

Study effect of temperature on enzyme activity

Enzymes are generally globular proteins, acting alone or in larger complexes. Like all proteins, enzymes are linear chains of amino acids that fold to produce a three-dimensional structure. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme. Although structure determines function, a novel enzyme's activity cannot yet be predicted from its structure alone. Enzyme structures unfold (denature) when heated or exposed to chemical denaturants and this disruption to the structure typically causes a loss of activity.

Protein folding is key to whether a globular protein or a membrane protein can do its job correctly. It must be folded into the right shape to function. But hydrogen bonds, which play a big part in folding, are rather weak, and it does not take much heat, acidity, or other stress to break some and form others, denaturing the protein. This is one reason why tight homeostasis is physiologically necessary in many life forms.

Denaturation

Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), radiation or heat. If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death. Denatured proteins can exhibit a wide range of characteristics, from conformational change and loss of solubility to aggregation due to the exposure of hydrophobic groups.

Experimental procedure : Day 1

Enzyme alkaline phosphatase as in the previous chapter .

Substrate p- nitrophenyl phosphate solution following the previous chapter .

3. Enzymes at a particular aliquot taken in Eppendorff tubes are kept at particular temperature like 30, 40 ,50, 60, 70 ,80 degree Celsius for 5-10 mins .Then are to be used for enzyme activity .Time in temperature bath should be fixed for a whole set of experiment

4. Draw a graph Temperature vs. Reading at 410nm . Graph may look like the first figure as Alkaline Phosphatase is a thermostable enzyme .

