AP Bio- Enzyme Lab

Practically all of the numerous and complex biochemical reactions that take place in animals, plants, and microorganisms are regulated by enzymes. Catalase -- found throughout nature in the cells of organisms that grow in the presence of oxygen -- is an enzyme with potent antioxidant properties. The enzyme facilitates the breakdown of hydrogen peroxide into its harmless components -- water and oxygen. Without catalase, the hydrogen peroxide that your body produces during the metabolic process would build up to toxic levels. Catalase occurs naturally in a wide array of plant-based foods, ensuring its availability to vegetarians and vegans.

The cells in your body can produce catalase as well as other antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase, or SOD. These enzymes are housed in the organelle called the peroxisome. These enzymes help to neutralize free radicals. Free radicals are atoms or molecules with unpaired electrons that are the byproduct of human biological reactions such as metabolism. Scientists cite free radicals as causative factors in disease and aging-related cell damage. As you age, your body gradually produces less catalase, which results in a buildup of free radicals and an increase in the damage they can cause. To help supplement your body’s declining output of catalase, some scientists recommend consuming foods that are rich in this vital enzyme.

Remember, most enzymes are proteins. Each enzyme is able to promote only one type (or a small number) of chemical reaction. Enzymes can be classified into several broad categories, such as hydrolytic, oxidizing, and reducing, depending on the type of reaction they control. In an enzyme catalyzed reaction, the compounds on which the enzyme acts are called substrates and the resulting compounds are called products. Today, we will quantitatively investigate the reaction catalyzed by the enzyme Catalase.

The enzyme Catalase carries out the following reaction:

http://bioweb.wku.edu/courses/Biol114/enzyme/Catalase_Rxn.gif

Like any other enzyme, catalase can be affected by various factors including: temperature, pH, salinity and concentration of substrate or enzyme. Temperature can directly affect the rate of an enzymatic reaction. Firstly, all chemical reactions are affected by temperature, according to the laws of thermodynamics. The increased molecular motion that occurs as a result of increased temperature makes collisions between the enzyme and substrate more likely, and therefore the reaction will occur at a greater rate.

So generally, as temperature increases so does the rate of reaction. However one must also bear in mind that high temperatures can cause thermal denaturation of the enzyme and freezing may also damage an enzyme. Denaturation is a change in the tertiary structure of an Enzyme. An enzyme's function is related to its 3-dimensional structure. This structure can be altered by heat, thus causing the enzyme to lose function.

Enzymes are also sensitive to changes in pH. The protein chain the enzyme molecule is made from is coiled and folded into a very specific shape. This shape is held together by the attraction between positive & negative charges on different parts of the molecule. The pH of the surrounding environment can affect the charges on the enzyme molecule, causing them to be changed. This can cause the protein chain to unfold or uncoil. Even a slight change in an enzyme molecule's shape can prevent it from working properly. This change of shape is called denaturing.

Pre- Lab Questions:

1. Which enzyme breaks down hydrogen peroxide in aerobic cells?
2. What is the substrate of the enzyme catalase?
3. What is an anti-oxidant?
4. Why does hydrogen peroxide disinfect wounds?
5. How does temperature affect enzymes?
6. How does pH affect enzymes?
7. How does concentration of substrate affect enzymatic rate?
8. How does concentration of enzyme affect enzymatic rate?

Part I- Which plants have the most catalase?

Materials:

* Bananas
* Sweet Potatoes
* Mushrooms
* Zucchini
* Apples
* Carrots
* Graduated Cylinder
* Stoppers
* Timers
* Digital Balance
* Hydrogen Peroxide

1. Measure out 5 grams of assigned type of plant material.
2. Score the plant material (make grooves on it to maximize surface area). Make sure to cut it into pieces that will fit in graduated cylinder.
3. Place 50 ml of hydrogen peroxide solution into graduated cylinder.
4. Drop the plant material into the solution, gently stopper the cylinder and immediately record the initial volume.
5. Allow the plant to catalyze the H2O2 for ten minutes, taking a volume reading at the end of each minute. Subtract the initial volume from the greatest volume reached, and record this as the change in volume. Measure at the top of the bubbles for each reading.

TABLE 1: Group Volume changes and Rate of Catalase Activity in \_\_\_\_\_\_\_\_\_\_\_\_\_.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type of Plant Material | Initial Volume (ml) | Final Volume (ml) | Change in volume | Rate (change in volume over time) ml/minute |
|  |  |  |  |  |

1. Obtain other groups info and record in table below.

TABLE 2: Class Volume Changes and Rate of Catalase Activity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type of Plant Material | Initial Volume (ml) | Final Volume (ml) | Change in volume | Rate (change in volume over time) ml/minute |
|  |  |  |  |  |
|  |  |  |  |  |
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Questions:

1. Looking at the results which group had the fastest rate?
2. Which fruit or vegetable must have the most catalase?
3. Which fruit or vegetable has the most antioxidant per serving?
4. Which fruit or vegetable has the least antioxidant per serving?

Part II- Design your own experiment

Your challenge is to design an experiment to show factors that affect enzyme activity. You can use any of the fruits or vegetables. Below are the materials available for use and helpful formulas. YOU MUST have a control group, controlled variables, and at least three treatment groups.

* Hydrogen Peroxide
* Plant
* Balance
* Graduated Cylinders
* Beakers
* Acid (HCl)
* Base (NaOH)
* Microwave
* Water
* Timer

Formulas:

Rate= dY/dt (change in product over time)

Dilution = CiVi= CfVf

C= concentration, V= Volume, i= initial, f= final

Your Procedure:

Data Table:

Rate Calculation Table (Calculate at each time interval):

Graph of rate for each treatment group:

**How Free Radicals are Formed**

Normally, bonds don?t split in a way that leaves a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are formed. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell.

Some free radicals arise normally during metabolism. Sometimes the body?s immune system?s cells purposefully create them to neutralize viruses and bacteria. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals.

Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur. Of particular importance is that free radical damage accumulates with age.

**How Antioxidants May Prevent Against Free Radical Damage**

The vitamins C and E, are thought to protect the body against the destructive effects of free radicals. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-"stealing" reaction. The antioxidant nutrients themselves don?t become free radicals by donating an electron because they are stable in either form They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease.

Vitamin E ? The most abundant fat-soluble antioxidant in the body. One of the most efficient chain-breaking antioxidants available. Primary defender against oxidation. Primary defender against lipid peroxidation (creation of unstable molecules containing more oxygen than is usual).

Vitamin C ? The most abundant water-soluble antioxidant in the body. Acts primarily in cellular fluid. Of particular note in combating free-radical formation caused by pollution and cigarette smoke. Also helps return vitamin E to its active form.