The First Ever Refolding of Bovine Thrombin!

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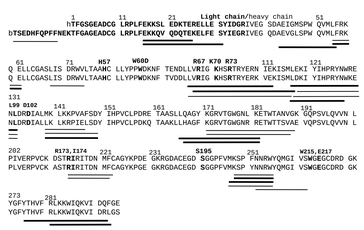
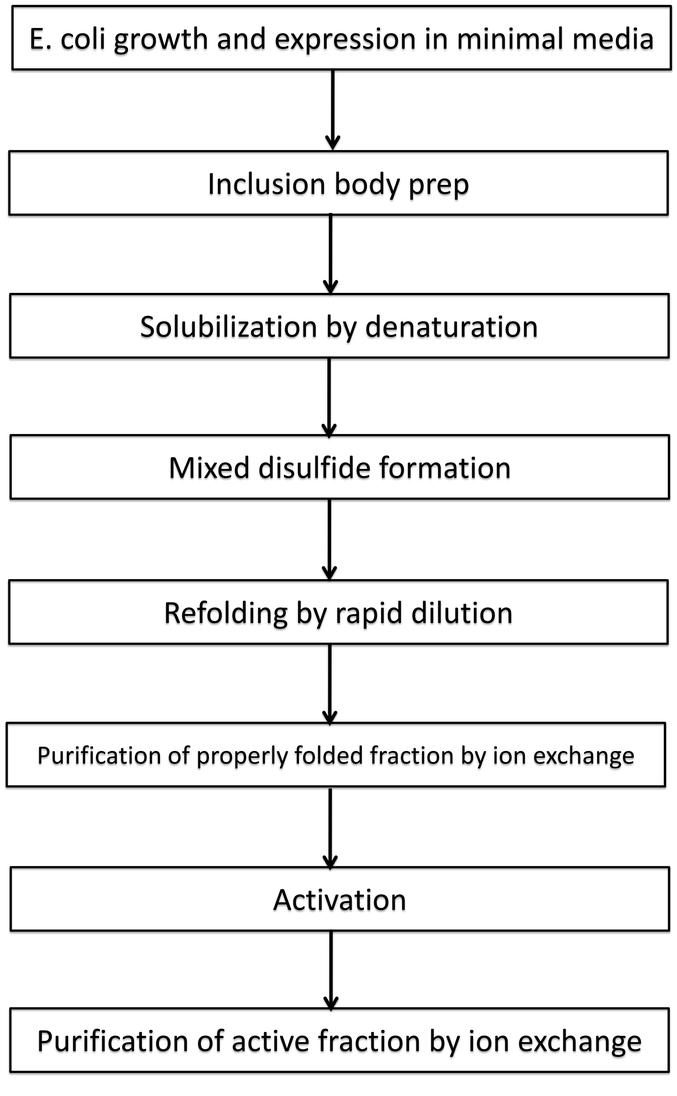
Academic Connections: Research Scholar 2010 Komives Labs

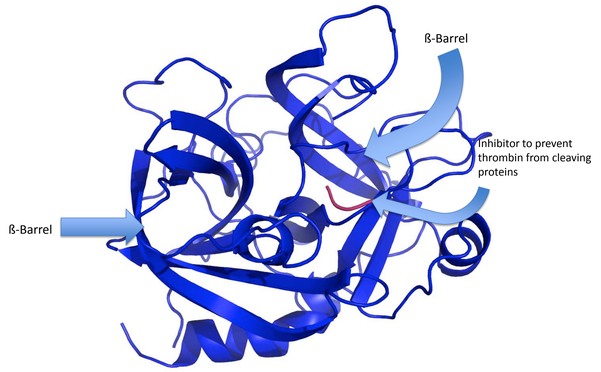
Thrombin is one of the many proteins found in blood that contribute to the formation of fibrin clots. It is the last protein in the blood clotting chain, and it operates by cleaving fibrinogen to create fibrin which activates platelets, which then allow the fibrin to make a plug that prevents and blocks blood loss. By itself, thrombin is a procoagulent, but upon binding with thrombomodulin, it becomes an anicoagulent, cleaving protein C instead. As blood conditions are some of the most commonly encountered medical problems, the study of thrombin, including that of its structure, function, dynamics, and other properties, promises much for the biochemistry and health communities.



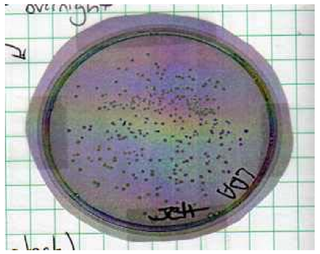
The Process of Thrombin Preparation



Thrombin contains a double ß-barrel structure



There are four tiers of protein structure; the primary, secondary, tertiary, and quaternary. The primary is what is known as an amino acid chain. The second is the formation of α-helices and ß-sheets from those peptide chains. The tertiary structure is characterized by the three-dimensional shape formed when the helices and sheets fold upon each other. The quaternary structure is characterized by more than one amino acid chain folding together, which makes a more complex structure. Thrombin has ß-sheets that fold to make double ß-barrels.



