“Urease Purification by Filtration and Salting Out"

Overview

- Introduction
- Scheme of process
- Method of purification
- Quantity and quality

Introduction

1. Intracellular/exracellular protein
2. Check the existence of protein by activity
3. Cell distruption
4. Purification
    a. Filtration
    b. Salting out(Ammonium sulfate precipitation)
    c. Other methods

5. Quantity
   a. Lowry method
   b. Activity
   c. Other methods
6. Quality
   a. Chromatography
   b. Electrophoresis
7. Summary

1. Scheme of process

- We have to confirm the existence of the protein by activity at each step.

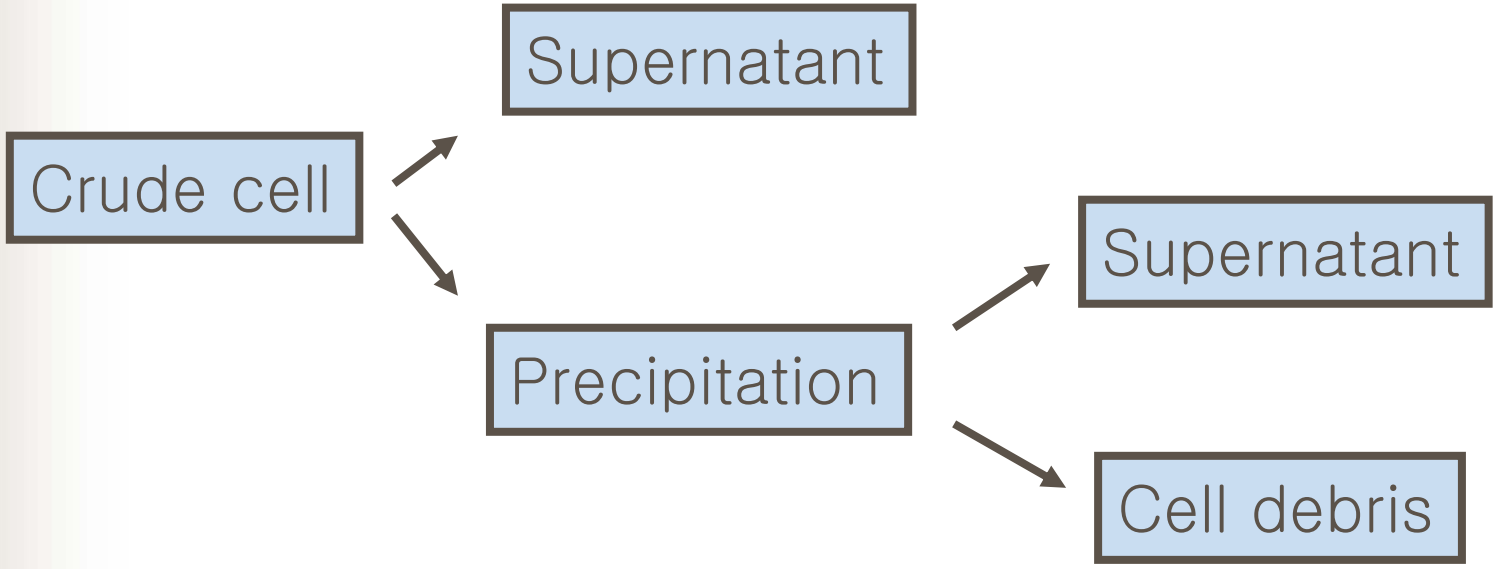

Fig. 1. Checking the existence of the protein.

2. Activity

1. Correlation with purity and concentration.
2. Substrate and enzyme reaction.
3. Expressed in activity unit[U]or[U/mg].
4. Check the activity with color change.

When enzyme exists, the color changes from red to pink.

3. Protein Extraction

Table. 1. Cell disruption methods for various tissues.

