Emission Properties of 1,2,3-Triazine Fluorophores

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Abstract

Fluorescence is probably the most widely used spectroscopic technique for the life sciences. There is a continual demand for customized fluorophores that can be fine tuned to sense particular environmental variables or to explore protein localization in cells.[1] *A new class of 1,2,3-triazine based fluorophores have been developed in NUI Galway and Maynooth.*[2] *A detailed exploration of the fluorescence properties of these fluorophores in aqueous solutions and micelles is required to determine their suitability for protein labelling.*

1. Introduction

Fluorescence methods are non-contact, quantitative, and highly sensitive making them ideal for the analysis of biological molecules. In many cases it is necessary to introduce an additional molecule, which has distinct, invariant photophysical properties that can act as a reporter of the biomolecule's concentration in different environments.[3] This 1,2,3-triazine based family of fluorophores have the potential to be used for such applications.[2,4] The fluorescence emission is complex with three distinct emission bands, and a wavelength dependant lifetime which enables unique imaging modalities. BSA was used as a model protein system to determine the suitability of these fluorophores as viable biological labels.

2. Materials and Methods

BSA solutions in PBS buffer of pH 9 and 10 were made up. BSA concentration was maintained at 3×10^{-5} M throughout sampling. An appropriate volume of a PhTr/EtOH solution was added with stirring to achieve the desired PhTr/BSA reaction ratio. The final ethanol concentration was 3 % v/v. The sample was placed on a stir-plate in the fridge overnight. The reaction was performed at a number of PhTr-BSA ratios. Immediately before testing, the sample was diluted by a factor of three.

The PhTr-BSA ratio of 1:4 was selected for a further time study in which the steady-state fluorescence and fluorescence anisotropy were monitored over a period of three hours at a constant temperature of 25°C.

3. Results and Conclusions

The hydrophobic character of the triazine molecule means it has a high affinity for the protein's interior. This was confirmed by a steady-state anisotropy investigation, which showed a significant increase in anisotropy at 480 nm as the fluorophore became incorporated into BSA. The steady-state fluorescence emission spectra remain unaffected despite the changing microenvironment of the fluorophore. Band positions, full-width half-maxima, and the integrated area of the three fluorescence emission bands remain constant with time and do not vary over a pH range of 9-10.

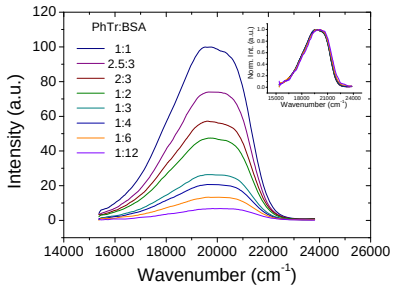

Fig 1: Raw fluorescence emission spectra of PhTr-BSA mixtures of varying ratios at pH 9. Normalised spectra appear inset.

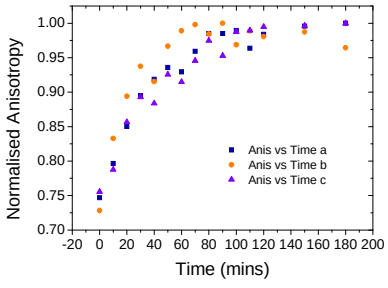

Fig 2: Normalised steady-state fluorescence anisotropy (λ_{ex} 405, λ_{em} 480) of PhTr-BSA mixtures with a ratio of 1:4 at pH 9 over a time period of 180 minutes.

4. References

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(4) St. Mart, D.; Togashi, D.M.; Stephens, J.; Burke, L.A.; Ryder, A.G., *in preparation*.