Lodz University of Technology
Institute of Materials Science and Engineering

Laboratory 2

Inactivation of enzymes

Instruction for the Laboratory of Biophysics

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I. THEORETICAL INTRODUCTION

Enzymes are biomolecules that catalyze (i.e. increase the rates of) chemical reactions. Almost all enzymes are proteins. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Proteins are long strands of amino acids linked together in specific sequences. A protein is created by ribosomes that "read" codons in the gene and assemble the requisite amino acid combination from the genetic instruction, in a process known as translation. The newly created protein strand then undergoes post-translational modification in which additional atoms or molecules are added, for example copper, zinc, iron. Once this post-translational modification process has been completed, the protein begins to fold (spontaneously, and sometimes with enzymatic assistance), curling up on itself so that hydrophobic elements of the protein are buried deep inside the structure and hydrophilic elements end up on the outside. The final shape of a protein determines how it interacts with its environment. If the shape of the protein is altered through some form of external stress (for example, by applying heat, acid or alkali) then it will no longer be able to carry out its cellular function. This is called denaturation of the protein.

II. EXPERIMENTAL PART

1. Wash the potato and dry it using blotting paper

2. Grate 50 g of a potato and put it into a glass beaker

3. Put the homogenate of potato to 4 centrifuge tubes – all tubes must have the same weight. Next, place tubes in centrifuge and turn on machine by following parameters – 2 min. and 7 000 RPM
4. Gently take out tubes and add 500 µl of supernatant to 8 Eppendorf tubes
5. From 8 Eppendorf tubes create 4 pairs:

   1 - control conditions
   2 - put to 60° C,
   3 - put to 100° C,
   4 - add 500 µl of methanol.

   Incubation time – 15 min.
6. Take one Eppendorf tube from each pair and centrifuge (1 min, 11 000 RPM). Then take 50 µl of supernatant and put it to new 4 Eppendorf tubes. From remaining 4 tubes take 50 µl homogenate (uncentriguge) to 4 new tubes.
7. For each tubes add 1000 µl 5% H_2O_2 (made from 30% stock solution). Incubate 30 min in room temperature.
8. Measure a absorption of sample at wavelength 290 nm. Results put to the table.

Remember to calibrate the spectrophotometer!!!!

	Absorbance at 290 nm	
	centrifuged	Non centrifuged
Control		
60° C		
100° C		
500 µl of methanol		

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III. THE REPORT MUST CONTAIN

1. A short theoretical introduction.

2. Table with data

3. Comparison of enzyme activity after different condition.

VI. LITERATURE

1. Harper's Biochemistry by Daryl K. Granner, Peter A. Mayes, Robert K. Murray, Victor W. Rodwell (1996)

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