Application Note

CD-0013

Change of fluorescence anisotropy spectrum of α-lactalbumin by denaturation

Introduction

CD spectrum measurement is one of the leading techniques in protein structure analyses, while fluorescence spectra and fluorescence anisotropy spectra can give complementary information to CD spectra. CD spectra provide information about the secondary structure of proteins, while fluorescence spectra and fluorescence anisotropy spectra provide information about the local environment of the fluorophore, such as the tryptophan residue. In particular fluorescence anisotropy spectra provide information about rotational movement which cannot be obtained by fluorescence spectra alone.

JASCO J-815 CD spectrometer enables measurement of CD spectra, absorption spectra, Ex/Em spectra and fluorescence anisotropy spectra, thereby, allowing not only secondary structure estimation but also the analysis of protein-ligand binding and rotational movement of protein.

Here, the change of fluorescence anisotropy spectrum of α-lactalbumin by denaturation[1] using J-815 is explained.

Keywords: Fluorescence anisotropy spectra, a-Lactalbumin, Denaturation

<System>

J-815 CD spectrometer
LD-403 LD attachment
CDF-426 CD/Fluorescence Measurement unit (Peltier type) with Polarizer (optional)

<Samples>

0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in H_2O
0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in 3.4 M GuHCl:

<Parameters>

Measurement range:	310 to 245 nm	Scan speed:	100 nm/min
Response:	2 sec	Data interval:	0.1 nm
Ex Bandwidth:	7 nm	Cutoff filter:	UV34

<Fluorescence anisotropy spectrum of α-lactalbumin >

Fluorescence anisotropy spectra of native-state a-lactalbumin in H_2O and unfolded a-lactalbumin in 3.4 M GuHCl are shown in Fig. 1. These two spectra show a peak maximum at 267 nm and two peak minima at 283 nm and 291 nm originating from the tryptophan residue[2]. Denaturation of α-lactalbumin clearly results in a decrease in fluorescence anisotropy originating from the tryptophan residue.

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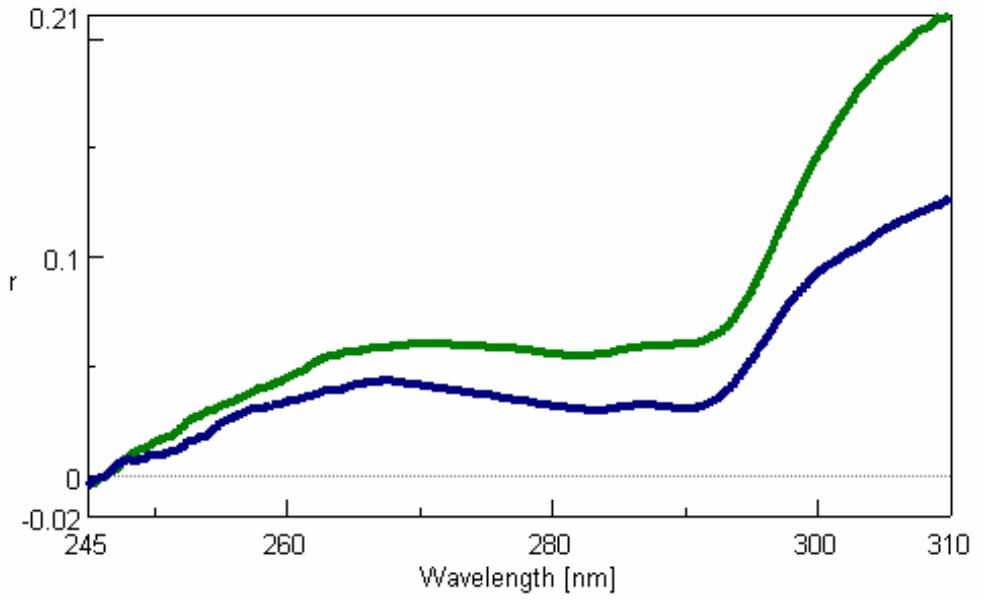

Fig. 1 Change of fluorescence anisotropy spectrum of α-lactalbumin by denaturation

0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in H_2O:
0.02 mg/mL α-lactalbumin, 0.1 mM EDTA in 3.4 M GuHCl:

<References>

(1) Denis Canet, Klaus Doering, Christopher M. Dobson, and Yves Dupont, *Biophysical Journal,* **80**, 1996-2003, (2001)

(2) Protein fluorescence. *In* Principles of Fluorescence Spectroscopy. J. R. Lakowicz, editor. Kluwer Academic/Plenum Publishers, New York. 446-487.

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