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Thermal Denaturation Analysis of Bovine Serum Albumin over Wide Concentration Range by Microfluidic Modulation Spectroscopy

Introduction

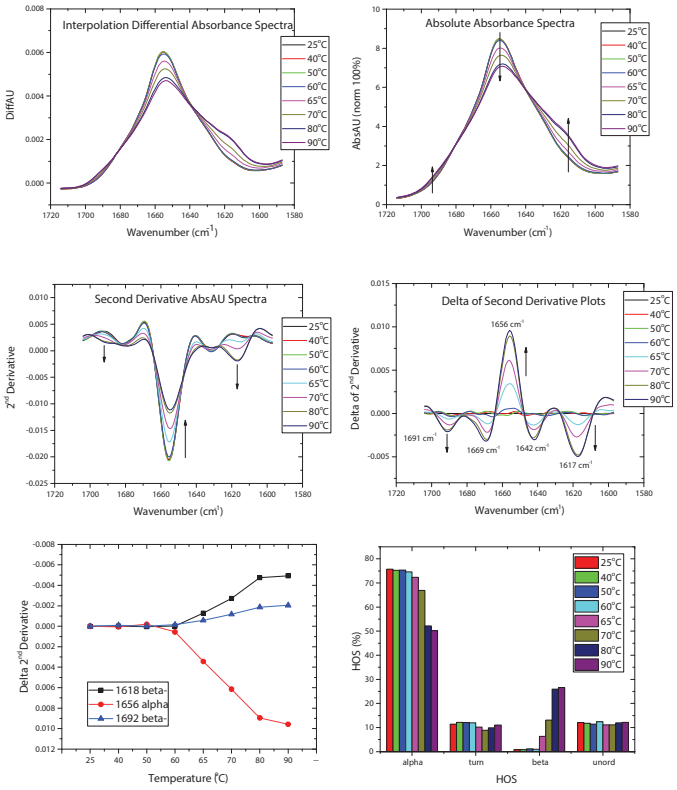
Microfluidic Modulation Spectroscopy (MMS) is a powerful new infrared spectroscopy tool for protein structural analysis developed by RedShift BioAnalytics. This technology provides significant increases in sensitivity, dynamic range, and accuracy for determination of protein secondary structure relative to conventional mid-IR and far-UV CD techniques. The analyzer utilizes a tunable quantum cascade laser to generate an optical signal > 100x brighter than the conventional sources used in FTIR spectroscopy. Brighter sources also allow the use of simpler detectors without the need for liquid nitrogen cooling. Additionally, the sample (protein) solution and a matching reference buffer stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (1-4 Hz) across the laser beam path to produce nearly drift-free background compensated measurements.

Methods

BSA samples at different concentrations (1 mg/mL, 20 mg/mL, and 100 mg/mL) were incubated for 20 minutes at temperatures ranging from 25°C to 90°C and then cooled to room temperature for MMS analysis. Protein differential absorption spectra were automatically acquired across the amide I band from approximately 1700 to 1600 cm-1 in 4 cm-1 steps and were analyzed using the AQS³delta Analytics software. All measurements were performed at room temperature and all samples were measured in native form with no dilution, dialysis, or other sample preparation required.

