A Preliminary Characterization of Hia, an Auto-secreting Protein of *Brucella abortus*

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EPSCoR and Senior Honors Project

Why investigate Hia?

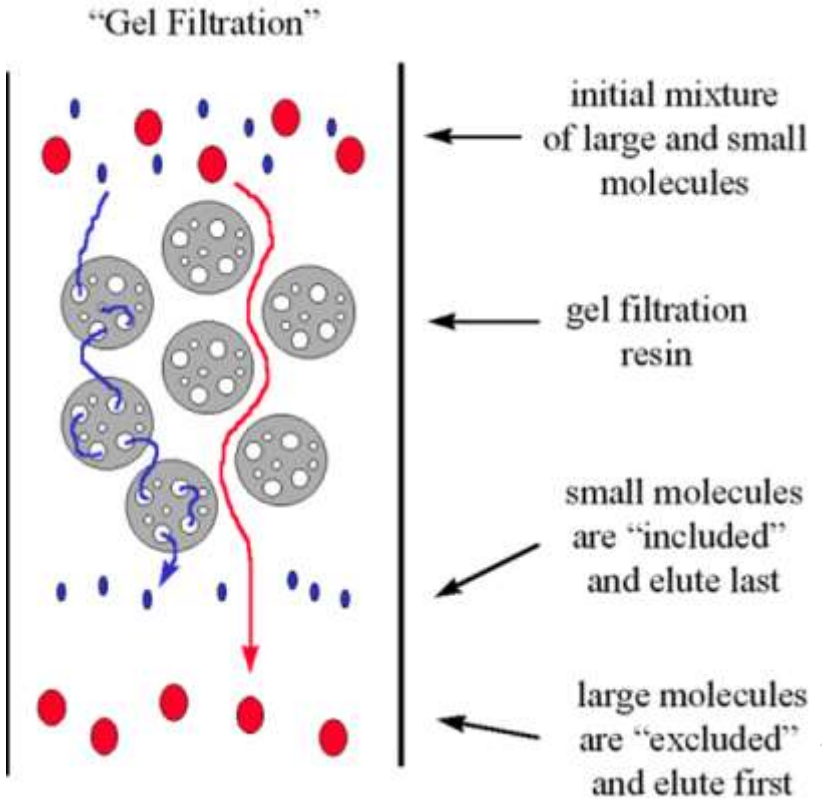
- *Brucella abortus*, causative agent of brucellosis
- Type V, auto-secreting protein
- Is modified in the attenuated strain S19
- Can trimerize *in vitro*

Preparing Hia

- Recombinant *E. coli* strain with pET vector containing coding sequence for Hia
- BugBuster Protein Extraction Kit (Novagen)
- HisPur Cobalt Spin Columns (Thermo Scientific)
  - Utilized Histidine tag fused to N-terminal of Hia

