

Application Note

Keywords

- Proteins
- Protein folding
- Amino acids
- UV LEDs

Techniques

- Fluorescence
- Modular spectroscopy
- Absorbance

Applications

- Protein determination
- Intrinsic fluorescence measurement

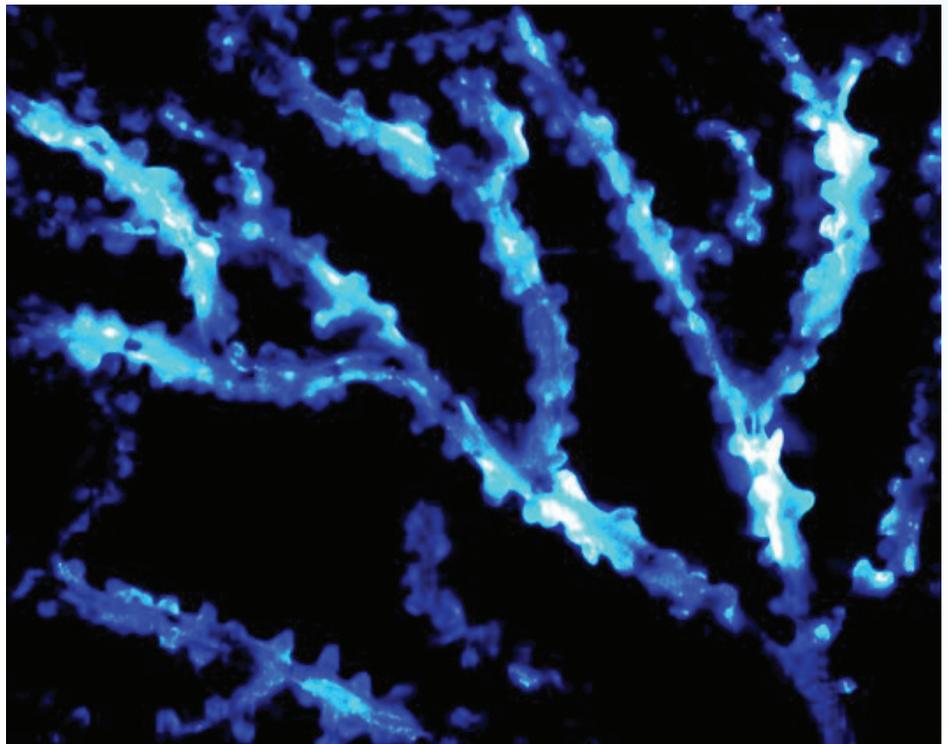
Modular Spectroscopy Tools for Measuring Intrinsic Protein Fluorescence

Written by Yvette Mattley, PhD

Intrinsic fluorescence is a powerful indicator of protein structure and function. The amount of fluorescence can often give the researcher insight into the protein's conformational states or activity under different biological conditions including changes in temperature, pH and ion concentration.

Introduction

For fluorescence measurements, a reliable excitation source is required. Light emitting diodes (LEDs) are a cost-effective choice for these measurements, with high-power UV LEDs available today that can be used to yield valuable information about proteins and amino acids.



To demonstrate, we used a 280 nm UV LED and a back-thinned CCD array spectrometer to measure intrinsic fluorescence from samples of lysozyme and Bovine Serum Albumin (BSA) in different conformational states. Lysozyme is a naturally occurring enzyme often used as a bacterial agent; BSA is a protein important to biochemical functions in the body. The results illustrated the power of the modular spectroscopy approach to monitoring fluorescence from proteins, where flexibility among components allows users to configure setups for biomolecules and even non-biological samples that require UV excitation to fluoresce.

Background

Most proteins contain aromatic amino acids that fluoresce when excited with UV light. The fluorescence spectrum of the sample depends on the amino acid composition and conformational state of the protein. As the protein goes from a native (folded) to a denatured (unfolded) state, the local environment surrounding the aromatic amino acids changes, which affects the fluorescence properties of the amino acids. These changes in intrinsic protein fluorescence can be used to monitor protein unfolding. This is valuable information for applications such as medical diagnostics, where researchers are investigating neurodegenerative and other diseases associated with improper protein unfolding.

The native state of a protein can be altered using elevated temperature, chaotropic or other chemical agents such as urea or guanidine hydrochloride, and adjustments in pH. As the protein unfolds, amino acids that were previously buried in the hydrophobic core of the protein are exposed to the solvent. Solvent exposure and the resulting susceptibility to quenching agents reduce the fluorescence of tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe).

A 280 nm UV LED was used with a high-sensitivity spectrometer to measure fluorescence from samples of lysozyme and BSA. To show the impact of protein conformation, fluorescence spectra were measured for the proteins diluted in phosphate buffered saline (1X PBS pH 7.4) and 0.1 M HCl/KCl (pH 1).

Measurement Conditions

Samples of 3 mg/mL lysozyme (L6876 Sigma) and 12 mg/mL BSA (A2153 Sigma) were prepared in 1X PBS and 0.1 M HCl/KCl. PBS is a buffer solution used to maintain a consistent sample pH. Protein suspended in 1X PBS should remain in its native (folded) state. As the protein is suspended in the lower pH 0.1 M HCl/KCl solution, the protein begins to denature and exposes amino acids previously contained within the core of the protein to the solvent environment.

