Revisiting 2nd derivative UV spectroscopy for real-time monitoring of structural changes and complexations of macromolecules

Introduction

UV-Vis is a well established analytical technology commonly used in the pharmaceutical industry as a reliable and mature analytical technique. We used Pion's extensive experience in UV-Vis to revisit the use of the 2nd derivative spectroscopy for monitoring *in real time* structural changes and complexations of macromolecules (proteins and nucleic acids). This non-destructive method helps to overcome or reduce limitations of current measurement techniques such as a lag time between sample preparation and analysis as well as volume, mixing and concentration constraints.

Background

The kinetics of protein denaturation is of interest due to protein instability when exposed to chemical and physical stress. UV spectroscopy can detect changes in protein folding due to spectral differences between native and denatured protein forms 1-3. In this work we show how protein's reversible unfolding can be monitored in real-time.

Method

Figure 1. An artistic expression of chemically induced unfolding and refolding of BSA. Green shapes - BSA, blue spheres – SDS molecules below CMC; yellow conical frustums – HP-β-CD molecules.

BSA solutions at pH 7.4 were titrated with SDS, followed by titration with HP-β-CD. BSA spectra during unfolding/refolding processes were characterized by UV-Vis and Circular Dichroism (CD) spectroscopy. UV-Vis spectra were collected in situ using the Rainbow® fiber optic spectrometer (Pion Inc., Billerica, MA, USA). Changes in 2nd derivative spectra were monitored by Zero Intercept Method (ZIM) in-built in AuPro software.

CD spectra were collected using a Chirascan™ plus spectrometer. BSA mean residue ellipticity (MRE) at 222 nm was determined and secondary structure (alpha helix, beta sheet and random coil content) calculated using CDNN software.

Results

ZIM point, the wavelength where the BSA 2nd derivative spectrum crosses the abscissa (λ_{ZIM}^{native} 289.6 nm) measured in the absence of SDS, sequentially shifted ~ 0.1 – 0.3 nm, reaching a minimum $\lambda_{ZIM}^{unfolded}$ 288.5 nm at C max SDS. Addition of HP-β-CD, shifted $\lambda_{ZIM}^{unfolded}$ in the opposite direction, showing the ability of the ZIM method to monitor the unfolding/refolding processes.

ZIM points of controls, caffeine and tryptophan under the same conditions, were unaltered, fluctuating within 0.03 – 0.06 nm across the range of SDS and HP-β-CD concentrations studied.

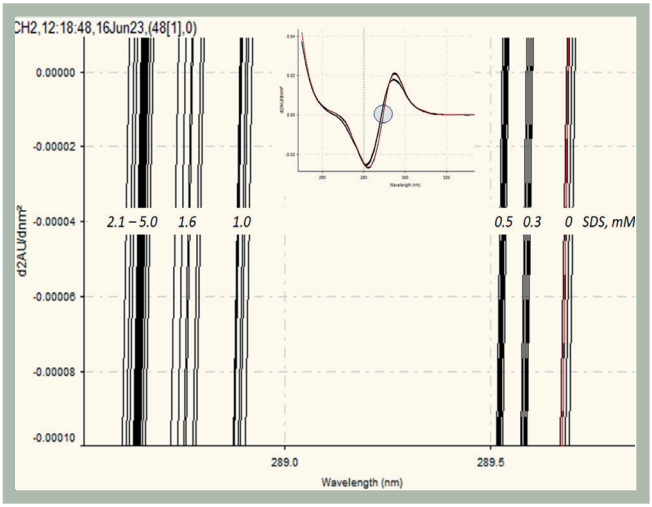

Figure 2. 2nd derivative UV profiles BSA in the presence of SDS. Zoom-in on wavelength scale illustrates drift of BSA ZIM289 during SDS induced unfolding. Each bench of vertical line represents partial view of 5-7 overlaying spectra at different concentrations of SDS. For the graphs: abscissas - wavelength, nm; ordinates - intensity of 2nd derivative signal (AU).

CD spectra monitored BSA unfolding, due to SDS addition, via increases in BSA mean residue ellipticity (MRE) at 222 nm and % beta-sheet content. This behavior was reversed upon the addition of HP-β-CD, demonstrating BSA refolding.

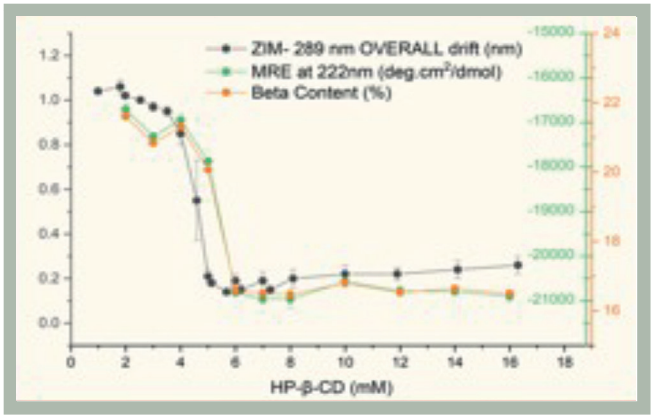

Figure 3. BSA-unfolding (Top) and refolding (bottom): Mean Residual Ellipticity and CDNN determined beta sheet content (right axes) and the drift of ZIM289 (left axis) as a function of SDS and HP-β-CD concentration correspondingly.

Conclusion

Our results demonstrate the implementation of UV spectroscopy for monitoring protein structural changes at timescales from 2 seconds to days. This method can be applied to a broader variety of tryptophan containing proteins, although potential limitations are yet to be studied.

Pion stands behind the science

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