equipment. Contact the Department of Environmental Health and Safety.

Use liquid disinfectant traps and in-line vacuum filters on all vacuum lines. This protects the vacuum system from contamination and minimizes the risk to those who service and repair the system.

Decontamination

Work surfaces must be decontaminated with a suitable disinfectant at the end of each day and after any spill of potentially dangerous material.

Contaminated or infectious liquid or solid materials must be decontaminated before disposal or reuse. Decontaminate by autoclaving or chemical disinfection all biologically contaminated materials (such as glassware, animal cages, laboratory equipment, etc.) before washing, reuse or disposal. When materials have been decontaminated properly, drain off liquids, remove the biohazard designation, and place them in the regular waste stream (trash).

Decontaminate laboratory equipment before it leaves the lab for repair, recalibration, resale, or other destinations.

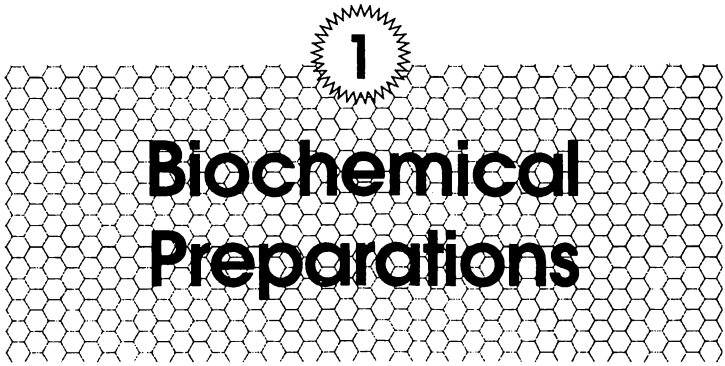
Some symbols and their meaning

Corrosive substances

Explosive substances

Irritant

Toxic substance



INTRODUCTION

The enzymes are critical in the functioning of cells. Life is a dynamic process that involves constant changes in chemical composition. These changes are regulated by catalytic reactions, which are regulated by enzymes. At one time, the cell was actually conceived of as a sac of enzymes. It was believed that on the basis of the reactions and their rates of action, cell and indeed, life itself could be defined. Few biologists continue to think of this as a simple task, but we know that life could not exist without the function of enzymes. Ideally, enzymes could be examined within an intact cell, but this is difficult to control. Consequently, enzymes are studied *in vitro* after extraction from cells. In a simpler way enzymes can be defined as biocatalysts. A catalyst is a substance that accelerates the reaction but does not get itself modified in the process so that it can be used again and again. Enzymes are the largest and most specialized class of protein molecules. More than a thousand different enzymes have been identified of which many of them have been obtained in pure and even in crystalline form.

An important feature of enzyme activity is its substrate specificity, i.e. a particular enzyme will act only on a certain

inversion. Then pipette out 1 ml of the 1/10 dilution into the second tube (labeled as 1/100) and mix by inversion. Pipette 1.0 ml of the 1/100 dilution into the third tube (labeled as 1/1000) and mix by inversion.

- Place all of the dilutions in the ice bath until ready to use. Turn on a spectrophotometer, adjust to 475 nm and with a blank in a tube containing 2.5 ml of citrate buffer and 0.5 ml of enzyme extract. Add 2.5 ml of 8 mM DOPA to each of 4 cuvettes or test tubes. Note that each tube contains 0.0025 × 0.008 moles or 20 µM of DOPA.
- Pipette 0.5 ml of undiluted enzyme extract to one of the tubes containing 8 mM DOPA. Mix by inversion, place into the spectrophotometer and immediately begin timing the reaction. Carefully measure the time required for the conversion of 8 µM of DOPA into dopachrome. Note that since the cuvette will contain a volume of 3.0 ml, the concentration when 8 µM are converted will be 8/3.0 or 2.67 mM dopachrome. Use the data from the standard curve to determine the absorbance equal to 2.67 mM dopachrome. This absorbance value will be the end point for the reaction.
- As the reaction takes place within the cuvettes in the spectrophotometer the absorbance will increase with dopachrome formation. When the absorbance reaches the value above, note the required time from the mixing of the enzyme extract to the 10 µM DOPA.
- Express the time as a decimal rather than minutes, seconds. The time should be between three and five minutes. If the end point is reached before three minutes, repeat this step using the next dilution of enzyme (i.e. the 1/10 after the undiluted, the 1/100 after the 1/10 and the 1/1000 after the 1/100). For the enzyme dilution, which reaches the end point between 3 and 5 minutes, calculate the velocity of reaction.
- Divide the amount of product formed (10 µM) by the time required to reach the end point.

- Repeatedly thaw the frozen solid and freeze few times to lyse the erythrocytes.
- Determine the amount of hemoglobin released in the supernatant by centrifuging for 5 minutes at 100g and measure the absorbance/OD of the supernatant at 560 nm.
- Emulsify 100 mg of lecithin in saline (100 µg/ml) under vigorous shaking.
- Add some enzyme source to 1 ml of erythrocyte suspension and make up the final volume to 2.0 ml with veronal buffer.
- Incubate the mixture at 37°C for 30 minutes.
- Keep the mixture at 4°C for 20 minutes.
- Centrifuge and determine the amount of hemoglobin released in the supernatant by measuring the absorbance/OD at 560 nm and compare to that of completely lysed sample.

Phospholipase D

Principle

The enzyme releases choline by splitting lecithin (phosphatidyl choline), which forms a complex with iodine. The formed complex is measured spectrophotometrically at 365 nm.

Materials

Iodine reagent (as described in official Pharmacopoeias)

Enzyme Source

Following protocol is followed for the processing of the enzyme.

- Grind and homogenize 100 g fresh cabbage leaves with 75 ml distilled water in a blender.