Application Note
CD Spectroscopy

Lysozyme and N-acetyl glucosamine interaction probed by circular dichroism

Introduction

Changes in a protein's CD spectrum directly reflect the changes in its structure. The absorption derived from the protein's peptide bonds can be seen below 240 nm of the CD spectrum. Therefore, by measuring the CD spectrum in the far-UV wavelength region, information regarding a protein's secondary structure can be obtained. On the other hand, absorption due to aromatic amino acid side chain residues are seen in the 210-220 nm wavelength region of the spectrum. There are additional absorption bands at wavelengths beyond 240 nm which do not overlap with the absorption band of the peptide bonds. For this reason, in order to study side chain residues the CD spectrum is usually measured in the wavelength region longer than 240 nm which is known as the near-UV.

In this application note, the interaction between lysozyme and its inhibitor, N-acetyl-(D+)-glucosamine (NAG) was measured in the near-UV region using a J-1500 CD spectrometer and a ATS-530 automatic titrator.

ATS-530 automatic titrator
View product information at www.jascoinc.com

Keywords

J-1500, circular dichroism, near-UV, side chain residues, aromatics, ATS-530, automatic titrator, biochemistry

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

Experimental

Measurement conditions	
Data acquisition interval	1 sec
Data pitch	0.1 nm
Spectral bandwidth	1 nm
Scan speed	50 nm/min
Accumulations	4
Path length	10 mm

The titration of 225 mM NAG to 0.07 mM lysozyme solution was measured between 260-320 nm. NAG was slowly titrated in 50 µL increments at a constant temperature of 20°C.

Results

The CD spectra of lysozyme, lysozyme + NAG, and NAG are shown in Figure 1. The lysozyme CD peaks are positive and observed at 293.5, 289, and 283.2 nm. The addition of NAG to lysozyme increases the CD intensity between 300-270 nm while the peaks in the CD spectrum of NAG only are barely observable.

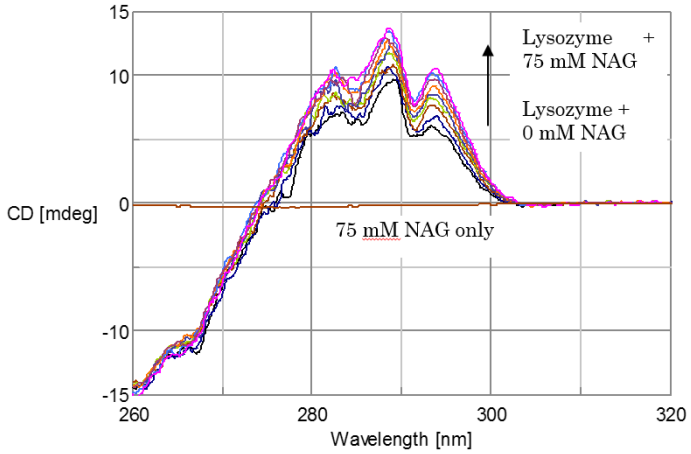

Figure 1. CD spectra of lysozyme only, NAG only, and NAG titrated into the lysozyme solution in the near-UV region.

Figure 2 shows a 3-D graphical representation of the changes in the CD spectra reflected by the titration of NAG to the lysozyme solution. Figure 3 illustrates the change in the CD intensity at 293.5 nm during the titration of lysozyme. X-ray crystallography data illustrates that there is a tryptophan residues in the substrate bonding region of lysozyme. The X-ray data suggests that the CD data can be interpreted as increasing in CD intensity due to the interaction of NAG with lysozyme's tryptophan residue.

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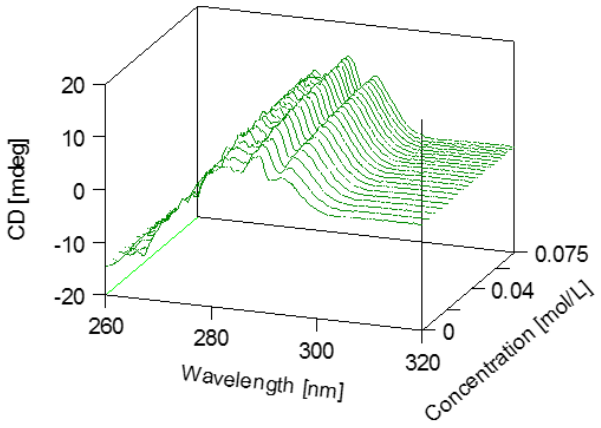

Figure 2. 3-D graphical representation of the CD spectra changes due to the titration of NAG to lysozyme.

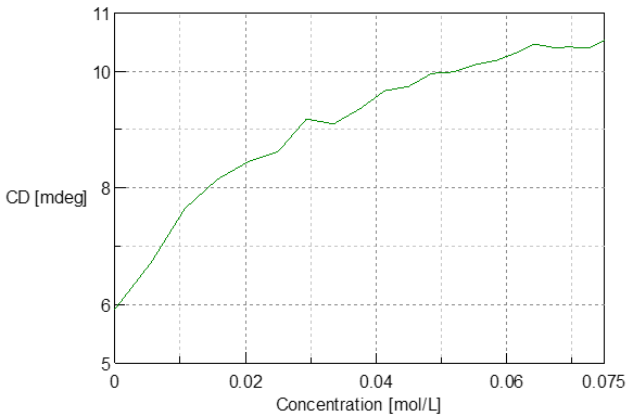

Figure 3. CD spectrum at 293.5 nm as a function of the NAG concentration.

By conducting this CD measurement, the change in the conformation of the enzyme's side chains can be observed during interactions with the substrate and when the substrate bonding region is known, the type of side chain can be estimated using the CD spectrum obtained.

Conclusion

This application note demonstrates the use of the J-1500 CD spectrometer and ATS-530 automatic titrator in probing the near-UV region of the CD spectrum in order to elucidate conformational changes in specific aromatic side chain residues of proteins.

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