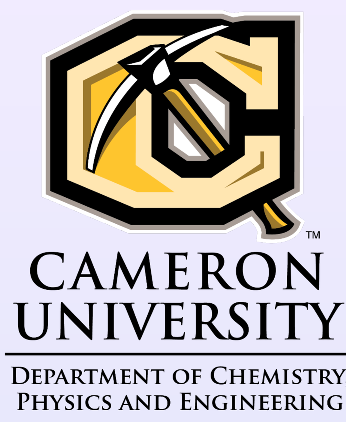

Analysis of Heme-Binding Proteins from *Listeria monocytogenes* Using Differential Scanning Calorimetry

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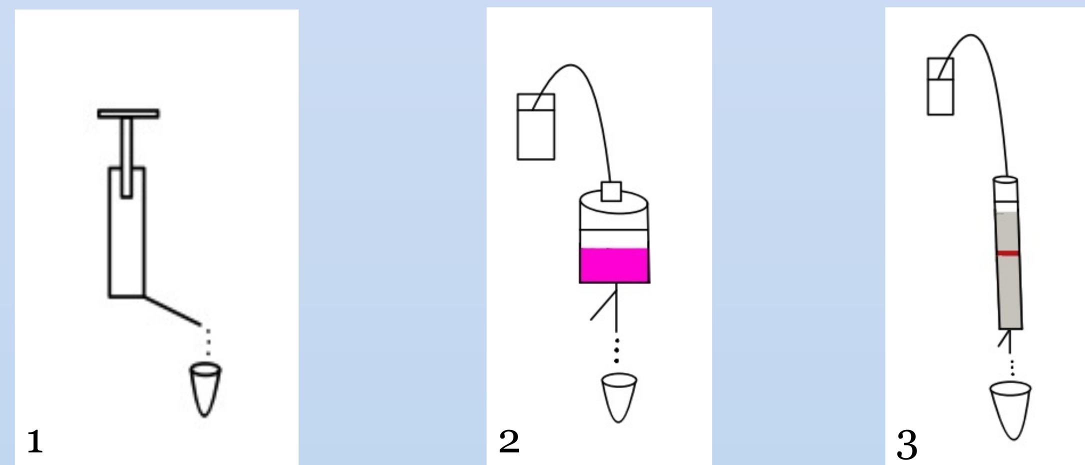
Objectives

- Determine the melting points, T_m, for Hbp1 and Hbp2 in the presence and absence of heme
- Examine the effects of small molecules on the melting points of the two proteins

Introduction

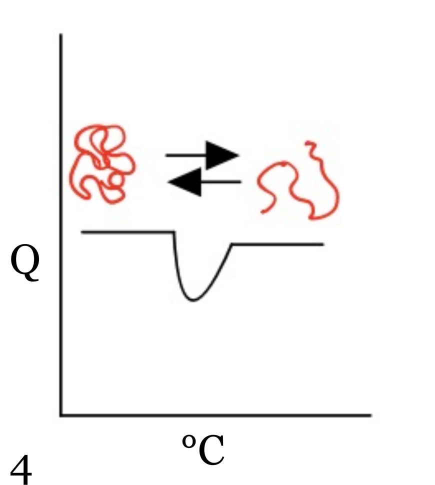
- *Listeria monocytogenes* is a gram-positive bacterial pathogen that spreads intracellularly by modified actin filaments from the cytoskeleton to propel itself into neighboring cells
- Heme binding proteins 1 and 2, or Hbp1 and Hbp2, are two proposed proteins that function as hemophores – iron scavengers
- Hbp1 and Hbp2 allow listeria to grow even in an iron poor environment
- Differential Scanning Calorimetry – fluctuations in heat flow to track endothermic and exothermic events

Methods



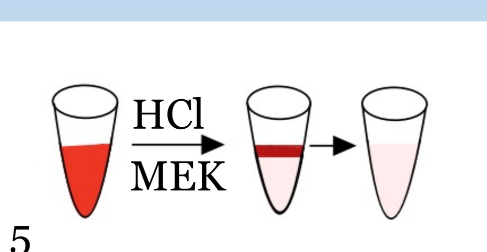
Preparation of Lysate and Protein Purification

- Fig.1 – A French press is used to lyse cells open to collect the overexpressed protein
- Fig.2 – An Affinity Chromatography is performed to extract the protein out of the lysate as it binds to the Talon resin (pink)
- Fig.3 – A size exclusion chromatography allows for an elution containing only the protein of interest



