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ADVANCEMENTS OF ENZYME PURIFICATION

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Abstract: The goal of enzyme purification is to obtain a substantial amount of a specific enzyme while conserving its function in vivo. Enzyme purification consists of three phases, which can be elaborated into four general steps. Depending on the physical and chemical properties, downstream application, and the source of the target enzyme, each enzyme requires its unique purification strategy. The purpose of purification is to isolate specific enzymes from a crude extract of cells containing many other unwanted components in order to obtain the maximum specific activity with the best possible recovery of the initial activity. The following article discusses enzyme purification techniques and the advancements of enzyme purification are also discussed.

Keywords: enzyme purification, aqueous two phases, hybrid nanoflowers, three phase partioning.

Introduction

Enzymes are biological catalysts that are essential in a variety of metabolic processes in living organisms. Substrates are molecules with which enzymes can interact, and enzymes convert substrates into other molecules known as products. They're common in industries including food, pharmaceuticals, and biotechnology. [1]

However, enzymes derived from natural sources are frequently impure and must be purified before they can be employed efficiently in commercial applications. Enzyme purification is the separation and isolation of enzymes from other biological components in order to get pure enzymes. When selecting a purification procedure, the goal is to produce the highest yield of the needed enzyme with the highest catalytic activity and purity.

The development of techniques and methods for enzyme purification has been an essential pre-requisite for many of the advancements made in biotechnology. Enzyme purification varies from simple one-step precipitation procedures to large scale validated production processes. To attain the needed purity, more than one purification process is frequently required. The key to successful and efficient Enzyme purification is to select the most appropriate techniques, optimise their performance to suit the requirements and combine them in a logical way to maximise yield and minimise the number of steps required. More than one purification step is typically necessary to

achieve the requisite purity. As a result, chromatography has become an indispensable tool in any laboratory that requires protein purification. Different chromatography techniques with varying selectivities can be combined to generate powerful purification combinations for any biomolecule. [2-3] The development of recombinant DNA techniques has revolutionized the production of proteins in large quantities. Recombinant proteins are frequently created in ways that make subsequent chromatographic purification easier. However, this has not eliminated all difficulties. Host contaminants are still present, and issues with solubility, structural integrity, and biological activity may persist.

One of the oldest and most important activities in biochemical and physiological research is enzyme purification. As a result, an astounding number of enzymes have been purified to a great degree.

In the planning of enzyme purification, the work to be devoted to the preparation should be balanced against the ultimate aim. A researcher who is just interested in the metabolic function of an enzyme may be content with a preparation that is essentially free of "surrounding" enzymes in the same pathway, and he may be able to explicate the reaction sequence without having to prepare the enzymes again.

On the other hand, a worker intersted in the chemistry of the catalytic action of an enzyme will need a highly purified enzyme and will probably consume considerable quantities. A third possibility would be that the enzyme is required as an analytical tool, and this situation would stress the need for a high degree of purity, but perhaps not the need for large quantities.[4]

Separation procedures for enzymes are classified into two types: those that rely on the movement of substances within a liquid phase (such as gel filtration or electrophoresis) and those that rely on the transition of substances from one phase to another (such as chromatography or precipitations). Separations using methods from the two categories, on the other hand, may rely on the same molecular features. If electrophoresis yields an insufficient separation in a buffer of a specific pH, chromatography on DEAE cellulose should yield a comparable result, especially if the same pH and buffer are utilised. The reason for this is that both approaches rely heavily on the electrical charge of the molecules.[4]

Recent Advancements

Aqueous two-phase system (ATPS)

It is a liquid-liquid fractionation technique and has gained an interest because of great potential for the extraction, separation, purification and enrichment of proteins, membranes, viruses, enzymes, nucleic acids and other biomolecules both in industry and academia. Although, the partition behavior involved in the method is complex and difficult to predict. Current research shows that it has also been successfully used in the detection of veterinary drug residues in food, separation of precious metals, sewage treatment and a variety of other purposes. The ATPS is able to give high recovery yield and is easily to scale up. It is also very economic and environment friendly method. The aim of this review is to overview the basics of ATPS, optimization and its applications. [5]