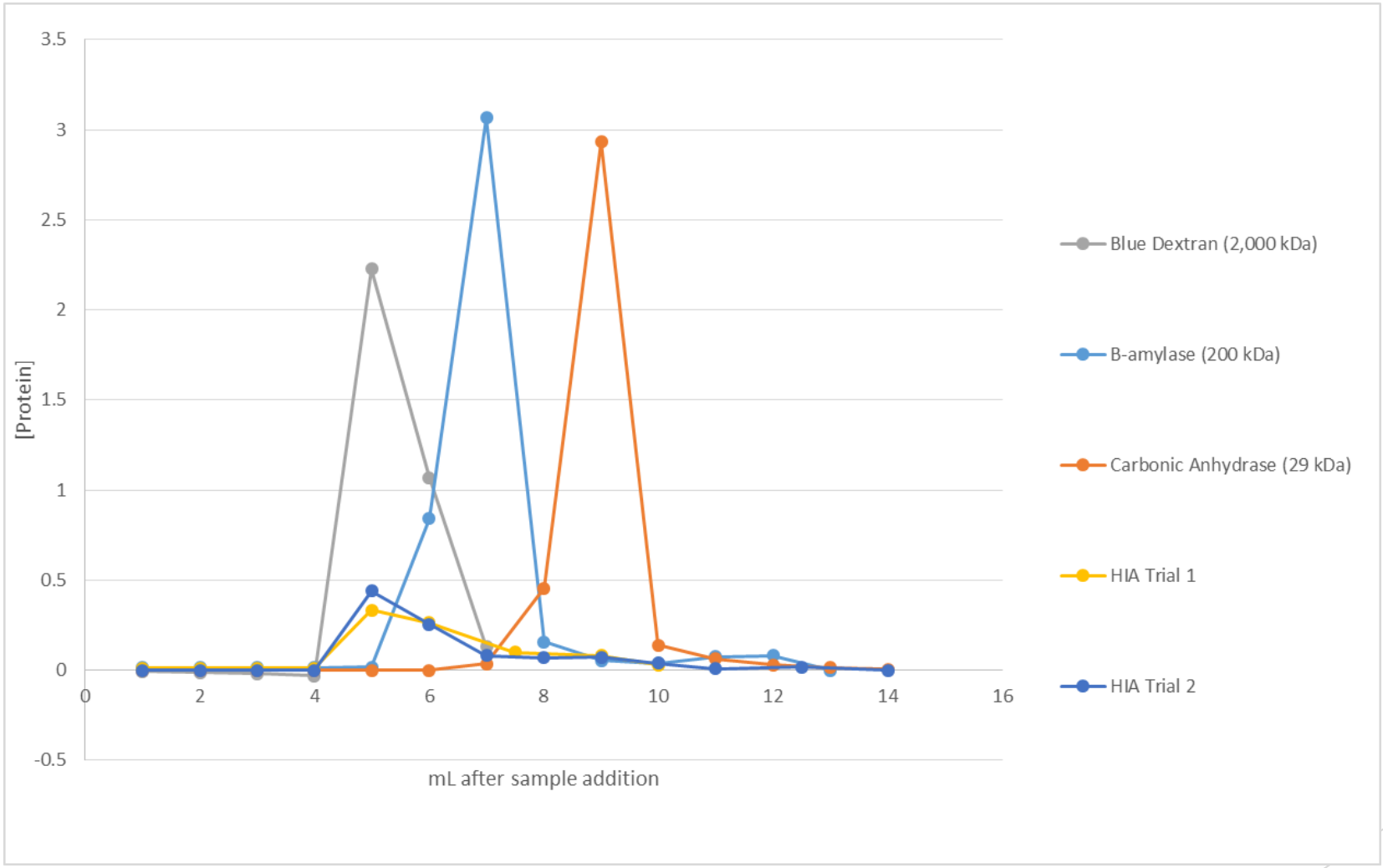
Gel Filtration Column

- Superdex 200 resin (Sigma) degassed under 1X PBS + 6 M Urea
- 15.5 cm tall
- 12.17 cm^3 volume
- Blue Dextran, Carbonic Anhydrase, and ß-amylase (Sigma-Aldrich) as molecular weight standards



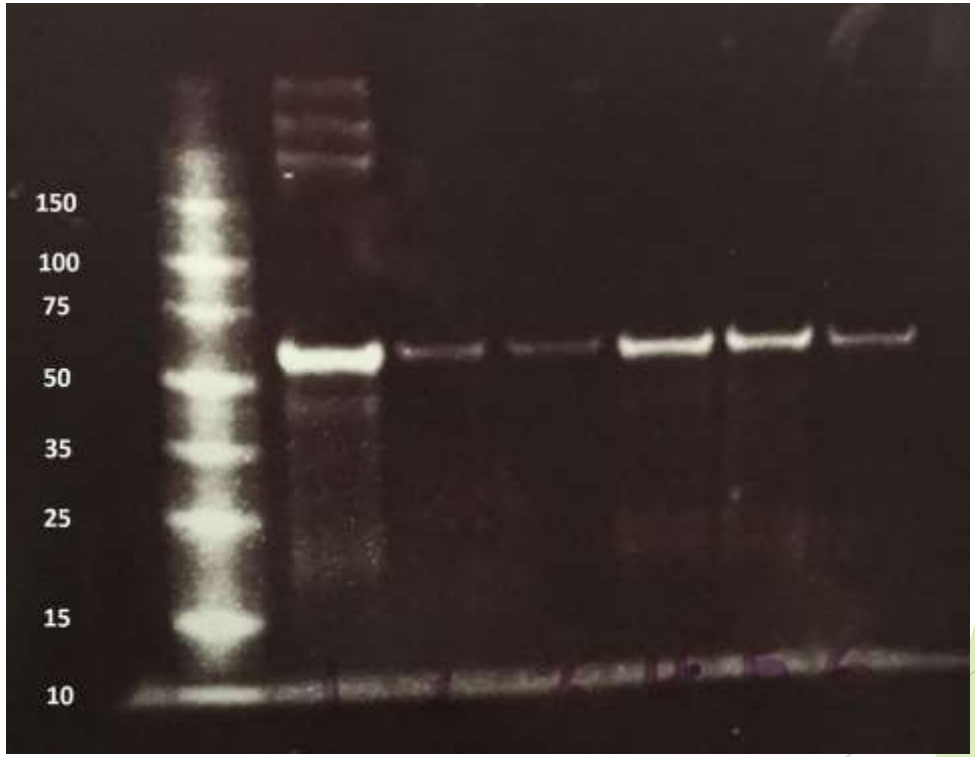
http://www.mikeblaber.org/oldwine/bch5425/lect31/gelfiltr.gif