Differential Scanning Calorimetry

- Fig.4 – The purified protein sample is compared to a 1X TBS Buffer producing a graph similar to this schematic
- Where the endothermic event produced where protein folding, and unfolding are in equilibrium



Methyl-Ethyl-Ketone Extraction of Heme

- Fig.5 – Heme can be extracted into an organic layer by adding HCl until pH 2
- Addition of MEK separates the protein without heme into the aqueous layer

Results

- Protein concentration must be above 3 mg/mL to produce consistent DSC results
- MEK Extraction yields concentrations too low to be detected by the DSC

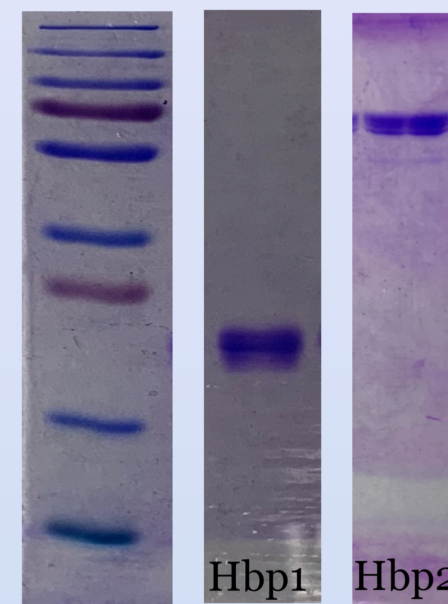


Fig.6 SDS PAGE results for Hbp1 and Hbp2 samples. Hbp1 is roughly 19 kDa and Hbp2 is around 60 kDa. These are separate gels where similar fractions were concentrated prior to placing on the DSC Instrument.

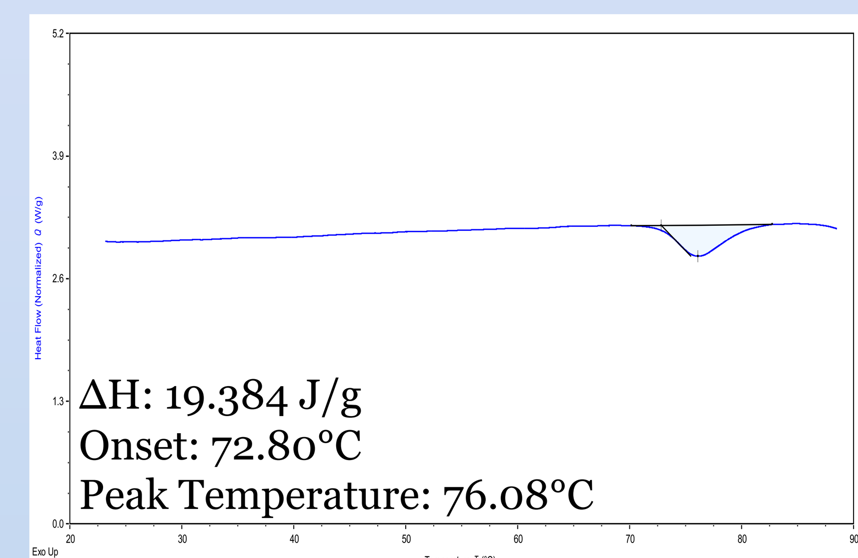


Fig.7 Conventional DSC run for Myoglobin control. The melting temperature, or denaturation event, is consistent with previously published literature.

Table 1. Thermodynamic values containing both control and experimental samples. Enthalpy was calculated by averaging the data from several DSC runs and converted to kJ/mol. Entropy was calculated by using the standard state free energy of reaction equation (ΔG=ΔH-TΔS), where T was measured by the DSC. BSA, Lysozyme, and Myoglobin data are consistent with previous literature.

