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Activity

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Approaching a Conceptual Understanding of Enzyme Kinetics and Inhibition: Development of an Active Learning Inquiry Activity for Prehealth and Nonscience Majors

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Supporting Information

ABSTRACT: Presented is a guided inquiry activity designed to be conducted with prenursing students using an analogous system to help develop a conceptual understanding of factors impacting enzyme kinetics and the various types of enzyme inhibition. Pre- and postconceptual understanding evaluations and effectiveness of implementation surveys were given to the students ($N = 55$). The results indicate the students did slightly increase their conceptual understanding of the material after completing the activity. The result of the implementation survey provided insight into student-identified strengths and areas of improvement.

KEYWORDS: *First-Year Undergraduate/General, Biochemistry, Curriculum, Laboratory Instruction, Enzymes, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Kinetics, Catalysis*

Enzyme−substrate interactions and inhibition have been shown to be a great area of student conceptual difficulty but are hugely important when understanding enzyme kinetics.[1] One way in which research has shown to address such difficulties is through the use of analogies.[2−6] Analogies are commonly utilized to explain complex or complicated subject matter, such as enzyme−substrate interactions, in simplified, familiar terms, frequently by reducing the number of components or by making near-to-reality assumptions, respectively. In biochemistry, what can be directly observed are the effects of chemical and enzymatic reactions despite the inability to watch the molecules themselves, leading to inferences about the mechanics of molecular and atomic interaction. The purpose of this study was to implement an analogical laboratory activity that would strengthen students' conceptual understanding of biochemistry fundamentals such as enzyme kinetics and inhibition, essential for the intellectual growth of nonscience and prehealth majors.

To build a strong foundation of understanding, the activity was designed following the format of structured inquiry, which allows students to draw conclusions from the background information, procedure, and problems.[7] In addition, each segment of the activity follows the learning cycle where students must first *explore* a given model or data they collect, followed by answering a set of questions that help them *invent* the concept presented by the model, and finally they *apply* the newly developed concept.[8] These models of inquiry follow the constructivist framework where the teacher acts as a guide, and students are an active participant while using the information in the activity to construct their own mental model of enzyme kinetics and inhibition.[9] The three learning objectives addressed by the developed activity include:

(1) To distinguish competitive, uncompetitive, noncompetitive, and irreversible inhibition.
(2) To explain how temperature, pH, and substrate concentration affect enzyme function.
(3) To reproduce the graphs of the rate of enzyme-catalyzed reactions that are affected by temperature, pH, and substrate concentration.

There are currently only two other modeling activities that present enzyme kinetics and inhibition at a conceptual level appropriate for nonscience majors through use of common supplies (e.g., beans, nuts, bolts) to represent substrates and inhibitors with the student representing the enzyme.[10,11] The first exercise only focuses on the impact of changing substrate concentration and also sacrifices accurate representation of fundamental concepts of enzyme function and mass action of molecules by the removal of beans from a bag disregarding the unrealistic condition of removing all the beans from the bag and continually concentrating the remaining beans at the bottom of the bag, never increasing the difficulty of finding fewer beans.[10] The activity using nuts and bolts to represent the substrates goes further than simply modeling substrate

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concentration, as it also addresses competitive and noncompetitive inhibition.[11] Others have adapted the activity to show the actual calculations of the model and also adding product inhibition and the impact of chirality on enzyme specificity.[12,13] While the proposed activity presented here does diverge in some respects from the reality of enzyme, substrate, and inhibitor interactions, the simplifications do not detract from the learning objectives or misrepresent key concepts, and it may be expanded to provide a more in depth exercise for science majors. Moreover, the proposed activity models additional processes such as the impact of pH on enzyme activity and uncompetitive inhibition that are absent from previously developed activities.

