Purifying and Analyzing Spider Silk Proteins for Commercial Use
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Introduction
Spider silk proteins’ gene sequences have been obtained from Nephila clavipes. The proteins from those genes have been produced in E. coli, alfalfa, and in the milk of goats. Before the proteins can be used, they must go through a series of purification steps. The AKTA Pilot instrument purifies the E. coli based protein by using an affinity resin. It is important in determining protein purity to quantify the amino acids in samples. Purification and identification of the spider silk proteins provide the basic steps in commercial usage.

Amino Acid Analysis
• Importance: To analyze amino acids using AccQ-Tag derivatization method and to quantify amino acids via UV light.
• Method:
  • Samples are washed with acetone and concentrated in a spin dryer and hydrolyzed
  • Dried samples are reconstituted in borate buffer and amino acids are labeled with fluorescent AccQ-Tag
  • Samples are run through a UPLC machine and compared with standard curve to determine concentration
  • Quantified amino acids are presented in trace graphs for analysis

AKTA purification
• Importance: To purify and concentrate spider silk protein produced by bacterial fermentation
• Method:
  • Bacterial pellet is sonicated in lysis buffer to rupture cells
  • Centrifuged to separate protein from cell waste
  • pH and conductivity of supernatent is adjusted to ensure His-tag exposure
  • Supernatent is passed through nickel column (cross-linked agarose resin)
  • Protein bound to column is eluted with high imidazole concentration
  • Strip buffer is ran through the column to strip off the nickel followed by NaOH for proper cleaning

Future Studies
Finding the right conductivity and pH for the AKTA to get the best protein yield.