Thermodynamic Values For Protein Denaturation

Protein	ΔH (kJ/mol)	ΔS (kJ/mol)	Melting Temperature °C (T_m)
BSA	875	2.49	78.00
Lysozyme	468	1.35	72.63
Myoglobin	333	4.38	76.08
Hbp1	96± 20	1.60	59.63
Hbp2	458 ± 30	6.98	65.60

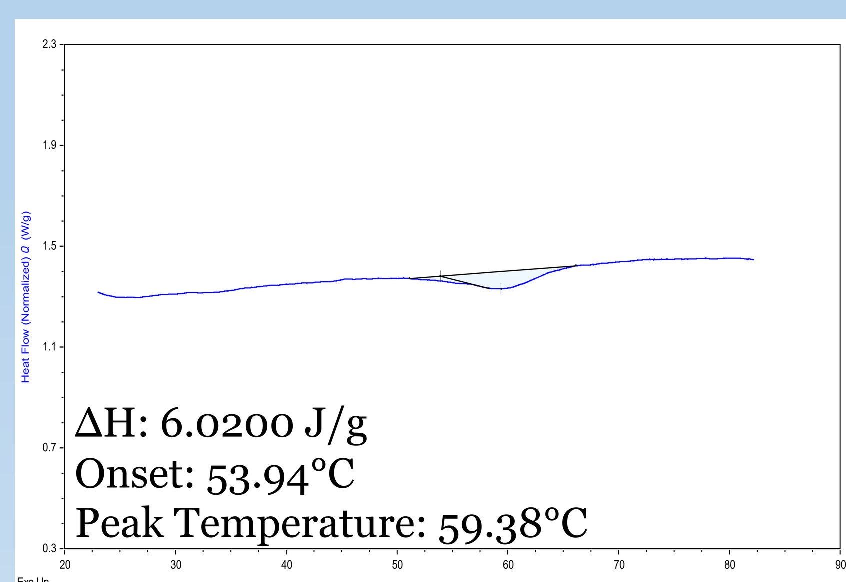


Fig.8 Conventional DSC run for Hbp1 showing an endothermic event occurring at a temperature 59.38°C.

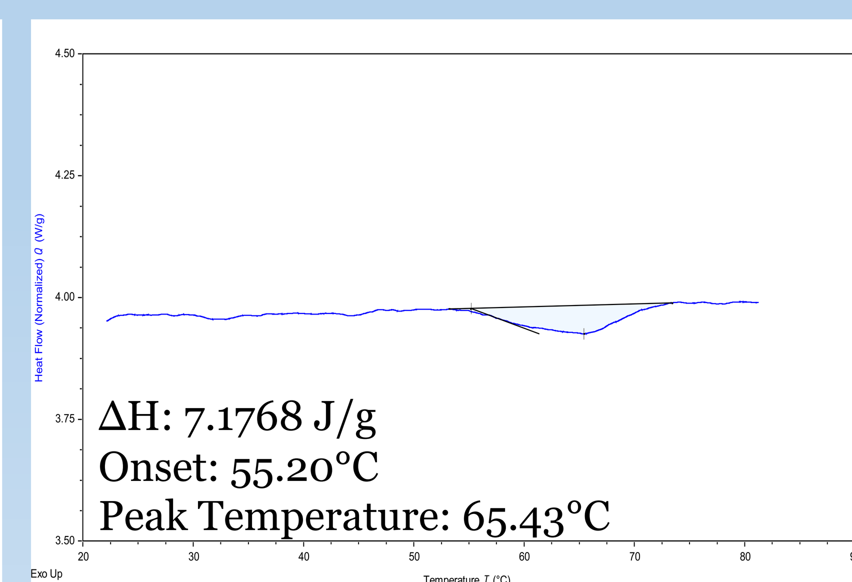


Fig.9 Conventional DSC run for Hbp2 showing an endothermic event occurring at a temperature 65.43°C..

Future Directions

- The use of non-ionizing surfactants to safely remove heme without damaging the protein
- Tween-20 functions to expose hydrophobic areas of the protein where we can remove functional groups by Gel Filtration or Ionic Exchange Chromatography
- The use of small spin chromatography columns and detergents will be used on myoglobin first to test if the method is working
- We expect the heme and protein to elute in two separate collections, which can both be dialyzed and concentrated

Conclusion

- DSC is a qualitative tool to determine a protein's denaturation point
- Nonionic surfactants, like Tween-20, will be used in the future to remove bound heme from the protein complex
- MEK Extraction functionally destroyed proteins and we were unable to analyze samples on the DSC following an extraction

Societal Impacts

- These findings can be applied to pharmaceutical studies to understand the role of heme binding proteins Hbp1 and Hbp2 as virulence factors for disease
- The presence of small molecules could potentially disrupt protein denaturation and can be potential therapeutic targets
- This ongoing project will help showcase the role of ligands in improving entropy, stability, and overall kinetics of the protein

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