Enzyme purification by ammonium sulfate precipitation

The raw materials for the isolation of enzymes are animal organs, plant material and M.O.s. The degree of purity of commercial enzymes ranges from raw enzymes to highly purified forms and depends on the application.

Downstream processing is a very important step in biotechnology because costs for collection, concentration and purification of the final product are substantial. High product concentrations in the supernatant or inside the cells and efficient purification are therefore important aspects in the overall economy of enzyme manufacture.

The choice of procedures for enzyme purification depends on their location. Isolation of intracellular enzymes often involves the separation of complex biological mixtures. While extracellular enzymes are generally released into the medium with only a few other components.

Cell disruption occur by **mechanical** methods such as high-pressure homogenization & the wet grinding of cells in a high-speed bead mill, and by **non-mechanical** methods ex. cells may frequently be disrupted by chemical, thermal, or enzymatic lysis.

After cell disruption, the next step is separation of extracellular or intracellular enzymes from cells or cellular fragments, respectively.

Purification is to be achieved, the volume of starting material must be decreased by one of the following concentration methods;

A- Thermal methods, B- Ultrafiltration, C- Precipitation

Precipitation with Salts

Ammonium sulfate precipitation

Is a method used to concentrate and purify proteins by altering their solubility. The solubility of proteins varies according to the ionic strength of the solution, and hence

according to the salt concentration. At low salt concentrations, the solubility of the protein increases with decreasing salt concentration, an effect termed salting in.

As the salt concentration (ionic strength) is increased further, the solubility of the protein begins to decrease. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution (salting out).

The ammonium sulfate amount to add can be determined from special tables. Each protein precipitate is dissolved individually in fresh buffer and assayed for total protein content and amount of desired protein. Salting-out is a very useful procedure to assist in the purification of a given protein. Ammonium sulfate is commonly used salt as it is very water soluble and has no adverse effects upon enzyme activity. It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution).

Practical part

1- Extraction of peroxidase from horseradish by using buffer (pH 6) in ratio 1:10.
2- To precipitation the desired protein (peroxidase) using ammonium sulfate at concentration 50% through addition of salt gradually to your sample to get the desired concentration, then stir for 1 hour to fully equilibrate.
3- Centrifuge at 10000 rpm for 30 minutes to pellet out protein.
4- Dissolve pellets in buffer to analyze proteins.

Purification

For many industrial applications, partially purified enzyme preparations will suffice; however, enzymes for analytical purposes and for medical use must be highly purified. Chromatography, is of fundamental importance to enzyme purification, molecules are separated according to their physical properties (size, shape, charge, hydrophobic

interactions), chemical properties (covalent binding), or biological properties (bio specific affinity).