Gel Filtration Column

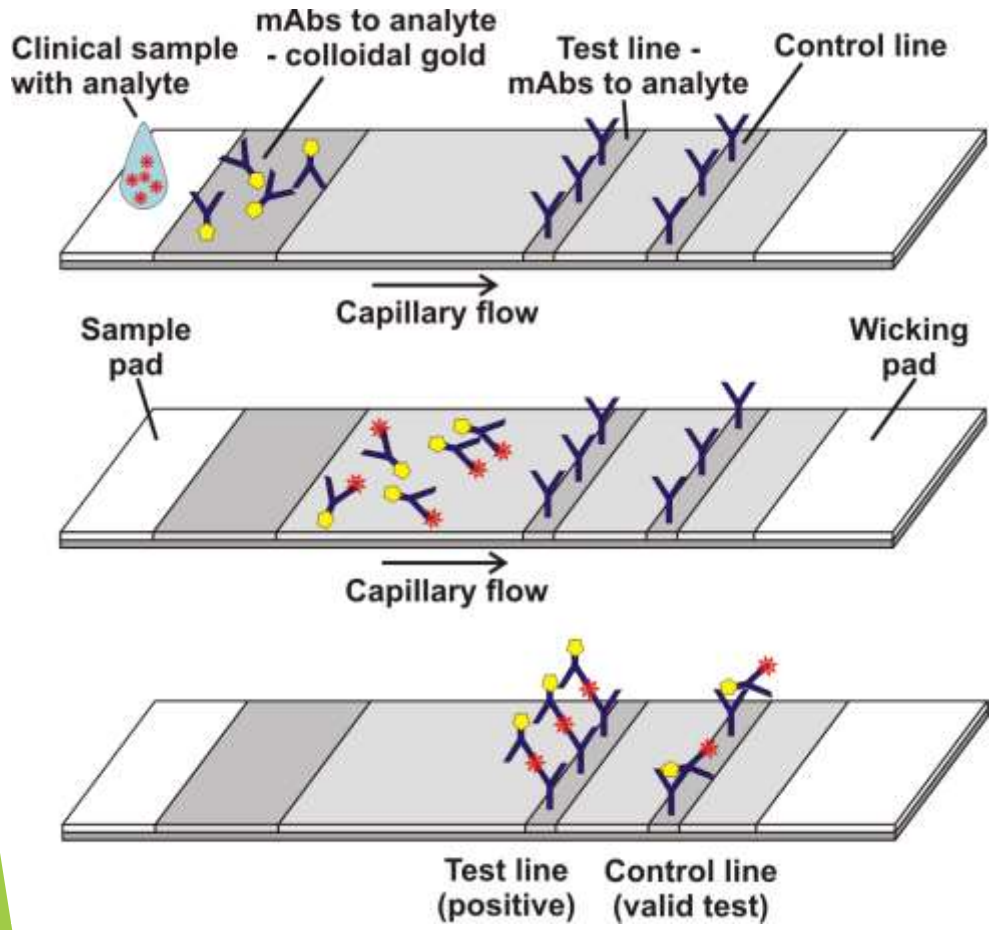


SDS-PAGE, and Silver Stain Gels

- Hia monomers are approximately 60 kDa in size
- Investigated effects of pH change on protein conformation.
- Fewer aggregates in samples which had been eluted from the column than those which had flowed straight through
- Does the Histidine tag (which is used to elute Hia) interfere with the protein binding properties?



Lateral Flow Device



http://medicine.nevada.edu/ddl/technology/lateral-flow-immunoassay

- Samples were prepared with dialysis and sent to Arista Biologicals
- Tests revealed poor sensitivity and specificity to confirmed cattle serum samples
- Aggregated samples showed reduced sensitivity to antibody capture
- Aggregates also are resistant to detergents, pH changes, and various temperature conditions

Future Work

- Investigate affect of Histidine tag on binding properties of Hia.
- Testing Hia at 37^{0} C could illuminate properties it displays during an infection
- Laboratory modification of Hia to improve sensitivity in the Lateral Flow platform
- Additional Type V proteins as diagnostic targets in the LF platform

Conclusions

- The physical properties of Hia *in vitro* are consistent with the previous hypotheses topology of the protein when secreted to the cell surface
- Aggregates of Hia are high MW complexes stable under variety of pH ranges, heat.
- Seroreactivity of Hia appears to decline with the increasing aggregation state.

Works Cited

- 1.) Andrews, G.P., Leonhardt, J.A., Dougherty, A.M., Lowry, J.E., and Bowen, R., "*Brucella abortus* recombinant outer membrane proteins induce clearance immunity against virulent challenge in BALB/c mice." 2012. The 93rd Annual Meeting of the Conference of Research Workers in Animal Diseases (CRWAD), Chicago, IL. Poster Session.
- 2.) Cotter, S.E., Surana, N.K., and St. Geme, J.W., "Trimeric autotransporters: a distinct subfamily of autotransporter proteins." *Trends in Microbiology*. 13.5 (2005): 199-205. Web. 10 Dec. 2013.
- 3.) Crasta, O.R., Folkerts, O., Fei, Z., Mane, S.P., Evans, C., Martino-Catt, S., Bricker, B., Yu, G., Du, L., Sobral, B.W., "Genome Sequence of *Brucella abortus* Vaccine Strain S19 Compared to Virulent Strains Yields Candidate Virulence Genes." 2008. PLoS ONE 3(5): e2193.
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