Salt fractionation of plasma proteins | lab report

Most enzymes are protein in nature and it is useful to be used for understanding the basic aspects in determining structure and physiochemical properties of proteins. Solubility of proteins determine on the salt concentration in the solution. At low concentrations, the presence of salt stabilizes the various charged groups on a protein molecule, thus attracting protein into the solution and enhancing the solubility of protein. This is commonly known as salting-in. However, as the salt concentration is increased, a point of maximum protein solubility is usually reached. Further increase in the salt concentration implies that there is less and less water available to solubilize protein. Finally, protein starts to precipitate when there are not sufficient water molecules to interact with protein molecules. This phenomenon of protein precipitation in the presence of excess salt is known as salting-out.

Ammonium sulfate has been the most widely used chemical because it has high solubility and is relatively inexpensive. Because enzymes are proteins, enzyme purification can be carried out by following the same set of procedures as those for protein, except that some attention must be paid to the consideration of permanent loss of activity due to denaturation under adverse conditions.

There are two major salting-out procedures. In the first procedure, either a saturated salt solution or powdered salt crystals are slowly added to the protein mixture to bring up the salt concentration of the mixture. The precipitated protein is collected and categorized according to the concentration of the salt solution at which it is formed. This partial collection of the separated product is called fractionation. The protein fractions

collected during the earlier stages of salt addition are less soluble in the salt solution than the fractions collected later.

Whereas the first method just described uses increasing salt concentrations, the following alternative method uses decreasing salt concentrations. In this alternative method, as much protein as possible is first precipitated with a concentrated salt solution. Then a series of cold (near 0°C) ammonium sulfate solutions of decreasing concentrations are employed to extract selectively the protein components that are the most soluble at higher ammonium sulfate concentrations. The extracted protein is recrystallized and thus recovered by gradually warming the the cold solution to room temperature. This method has the added advantages that the extraction media may be buffered or stabilizing agents be added to retain the maximum enzyme activity. The efficiency of recovery typically ranges from 30 to 90%, depending on the protein. The recrystallization of protein upon transferring the extract to room temperature may occur immediately or may sometimes take many hours. Nevertheless, very rarely does recrystallization fail to occur. The presence of fine crystals in a solution can be visually detected from the turbidity.

Materials used:

- Bovine plasma
- Dialysis tubing
- Ammonium sulphate
- Visible spectrophotometer
- Centrifuge
- PBS(6. 1g KH_2PO_4, 1g NaOH , 8. 75g NaCL per liter)

- 2L beaker
- String
- Scissors
- Aluminium foil
- 50ml centrifuge tube

Procedure (1st session)

- 10ml bovine plasma diluted 1: 3 + phosphate buffer + ammonium sulphate (mixed on ice for 10 minutes)
- Centrifuged at 12, 000 RPM
- Supernatant decanted, made up to 90% saturation
- Mixture was centrifuged again, supernatant decanted
- Pellet washed with ammonium sulphate, then dissolved in distilled water
- Centrifuged
- Dialysed against distilled water (4 degree Celsius)

Procedure (2nd session)

1ml of protein sample was added to 4ml of biuret reagent. (2 tubes)

kept for 20 minutes

absorbance measured at 540nm

standard curve constructed and concentration of bovine proteins was determined

Results

Calculations

Calculate the concentration of bovine proteins (mg/ml) by referring to the standard curve.

Tube 1 = 0 mg/ml

Tube 2

M1V 1= M2V2

10 x 0. 1 = M2 x 5

5M = 1

M = 0. 2 mg/ml

Tube 3

M1V 1= M2V2

10 x 0. 2 = M2 x 5

5M = 2

M = 0. 4 mg/ml

Tube 4

M1V 1= M2V2

10 x 0. 3 = M2 x 5

5M = 3

M = 0. 6 mg/ml

Tube 5

M1V 1= M2V2

10×0. 4 = M2 x 5

5M = 4

M = 0. 8 mg/ml

Tube 6

M1V 1= M2V2

10×0. 5 = M2 x 5

5M = 5

M = 1. 0 mg/ml

Discussions

Questions

Explain why you need to dialyse the sample before you determine the protein concentration?

The sample needs to be dialysed to eliminate small molecular weight substances such as reducing agents , non reacted crosslinks, labelling agents, or preservatives that might interfere with the process to determine the protein concentration and might produce a fake result or reading. The presence of this compounds might cause the reading to be more higher than the actual amount of protein present and so the data will not be reliable anymore.

Other reagents such as ethanol and acids can also be used to precipitate proteins from solutions. How do they work?

Precipitation of proteins occurs primarily by hydrophobic aggregation, either by subtly disrupting the folded structure of the protein and exposing more of the hydrophobic interior to the solution, or by dehydrating the shells of water molecules that form over hydrophobic patches on the surface of properly folded proteins. Once the proteins start aggregating into larger structures, the amount of water per protein drops, enhancing the density differences between the proteins and the solute(Protein Precipitation, 2011). Ethanol causes protein to precipitate by the solvation layer around protein will decrease when the organic solvent displaces water from the protein surface and binds it in hydration layers around the organic solvent molecules. the smaller hydration layers causes the proteins to aggregate and temperature should be less than 0 degree to avoid protein denaturation, acids compresses solvation layer which increases in protein interactions. This causes the charges on the surface of protein to act with salt and not water and results in protein precipitation. Its known as salting out.

Why can bovine serum albumin be used as a standard in the quantitation of proteins by the Biuret method? Do you know of any other protein property that can be used for its quantitation? Explain.

Selection of a protein standard is potentially the greatest source of error in any protein assay. The best choice for a standard is a purified, known concentration of the most abundant protein contained in the samples being tested. Often, a highly purified, known concentration of the protein of interest is not available or it is too expensive to use as the standard, or the sample itself is a mixture of many proteins (e. g., cell lysate). In such cases, the best standard is one that will produce a normal (i. e., average) color

response curve with the selected protein assay method and is readily available to any researcher. BSA is such a protein, and the Pierce Albumin Standards are the most convenient source of ready-to-use BSA standard.

For greatest accuracy in estimating total protein concentration in unknown samples, it is essential to include a standard curve each time the assay is performed. This is particularly true for the protein assay methods that produce non-linear standard curves. Deciding on the number of standards and replicates used to define the standard curve depends upon the degree of non-linearity in the standard curve and the degree of accuracy required. In general, fewer points are needed to construct a standard curve if the color response is linear. Typically, standard curves are constructed using at least two replicates for each point on the curve.

Why can Cu2+ in an alkaline medium be used to detect the presence of proteins in solution?

Copper ion based protein assays is by mixing a protein solution with alkaline solution of copper salt. The cu2+ ions then chelate with the peptide bonds resulting in reduction of cupric (Cu2+) to cuprous ions (Cu+). If there are more alkaline copper than peptide bonds then some of the cupric ions will be unbound and will be detected. There are 2 methods in using this assay which is by measuring the reduced cuprous ions (Cu+) or assays that detect the unbound cupric (Cu2+) ions. Cuprous ions (Cu+) reduction of Folin Reagent produces a blue color that can be read at 650-750nm. The amount of color produced is proportional to the amount of peptide bonds. For the detection of unbound cupric ions, the protein solution is mixed with an amount of alkaline copper that is in excess over the amount of peptide bond. The

unchelated cupric ions are detected with a color-producing reagent that reacts with cupric ions and the amount of color produced is inversely proportional to the amount of peptide bond(Protein Assays , n. d)