NAME: ______________________________ PERIOD: ____ DATE: ____________

Toothpickase Activity

Adapted from "Investigating Enzyme Reaction Rates Using Toothpickase" by Peggy O'Neill Skinner

Background

This exercise concerns enzymes and enzymatic regulation. More specifically, this lesson considers how the structure of an enzyme is related to its function. Enzymes have at least one active site. The substrate binds to the enzyme at the **active site**, but the enzyme will only be able to bind to the substrate at the active site if both have a **complementary three-dimensional structure**. Once the substrate has been bound to the enzyme's active site, the enzyme will then be able to effect a **lowering of the activation energy** thereby **increasing the rate of the reaction**.

The active site of an enzyme may be blocked. When the active site of an enzyme is blocked, the enzyme is unable to bind with the substrate. If the enzyme is unable to bind with the substrate, then the enzyme cannot catalyze the reaction. If the active site of an enzyme is blocked, then the enzyme is **inhibited**. If the enzyme is inhibited, then it will not be able to catalyze or accelerate the rate of the reaction. Inhibition may be transitory or long lasting.

Typically, inhibitor molecules are used to regulate enzymes in biochemical pathways such as Phosphofructokinase or PFK. Inhibition of an enzyme in a biochemical pathway will essentially shut down the pathway. Hence, it is crucial to understand enzymatic inhibition, as it is the primary method of biochemical pathway regulation.

The main way that enzymes are inhibited is through **allosteric inhibition**. Allosteric inhibition is when an inhibitor molecule binds **somewhere other than the active site**. This binding causes the entire enzyme to **change shape**, called a conformational change. A conformational change in the enzyme affects the **structure of the active site**. If the shape of the active site is altered, then it will likely **not be able to bind the substrate**.

Vocabulary

Enzyme – structure that holds the substrate in place so that it can lower the activation energy of the reaction and therefore speed up the reaction rate
Quaternary structure – level of protein structure consisting of multiple subunits or chains
Active site – where the substrate actually binds
Substrate – binds to the enzyme at the active site (like a reactant in a chemical reaction)
Product – the end result of a chemical reaction
V-max – maximum rate of reaction

Background to Lab

Enzymes are agents that change the rate of a reaction without being changed themselves. In this activity, your hands become the functional part of an enzyme that we will call toothpickase. You will be called toothpickase since you will be breaking toothpicks and every enzyme ends in "-ase". Your substrate is the toothpick. When you find a toothpick, you react with it and break it into two pieces. Your goal is to break toothpicks quickly and efficiently (without damaging the toothpickase in the process).

Procedures

PART 1

1. Groups of 3 people
    a. 1 person will be breaking toothpicks
    b. Another person will be keeping track of how many toothpicks are broken
    c. Last person will be keeping time and recording data

2. Select approximately 250 toothpicks and divide them into 6 equal sized piles
3. You will be breaking toothpicks by holding the toothpick between your index finger and thumb of each hand.

4. The class will start metabolizing toothpicks at the same time
5. There are NO breaks in timing
6. Since you will be breaking toothpicks, make sure that the toothpick piles are conveniently located as speed is the main measurement

7. RULES
    a. You can only BREAK ONE (1) toothpick at a time
    b. You must BREAK the toothpick CLEANLY – if there is any attachment, it does not count

8. RECORD the total number of toothpicks broken at
    i. 0 sec. 10 sec. 30 sec. 60 sec. 120 sec. 180 sec.

PART 2

1. Same Groups, but switch responsibilities
2. Same Rules as in Part 1
3. Select approximately 250 toothpicks and divide them into 6 equal sized piles

4. **NOW: the student breaking toothpicks can ONLY USE index and middle fingers on each hand**

5. The class will start metabolizing toothpicks at the same time
6. RECORD the total number of toothpicks broken at the same time intervals as in the previous part

PART 3

1. Same Groups, but switch responsibilities again
2. Same Rules as in Part 1 & Part 2
3. Select approximately 150 toothpicks and divide them into 6 equal sized piles

4. Select approximately 100 **round** toothpicks and divide them into the existing 6 piles

4. **NOW: there are 2 types of toothpicks in each pile: Round and flat toothpicks**
    a. If the breaker selects a ROUND toothpick, it must NOT be broken & placed in a separate pile at the end of the table

5. The class will start metabolizing toothpicks at the same time

6. RECORD the total number of toothpicks broken at the same time intervals as in the previous parts - DO **NOT** RECORD THE NUMBER OF ROUND TOOTHPICKS

Data Collection Tables

Toothpick Metabolism Data

Time (seconds)	Toothpicks Metabolized **PART 1**	Toothpicks Metabolized **PART 2**	Toothpicks Metabolized **PART 3**
0			
10			
30			
60			
120			
180			

Reaction Rates
For each of the following time intervals, calculate the rate of reaction of the toothpickase enzyme using the following formula for the slope of the line graphed above:

$$\text{Reaction Rate Calculation} = \frac{M2 - M1}{T2 - T1}$$

M = number of toothpicks metabolized by a given point in time

T = time

Reaction Rate Data

Time Period (seconds)	Rate of Reaction **PART 1**	Rate of Reaction **PART 2**	Rate of Reaction **PART 3**
0 to 10			
10 to 30			
30 to 60			
60 to 120			
120 to 180			

GRAPHING

1. Graph the Toothpick Metabolism data in PART 1
2. Graph the Toothpick Metabolism data in PART 2
3. Graph the Toothpick Metabolism data in PART 3

** There must be **<u>3 separate graphs</u>**
** Don't forget to appropriately label the axes & provide a title
** You may use a computer program to graph the data or
construct the graph by hand

ASSESSMENT QUESTIONS

1. In the Toothpickase activity, identify the following:

   a. Substrate:

   b. Enzyme:

   c. Active Site:

   d. Product:

2. How many subunits does Toothpickase have?

3. What is the quaternary structure of Toothpickase?

4. Explain how the Toothpickase activity modeled competitive inhibition?

5. Explain how the Toothpickase activity modeled allosteric (non-competitive) inhibition?

6. Explain how structure is related to function in the Toothpickase activity

7. What is the V-max in each of the three parts?