Designing a Lab Around Protein Thermodynamics

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Introduction

- Goal was to design a lab protocol utilizing aspects from physical chemistry and biochemistry to help student understanding.
- Lab utilizes circular dichroism thermal denaturation to evaluate the protein unfolding process and the wavelength scan to determine secondary structure composition.
- Thermodynamic data analysis developed from physical chemistry is used to find entropy (ΔS), enthalpy (ΔH), and Gibbs free energy (ΔG).
- Students taking this lab have to compare their final entropy, enthalpy and Gibbs free energy to literature values to determine validity.

Hypothesis

- Protein thermodynamic properties can be determined while observing and evaluating the unfolding process.
- Students who take this lab that utilizes aspects from both Biochemistry and Physical Chemistry will bolster their understanding of the protein unfolding process and the thermodynamic changes associated with it.

Proteins

- Proteins used for thermodynamics were B-amylase, Chymotrypsin, Papain, BSA, RNAse A, Trypsin, and Lysozyme.
- Proteins were chosen because they all exhibit a wide variety of secondary structures.

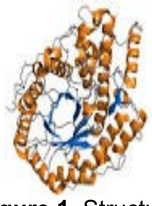


Figure 1, Structure of B-Amylase

References

(1) Immunoglobulin Light Chain Enrico Rennella, Gareth J. Morgan, Nicholas Yan, Jeffery W. Kelly, and Lewis E. Kay Journal of the American Chemical Society 2019 141 (34), 13562-13571 DOI: 10.1021/jacs.9b05499

(2) Lysozyme Thermal Denaturation and Self-Interaction: Four Integrated Thermodynamics Experiments for the Physical Chemistry Laboratory
Christopher A. Clark, Jeffrey J. Schwinefus, Nathaniel J. Schaefle, Gregory W. Muth, and Gary L. Miessler Journal of Chemical Education 2008 85 (1), 117 DOI: 10.1021/ed085p117

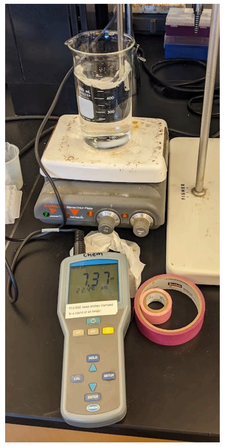
(3) Vibrational Circular Dichroism Spectra of Lysozyme Solutions: Solvent Effects on Thermal Denaturation Processes Alessandra Giugliarelli, Paola Sassi, Marco Paolantoni, Assunta Morresi, Rina Dukor, and Laurence Nafie The Journal of Physical Chemistry B 2013 117 (9), 2645-2652 DOI: 10.1021/jp311268x

(4) Quercetin Influence on Thermal Denaturation of Bovine Serum Albumin
Aurica Precupas, Romica Sandu, and Vlad T. Popa
The Journal of Physical Chemistry B 2016 120 (35), 9362-9375
DOI: 10.1021/acs.jpcb.6b06214

(5) Reeb, J.; Rost, B. Secondary Structure Prediction. In Encyclopedia of Bioinformatics and Computational Biology; Ranganathan, S., Gribskov, M., Nakai, K., Schönbach, C., Eds.; Academic Press: Oxford, 2019; pp 488–496. https://doi.org/10.1016/B978-0-12-809633-8.20267-7.

Protocol Design

Preparation of Phosphate Buffer:



- 10 mM Potassium Phosphate buffer at pH 7.5 was created to ensure proper protein suspension for both wavelength and thermal denaturation scans.
- This buffer restricts the protein from unfolding naturally. This is to ensure unfolding occurs primarily due to heating up.
- **Figure 2** (right) shows creation of the potassium phosphate buffer.

Monitoring Unfolding Using CD Spectrometer:

- Wavelength scan was used to determine the secondary structure of proteins.
- **Figure 3** (right) shows the circular dichroism instrument available at WSU.
- Wavelength scan used phosphate buffer as a baseline while running through the wavelength range of 220 nm – 350 nm.
- Thermal Denaturation (TD) scan ran each protein from 20-90 ℃ with a ramp rate of 1 ℃/min running at wavelengths 210 nm and 220 nm for each data point.
- Converted data to molar ellipticity to start determination of thermodynamic properties.

