ISOLATION OF ENZYMES

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Introduction

- Enzyme is not a stable molecules and, therefore a small alteration in its definite physicochemical organization diminishes the enzymatic activity & sometimes the enzymatic activity is fully deactivated.

- Owing to aforementioned facts, the enzyme must be isolated in controlled environments of pH, ionic strength, temperature, etc.

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.

Enzyme Extraction

Fresh tissue is crushed to paste using extraction medium (often a buffer) in a mortar & pestle/ tissue homogenizer/ blender/ ultrasonic vibrations (sonication).

The pH of the buffer is appropriately adjusted that may differ for different enzymes to attain maximum solubility & enzymatic activity.

Ethylene diamine tetra acetic acid (EDTA) is frequently added to the extraction medium so as to get rid of heavy metals (that else block enzyme activity) & towards disruption of the cell membranes/cell organelles. Occasionally, detergents like Triton-X are too exploited to solubilize the membranes

Several enzymatic protein bear disulfide (-s-s-) linkages owing to the occurrence of cysteine residues that are simply disrupted in the course of enzymatic extraction, thereby causing loss of enzymatic activity.

The aforementioned issues are resolved by the incorporation of thiols like mercaptoetanol whose sulfhydryl (-SH) group is capable to preserve the -enzymatic S-S- bond.

If the extract is not homogenous, the homogenate (extract) is filtered to remove cell debris, fibres, etc., otherwise filtration may be avoided.

All extraction operations & purification are usually performed at 0-4 ^{0}C as most of the enzymatic activity get lost at higher temperature.

Preparation of crude enzymes

- The formation of enzymatic crude extract involves many steps followed by enzyme purification.

(1) Centrifugation: The enzymatic extract is subjected to centrifugation for eliminating cellular debris/ organelles & other molecular aggregates, thereby lead to partial purification of enzymes.

- **It also assist towards the enzymatic characterization as depending on the mass as well as shape, the enzyme will travel over solution with a definite velocity & occupy a distinctive position in the centrifuge tube.**

- In case of most cytosolic enzymes, centrifugation at 30,000g for 30 min is adequate for attaining a reasonable amount of enzymatic activity in the supernatant.

- If enzyme is situated in a particular cellular organelle, an extract rich in that organelle is prepared by preparative centrifugation. Centrifugations for different period with different speeds permits the cellular organelle to sediment as per their sizes.

- All centrifugation operations are carried out 0-4 ^{0}C (cold)

(2) Precipitation: The enzymes including other protein molecules are highly charged & they can be precipitated with suitable charge neutralizing agents. Once their charge are neutralized, they produce aggregate & settled down in the form of precipitate.

- If an acid/ base is supplemented, the enzyme can be carried upto its isoelectric pH, where no net charge present on enzymes & low electrostatic repulsion exist amongst them, which result in aggregation. This indicates that pH adjustment towards isoelectric point of a certain protein results in precipitation

- The acids as well as bases are often deactivate the enzymatic activity & thus, they are not recommended as enzyme precipitating agent in utmost cases.

- Ammonium sulfate including other salts are exploited towards precipitation in a process known as "salting out"

- Salts can transform the solvent structure in such a way that ultimately result in huge alterations towards the conformation of protein via changing the electrostatic interactions amongst charged groups of protein surface.

- The salts also involve in competition with the protein molecules for solvent molecules and thereby lowers their solvation.

- For large-scale precipitation of enzymes, exploitation of several other neutral salts is recommended compared to ammonium sulfate as it is corrosive & liberates ammonia at higher temperature.

- Some organic solvents such as acetone, ethanol, etc., are also exploited as enzyme precipitating agent as water miscible solvents reduces the solvation of proteins, leading to precipitation. This process is performed at 0 ^{0}C as room temperature precipitation results in enzyme denaturation in most cases.

References

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