Application Note
CD Spectroscopy

Change in the fluorescence anisotropy spectrum by the denaturation of α-lactalbumin

Introduction

CD spectroscopy is one of the leading techniques in protein structure analysis, with fluorescence spectroscopy and fluorescence anisotropy providing complementary information. While CD spectra provide information regarding the secondary structure of proteins, fluorescence and anisotropy spectra provide information about the local environment of fluorophores. In particular, fluorescence anisotropy offers insight about the rotational movement of these fluorophores which cannot be obtained by fluorescence spectroscopy alone.

The JASCO J-1500 CD spectrometer can be used to measure CD, absorption, excitation and emission, and fluorescence anisotropy. This variety of techniques not only allows for secondary structure estimation but also the analysis of protein-ligand binding and rotational movement in proteins.

This application note describes the changes in fluorescence anisotropy measurements of the denaturation of α-lactalbumin by guanadinium hydrochloride (GuHCl).

Keywords

J-1500, circular dichroism, CDF-426, fluorescence, anisotropy, α-lactalbumin, GuHCl, secondary structure, denaturation, biochemistry

JASCO J-1500 CD spectrometer
View product information at www.jascoinc.com

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Experimental

Measurement conditions	
Data acquisition interval	0.1 nm
Excitation bandwidth	7 nm
Response time	2 sec
Scan speed	100 nm

Results

The fluorescence anisotropy spectra of both the native-state α-lactalbumin in H_2O and the unfolded α-lactalbumin in 3.4 M GuHCl are shown in Figure 1. Both spectra show a peak maxima at 267 nm and peak minima at 283 and 291 nm. All three peaks result from the tryptophan residue of the protein. Figure 1 illustrates that the denaturation of α-lactalbumin clearly decreases the fluorescence anisotropy, indicating a more freely rotating tryptophan residue.

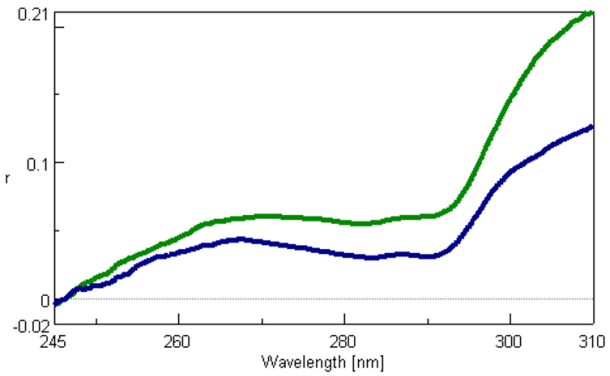

Figure 1. Fluorescence anisotropy spectra of the denaturation of α-lactalbumin by GuHCl. The green line indicates 0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in the absence of GuHCl, and the blue line is 0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in 3.4 M GuHCl.

Conclusion

This application note demonstrates that the J-1500 CD spectrometer can be used to measure samples for a variety of spectroscopic techniques. The addition of the FPA-580 polarizer to the J-1500 spectrometer allows users to obtain CD and fluorescence anisotropy data all on the same instrument.

References

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2. J. R. Lakowicz, Principles of Fluorescence Spectroscopy, New York, 446-487.

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