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Opinion Article

A Note on Enzyme Purification

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DESCRIPTION

Purification of enzymes is often a complicated process, requiring a combination of techniques to achieve sufficiently high purity levels. 1) A high final degree of purity is one of the most important requirements for an efficient enzyme purification strategy. 2) High overall enzyme activity recovery. 3) Reproducibility. Recall that extraction procedures release the desired enzyme into the medium along with a variety of other cell components, such as other enzymes and proteins, nucleic acids, and polysaccharides, which, due to their polymeric structure, tend to raise the viscosity of the solution. Following homogenization of the original raw material, the first stage in purification is to separate any leftover cell debris (if any) using differential sedimentation or supernatant precipitation, or centrifugation or filtration for a faster and clearer extract.

Purification and separation of enzymes are usually based on solubility, size, polarity, and binding affinity. The production scale, timeline, and properties of the enzymes should all be deliberated when choosing the appropriate separation method.

Types of enzyme separation

Solubility based separation: The principle of the kind of separation is that enzyme solubility changes drastically when the pH, ionic strength, or dielectric constant changes.

Mass based method: Enzymes are relatively large molecules, separation based on the size or mass of molecules favors purification of enzymes, particularly the ones with high molecular weight. Dialysis is a frequently used method, where semipermeable membranes are used to eliminate salts, small organic molecules, and peptides.

Polarity based separation: Like other proteins, enzymes can be alienated on the basis of polarity, more specially, their net charge, charge density, and hydrophobic interactions. In ion-exchange chromatography, a column of beads comprising negatively or positively charged functional groups are used to separate enzymes. The cationic enzymes can be alienated on anionic columns, and anionic enzymes on cationic column.

Affinity or ligand based purification: Affinity chromatography is additional powerful and commonly pertinent means of purifying enzymes. This method takes benefit of the high affinity of many enzymes for particular chemical groups. In general, affinity chromatography can be successfully used to isolate a protein that recognizes a definite group by covalently attaching this group or a derived of it to a column, adding a mixture of proteins to this column, which is then washed with buffer to eliminate unbound proteins and eluting the required protein by adding a high concentration of a soluble form of the affinity group or altering the circumstances to diminution binding affinity.

Affinity adsorbents based on immobilized triazine dyes offer significant advantages circumventing many of the difficulties associated with biological ligands. The main drawback of dyes is their moderate selectivity for proteins. Rational attempts to tackle this problem are realized through the biomimetic dye model according to which new dyes, the biomimetic dyes, are designed to mimic natural ligands. Biomimetic dyes are estimated to exhibit augmented affinity and purifying capability for the targeted proteins. Biocomputing offers a predominant method to biomimetic ligand design. The successful corruption of contemporary computational techniques in molecular design needs the knowledge of the three-dimensional structure of the target protein, or at least, the amino acid sequence of the target protein and the three-dimensional structure of an extremely homologous protein. From such data one can then design, on a graphics workstation, the model of the protein and also a number of appropriate synthetic ligands which mimic natural biological ligands of the protein. There are numerous examples of enzyme purifications (trypsin, urokinase, kallikrein, alkaline phosphatase, malate dehydrogenase, formate dehydrogenase, oxaloacetate decarboxylase and lactate dehydrogenase) where synthetic biomimetic dyes have been used effectively as affinity chromatography tools.

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