Application Note

CD-0029

Secondary structure analysis of poly-L-glutamic sodium using titration with dilute sulfuric acid

Introduction

In the fields of fundamental protein research and pharmaceutical technology, studies into protein structure and peptides models are becoming of increasing interest; the function of proteins is intrinsically linked to their structure, and the structural analysis of proteins and peptide models is important in determining their bioactivity.

NMR and X-ray crystal structure analysis are both very effective methods in the elucidation of protein structure, but the requirement for large amounts of sample and equipment costs can be prohibitive for routine analysis.

By comparison, analysis using circular dichroism is very straightforward and can be done with small amounts of sample. These features make CD a useful tool for the estimation of secondary structure of proteins and peptides, analyzing the conformational changes caused by pH, temperature, and ligand binding.

In structural analysis using CD measurement, the abundance ratio of secondary structure motifs in proteins and peptides can be estimated using a least-square method with reference spectra including α-helix, β-sheet, turn, and random structure. The JWSSE-513 protein secondary structure analysis program uses a Classical Least Squares (CLS) method, which includes the reference spectra of Yang[1)] and Reed[2)]. Yang's reference spectra are extracted from CD spectra of proteins, and are best suited to protein secondary structure analysis[1), 3)]. Reed's reference spectra are extracted from the CD spectra of peptides, and are suited to the secondary structure analysis of peptides; because of lesser effects on CD caused by the side chains of aromatic amino acids often found in proteins. For the secondary structure estimation of peptides, the JWSSE-513 protein secondary structure analysis program using Reed's reference spectra is extremely effective. The data shown here is an example of CD spectral change in poly-L-glutamic acid sodium solution with titration with dilute sulfuric acid. The changes in secondary structure are reported below.

Keyword: J-1500, circular dichroism, protein secondary structure, polypeptide secondary structure, α-helix, β-sheet, turn, random coil, Yang's reference spectra, Reed's reference spectra, JWSSE-513

Measurement condition

2 ml of poly-L-glutamic acid sodium solution (0.02 mg/ml) was titrated with 10^{-5}N dilute sulfuric acid and the corresponding CD spectrum measured in the range from 260 nm to 190 nm.
The titration was repeated 20 times per 50 µl using the J-1500 CD spectropolarimeter fitted with ATS-530 Automatic Titration Unit.

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Results

Figure 1. CD spectral changes in poly-L-glutamic acid sodium solution with titration using dilute sulfuric acid.

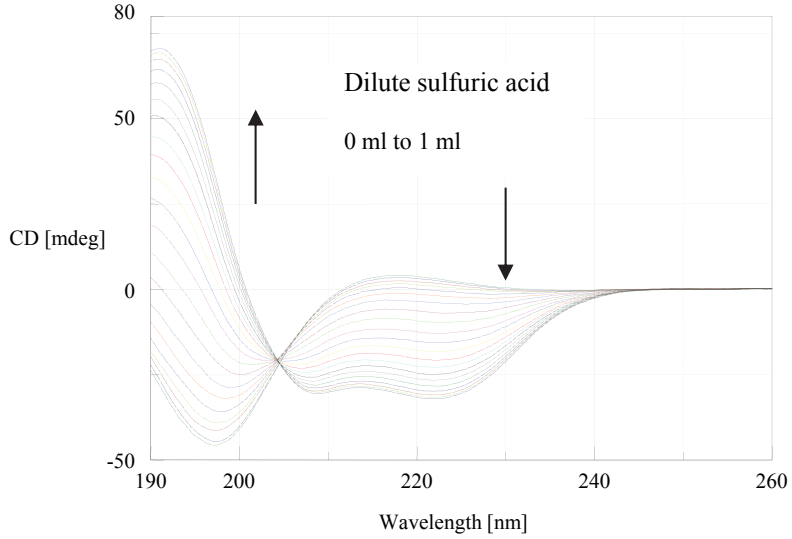

Fig. 1 CD spectra of poly-L-glutamic acid sodium solution with titration using dilute sulfuric acid

In a near-neutral pH solution, the CD spectrum shows that the protein has a random structure. With the addition of dilute sulfuric acid, the CD spectrum shows that the structure develops into a strong helix motif. The CD spectra for poly-L-glutamic acid sodium solution before the addition of dilute sulfuric acid and after the addition of 1 ml dilute sulfuric acid were both analyzed using Reed's reference spectrum as shown in Figure 2. For the analysis, the Y axis is converted to Molar ellipticity (Mol. Ellip). To illustrate the results of the analysis of secondary structure, the measured spectra, calculated spectra based on the measured spectrum and the residual error between the measured and calculated spectra are drawn on the same plot.

