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Application Note

CD-0036

CD/fluorescence anisotropy spectral measurement of apo-α-lactalbumin by Multi-probe measurement

Introduction

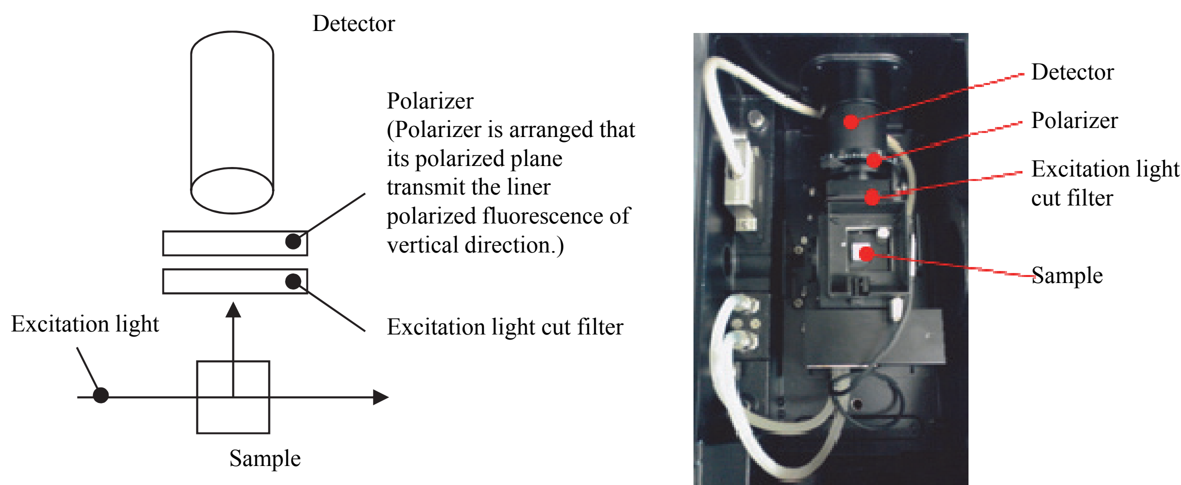
Many measurement methods such as CD measurement, fluorescence measurement and fluorescence anisotropy are complementarily used for protein structure analysis. With J-1500 circular dichroism spectrometer, multiple analysis for one sample can be realized because J-1500 corresponds to many measurement modes such as CD, absorption, fluorescence, florescence anisotropy and linear dichroism (LD) spectrum. (Multi-probe measurement)

Table 1 Structural information reflecting each measurement mode

Far-UV region CD spectrum	CD signal derived from a peptide bond reflecting the secondary structure of protein
Near-UV region CD spectrum	CD signal derived from the side chain of aromatic amino acids reflecting the tertiary structure of a protein
Fluorescence spectrum	Fluorescence signal reflecting environmental change of side chain of aromatic amino acids in protein
Fluorescence anisotropy	Signal reflecting the rotational motility of the side chain of aromatic amino acids in protein

In this report, changes in tertiary structure accompanying guanidine hydrochloride denaturation and changes of the rotational motility of the side chain of the tryptophan residue of apo-α-lactalbumin were observed by near-UV region CD spectral measurement and fluorescence anisotropy spectral measurement [1].

<Fluorescence anisotropy measurement system>



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Keywords
Near-UV CD, tertiary structure, fluorescence anisotropy

Measurement Condition

(Near-UV CD measurement)

Scan speed:	100 nm/min	Response:	1sec
Data interval:	0.1 nm	bandwidth:	1 nm
Accumulation:	9 times	Cell:	10 mm x 10 mm

(Fluorescence anisotropy measurement)

Scan speed:	100 nm/min	Response:	1 sec
Data interval:	0.1 nm	Excitation bandwidth *1):	5 nm
Accumulation:	9 times	Cut filter:	UV34
Cell:	10 mm x 10 mm		

Sample preparation

Sample solution was prepared by mixing solutions shown below. The concentration at the time of measurement is shown in ().

