Principles and Practice of Protein Purification

Lecture 1

Introduction of Protein Purification

San-Yuan Huang
Lab. Animal Proteomics
Dept. of Animal Science, NCHU

Outlines of this lecture

- Definition of protein purification, Why purify proteins?
- Protein purification in the post-genome era
- Types of protein purification
- Procedure/techniques of protein purification

(http://en.wikipedia.org/wiki/Protein_purification)
**Definition of Protein Purification:**
A series of processes intended to isolate a single type of protein from a complex mixture.

**Why Purify Proteins?**
For the characterization of the function, structure and interactions of the protein of interest.

(https://en.wikipedia.org/wiki/Protein_purification)

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**Protein Purification in the Post-genome Era**

- Several years ago everyone was talking about genomics, now the focus is on proteomics. More and more we are seeing research moving toward specific proteins. The challenge is trying to keep up with the technologies.” (Hall, 2006)

- Proteomics aims to identify the cellular functions of all proteins encoded by the genome of an organism.

(Bonetta, 2006)
Protein Purification in the Post-genome Era

- Proteomics raises new challenges in protein purification. Technologies well adapted to isolate individual proteins get a makeover to tackle large numbers of samples.

- Protein structure determination, proteome-wide functional screens, and the identification of protein interactions are just a few of the proteomics applications requiring thousands of purified proteins as a starting point.

Types of Protein Purification

Purification may be preparative or analytical.

1. Preparative purifications aim to produce a relatively large quantity of purified proteins for subsequent use.

Examples include the preparation of commercial products such as enzymes (e.g. lactase), nutritional proteins (e.g. soy protein isolate), and certain biopharmaceuticals (e.g. insulin).
2. Analytical purification produces a relatively small amount of protein for a variety of research or analytical purposes, including identification, quantification, and studies of the protein’s structure, post-translational modifications and function.

Among the first purified proteins were urease and Concanavalin A.

Analytical methods aim to detect and identify a protein in a mixture, whereas preparative methods aim to produce a large amount of the protein for other purposes, such as structural biology or industrial use.

Strategies of Protein Purification

An analytical purification generally utilizes three properties to separate proteins.

1. according to their isoelectric points by running them through a pH graded gel or an ion exchange column.
2. according to their size or molecular weight via size exclusion chromatography or by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis.
3. purified by using 2D-PAGE and are then analyzed by peptide mass fingerprinting and/or tandem mass to establish the protein identity.
Procedure/Techniques of Protein Purification

- Extraction
- Precipitation and differential solubilization
- Ultracentrifugation
- Chromatographic methods
  - Size exclusion chromatography
  - Ion exchange chromatography
  - Affinity chromatography
  - Metal binding
  - Immunoaffinity chromatography
  - High performance liquid chromatography (HPLC)

Questions and Comments?