Three phase partitioning

TPP is a simple, quick, and efficient and often a one-step process for the separation and purification of enzymes from complex mixtures. This elegant non-chromatographic process employs collective operation of principles involved in numerous techniques like i) salting out, ii) isoionic precipitation, iii) co-solvent precipitation, iv) osmolytic, and v) kosmotropic precipitation of enzymess. It is easily scalable and can be used directly with crude suspensions [6]. The principle of this fast emerging tool consists in mixing the crude enzyme extract with solid salt (mostly ammonium sulfate $(NH_4)_2SO_4$) and an organic solvent, usually *tert*-butanol (*t*-BuOH) in order to obtain three phases (Figure 2). The desired enzymes or proteins are selectively partitioned after centrifugation at 4°C to one phase while contaminants such as pigments and lipids to the other one (Figure 2). The upper organic phase which is containing non polar compounds (pigments, lipids etc.) is separated from the lower aqueous phase that containing polar compounds (proteins, carbohydrates etc.) by an interfacial enzyme precipitate [7-8].

As stated above, TPP is a simple and fast purification technique and found to be very effective for concentrating a wide range of enzyme solutions. By this process, some enzymes are stabilized and others are inhibited with *t* –butanol, allowing preliminary fractionations of crude extract. TPP enhance the enzymatic activity of several proteins.

In contrast, little quantities of $(NH_4)_2SO_4$ may need to be removed by dialysis. Some enzymes may lose their activity in the presence of high amount of t-butanol. Few studies reported that protein structure may be altered by *t* –butanol. TPP is not suitable for the isolation of IgG antibodies or proteins less present in the solution (<5 μg). Otherwise, a problem with tissue homogenization is the possible generation of artifacts, either by proteolysis or by other protein/protein interactions.

This technique is applicable for both upstream and downstream with unrefined samples, and downstream, when a simple and scalable step is required. The yield of the biomolecules reaches 90% and higher, and the maximum purification degree is 12–40-fold. Three-phase partitioning allows about 70% of potential savings over standard chromatographic procedures.[9]