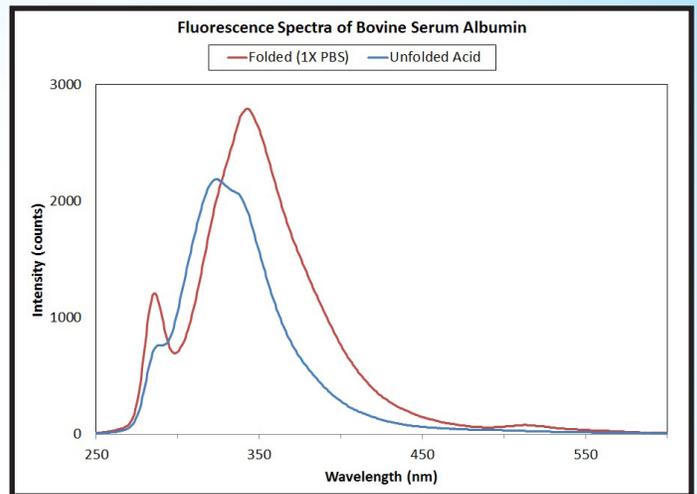


Figure 1: Fluorescence spectra of Bovine Serum Albumin, a major constituent of blood plasma, reveal changes in protein structure with exposure to a low-pH buffer.

A solution of 5 mg/mL L-Tryptophan (T90204 Aldrich) was prepared in deionized water for absorbance measurements to determine the optimal excitation wavelength for Trp and to provide a reference for Trp fluorescence. Both fluorescence and absorbance measurements were performed.

Results

Trp is a major contributor to the intrinsic protein fluorescence spectra and has the highest quantum yield of the aromatic amino acids. The Trp absorbance spectrum has a peak at ~280 nm arising from the indole group of Trp. The fluorescence spectrum excited with the 280 nm LED has a peak at ~350 nm. Tyr has a similar absorption spectrum to Trp with optimal excitation near 274 nm. Based on these absorbance properties, a 280 nm UV LED can be used to excite fluorescence from both Tyr and Trp for the protein unfolding measurements.

Fluorescence spectra for BSA diluted in 1X PBS and 0.1 M HCl/KCl also were captured (Figure 1). BSA is a major constituent of blood plasma, functioning as a carrier protein and regulator of osmotic pressure to maintain the proper distribution of body fluids. When BSA was exposed to the low pH of the 0.1 M HCl/KCl solution, the protein conformation changed, exposing the Trp and Tyr amino acids to a different environment. The fluorescence spectrum decreases in intensity and peak fluorescence shifts

to a shorter wavelength as the protein goes from folded in 1X PBS (red fluorescence curve) to an unfolded state (blue fluorescence curve).

The intrinsic protein fluorescence spectra for lysozyme diluted in 1X PBS and 0.1 M HCl/KCl are shown in Figure 2. Lysozyme is an enzyme that breaks down the cell walls of bacteria. The lysozyme used for these measurements was isolated from chicken egg whites where it serves as an anti-microbial agent. When lysozyme is exposed to the low pH solution, the protein conformation changes and exposes the Trp and Tyr amino acids to a different environment. The fluorescence spectrum decreases in intensity as the protein goes from a folded conformation in 1X PBS (red fluorescence curve) to an unfolded state (blue fluorescence curve).

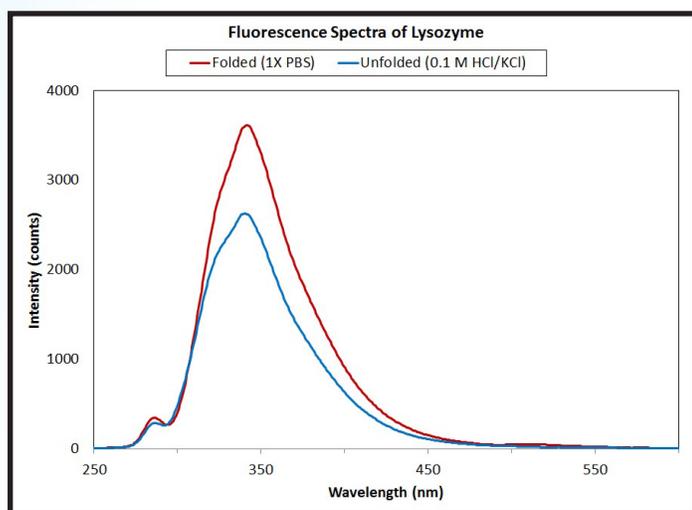


Figure 2: With exposure to a low pH solution, lysozyme protein conformation changes from folded to unfolded – a transformation revealed in fluorescence measurements.

Conclusion

The results described here for BSA and lysozyme are part of a much larger picture of protein conformation for these proteins. For example, chaotropic agents, like guanidine or DMSO, could be used in increasing concentrations to provide more granular information about the protein conformation. Also, pH could be increased more gradually to follow the unfolding process with fluorescence. Proteins could be gradually unfolded by slowly increasing the temperature of the sample using a temperature regulated cuvette holder.

The availability of powerful LED excitation sources covering the wavelength range from 240-627 nm provides many opportunities to investigate intrinsic protein fluorescence. The wide range of LED wavelengths enables testing with multiple excitation wavelengths, which allows the researcher to use the optimal wavelength for fluorescence excitation of each fluorophore. When UV LEDs are combined with a user configurable spectrometer and a flexible sampling device, a vast array of absorbance and fluorescence measurements is possible. 🔄

References

The experimental protocol used for these measurements is described at:

www.physics.nus.edu.sg/~Biophysics/pc3267/Fluorescence%20Spectroscopy2007.pdf

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