Lab Effectiveness

- The first draft of this lab protocol was offered to both a physical and biochemistry lab group.
- Both lab sections had the students perform the assigned lab protocol and design a lab report based on the thermodynamic data analysis results.

Physical Chemistry Group

- The physical chemistry students were given a pre-post quiz to determine if the lab is a proper learning tool.
- The results of the pre-post quiz showed an average of 31.1 % improvement for each student when retaking the quiz.

Biochemistry Group

- The biochemistry students were given a post lab quiz 3 weeks after the lab report was done to observe student learning. The average student score on this quiz was 67.67%.
- These students also took a satisfaction survey to determine if the lab was well received. Of the 45 responses, 73.3% were positive.

Results and Discussion

- From the Molar ellipticity data found on the TD scan, students developed a series of data plots in order to find melting temperature, enthalpy, entropy and Gibbs free energy
- Below is the process of data analysis using data found from the denaturation of Bovine Serum Albumin (BSA). Students were required to provide the data analysis for every protein evaluated.

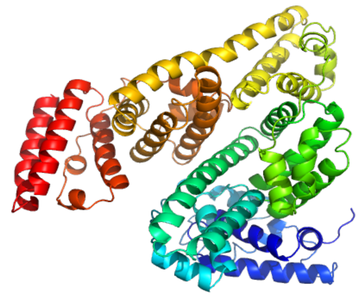


Figure 4” structure of BSA evaluated in PyMOL. Used to visualize secondary structure.

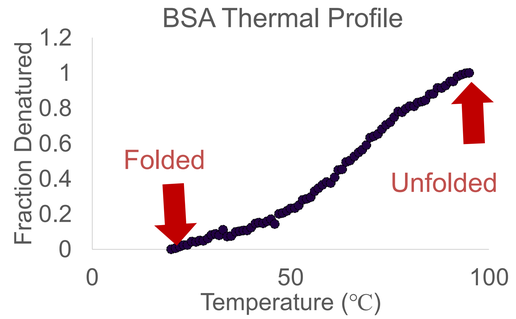


Figure 5: BSA thermal profile. Assuming start of TD scan , protein is 100% folded and end of TD scan, protein is 100% unfolded.

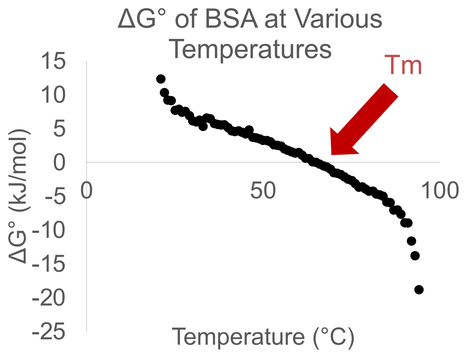


Figure 6: ΔG° Profile of BSA at various temperatures using the equation: $\Delta G = -RTlnK$. Melting temperature is the x-Intercept.

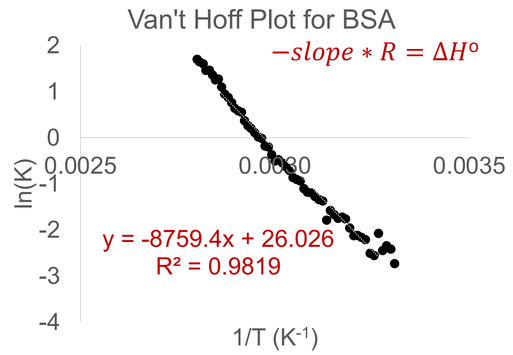


Figure 7: Van’t Hoff Plot for BSA used to determine ΔH of BSA using the equation $-slope * R = \Delta H$. ΔH is positive during the protein denaturation, an endothermic process.

Conclusion

- The thermodynamic properties of a variety of proteins can be determined using a thermo-denaturation scan on the circular dichroism instrument.
- Based on the lab effectiveness data and satisfaction survey, this general lab protocol proves to be an effective lab in both a physical and biochemistry setting.

Acknowledgments

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