

Studying the Interactions between Monosaccharide/Polysaccharide with Tryptophan Using Fluorescence Spectroscopy



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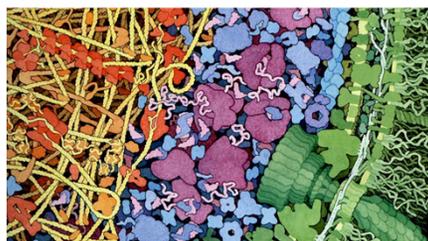
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Abstract

Fluorescence spectroscopy has become a pivotal tool in biochemical research by virtue of its robustness and high sensitivity. In particular, intrinsic protein fluorescence, that originates mainly from the aromatic amino acid tryptophan, have been extensively explored to study protein dynamics and conformational changes. Tryptophan has the strongest fluorescence quantum yield of all the amino acids found in proteins and its fluorescence is highly dependent on the surrounding environment. Therefore, properties including absorption and fluorescence maxima (λ_{max}), fluorescence intensity, and quantum yield have been used to probe conformational changes in a protein due to the change in external environment. Intrinsic tryptophan fluorescence spectroscopy is routinely used to study the impact of common metabolites and metal ions on proteins conformations and functions. However, the impact of some of these metabolites, specifically monosaccharides and polysaccharides, on free tryptophan has remained unknown. In our lab, we are performing a thorough investigation of the impact of monosaccharides and polysaccharides on free tryptophan using fluorescence spectroscopy. The preliminary results of our study are presented here.

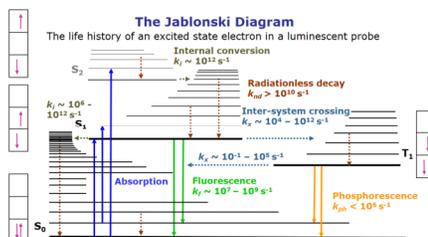
Background

- Most in vitro and in silico studies are performed in dilute conditions
- The intercellular environment is crowded with an assortment of macromolecules [1]
- This crowding can have an impact on the structure and function of proteins
- One way to analyze the effects of crowding on protein structure is through intrinsic tryptophan spectroscopy
- Fluorescent spectroscopy works through exciting electrons to a higher orbital, followed by a reemission at a different wavelength
- Tryptophan is one of three fluorescent amino acids. It has the best quantum yield, and occurs infrequently enough to be used as a marker [2]
- The fluorescent emissions of tryptophan are highly dependent on its surrounding, making it ideal for use when investigating the effects of macromolecular crowding [2]
- This study focuses on the effects of crowders on the fluorescence of free tryptophan, which will be valuable information when extended to the study of protein fluorescence



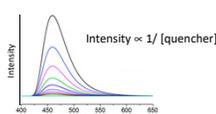
A cross section showing a portion of an *Escherichia coli* bacterium, including all macromolecules. Small molecules like ATP, glucose, and water are omitted for clarity. The coloring scheme is designed to highlight the functional compartments of the cell, with the cell wall and flagellar motor in green, soluble enzymes and other proteins in turquoise, ribosomes and tRNA in magenta, DNA in yellow, and proteins in the nucleoid in orange[3].

Fluorescence Spectroscopy



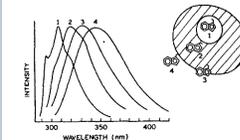
Fluorescence properties of aromatic amino acids in water at neutral pH [4]

	Lifetime (τ) (ns)	Absorption		Fluorescence	
		λ (nm)	Absorptivity (ϵ)	λ (nm)	Quantum Yield (Φ_f)
Tryptophan	3.1 (mean)	280	5600	348	0.2
Tyrosine	3.6	274	1400	303	0.14
Phenyl alanine	6.4	257	200	282	0.04



Two quenching processes:

- Dynamic (collisional) quenching
- Static (complex formation) quenching



Effect of Tryptophan Environment on the emission spectra of protein [2]. Exposure polar solvent leads to red-shift

Objective

To study the impact of synthetic crowding agents on tryptophan's fluorescence properties.

