Application Note
Dec 2020
AN-850-0118

Detecting Protein Conformational Change Due to Ligand Binding and Stabilization Using MMS

- Research
- Discovery
- Formulation and Development
- Quality Control
- Manufacturing

- Aggregation
- Quantitation
- Stability
- Structure
- Similarity

Introduction

Ligand binding can affect the function of proteins and often causes conformational change in the protein target. Since form fits function, determining the secondary structure of proteins with and without ligands is essential for a more complete understanding of how the ligand alters the protein's function. RedShiftBio has developed a new technology called Microfluidic Modulation Spectroscopy (MMS) that measures protein secondary structure by combining infrared spectroscopy with microfluidics to enhance sensitivity and accuracy. By using a Quantum Cascade Laser (QCL) that is 1000 times brighter than FTIR light sources, the RedShiftBio AQS^{3}pro enables the ability to probe secondary structure more sensitively and over a concentration range of 0.1 - >200 mg/mL that is wider than traditional IR techniques. Additionally, the AQS^{3}pro uses a flow cell that modulates between sample and reference buffer, enabling real-time, automated buffer subtraction that is highly accurate and compatible for use with any ligand. In this study, a protein was tested with two different ligands: one that completely stabilizes the protein against heat stress, and the other that moderately stabilizes it against heat stress.

Methods

All samples were prepared at 1 mg/mL protein concentration with a constant DMSO composition of 0.6%. Table 1 shows the three sets of samples that were prepared along with matching buffers: 1) Apo protein with 0.6% DMSO vehicle, matching the amount of DMSO in the samples with ligand; 2) Protein 1 with ligand 1; 3) Protein 1 with ligand 2.

Samples were then aliquoted into a control group that was kept at room temperature and a stressed group that was exposed to 50°C overnight. Samples that had visible particulates were filtered with a 0.2 μm filter. All samples were run in duplicate at 1 Hz modulation with 5 psi backing pressure on the AQS^{3}pro. The data was processed using the AQS^{3}delta Data Analysis package.

Results

I. Absolute Absorbance: The absolute absorbance spectra of the averaged replicates under each condition are shown in Figure 1. These spectra have been normalized for concentration and buffer contribution. It is clear in Figure 1A that heat stress impacts the absorbance spectrum of the Apo protein 1, however, the effect of heat stress on the sample with ligand 1 appears unaffected.

Sample	Ligand 1	Ligand 2
1	Control: Protein 1 Apo RT	
2	Control: Protein 1 Apo 50C	
3	Protein 1 - Ligand 1 RT	Protein 1 - Ligand 2 RT
4	Protein 1 - Ligand 1 50C	Protein 1 - Ligand 2 50C

Table 1: Sample Sets Prepared for Heat Stress Analysis

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This result indicates ligand 1 protects the protein from heat stress. Figure 1B shows protein 1 with and without ligand 2, demonstrating that the heat stress causes a significant decrease in the absorbance at the major peak of both samples with and without ligand 2. However, the decrease of the Apo protein is greater than the sample in the presence of ligand 2, indicating that the ligand somewhat stabilizes the protein against heat stress.

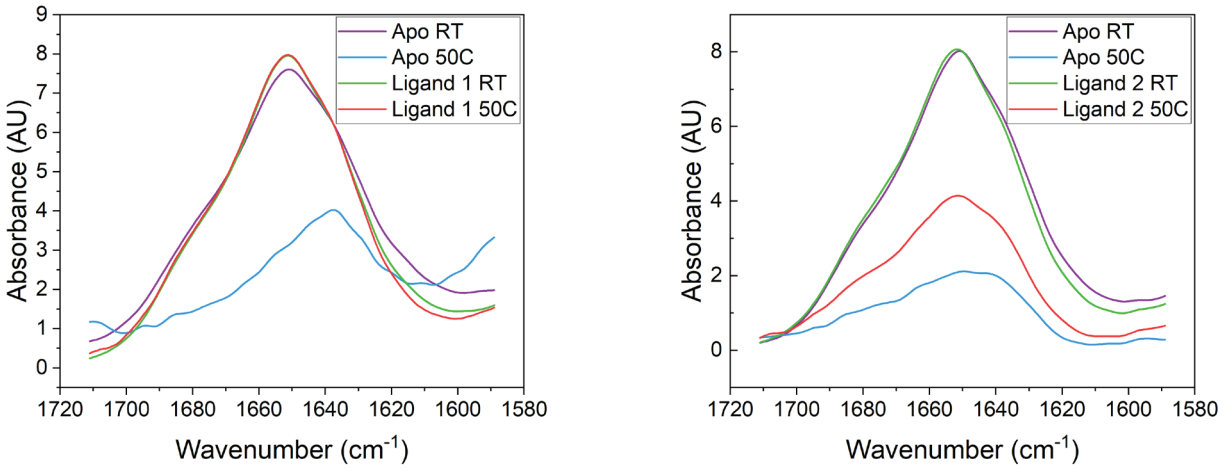

Figure 1: A) Absolute Absorbance spectra of protein 1 with and without ligand 1 at RT and 50°C (left).
B) Absolute Absorbance spectra of protein 1 with and without ligand 2 at RT and 50°C (right).