Cell lysis methods	Kind of tissue
Blade homogenization	Most animal, plant tissues
Hand homogenization	Soft animal tissues
Sonication	Cell suspension
Fench pressure cell	Bacteria, yeast, plant cells
Grinding	Bacteria, yeast, plant cells
Glass bead vortexing	Cell suspension
Enzyme digestion	Bacteria, yeast
Osmotic shock	Erythrocytes, bacteria

1. Sonication

   a. Disrupts tissue by creating vibrations which cause mechanical shearing of the cell wall.

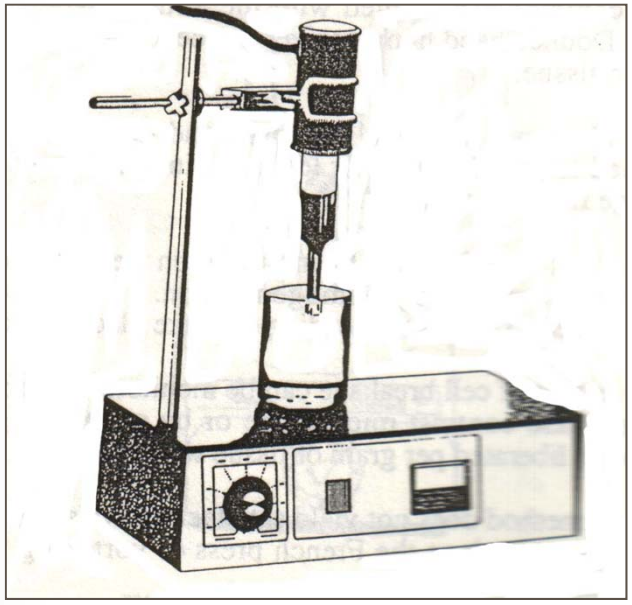

Fig. 2. Sonicator

4. Purification

1. Filtration
   a. Protein solution through a membrane which retains the protein of interest.
   b. This method is less likely to cause denaturation.

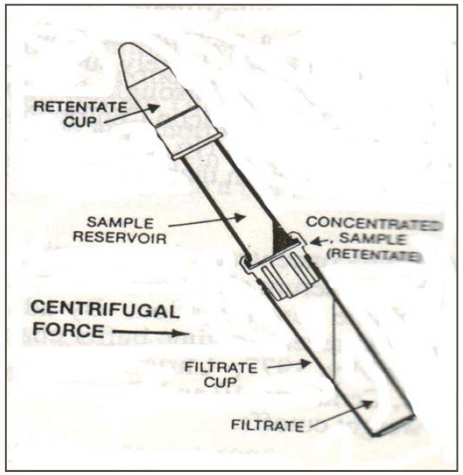

Fig. 3. Concentration of a protein solution using the Amicon Concentration system.

2. Salting out

(Ammonium sulfate precipitation)

a. Proteins tend to aggregate and precipitate from solution.
b. Different proteins precipitate at different salt concentration.
c. Important factor : pH, temperature, protein purity.
d. Making to X% solution from Xo% solution.

$g = [515(X-Xo0]/[100-0.27x]$

Table. 2. Ammonium sulfate precipitation table.

Starting Concentration	Final Concentration: 55%	60%	65%	70%	75%	80%	85%	90%	95%	100%
0%	351	390	430	472	516	561	608	657	708	761
5%	319	357	397	439	481	526	572	621	671	723
10%	287	325	364	405	447	491	537	584	634	685
15%	255	292	331	371	413	456	501	548	596	647
20%	223	260	298	337	378	421	465	511	559	609
25%	191	227	265	304	344	386	429	475	522	571
30%	160	195	232	270	309	351	393	438	485	533
35%	128	163	199	236	275	316	358	402	447	495
40%	96	130	166	202	241	281	322	365	410	457
45%	64	97	132	169	206	245	286	329	373	419
50%	32	65	99	135	172	210	250	292	335	381
55%		33	66	101	138	175	215	256	298	343
60%			33	67	103	140	179	219	261	305
65%				34	69	105	143	183	224	266
70%					34	70	107	146	186	228
75%						35	72	110	149	190
80%							36	73	112	152
85%								37	75	114
90%									37	76
95%										38

3. Other methods

- Dialysis, polyethylene glycol precipitation, Ion exchange chromatography and so on.