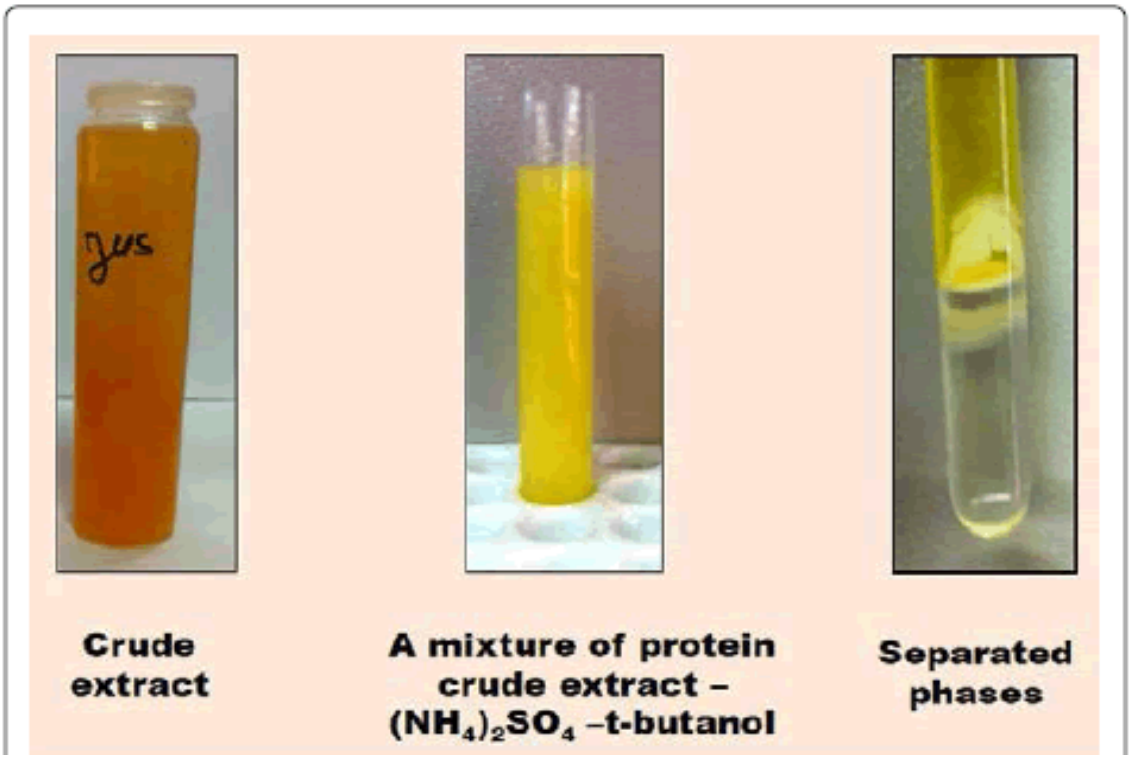

Figure 1: Three phases after Three Phase Partitioning experiment. From crude enzyme extract to three distinct separated phases

Organic–inorganic nanoflowers:

Given rapid developments in enzyme catalysis technology and nanoscience, enzyme-embedded nonmaterial's have attracted increased attention because of their special properties, particularly their functional and structural availability; these properties offer new opportunities with which to improve the biological functions of enzymes and expand their applications in areas such as industrial biocatalysis, biosensors, and bioanalytical devices [10]

These are hierarchical flower-shaped microstructures that are assembled by nanoscale petal like nanosheets composed of both organic and inorganic constituents. Using crude SBP as the organic component and Cu3 (PO4)2•3H2O as the inorganic component, researchers self-assembled a hierarchical flower-like spherical structure with hundreds of nanopetals. The structure of the hybrid nanoflowers was confirmed by Fourier-transform infrared spectroscopy, X-ray diffraction, and energy-dispersive X-ray spectroscopy. [11]

Reversed micelle

Surfactant action produces reverse micelles, which are nanometer-sized (1-10 nm) water droplets scattered in organic media. Surfactant molecules organise with the polar part on the inside, capable of dissolving water, and the apolar component on the outside, in contact with the organic solvent. Proteins can be dissolved in the reverse micelle water pool. Studies on the structure-function interactions of proteins in reverse micelles are critical because the milieu in which the protein is solubilized differs from a bulk aqueous solution in physicochemical qualities. [12]
Reverse micelles are single nanoscale particles made up of a water core encased in a surfactant. The amount of water in the core of reverse micelles can be easily changed to directly alter the particle size of the reverse micelle. When reverse micelles are subjected to low temperatures, their water loading capacity fluctuates with temperature, and water can be shed. The use of reverse micelle water shedding allows for accurate and complete control over the amount of water available to solvate host molecules. Proteins encapsulated within reverse micelles can be investigated to see how confinement and excluded volume affect them.[13]

Conclusion

Enzyme purification for in vitro application entails extracting the target enzyme from the source, eliminating non-target enzymes and other biological components, and increasing the concentration and purity of the target enzyme while keeping its function. The majority of successful enzyme purification relies on exploiting the enzyme's features, which can be used as a basis for purification strategies and separation technology selection. Aside from the inherent features of the enzyme, the sources from which the target enzyme is extracted, as well as the subsequent purification methods, all contribute to successful enzyme purification.
The growing interest in industrial enzymes necessitates the development of novel downstream techniques to maximize enzyme recovery. Significant work has gone into developing newly suited technologies for purifying enzymes in catalytically active form. Because of their versatility, lower cost, process integration capability, and ease of scale-up, aqueous two phase system (ATPS), three phase portioning (TPP), hybrid nanoflowers (HNFs) and reversed micelle have emerged as potent instruments for efficient enzyme extraction and purification. New purification procedures based on specialized, effective, and resilient methodologies and chromatographic materials are projected to influence the protein purification market's future.

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