II. Second Derivative: The Second Derivative Plots are shown in Figure 2. These plots show the individual features making up the Absolute Absorbance spectrum. It is clear that the major peak at 1652 cm^{-1} is the major feature affected by heat stress when not in the presence of ligand as well as in the presence of ligand 2. This feature is much more stable when in the presence of ligand 1.

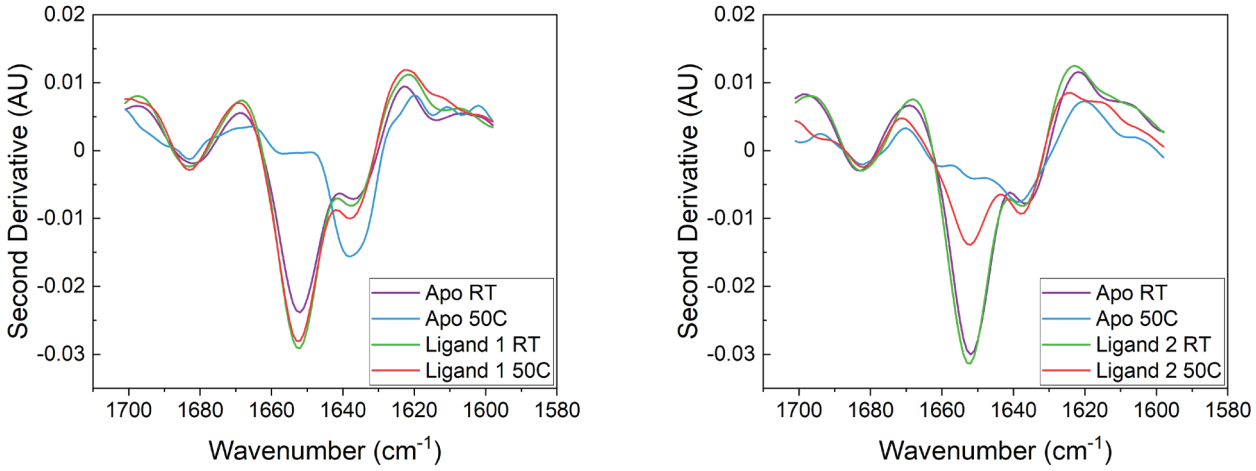

Figure 2: A) Second Derivative Plot of protein 1 with and without ligand 1 at RT and 50°C (left).
B) Second Derivative Plot of protein 1 with and without ligand 2 at RT and 50°C (right).

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III. Area of Overlap and Similarity: The Area of Overlap Plots, shown in Figure 3, have been derived from the baseline-subtracted second derivative of the Absolute Absorbance spectra. The Area of Overlap Plots show that heat stress causes an increase in the feature at 1638 cm^{-1}.

