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COS 7.433
Lesson plan for 3 May 2006: Enzymes, catalysts and inhibitors

Background:

This lesson assumes some initial presentation of enzymes and their different parts and activities (e.g., active site, specificity of action, conformation change, substrates and products, etc.). A quick review at the beginning re-establishes this baseline information. The lesson launches into a series of stations that allow the students to become an enzyme and perform a particular task in the presence of competitive and non-competitive inhibitors and a catalyst. The data are pooled since every group's numbers are important for the small group worksheet application at the conclusion of the lesson. Presumably, the time spent at each station could be longer and the worksheet could go into more about the catalyst to fill out a full 60-90 minute block of class time. The worksheet currently only emphasizes the competitive and non-competitive inhibitors and how they relate to normal enzyme activity in the absence of inhibition. This lesson involves a thought-swap, a hands-on activity to generate a dataset, a teacher-class interaction to pool the data and a worksheet that asks the student to apply the data to a series of questions about enzyme and inhibitor function.

Supplies:

- 130 pennies
- 130 dimes
- 70 nickels
- Two tennis balls
- Duct tape
- Masking tape

Initial review of enzyme-related vocabulary:

Quickly, in a thought swap, define the following:

1. Active site
2. Substrate vs. product
3. Conformational change

Preparation:

Across the room, mark out five 50 cm x 50 cm squares on the carpet with masking tape. Sort coins according to group in advance:

Group	# pennies	# dimes
1	6	6
2	10	10
3	24	24
4	40	40
5	50	50

Station 3 will have 20 nickels and station 4 will have 50 nickels.

Hands-on activity:

The class will be divided into five groups (2-3 students in each) and will be asked to follow the instructions at each of the 5 stations. Before beginning, there are a set of common instructions for all groups. Drop all pennies and dimes from your group (see table above for amounts) into the 50 cm x 50 cm square. At stations 3 and 4, drop nickels as well. Select one person in the group to be an enzyme called *depositinchase*. This enzyme's task is to start standing, kneel with their eyes closed and touch somewhere inside the square. If the enzyme's hand lands on a dime or penny, then they can open their eyes to find two dimes and two pennies on the ground, stack them in alternating order (with the dime on top) and bring them from the ground to the table by standing up fully where the stack will remain. If the stack spills, it will not count. If the hand lands on empty carpet, they are to stand up empty handed. The enzyme is to repeat this task over and over until time is called. All of the stacking and placing will be done with a single hand (left or right is fine).

[Q: What is the hand called in an actual enzyme? A: Active site. Q: What are the pennies and dimes on the ground? A: Substrate. Q: What are the pennies and dimes stacked on the table? A: Product.]

One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 90s of enzyme activity. The data recorder will keep track of the results on the data sheet (attached). Bring your group's pennies and dimes from station to station. Leave nickels behind at stations 3 and 4.

Station 1 ~ Normal enzyme activity: This station follows the oral directions exactly but will be written out as well:

0. Start the station by dropping all pennies and dimes from the group onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a dime or a penny, open eyes and
     - Find two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 90s of enzyme activity. The data recorder will keep track of the results on the data sheet.

Station 2 ~ Catalyst-enhanced enzyme activity:

0. Start the station by dropping all pennies and dimes from the group onto the ground within the tape square.

1. One person in your group will be a catalyst called *analretentive*. This catalyst will stack dimes and pennies in the proper order as quickly as they are able but they cannot transport them to the table.

2. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a dime or a penny, open eyes and
     - Find two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

3. The extra person (if there are 3 in your group) or the person playing the role of *analretentive* in the group will be the time-keeper that keeps an eye on the clock and times 90s of enzyme activity. A data recorder will keep track of the results on the data sheet.

Stations 3 & 4 ~ Enzyme activity & competitive inhibition, low & high inhibitor concentrations:

(The only feature that differs between stations 3 and 4 is the number of nickels that are mixed in with the pennies and dimes. In the low inhibitor concentration (station 3), there are 20 nickels and in high inhibitor concentration (station 4), there are 50.)

Competitive inhibitors bind the actual active site of the enzyme to interfere with enzymatic activity.

0. Start by dropping all pennies and dimes from the group and the nickels at the station onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a coin that is a dime or a penny:
     - Continue to search for two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully

    - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
- If hand lands on a coin that is a nickel:
    - Bring the quarter from the ground to the table by standing up fully
    - Leave the quarter on the table
- If hand lands on empty carpet, stand upright
- Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 90s of enzyme activity. The data recorder will keep track of the results on the data sheet.

Station 5 ~ Enzyme activity & non-competitive inhibition:

Non-competitive inhibitors do not bind to the active site of an enzyme. Rather, they bind to another region of the enzyme, usually causing a conformational change at the active site. The result is that the enzyme either cannot bind the substrate initially or it cannot release the substrate once it becomes bound.

