



Department of biochemistry



Protein purification

Introduction

- Protein purification is multistep process project to
- separate a single type of protein from mixture .
- It involved the separation of protein from non protein part and then from undesired protein .
- It is difficult and laborious to purify single type of protein from the mixture of protein .
- It is very important to purify the protein to understand the structure and function of protein .
- The use of highly selective procedure to allow the detection and isolation of only those micro organisms . Which are of interest from among a large microbial population .

Basis of separation

- Protein are present in very small amount in the curd samples .it may represent only 1 percent or less of starting material .
- Separation of protein is mainly basis on
- Size
- Shape
- Charge
- Hydrophobicity
- Solubility
- Density
- Biological activity

Strategy for protein purification

- Protein purification can be performed in three phase purification strategy .
- Capture
- Intermediated purification phase
- Polishing

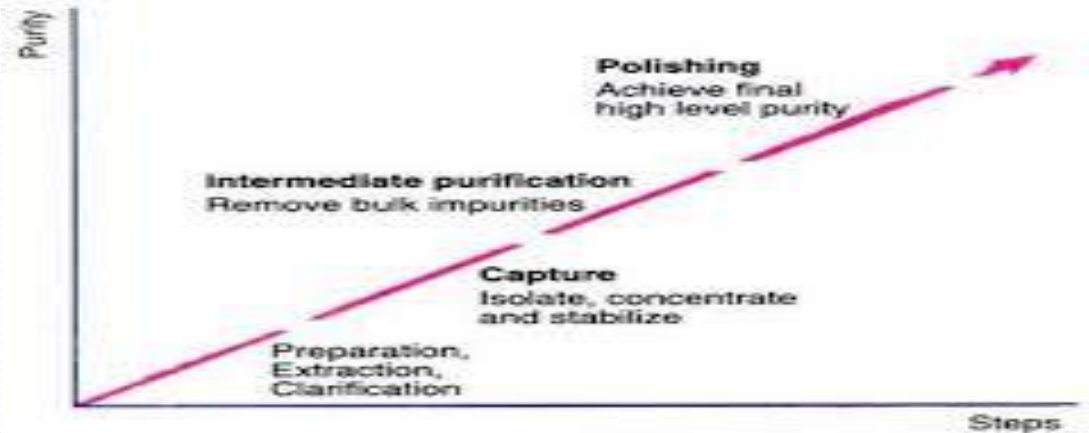


Fig. 14.1: Preparation and three-phase purification strategy

Preparative and analytical method

- Analytical method is used to detect identify a protein in a mixture
- Preparative method involved the production of large quantities of protein from other purposes.

Various step used for protein purification

- Defined objective –purity quality and quality of final product
- Identification of source of protein
- Developed enzyme assay from the protein
- Purification
 - centrifugation
 - bulk separation
 - chromatograph
 - electrophoresis
- Characterization

Important point of protein purification

- Carry out enzyme assay every step
- It is possible that contaminants at early stages can inhibit activity .
- Run an SDS gel to visualize specific contaminants .
- Minimize the sample binding at every step .
- Minimize use of additive it may interfere with other step or assay .
- To stabilize protein during extra action .

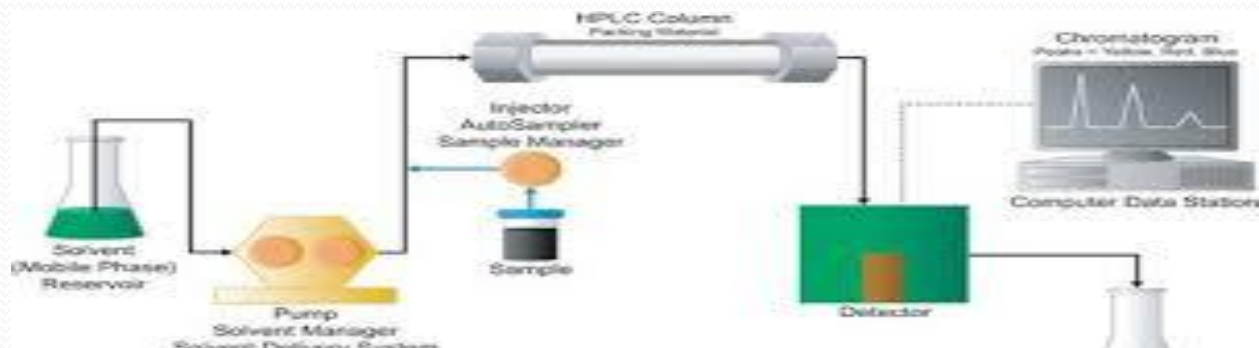
Purification table

- It's a kind of quantitative evaluation of the protein purification scheme
- Objective of the purification table is to monitor and development of the purification
- Determine protein concentration and enzyme activity to measure fold and relative purity of the enzyme
- Specific activity enzyme concentration and protein concentration
- Specific activity should increase as the purification progresses .



System of protein purification

- These are the automated system consist of assembly for loading of collection and detection.
- Such system are specifically designed to facility easy isolation of the target portion under simple complex condition.
- Such system designed from the ease fuse with software .
- Advance protein purification system design are modular which allow deferent pumps and values and other hardware to be integrated as need.





THANK

YOU

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