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Properties of an Enzyme: Wheat Germ Acid Phosphatase

Experiment #10

Pre-Lab Exercise

1. What is an enzyme? Give a description of what an enzyme is in terms of its chemical composition (what kind of biomolecule) and in terms of what it does (its function).
2. A chemical reaction catalyzed by the enzyme acid phosphatase is shown in the introduction. How would you classify this enzyme in terms of the enzyme classification scheme described in the text book, *i.e.*, oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase?
3. List at least four factors that can influence enzyme activity and indicate what is meant by the term "enzyme activity" in this context (see the text book).
4. Considering the biochemical (protein) nature of enzymes, what are some factors or chemical and physical agents that can destroy or denature enzymes? Consider the earlier experiment regarding properties of proteins. You should list at least three.

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5. Would you say that boiling water would be an effective way to destroy the enzymes essential for microbes to live? Explain.
6. Fluoride ion is considered to be toxic to most organisms at relatively high concentrations. There is currently a controversy over the use of fluoride in municipal drinking water. What is the basis for putting fluoride in drinking water? What would be the reason for not adding it? You may want to check the internet for discussion of this issue.

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Data & Report Sheet

Part A. Demonstration of Enzyme Action

Record absorbance at 410 nm of each solution after adding to 3.0 mL of 0.10 M sodium hydroxide.

| Tube | Conditions | Absorbance @ 410 nm |
|------|--|---------------------|
| 1 | On ice, 0°C | |
| 2 | Room Temperature, 22°C | |
| 3 | Body Temperature, 37°C, with Substrate | |
| 4 | Body Temperature, 37°C, No Substrate | |
| 5 | Boiled Enzyme, Incubated at 37°C, with Substrate | |

A-1. Comparing tubes 3 and 4, what conclusion can you make about the need for substrate in order to demonstrate enzyme activity?

A-2. Explain why tube 5 would have lower activity than tube 3.

A-3. Explain the relative absorbance for tubes 1, 2 and 3. What accounts for the differences?

Part B. Dependence of the Reaction on Enzyme Concentration and Fluoride Inhibition

| Tube | Conditions | Absorbance @ 410 nm |
|------|---|---------------------|
| 0 | No Enzyme Added | |
| 5 | 5 Drops of Enzyme Solution Added | |
| 10 | 10 Drops of Enzyme Solution Added | |
| F | 5 Drops of Enzyme Solution Added, with Fluoride Inhibitor | |

B-1. Would you expect any p-nitrophenol product to be formed when there is no enzyme present in the solution? Explain.

B-2. Would you expect fluoride to occur naturally in drinking water? Explain.

Part C. pH Dependence of the Enzyme Catalyzed Reaction

| pH | Absorbance @ 410 nm |
|----|---------------------|
| 3 | |
| 5 | |
| 8 | |

C-1. Discuss the observed effect of pH on the activity of acid phosphatase. Why is it called “acid” phosphatase?

C-2. Do most enzymes have the same optimum pH?