Proteomics Course

LECTURE-11
Sample preparation for proteomics applications:
Bacterial & Plant Proteome, Quantification

Dr. Sanjeeva Srivastava
IIT Bombay

Previous lecture

• Work-flow: protein sample preparation
• Precipitation methods
• Removal of interfering substances
• Specific examples:
  • Sample preparation for serum proteome analysis
  • Sample preparation for bacterial proteome analysis
Today’s lecture

- Specific examples:
  - Sample preparation for bacterial proteome analysis
  - Sample preparation for plant proteome analysis
  - Protein quantification

II. Bacterial proteome analysis
Bacterial Protein Sample Preparation

- Precipitated pellet of bacteria
- Bacteria grown in broth
- Pelleted, washed bacterial
- Sonication of bacteria
- Addition of trizol
- Centrifuged mixture
- Mixture showing separation of proteins, DNA and RNA
- Protein pellet
- Dried protein pellet
- Reconstitution of Pellet

Bacterial sample preparation

- 2% SDS and heat treatment
- Lysozyme and acetone precipitation method
- TCA-acetone precipitation method
- Direct extraction with solubilization buffer
- Trizol method
### Bacterial protein extraction: Trizol method

- Able to recover DNA, RNA and Protein
- Trizol having Guanidinium isothiocyanate is inhibitor of RNAase and gives good quality RNA
- No nucleic acid contamination
- No need of desalting
- No lipid contamination (since chloroform dissolves lipids)
- Proteins are easy to resolubilize

### Procedure:
- Add 1 ml trizol reagent to the bacterial suspension
- Add 200 ul chloroform to the mixture
- Vortex vigorously & incubate for 15 min at RT
- Centrifugation at 12000 g for 15 min
**Bacterial protein extraction (contd..)**

- Carefully remove upper layer containing RNA using a micropipette
- To bottom layer, add 300 ul ethanol
- Centrifuge at 5000 g for 5 min to remove DNA
- Remove supernatant containing protein collect into a fresh tube
- In supernatant, add 4 volumes of chilled acetone and incubate for ~ 4 hrs at – 20°C

**Bacterial protein extraction (contd..)**

- After incubation centrifuge at 12,000 g for 5 min
- Discard supernatant and retain pellet
- Wash protein pellet with 95% ethanol (4 times)
- Dry pellet at room temperature
- Reconstitute dried pellet in lysis buffer
III. Plant proteome analysis

Plants are very important because they are food source for humans and animals

A thorough understanding of plant proteome is crucial

- to reveal molecular mechanisms underlying plant growth, development and interactions with environment
Plant proteome analysis

• Analysis of plant proteome provides information
  • Protein abundance
  • Protein modification
  • Subcellular localization
  • Three-dimensional structure
  • Interaction with other biomolecules

Leaf protein extraction

• Take weight of 300 mg of leaves and homogenize using a clean mortar pestle with liquid nitrogen
• Add 1500 uL of TCA, acetone to ground tissue
• Incubate homogenous solution at -20°C for 1 hour
• Centrifuge mixture at 14,000 rpm for 30 min at 4°C
• Remove supernatant and wash pellet 3-4 times with chilled acetone containing 0.07% DTT
Leaf protein extraction

- Dry pellet at room temperature
- Reconstitute dried pellet in lysis buffer
- Centrifuge contents at 14,000 rpm for 15 min at 4°C
- Collect the supernatant

Plant Protein Sample Preparation

- Fresh Leaves
- Weigh leaves
- Transfer to mortar
- Grinding in liquid nitrogen
- Homogenized Plant Leaves
- Removal of supernatant
- Protein pellet
- Reconstitution of pellet
Protein quantification

Protein concentration determination by UV absorption

- Determination of protein concentration by absorbance measurement at 280 nm
  - oldest method
  - based on the absorbance of UV light by aromatic amino acids in protein solutions
  - due to tryptophan and tyrosine residues, to a lesser extent phenylalanine residues
Protein concentration determination

- $A_{280}$ method requires that protein contains tryptophan and tyrosine
- Due to variability in aromatic amino acid content the absorptivity at 280 nm will be variable
- Higher protein concentrations are necessary

Protein quantification assays

- Different reagent can be used to determine the concentration of proteins in solution
  - Lowry assay
  - BCA assay
  - Bradford assay
Colorimetric methods: to determine protein concentration

Lowry assay

• Common method for quantitation of soluble protein
• 1. Alkaline cupric tartrate forms complex with peptide bond of protein
• 2. A reduction step with Folin and Ciocalteu's reagent
• Reaction yields purple color and absorption is read between 500-800 nm

Lowry assay (2)

- Advantages:
  - Sensitivity, simplicity, precision
- Problems:
  - Unsuitable for proteins without tyrosine residues,
    - assay depends on reaction of tyrosine residues with reagent
  - Sensitive to interference of Tris, EDTA etc.
    - this limitation can be overcome by precipitation methods such as TCA

Bicinchoninic Acid (BCA) assay

- Proteins form complex with Cu\(^{2+}\) ions in alkaline solution
  - Cu\(^{2+}\) ions are reduced to Cu\(^{+}\) ions (Biuret reaction)
  - form a violet color complex with Bicinchoninic Acid (BCA)
  - Amount of reduction is proportional to protein present

**BCA assay (2)**

- **Advantages:**
  - More sensitive than Biuret or Lowry methods
  - Color complex is stable, less susceptibility to detergents
  - Useful for membrane proteins and detergents
- **Problems:**
  - Disrupted by high concentrations of complex-forming reagents EDTA, ammonium sulfate; reducing materials DTT

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**Bradford Assay**

- Assay is based on complex formation between Coomassie brilliant blue G-250 dye and protein
- Due to binding absorption max of color shifts
  - 465 nm without protein
  - 595 nm with protein
- Increase of absorption at 595 nm is use to measure protein concentration

Bradford Assay (2)

- Advantages:
  - Compatible with reducing agents and thiols, unlike Lowry, BCA
  - It is quick and compatible for microwell plate assay

- Problems:
  - Dye binds most readily to arginyl and lysyl residues of proteins
    - this specificity may lead to variation
  - Commonly used detergents such as TRITON-X-100, SDS and CHAPS interfere

Bradford assay to determine protein concentration

- Requirements
  - Standard protein solution (0.5 mg/ml BSA), 0.15 M NaCl, Coomassie brilliant blue solution, cuvette

- Standard preparation
  - Add 0.5 mg/ml BSA 5, 10, 15, 20, 25 µl
  - Dilute with 100 µl of 0.15 M NaCl (use NaCl alone as blank)
  - For unknown – take 10 µl of sample and dilute with NaCl
Bradford assay to determine protein concentration

- Add 1 ml Coomassie brilliant blue solution and vortex
- Incubate reaction for 2 min
- Measure absorbance at 595 nm
- Use standard curve to determine protein concentration of unknown protein sample

Summary

- Sample preparation: work-flow
- Specific examples
  - Human serum
  - Bacteria
  - Plants
- Protein quantification
REFERENCES


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• Lowry et al. J Biol Chem. 1951 Nov;193(1):265-75. Protein measurement with the Folin phenol reagent