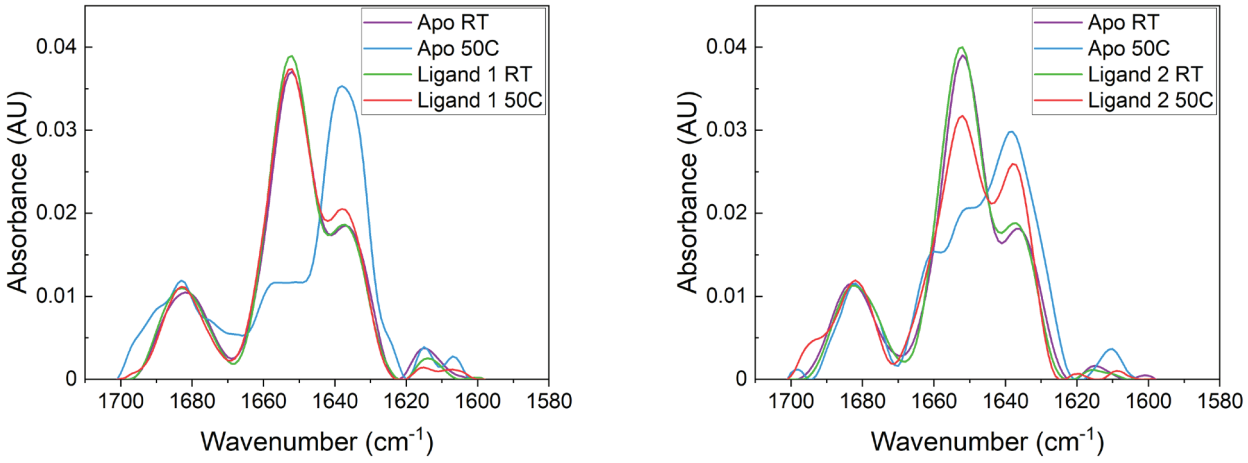

Figure 3: A) Area of Overlap Plot of protein 1 with and without ligand 1 at RT and 50°C (left).
B) Area of Overlap Plot of protein 1 with and without ligand 2 at RT and 50°C (right).

The percent similarity among samples compared to the Apo RT sample is shown in Table 2 as calculated from the Area of Overlap results.

Sample	% Similarity (Area of Overlap)
Control: Protein 1 Apo RT	100
Control: Protein 1 Apo 50C	70.6
Protein 1 - Ligand 1 RT	96.5
Protein 1 - Ligand 1 50C	95.8
Protein 1 - Ligand 2 RT	96.0
Protein 1 - Ligand 2 50C	88.8

Table 2. The percentage Similarity for all samples compared to the Apo + vehicle RT sample.

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IV. HOS: Using the Similarity Plot, the Higher Order Structure Plots (HOS) can be calculated by Gaussian Curve fitting and are shown in Figure 4. The HOS bar graph shown in Figure 4A depicts that ligand 1 stabilizes protein 1 and prevents the conversion of alpha-helix and unordered structure to beta-sheet under 50°C heat stress. Figure 4B shows that ligand 2 mitigates the aggregation process by minimizing the amount of alpha-helix and unordered structure that is converted to beta-sheet, but protein 1 is not completely resistant to heat stress and there are still some observable conformational changes.

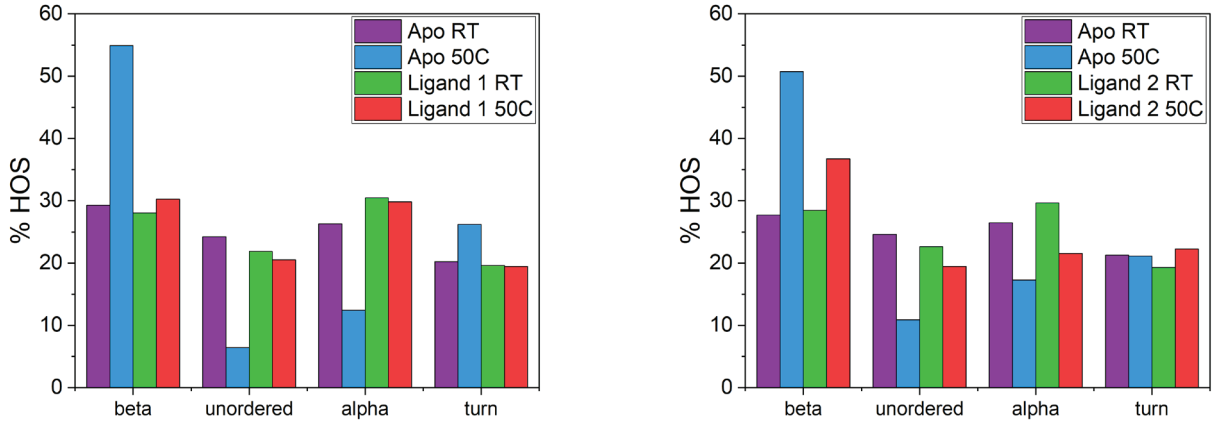

Figure 4: A) HOS bar graph of protein 1 with and without ligand 1 at RT and 50°C (left).
B) HOS bar graph of protein 1 with and without ligand 2 at RT and 50°C (right).

Conclusions

MMS was used to detect structural changes caused by heat stress on Protein 1 and the stabilizing effects of 2 different ligands. Protein 1 is completely stabilized against heat stress by ligand 1, as seen in the data presented. Ligand 2 also provides some increased stabilizing effects, however, there were some structural changes still observed due to heating when in the presence of ligand 2. Overall, MMS is a sensitive secondary structure characterization tool that can enhance the biophysical characterization toolbox by contributing secondary structural information to ligand binding applications.

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