5. Quantity

1. Lowry method
   a. A combination of the copper reaction with peptide bond.
   b. Folin-Ciocalteau reagent with phenol was found to give blue color with proteins.
   c. There are many modified methods.
   d. Obtain a value by spectrophotometer.

2. Activity

a. The total activity(U) is in proportion to the total protein(mg or μg).

b. Using a spectrophotometer, we can check the activity.

3. Other methods.

- Bradford Assay, bicinchoninic acid assay, Kjeldahl method, Warburg-Christian method etc.

6. Quality

1. Chromatography
   - Accomplished by the physical and chemical properties such as size, charge, hydrophobicity and affinity.

a. Gel filtration chromatography : separates proteins by size, column packed with porous polymeric bead.

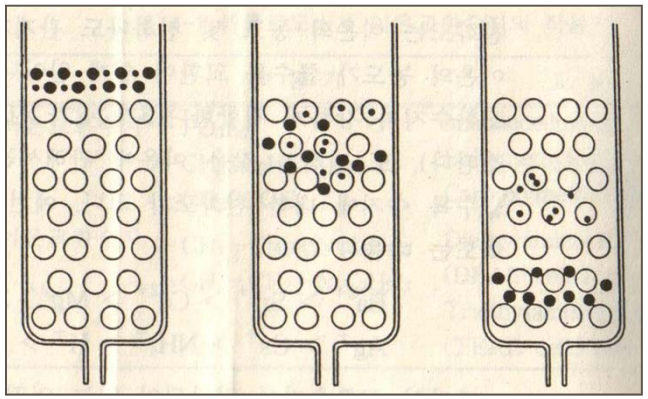

Fig. 4. Mechanism of gel filtration.

b. Ion exchange chromatography : proteins bind to ion exchangers by electrostatic force between the protein's surface and the charged group of exchangers

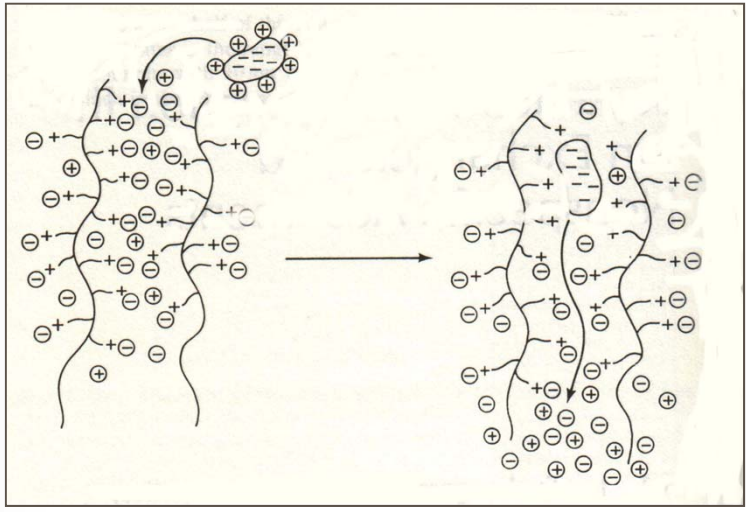

Fig. 5. Schematic of cation exchange mechanism.

2. Electrophoresis

- separates proteins based on their size and charge.
  a. SDS-PAGE – denaturing condition
  b. Native gel electrophoresis – nondenaturing condition

7.Summary

1. Urease is a intracellular protein.
2. Most of enzyme's size is greater than 300kDa.
3. Most of enzyme precipitate between 60% to 80% ammonium sulfate.
4. Activity is 0.86 APU.
5. Recovery is 30%.