REVISITING UV SPECTROSCOPY FOR REAL-TIME MONITORING OF REVERSIBLE PROTEIN UNFOLDING

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PURPOSE

Show the capability of derivative UV spectroscopy to monitor chemically induced protein unfolding/re-folding in real-time overcoming limitations of current measurement techniques such as a lag time between sample preparation and analysis, sample volume, mixing and concentration constraints.

OBJECTIVE(S)

- Characterize spectral differences between native and denatured forms of a model protein BSA using derivative UV spectroscopy [1-3]
- Monitor in real time reversible unfolding of a model protein BSA, induced by sodium dodecyl sulphate (SDS) and refolding induced by hydroxypropyl-β-cyclodextrin (HP-β-CD). [4-6.]

METHOD(S)

- BSA solutions (~ 3 μM) in phosphate buffer pH 7.4 were titrated with SDS (0 to 5 mM), followed by titration with HP-β-CD (0 – 16 mM)
- BSA spectra during unfolding/refolding processes were characterized by UV-Vis and Circular Dichroism (CD) spectroscopy.
- UV-Vis spectra (200-720 nm) were collected *in situ* using a Fiber Optic Rainbow® instrument (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 (300-190 nm at 60 nm/min, 1.0 nm bandwidth). 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.

FUNDING / GRANTS / ENCORE / REFERENCE OR OTHER USE

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2. UV-Vis spectroscopy of tyrosine side-groups in studies of protein structure. Part 1: basic principles and properties of tyrosine chromophore Jan M Antosiewicz, David Shugar Biophys Rev 2016 Jun;8(2):151-161
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4. DSC studies on bovine serum albumin denaturation Effects of ionic strength and SDS concentration C. Giancola, C De Sena, D. Fessas, G Graziano, G Barone, Int J Biol Macromol 1997 Jun;20(3):193-204
5. Protein unfolding and subsequent refolding: a spectroscopic investigation Uttam Anand, Chandrima Jash and Saptarshi Mukherjee, Phys. Chem. Chem. Phys., 2011, 13, 20418–20426
6. Reversibility in protein folding: effect of β-cyclodextrin on bovine serum albumin unfolded by sodium dodecyl sulphate. Uttam Anand, Saptarshi Mukherjee, PCCP, 2013, 15(23)
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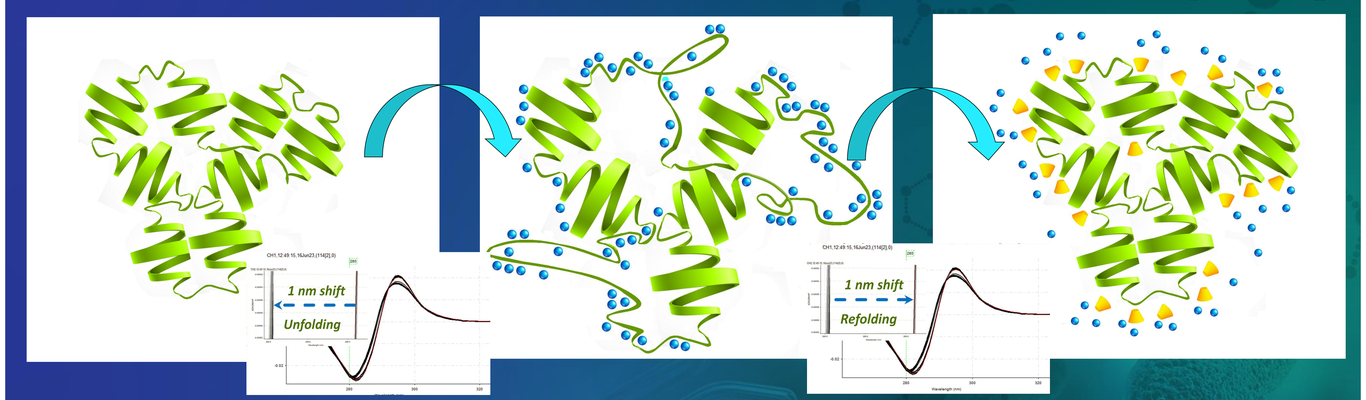
Fiber optic 2nd derivative UV spectroscopy combined with ZIM is a labor-saving tool to monitor reversible protein unfolding



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RESULT(S)

- The Zero Intercept (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 λ_{ZIM} ^{unfolded} 288.5 nm at 5 mM SDS.
- Addition of HP-β-CD (1 – 16 mM), shifted λ_{ZIM} ^{unfolded} in the opposite direction, showing the ability of the λ_{ZIM} method to monitor the unfolding/refolding processes. ZIM measurements of controls, caffeine (λ_{ZIM} 286.4 nm) and tryptophan (λ_{ZIM} 292.3 nm), were unaltered, fluctuating within 0.03 – 0.06 nm across the range of SDS and HP-β-CD concentrations studied.
- 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 behaviour was reversed upon the addition of HP-β-CD, demonstrating BSA refolding.
- CD MRE and % beta-sheet content data correlated with the drift of ZIM wavelength at 289 nm measured by UV spectroscopy.

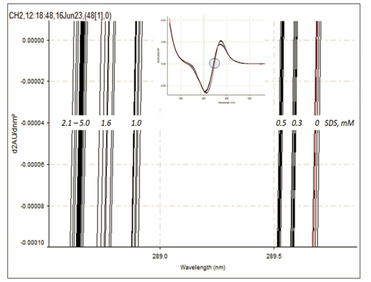

Figure 1. 2nd derivative UV profiles BSA in the presence of SDS. Zoom-in on wavelength scale illustrates drift of BSA ZIM^{289} during SDS induced unfolding. Each bench of vertical lines represents a partial view of 5-7 overlaying spectra at different concentrations of SDS. For the graphs: abscissas - wavelength, nm; ordinates - intensity of 2nd derivative signal (AU).

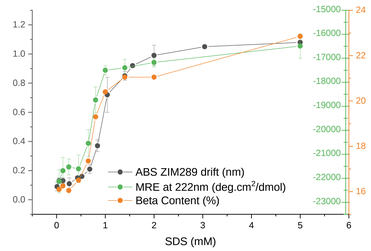

Figure 2. BSA-unfolding: Mean Residual Ellipticity and CDNN determined beta sheet content (right axes) and the drift of ZIM ^{289} (left axis) as a function of SDS concentration

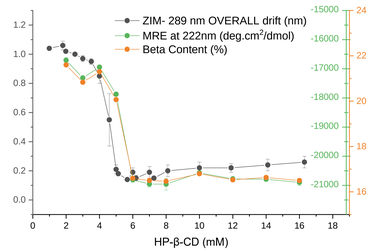

Figure 3. BSA-refolding: Mean Residual Ellipticity and CDNN determined beta sheet content (lright axes) and the drift of ZIM ^{289} (left axis) as a function of HP-β-CD concentration

CONCLUSION(S)

- 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.
- Fiber optic derivative UV spectroscopy can be employed as a labor-saving tool to monitor protein structural changes, allowing stable and flexible data collection, as well as fast and reliable data processing.