0. Start the station by dropping all pennies and dimes onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
    - Place a tennis ball in the active site and duct tape it in place
    - Start standing
    - Kneel with eyes closed. If hand lands on a dime or a penny, open eyes and
        - Find two dimes and two pennies on the ground
        - Stack them in alternating order (with the dime on top)
        - Bring them from the ground to the table by standing up fully
        - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
    - If hand lands on empty carpet, stand upright
    - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 90s of enzyme activity. The data recorder will keep track of the results on the data sheet.

Regroup and review:

I will plot the data on the board, asking the groups for their numbers. Q: Why was the task of the enzyme so specific? A: Enzymes are proteins with very specific tasks. Substrate orientation is very important for proper product formation. Q: What do they notice from this activity and the results on the board? A: Catalysts speed up enzymatic reactions while inhibitors slow them down or

prevent them entirely. Q: What are some of the differences between competitive and non-competitive inhibitors? A: Competitive inhibitors bind the actual active site of the enzyme to interfere with enzymatic activity. Non-competitive inhibitors do not bind to the active site of an enzyme. Rather, they bind to another region of the enzyme, usually causing a conformational change at the active site. The result is that the enzyme either cannot bind the substrate initially or it cannot release the substrate once it becomes bound.

Application:

Pass out worksheet and have students work in small groups of 2-3.

Enzymes & Inhibitors

Names of group members:

1. As a rate of reaction, enzyme velocity has units of product concentration (e.g., moles) over time:

$$\frac{\text{product concentration}}{\text{time}}$$

   What units could you use for the velocity of *depositinchase*?

2. Based on the setups at the stations, what ideas do you have for quantifying the substrate concentration for *depositinchase*?

3. Let's start with the free enzyme condition of station 1. Using the data on the board, plot the results (only from station 1) on the graph on the next page.

4. Using this graph, talk with your partner about what you expect the enzyme velocity to be at different substrate concentrations that you did not explicitly test at the stations. Draw a dotted line connecting your points along this expected curve of enzyme velocity as a function of substrate concentration.

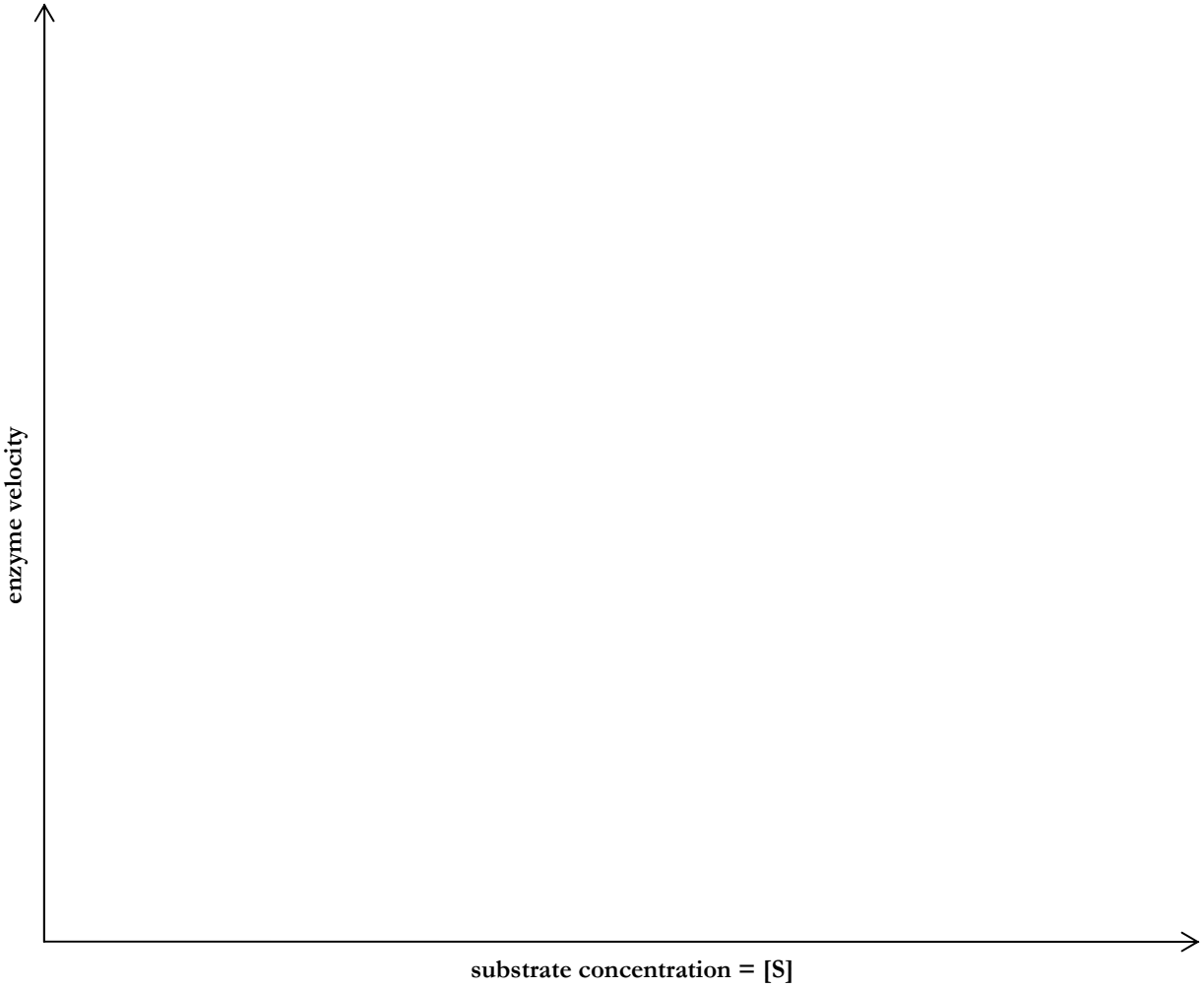
5. V_{max} is the maximum velocity of the enzyme. K_m is the amount of substrate required to reach half of the maximum velocity of the enzyme. Label these two quantities on the graph.