Methods

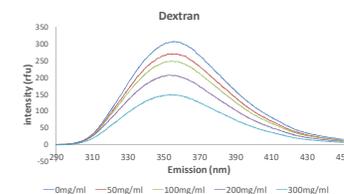
- All samples were run using a Cary Eclipse Fluorescence Spectrophotometer
- The sample was excited at 280 nm, and emissions collected from 250-450 nm
- The concentration of tryptophan was 10 μ M
- Change in intensity was measured by subtracting the maximum intensity of Tryptophan in a 300mg/ml crowder solution from the 0 mg/ml solution
- The blank contained no tryptophan to account for manipulation of the light from sources other than tryptophan
- Phosphate buffer (0.03 M, pH 8) and NaCl (0.1 M) were also added to the solution to control the pH and salinity
- A linear Stern-Volmer plot indicates purely dynamic or static quenching
- A Stern-Volmer plot with a quadratic form indicates both dynamic and static quenching

References-

1. Zimmerman and Trisch, *J. Mol. Biol.* (1991) 222, 599-620
2. Lakowicz, *Principles of Fluorescence Spectroscopy* (2006) 529-576
3. Goodsell, *Bioessays* (2012) 34, 718-720
4. Ghilardi and Chung, *Int. J. Biol. Sci.* (2014) 15, 2319-2338
5. Schuttz and Solomon, *Gen. Physiol.* (1961) 44, 1189-99
6. Ling, Hong, and Zhang, *Nucleic Acids Res. Lett.* (2013) 8, 30
7. Kuznetsov, Todorova, and Ulevsky, *J. Mol. Sci.* (2014) 15, 23090-23140
8. Armstrong, Wenby, Malaisolan, and Fisher, *Biophys. J.* (2004) 87, 4259-70.

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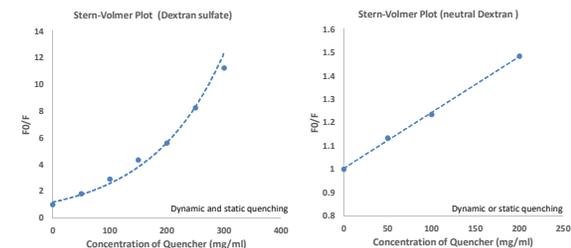
Results



Tryptophan fluorescence parameters in the presence of crowding agents

Crowding agents	Structure	R_h (\AA)	Conc. (mg/ml)	% decrease in intensity	$\Delta\lambda_{DCM}$ (nm) ^a
Dextrose		4.2 ^b	300	5.11 \pm 0.7	-0.047 \pm 0.072
Sucrose		5.2 ^b	300	28.58 \pm 1.20	1.75 \pm 0.041
Ficoll 70		40 ^c	300	48.23 \pm 1.26	0.061 \pm 0.245
Dextran-40,000		4.78 ^b	300	52.26 \pm 2.60	0.902 \pm 0.915
Dextran sulfate-40,000		4.78 ^b	300	84.45 \pm 0.44	52.044 \pm 12.000
Polyvinyl-pyrrolidone-10, 000		2.16 ^d	100	83.20 \pm 0.39	0.039 \pm 0.132
Polyethylene glycol-8000		27.5 ^e	300	5.91 \pm 1.14	-3.21 \pm 0.307

^a Red-shift and blue-shift in barycentric wavelength is shown in red and blue color, respectively. Measurements are performed in triplicate.



Conclusions

- The presence of macromolecular crowders decreases the fluorescence intensity of free tryptophan
- The decrease in fluorescence intensity is proportional to the concentration of the crowder
- Impact of crowding on fluorescence intensity is independent of crowder's size [hydrodynamic radius (R_h)]
- Dextran sulfate has maximum impact on barycentric mean wavelength (λ_{DCM})
- Stern-Volmer plots revealed that quenching in presence of dextran sulfate is due to steric and dynamic quenching

Future Directions

- The effect of varying concentrations of crowders will be extended to the other crowders
- Temperature variation studies will be performed to examine the molecular mechanism of quenching