PURIFICATION OF ENZYMES

Dr. Akhilesh Kumar Singh
(Assistant Professor)
Department of Biotechnology
Mahatma Gandhi Central University,
Motihari, Bihar, India

Introduction

- Purification of enzymes includes the following approaches:

(1) Dialysis

(1) Chromatography

(2) Electrophoresis

Being proteinaceous nature, the standard extraction & purification approaches of enzymes are similar to that of protein molecules except that the enzymatic activity is evaluated during following stages of extraction & purification:

(a) Enzyme extraction

(b) Crude extract formation

(c) Enzyme purification &

(d) Final enzyme processing

Dialysis

Dialysis is an technique for the removal of small molecules from enzyme. Enzyme precipitate achieved as a result of precipitation techniques like salting out, etc., is dissolve in small amount of buffer solution, where the enzyme was previously extracted.

The resulting solution is then transferred to a dialysis bag followed by sealing tightly. The bag is then suspended either in distilled water or buffer of known molarity & ionic constituents. Several others salts/ chemical substances need to be added occasionally in the exterior solution so as to inhibit the inactivation of enzymatic activity in the course of dialysis.

The dialysis is performed for few hours with regular change of the exterior solution/ distilled water.

On large-scale enzyme purification, dialafiltration approach is exploited as an alternative to dialysis technique. The enzyme solution is filtered for tiny substances by a membrane usually fixed on a fibrous support by pressure driven operations.

Chromatography

- Separation of protein molecules based on chromatography is the most common approach for enzyme purification.

- Following kinds of chromatographic techniques are accessible towards enzyme purification:

  (1) Adsorption/column chromatography
  (2) Ion exchange chromatography
  (3) Gel filtration chromatography
  (4) Affinity chromatography

Adsorption/ column chromatography

- In this, the protein/ enzyme solution having other protein impurities is allow to move through a column of inert substance (like charcoal, silica, alumina, and so on) packed in a glass/ steel tube.

- The effluent solution is uninterruptedly collected in small fractions of 1 or 2 ml followed by estimation of protein in each fraction through evaluating the absorption at 280 nm with the help of UV spectrophotometer. The enzyme is also assayed in each fraction.

- Different spleen enzymes like RNAase, DNAase, phosphodieesterase, phosphomonoesterase and so on are frequently separated from each other by Adsorption chromatography.

- For large-scale purification of enzyme, this technique is equipped with motors & additional mechanical devices in order to pack the column, load the enzyme on the column & elute the enzyme

Gel filtration chromatography (GFC)

- This technique involves the separation of different proteins/ enzymes based on differences in their molecular sizes.

- The basic arrangement of GFC is identical to that for adsorption chromatography. Here, the column composed of glass/ steel is taken and packed with a gel like sephadex. Different kinds of sephadex like G-10,G-30, G-50, G-100 and so are obtainable that vary according to their pore sizes.

- When a mixture of enzymes/ proteins is added on the topmost of the column, diverse protein molecules move down based on their sizes & come out from the column in order of reducing sizes, i.e., larger molecules are eluted first.

- GFC can be exploited to determine the molecular weight of protein molecules through calibrating the column with protein molecules of known molecular weight.

Affinity Chromatography (AC)

- This technique involves the purification of enzymes based on their specificity to a particular substrate or cofactor.

- The basic needs of AC are the similar to that of adsorption or GFC, however, in this case, the packing gel should contain constituent that can specifically bind to interest of component present in mixture but not with other.

Electrophoresis

- **Electrophoresis approach involves separation of molecules like enzymes, proteins, etc., by differences in their net charge in the presence of an externally applied electric field.**

- **In the lab., electrophoresis is normally exploited for enzyme purification & isozyme separation**

- **This technique has restricted use at large-scale as it is time consuming & is a bit costly**

Final Processing of Enzymes

- Most of commercially accessible enzyme formulations, purified as above are concentrated & sterile filtered after purification. This is carried out so as to decrease the volume & micobial contamination of the sample. Frequently, before storage & transport, the sample is freeze dried with additives like sugar substrate and dextrans

References

- Gupta P. K. (2016) Elements of Biotechnology, Rastogi Publications, Meerut, New Delhi, India.

- Bugg, T. D.H. (2012) Introduction to Enzyme and Coenzyme Chemistry, Blackwell Publishing Ltd, UK.