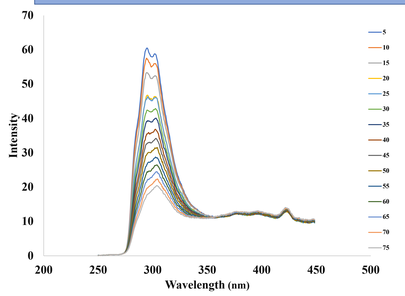

Fig. 3. Temperature-dependent fluorescence scan of PheCN-G3 LEA control in w_0 = 10 reverse micelles with a concentration of ~200 µM.

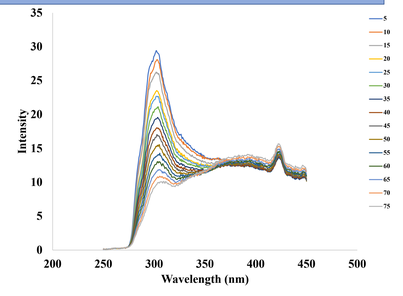

Fig. 4. Temperature-dependent fluorescence scan of mix solution of PheCN-G3LEA and Trp-G3 LEA in w_0 = 10 reverse micelles with a concentration of ~200 µM for both peptides.

Problem: The unexpected broad bands were caused by the AOT reverse micelles. They interfered with the FRET data because they served as an acceptor of energy from the PheCN labeled peptide, inhibiting energy transfer to the Trp-G3 LEA.

Solution: Reverse micelles made of cetyltrimethylammonium bromide (CTAB) could be used instead of AOT to simulate conditions of low peptide hydration for the FRET scans.

Experimental Methods

To prepare 10-mL of a reverse micelle solution with ω_0=5 and P_0=8, 0.212 g CTAB, 9.60 mL hexanes, 0.049 mL DI water, and 0.480 mL 1-pentanol were added to a bottle and was shaken until all the CTAB dissolved.

UV-Vis spectroscopy was used to determine absorption of the CTAB reverse micelle solution at room temperature between wavelengths 200 nm to 400 nm. Fluorescence emission and excitation scans using luminescence spectroscopy were performed on the CTAB solution as well. Next, samples with PheCN in the CTAB reverse micelles (363.6 µM PheCN) were prepared by adding 300 µL of the CTAB/hexanes/water/pentanol solution to a previously frozen and lyophilized PheCN sample. A thermal absorption scan was performed on the PheCN/CTAB sample.

Results

The CTAB reverse micelle solution does not show absorbance at or around 290 nm (Fig. 5), where it would interfere with Phe-CN energy transfer, and it shows very little to no emission and excitation at the wavelengths where Phe-CN and tryptophan emit light and are excited (Fig. 6-9). This indicates that CTAB is a suitable surfactant to use in future experiments with G3LEA tagged with Phe-CN and tryptophan probes. The steadiness in the absorption spectrum of Phe-CN in CTAB reverse micelles solution (Fig. 10) gives further indication there is no interference between the probe and the reverse micelle solution at various temperatures.

Results

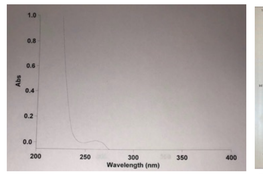

Fig. 5.

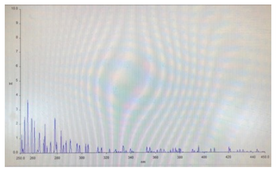

Fig. 6.

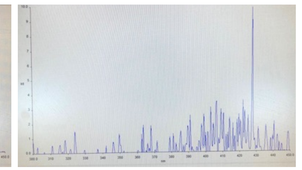

Fig. 7.

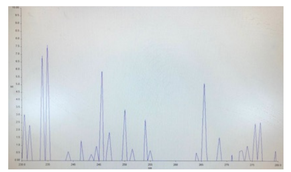

Fig. 8.

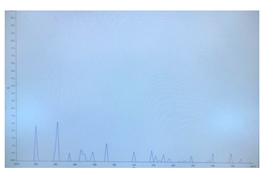

Fig. 9.

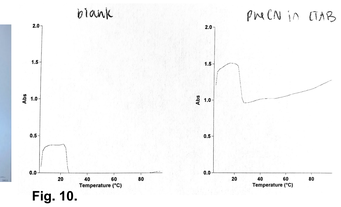

Fig. 10.

Fig. 5. Absorption spectrum of CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8).
Fig. 6. Emission scan of CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8) at excitation wavelength 240 nm.
Fig. 7. Emission scan of CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8) at excitation wavelength 290 nm.
Fig. 8. Excitation scan of CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8) at emission wavelength 290 nm.
Fig. 9. Excitation scan of CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8) at emission wavelength 350 nm.
Fig. 10. Absorption spectrum of wavelength 240 nm of blank (CTAB/hexanes/water/pentanol) and Phe-CN in CTAB/hexanes/water/pentanol solution (ω_0=5 and P_0=8) between 5°C and 95°C.

Future Work

In the future, FRET should be performed with the Trp in CTAB reverse micelle solution to further test if the reverse micelles interfere with its energy absorption and emission. Then, FRET with the PheCN-G3 LEA and Trp-G3 LEA peptides can be used in this new reverse micelle system to examine their potential dimerization into coiled-coils. Additionally, pH is an important factor in protein structure and function. Current experiments do not take pH into account. Future work can focus on controlling the pH in the reverse micelles and studying subsequent G3LEA behavior in the new environment.

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