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Research exercise: The Isolation and Purification of a Structural Polypeptide from the Ascidian Tunicate Styela plicata

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The Isolation and Purification of a Structural Polypeptide from the Ascidian Tunicate *Styela plicata*

Name: Yun Liu
Advisor: Dr. Douglas C. Hansen

**Methods & Technique**
- PAGE (Polyacrylamide Acid Gel Electrophoresis) Urea Gel: is a technique that separates proteins on the basis of charge and molecular weight. *S. plicata* blood cell extract followed by parallel staining with Coomassie blue for polypeptide.
- Nitroblue Tetrazolium (NBT) Gel: *S. plicata* cells followed by parallel staining with nitroblue tetrazolium for redox active amino acids (i.e., L-DOPA containing peptides).
- SDS Gel: In this system, proteins are denatured by heating them in buffer containing sodium dodecyl sulfate (SDS) and a thiol reducing agent such as 2-mercaptoethanol (BME). The resultant polypeptides take on a uniform charge-to-mass ratio and rod-like dimensions imparted by the SDS, proportional to their molecular weights.
- High Performance Liquid Chromatography (HPLC): is a chromatographic technique used to separate a mixture of components in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of mixture.

**Problem**
- Candidate biomolecule: *Styela plicata* 
  - *Styela plicata* tunicate peptide contains DOPA, Glycine, Proline and decarboxy-dehydro-DOPA.
  - *Sp-1*, a tunichrome from *S. plicata*, has metal binding capability which could serve as a potential corrosion inhibitor.

**Objective**
- Find aqueously soluble candidate corrosion inhibitor that is environmentally friendly
- Act as an adhesion promoter for any primer-coating system applied after the surface preparation process.

**Isolation & Purification**
1. Centrifuge collected blood cells at 800g for 20 minutes (*S. plicata*)
2. Homogenize resulting pellet in 5% acetic acid/8M Urea on ice
3. Centrifuge homogenate at 15,000g for 5 minutes @ 10 ºC
4. Immediately visualize supernatant on 5% acid-urea polyacrylamide gels
5. Purify tunichromes using C-8 reverse phase column eluted with acetonitrile/0.1% (v/v)-water gradient
6. Collect 1 ml fractions
7. Visualize fractions on 5% acetic acid-urea polyacrylamide gels
8. Lyophilize pooled fractions
9. Store lyophilized protein at -80ºC

**Conclusion**
- Partial HPLC purification of *Sp-1* has been accomplished

**Future Research**
- Find *Sp-1* molecular weight from SDS gel analysis and use selective molecular weight cut off (MWCO) membrane filtration to further separate them from remaining HPLC fractions and concentrate them
- Subject concentrated *Sp-1* fraction to further HPLC separation using modified gradient.

**References**
2. Douglas C. Hansen, Multi-Functional Biologically In spired Corrosion Inhibitor, 4 Jan. 2011, R-18815