6. Now, using a different color pen, plot the data from station 3 on the plot. Again, draw a dotted line connecting the points along an expected curve for the substrate concentrations not explicitly tested. Label V_{max} and K_m.

7. Do the same thing for station 4, and then for station 5.

8. How are V_{max} and K_m affected by competitive versus non-competitive inhibitors? Why do you think this is?

9. Based on the data and your experience at the different stations, how do competitive and non-competitive inhibition differ?


enzyme velocity
substrate concentration = [S]

Station 1
Normal enzyme activity:

0. Start the station by dropping all of the pennies and dimes from your group onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a dime or a penny, open eyes and
     - Find two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 60s of enzyme activity. The data recorder will keep track of the results on the data sheet.

Station 2
Catalyst-enhanced enzyme activity:

0. Start the station by dropping all of the pennies and dimes from your group onto the ground within the tape square.

1. One person in your group will be a catalyst called *analretentive*. This catalyst will stack dimes and pennies in the proper order as quickly as they are able but they cannot transport them to the table.

2. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a dime or a penny, open eyes and
     - Find two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

3. The extra person (if there are 3 in your group) or the person playing the role of *analretentive* in the group will be the time-keeper that keeps an eye on the clock and times 60s of enzyme activity. A data recorder will keep track of the results on the data sheet.

Station 3

Enzyme activity & competitive inhibition, <u>low</u> inhibitor concentration:

0. Start by dropping all pennies and dimes from the group and the 20 nickels at the station onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a coin that is a dime or a penny:
     - Continue to search for two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on a coin that is a nickel:
     - Bring the quarter from the ground to the table by standing up fully
     - Leave the quarter on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 60s of enzyme activity. The data recorder will keep track of the results on the data sheet.

3. When finished with this station, return the 20 nickels to the table where you found them.

Station 4

Enzyme activity & competitive inhibition, <u>high</u> inhibitor concentration:

0. Start by dropping all pennies and dimes from the group and the 50 nickels at the station onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Start standing
   - Kneel with eyes closed
   - If hand lands on a coin that is a dime or a penny:
     - Continue to search for two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on a coin that is a nickel:
     - Bring the quarter from the ground to the table by standing up fully
     - Leave the quarter on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 60s of enzyme activity. The data recorder will keep track of the results on the data sheet.

3. When finished with this station, return the 50 nickels to the table where you found them.

Station 5
Enzyme activity & non-competitive inhibition:

0. Start the station by dropping all of the pennies and dimes from your group onto the ground within the tape square.

1. Choose one person in your group to be the enzyme called *depositinchase*.
   - Place a tennis ball in the hand serving as the active site and duct tape it in place
   - Another person in the group should hold the other hand of the enzyme (and do nothing else to help or hinder). This hand-holding is not meant to interfere with the activity of the enzyme
   - The enzyme should start standing
   - Kneel with eyes closed. If hand lands on a dime or a penny, open eyes and
     - Find two dimes and two pennies on the ground
     - Stack them in alternating order (with the dime on top)
     - Bring them from the ground to the table by standing up fully
     - Leave the stack on the table. If the stack spills, it will not count. *Depositinchase* cannot restack a spilled coin stack once on the table
   - If hand lands on empty carpet, stand upright
   - Repeat this task (of kneeling and searching) over and over until time is called

2. One (or two) person(s) in the group will be the time-keeper and data recorder. The time-keeper will keep an eye on the clock and time 60s of enzyme activity. The data recorder will keep track of the results on the data sheet.

Enzyme data sheet

Group members:

Group number:

Number of pennies:

Number of dimes:

For each station, write how many successful penny/dime stacks you were able to form within the 90s.

Station number	Number of successful penny/dime stacks formed
1	
2	
3	
4	
5	