Thermal Denaturation Analysis of Bovine Serum Albumin by Microfluidic Modulation Spectroscopy

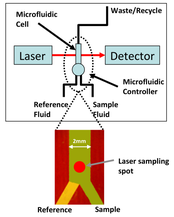
Libo Wang, Jeff Zonderman and Eugene Ma, RedShiftBio

Abstract

The heat-induced secondary structure change of Bovine Serum Albumin (BSA) during thermal denaturation was investigated using Microfluidic Modulation Spectrometry (MMS), a novel technique that combines a microfluidic cell and a tunable mid-IR Quantum Cascade Laser source. BSA samples at 1 mg/mL, 20 mg/mL and 100 mg/mL in water solution were incubated over a temperature range of 25 to 90 ºC. The differential absorbance spectra across the amide-I band for the samples at different concentrations and different incubation temperatures were then measured. For BSA samples at 20 mg/mL, at temperatures below 65 ºC, BSA maintained a native structure that consists of three major secondary structure components, specifically alpha helix at 1656 cm^{-1}, beta turn at 1682 cm^{-1} and extended structure at 1631 cm^{-1}. At 65 ºC, BSA began to exhibit signs of denaturation with a slight decrease in alpha helix and the appearance of irreversible anti-parallel beta-sheet structures at 1618 cm^{-1} and 1692 cm^{-1}. Above 65 ºC BSA lost more alpha helix structure and gained more beta-sheet structure, a trend that continued up to 80 ºC where BSA reached fully denaturation. For BSA at 1 mg/mL, a similar thermal denaturation trend was observed except the onset of denaturation appeared earlier, around 60 ºC. For BSA at 100 mg/mL, the onset of denaturation was observed at 65 ºC. At this higher concentration, only samples incubated at temperatures 25 through 70 ºC were tested due to the gelation behavior that occurred at higher temperatures. Our results show that Microfluidic Modulation Spectroscopy is a powerful technique for the measurement and analysis of protein secondary structure in samples over the concentration range between 1 mg/mL and 100 mg/mL.

Introduction

RedShift BioAnalytics has developed a powerful new infrared spectroscopy tool for protein structural analysis based on Microfluidic Modulation Spectroscopy (MMS). This technology achieves 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 mid-IR quantum cascade laser to generate an optical signal 1000X 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 buffer reference stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (e.g. 5 Hz) across the laser beam path to produce nearly drift-free background compensated measurements. A simplified diagram of the instrument is shown in the figure below.

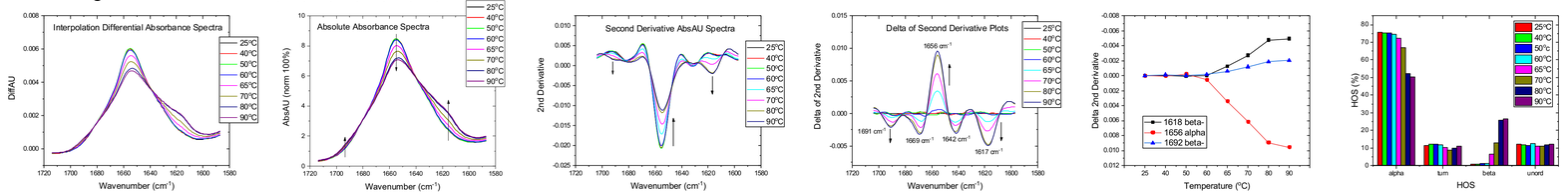


Methods

BSA samples at different concentrations (1 mg/mL, 20 mg/mL and 100 mg/mL) were incubated for 20 minutes at temperatures ranging from 25ºC to 90ºC and then cooled to room temperature for MMS testing. Protein differential absorption spectra were acquired across the amide I band, from approximately 1700 to 1600 cm^{-1} in 4 cm^{-1} steps and were analyzed using proprietary analytical software. All measurements were done at room temperature.