(Native apo-α-lactalbumin)

5 mg/mL apo-α-lactalbumin/buffer:	0.5 mL (0.5 mg/mL)
10 mM EDTA/buffer:	0.5 mL (1 mM)
Buffer:	4 mL

(Denatured apo-α-lactalbumin)

5 mg/mL apo-α-lactalbumin/buffer:	0.5 mL (0.5 mg/mL)
10 mM EDTA/buffer:	0.5 mL (1 mM)
4.3M Guanidine hydrochloride aqueous solution:	4 mL (3.44 M)

Buffer: 50 mM phosphate buffer, pH6.9

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Measurement Results

Near-UV region CD spectra of native and denatured apo-α-lactalbumin solution were shown in Fig 1. The difference of these spectra indicate that the addition of guanidine hydrochloride caused the denaturation of apo-α-lactalbumin and the change of tertiary structure.

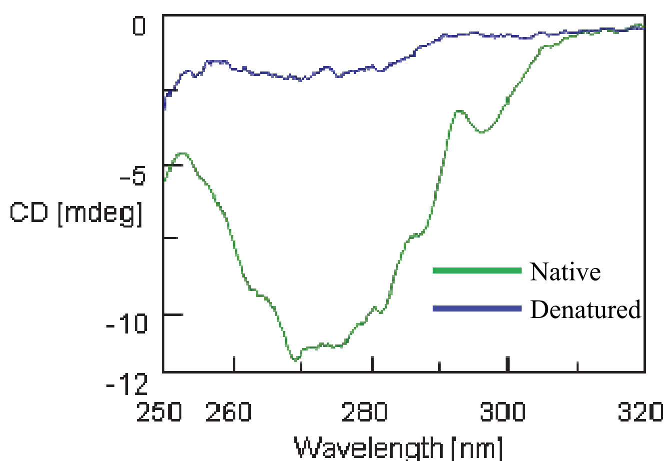

Fig. 1 The change of near-UV region CD spectrum of apo-α-lactalbumin (20ºC)

Fluorescence anisotropic excitation spectra of native and denatured apo-α-lactalbumin solution were shown in Fig 2. The maximum peak (267 nm) and the minimum peak (283 nm, 291 nm) derived from tryptophan residue was observed which are determined that fluorescence anisotropy derived from the fluorescence of tryptophan residue was observed [2)]. This result indicates that the intensity of fluorescence anisotropy was decreased by the denaturation by guanidine hydrochloride, and rotational motility of side chain of tryptophan residue was increased by the denaturation of apo-α-lactalbumin.

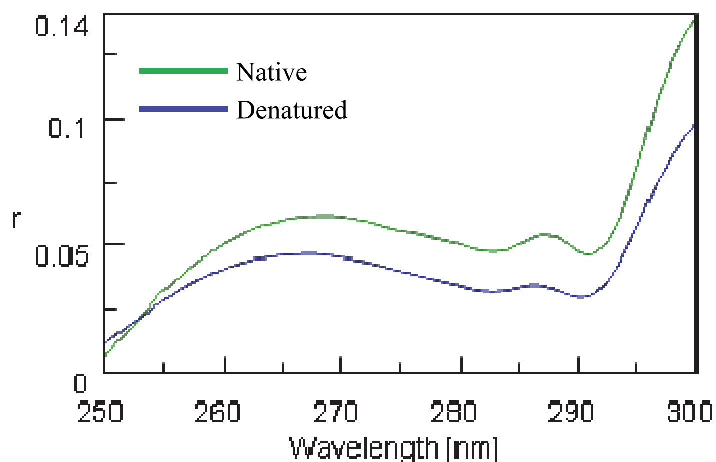

Fig. 2 The change of fluorescence anisotropy spectrum of apo-α-lactalbumin (20ºC)

*1) Since near-UV CD spectral measurement and fluorescence anisotropy measurement have different optimal bandwidth (excitation wavelength), they were measured separately.

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Reference

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

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

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