

ANALYSIS OF BOVINE GAMMA GLOBULIN USING THE CONCENTRATIR2™

FREDERICK BOSOY

HARRICK SCIENTIFIC PRODUCTS, INC, BOX 277, PLEASANTVILLE, NY 10570

INTRODUCTION

Infrared spectroscopy has recently gained recognition as a viable technique for protein structure analysis due to its high information content for biological systems.¹ Specifically, much focus has been placed on the application of infrared spectroscopy to the study of protein unfolding. The process of protein unfolding has been examined extensively by other methods such as fluorescence, circular dichroism, or visible absorption spectroscopy. However, infrared spectroscopy is unique in that it allows analysis of protein unfolding through secondary

structure alteration on a timescale unachievable by other techniques, while retaining exceptionally high structural resolution.¹

ATR (attenuated total reflectance) has proven to be a particularly advantageous infrared technique for analysis of proteins, with its high sampling rate and convenient sample preparation.² The ConcentratIR2™ is a multiple reflection ATR accessory capable of analyzing small amounts of liquid on a timescale small enough for effective study of the protein unfolding process.

This work examines the use of the ConcentratIR2™ to analyze small amounts of protein undergoing secondary structural change in response to thermal perturbation.

EXPERIMENTAL

This work examines unfolding of BGG protein assay standards. The solution consisted of 750 ug/mL BGG in a 0.9% sodium chloride solution with sodium azide. Measurements were carried out using a commercially available FTIR spectrometer equipped with KBr beam-splitter and MCT detector.

The ConcentratIR2™ was configured with a multiple reflection silicon ATR element.



Figure 1. The Harrick ConcentratIR2™ multiple reflection ATR accessory.

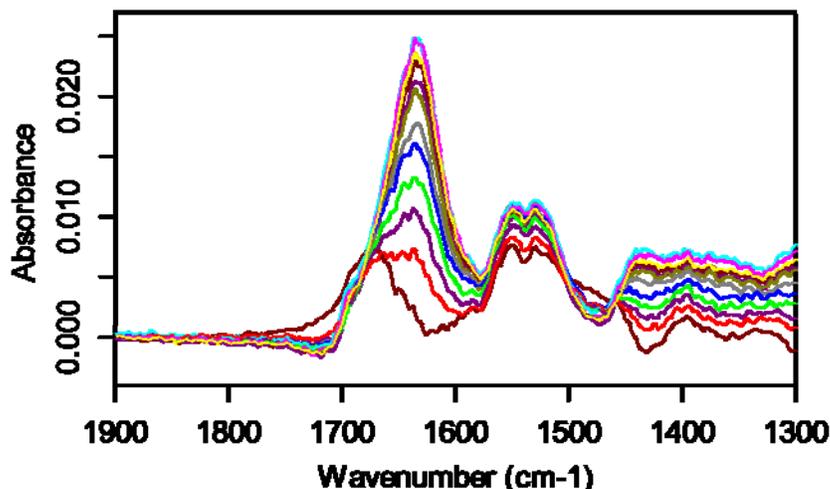


Figure 2. The ATR spectra of BGG at 100°C at 0 (lowest peak), 1, 2, 3, 4, 5, 6, 7, 8, 9, 20, and 30 (highest peak) minutes after injection of sample into cell.

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Data were collected over a wavelength range of 4000-650 cm^{-1} . Parameters for data collection were as follows: aperture was set to 100%, number of scans was 64, gain was set to 8, and resolution to 4 cm^{-1} . The ConcentratIR2™ was connected to its heated liquid cell that was modulated by a Harrick Temperature Controller. The temperature of the heated cell was held at 100 °C throughout data collection.

Protein samples were injected by syringe through the Luer fittings on the heated cell, with the temperature stable at 100 °C. Enough sample was loaded to ensure the interior of the cell was completely filled. The spectrum was collected immediately upon injection into the cell and subsequently every minute for nine minutes. Two additional spectra were run 20 and 30 minutes after injection. The fittings of the cell were capped during data collection to prevent evaporation.

RESULTS AND DISCUSSION

The collected spectra exhibit clear spectral changes in relation to one another, indicating that unfolding of the protein was occurring in response to a significant increase in environmental temperature. Changes in the characteristic amide I band around 1600-1700

cm^{-1} were of primary focus. The peaks in this region offer the most information regarding the structure of the protein backbone which is the key determinant of protein secondary structure.³ Within this region, a concomitant increase in wavelength of absorption and intensity of absorption is observed over time. The largest, most dramatic shift in wavelength, a shift from about 1670 cm^{-1} to 1620 cm^{-1} , is seen between the time of injection ($t=0$) and one minute after ($t=1$). However, the intensity of absorption changed very little during this period. This suggests that the majority of secondary structure change may have occurred in the moments immediately following exposure of the protein to heat.

As time progresses, the spectra seem to reverse this initial trend- increasing in absorption, yet remaining fairly constant in peak position. The spectra collected every minute from two minutes on show an increase in absorption but a slightly lower increase with each passing minute. Moreover, comparing these to the spectra collected outside this period - namely, $t=20$ and $t=30$ - offers insight into protein unfolding in the longer-term. These later spectra are almost identical in peak position and intensity in the amide I region, suggesting that

protein conformation is not significantly altered beyond a certain period of time. These results raise the prospect for future studies which could use the ConcentratIR2™ to generate a more detailed profile of the rate of secondary structure change associated with thermally induced protein unfolding.

Changes in base protein conformation brought on by heating result in certain known alterations in secondary structure composition that produce characteristic peaks in the infrared.⁴ The amide I band consists of overlapping component bands that reflect particular secondary structures such as alpha helices, beta sheets, turns, and random structures.⁴ Within the amide I region, globular proteins tend to produce prominent bands corresponding to alpha helices and beta sheets in particular.⁴ These bands consistently fall at wavenumbers around 1660 cm^{-1} and 1620-1640 cm^{-1} for alpha helices and beta sheets, respectively with slight variation possible depending on the specific protein in question.

Examination of the $t=0$ spectrum reveals a prominent alpha helix peak, spanning from about 1670-1650 cm^{-1} ; whereas, very little contribution from beta sheets is seen. As described earlier, the most significant



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141 Tompkins Ave., 2nd floor • PO Box 277 • Pleasantville, NY 10570

www.harricksci.com • E-mail: info@harricksci.com

Phone (international): 914-747-7202 • Phone (in USA): 800-248-3847 • Fax: 914-747-7209

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change in secondary structure appeared to occur in transition from $t=0$ to $t=1$. Upon this transition, there is an obvious disappearance of the aforementioned alpha helix peak and a concomitant appearance of a prominent beta sheet peak around $1620-1640\text{cm}^{-1}$, signifying a transition in the overall secondary structure composition to primarily beta sheets, agreeing with the findings of another infrared study that also saw a decrease in alpha-helices and increase in beta-sheets upon thermal perturbation.⁵

This work demonstrates the ease and efficacy with which the ConcentratIR2™ can visualize thermally induced secondary structure change in small amounts of a protein such as BGG. The above findings speak to the high level of structural detail that IR spectroscopy - as opposed to other methods of protein visualization - can achieve, especially in recording minute-to-minute changes in structure associated with protein unfolding.

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