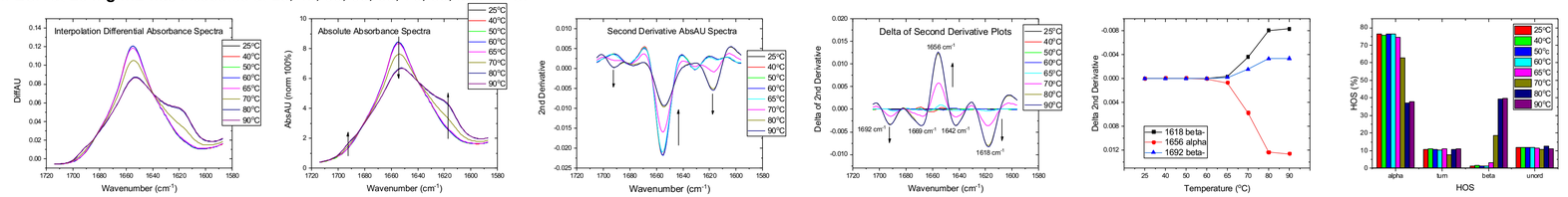
Results

I. **BSA at 1 mg/mL** was incubated at 25, 40, 50, 60, 65, 70, 80, and 90°C.



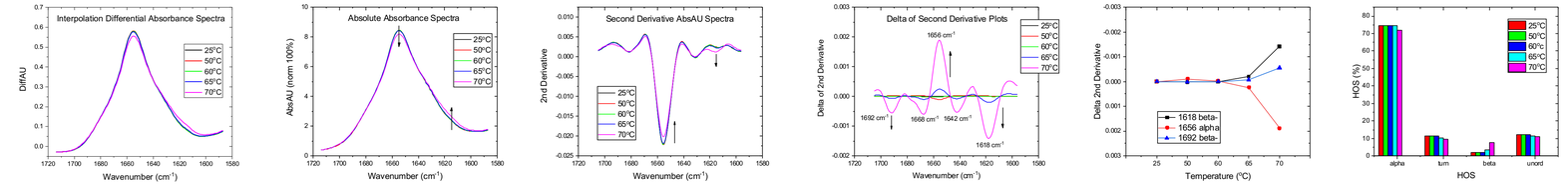
When the temperature increases, alpha helix structure (1656 cm^{-1}) decreases and beta structures (1617 and 1691cm^{-1}) emerge and increase. The secondary structure change starts at 60°C and reaches the full denaturation at 80°C.

II. **BSA at 20 mg/mL** was incubated at 25, 40, 50, 60, 65, 70, 80, and 90°C.



When the temperature increases, alpha helix structure (1656 cm^{-1}) decreases and beta structures (1618 and 1692cm^{-1}) emerge and increase. The secondary structure change starts at 65°C and reaches the full denaturation at 80°C.

III. **BSA at 100 mg/mL** was incubated at 25, 50, 60, 65, and 70°C.



When the temperature increases, alpha helix structure (1656 cm^{-1}) decreases and beta structures (1618 and 1692cm^{-1}) emerge and increase. The secondary structure change starts at 65°C.

Conclusions

- RedShift's MMS Platform was used to study the heat-induced thermal denaturation of BSA. The results show that at 1 mg/mL, BSA starts unfolding at 60°C while at 20 and 100mg/mL, BSA starts unfolding at around 65°C. Replicates measurements show very reproducible data;
- MMS analysis shows similar results with reported FTIR study of thermal denaturation of BSA in D_2O (Murayama and Tomida, Biochemistry 2004, 43, 11526-11532) with more structure change details;
- MMS provides accurate secondary structure measurements for protein samples at wide concentration range, 1 to 100 mg/mL in current study, and in other studies, we have tested samples at >200 mg/mL;
- MMS delivers comprehensive analytical information on protein secondary structure, stability, aggregation, similarity, and quantitation;
- 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 also in manufacturing.

For more information email Jeff Zonderman: jzonderman@RedshiftBio.com