The ratio of helix, β-sheet, turn, and random structure, and RMS value between the measured and calculated spectrum can also be calculated.

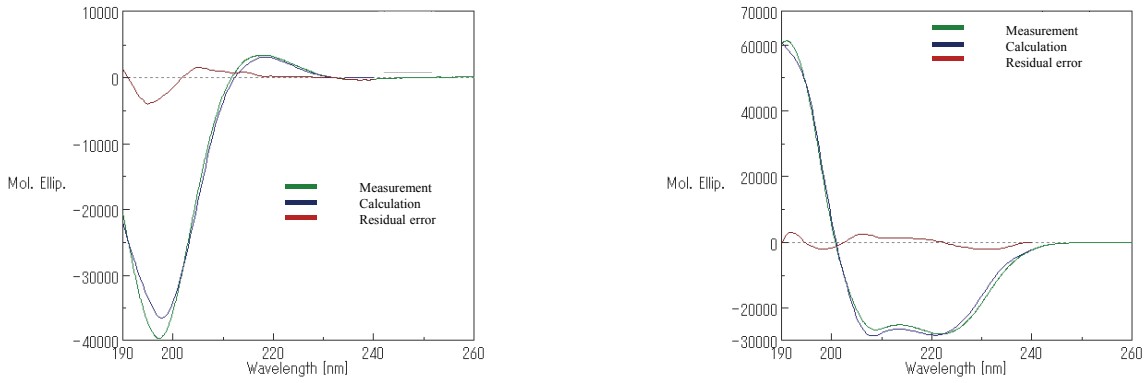

Fig. 2 CD spectrum comparison of measurement and calculated spectrum
Left: before titration
Right: after titration with 1 ml diluted sulfuric acid

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The change in secondary structure abundance ratio is shown in Figure 3. As the solution tends to acidity, the ratio of the helix motif is increased to a maximum of 82.4% of the total structure of the poly-L-glutamic acid, and the random motif is decreased to 3.2% at the 6.6 µM sulfuric acid concentration.

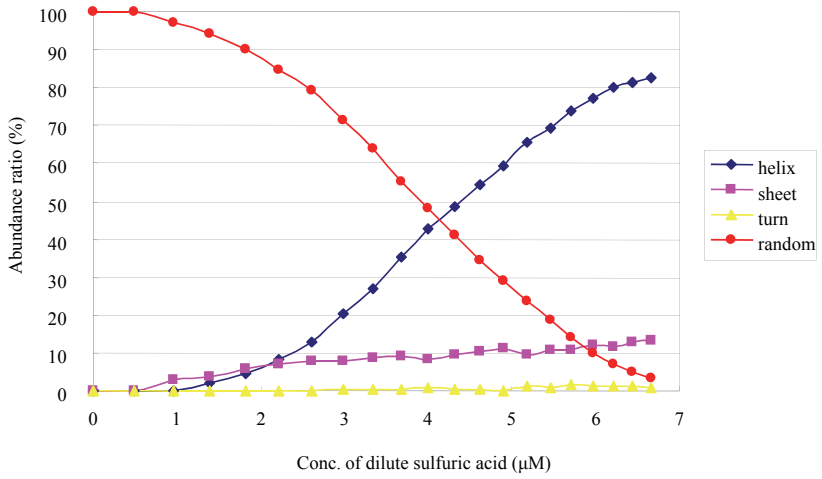

Fig. 3 The abundance ratio change in secondary structure of poly-L-glutamic acid

Reference

(1) Jen Tsi Yang, Chuen-Shang C. Wu, and Hugo M. Martinez, *Methods in Enzymology*, 130, 208-269, (1986)
(2) J. Reed, and T. A. Reed, *Anal. Biochem*., 254, 36-40, (1997)
(3) C. T. Chang, C-S. C. Wu, and J. T. Yang, *Anal. Biochem*., 91, 13-31, (1978)

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