After the transformation of the cells using the BL broth, we spread the cells onto a plate with minimal media to ensure that they kept the human plasmid and to allow the cells to grow further. To the left is a picture of different colonies grown using the minimal media solution containing ampicilin.

Cysteines form disulfide bonds

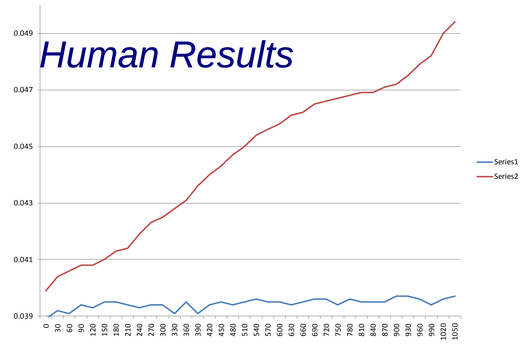
In thrombin, there are eight cysteines, which are amino acids unique for their sulfur atom. Each cysteine has the ability to make disulfide bonds with another cysteine, which greatly affects the final, quaternary structure of a thrombin molecule. When prepping the protein, this presents a problem because each cysteine has an equal affinity for another cysteine, which can lead to the creation of misfolded and, subsequently, useless thrombin. To prevent this problem, we used a molecule known as DTT to prevent the bonding, and although DTT was used a vast majority (as much as 85%) of the protein still had misfolding due to incorrect disulfide bonds.To allow the bonding to occur, we used a molecule known as glutathione, creating a facilitated disulfide bond

Venom activates thrombin

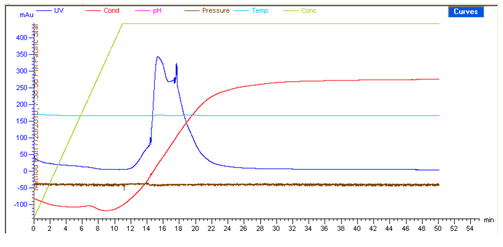
In both human and bovine preparation, we used a modified venom from the echis carinatus snake to activate thrombin. The venom from the snake cleaved a portion of the protein, thereby activating it. The venom cuts between the light and heavy chains by recognizing the DGRIVE sequence and cutting between the R and the I.

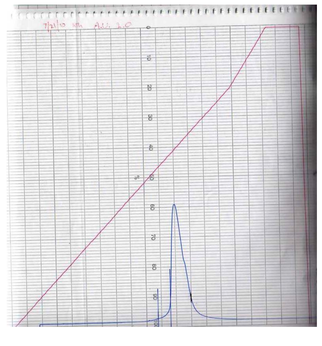
Activity Assay for Thrombin

We tested for thrombin activity using a chromagenic substrate. The red line was obtained with .5 nM thrombin and the blue line is the control with no thrombin added. The graph proves that the thrombin we produced was active.

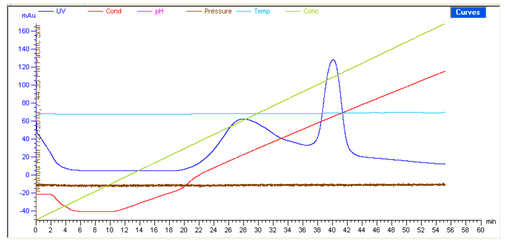


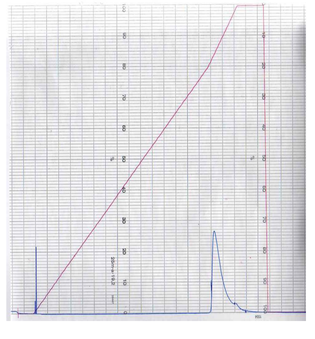
Human Results

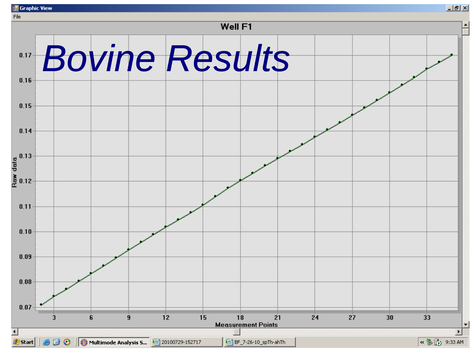




Bovine Results







To the left are the results from HiLoadS (top) and MonoS (bottom) column runs. In the HiLoadS run, the properly-folded thrombin was separated from the misfolded thrombin. In the MonoS run, the activated thrombin was separated from the inactivated thrombin. It is important to note some differences between the human and bovine thrombin found in both runs. In the HiLoadS column run, we saw sharper peaks with the human thrombin than the bovine thrombin. This might have been due to the quicker increase in concentration gradient in the human bovine experiment. Dually noted is the fact that the bovine thrombin peaked later in the MonoS column that the human thrombin. This means that the bovine thrombin is more positively charged than human thrombin.