Results

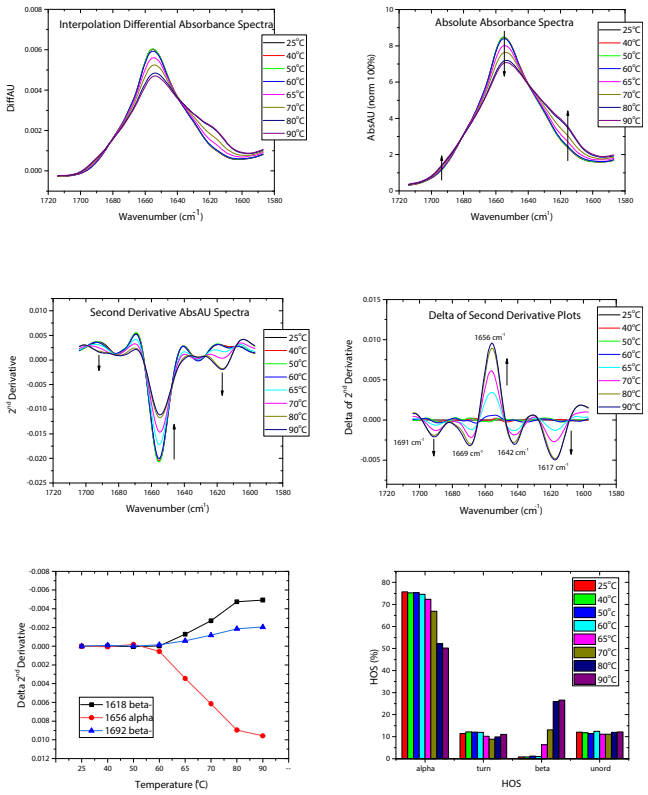
I. BSA denaturation at 1 mg/mL. As temperatures increased, a loss in alpha helical structure (1656 cm-1) was observed, signifying protein denaturation, and a gain in beta structures (1618 and 1692 cm-1) was observed, signifying the onset of aggregation. These secondary structural changes began at 60°C and appeared to plateau by 80°C.



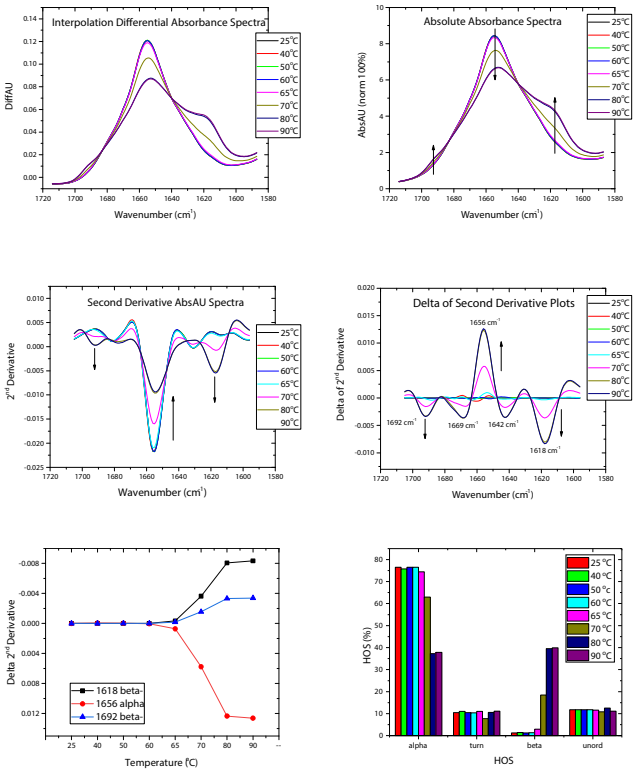
AQS³pro Microfluidic Modulation Spectroscopy System

Results, continued

II. BSA denaturation at 20 mg/mL. Structural changes were similar to the results observed at 1 mg/mL.



III. BSA denaturation at 100 mg/mL. Structural changes were similar to results observed at 1 mg/mL and 20 mg/mL at lower temperatures. Data is not shown for temperatures above 70°C due to sample gelation. Anti parallel beta-sheet absorbance increases were observed in solution prior to gelation.



Conclusions

- MMS was used to study the heat-induced thermal denaturation of BSA. The results show replicate measurements are very reproducible (99% similarity).

- MMS provides accurate secondary structure measurements for protein samples over a wide concentration range (1 to 100 mg/mL).

- MMS is a powerful and versatile tool for direct, label- free characterization of proteins through all phases of biologic drug development, from discovery through formulation and manufacturing.

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