THE ACTIVITY

Materials

The student handout begins with introductory information discussing enzymes as catalysts because this is often the first introduction to the content for the students in the laboratory. The activity then moves students through a series of six models where students work in pairs to collect data, explore a concept, and then answer questions related to the data to invent the concept of that model. Each student pair is provided the following materials: a 50 mL Falcon tube with screw-cap including a slit sized to allow passage of pennies but not larger coins, blindfold, stopwatch, 80 pennies, two knitted glove fingers, and 20 nickels. The knitted glove fingers are simply individual fingers that have been cut from the palm section of the glove.

Methodology

Model 1 introduces the model to students by investigating the effects of decreasing the number of pennies on the rate of reaction. Students begin with 80 pennies randomly spread over a 2′ × 2′ space on their lab bench. One partner keeps time while the other partner remains blindfolded throughout the activity and has 30 s to get as many pennies into the tube while having to lift and drop every other penny they pick up with only their thumb and index finger. Students are instructed to be as consistent as possible in picking up the pennies. To focus on the consistency, a practice round is built into the activity. The same instructions are followed when the number of pennies is decreased to 40, 20, 10, and 5. While exploring the data that are collected, students are asked to determine how objects and actions correspond to the enzymatic reaction that is found in Figure 1. This is a key distinction between this activity and previously developed activities as the students are specifically asked to correlate the base (e.g., the activity) with the target (e.g., the enzymatic reaction). This has shown in previous research to be an essential part of effective use of analogies in developing understanding.[3,4] Students come to the conclusion that the blindfolded partner with tube represents the enzyme, pennies represent substrates, and pennies in the tube represent products. Actions such as picking up and dropping the penny are interpreted to represent binding and release of substrate, respectively, and placing the penny in the tube is conversion of substrate to product. The connection between the model and the enzymatic reaction is an important point of the activity because, in subsequent models, new variables are added to the reaction. Therefore, instructors should stop students at this point and discuss the answers with the class as a whole. A common response from students is the initial impression that the tube alone represents the enzyme. While an additional explanation of cofactors, apoenzymes, and holoenzymes could be modeled via the tube in a more advanced course, a simpler clarification for nonscience majors is to ask the student if the tube alone could perform the "reaction" of converting substrate to product. Students then generally arrive at the conclusion that the blindfolded person along with the tube represents a functional enzyme.

$$S + E \leftrightarrows ES \rightarrow E + P$$

Figure 1. Enzymatic reaction used as the target in Model 1.

A Michaelis−Menten plot is the focus of Model 2. Students investigate the model and label where V_{max}, 1/2 V_{max}, and K_M are in the graph. If the concepts of maximal velocity and binding affinity have not been previously conferred, these can be gleaned from a brief discussion of a rectangular hyperbolic curve prior to beginning the exercise. Students are instructed to plot graphs based on the data collected from Model 1 and state differences and similarities between their graph and the Michaelis−Menten plot presented in the Model. It is critical that students grasp the concept of substrate saturation resulting in a finite maximal velocity prior to investigating the effects of inhibitors.

Model 3 investigates the effect of pH on the rate of reaction with the use of two knitted glove fingers. Students collect data similar to Model 1 starting with 80 pennies spread out on the lab bench. The difference is that the blindfolded partner completes the first round with only a glove-covered thumb. Once this round is complete, a second round is performed with both the thumb and index finger covered. Because the blindfolded partner can only use thumb and index finger to pick up pennies, these fingers represent amino acids in the active site responsible for substantive substrate binding. Instructors should emphasize here that models, no matter how well designed, will have limitations. Students find that decreasing (or increasing) the pH of the system by "protonating" (or "deprotonating") their thumb decreases the rate of the reaction as fewer pennies are placed into the tube. Further deviations from optimal pH by "protonating" (or "deprotonating") both the thumb and index finger cause a further decrease in reaction rate to the point of abolition of binding. Students may not immediately correlate changes in protonation state of the enzyme with the glove fingers altering the ability to pick up pennies. A limitation of the glove fingers serving as protonation or deprotonation and something instructors need to be cognizant of and address with students is that pH impacts all acidic and basic amino acid side chains and not just those in the active site causing denaturation of the enzyme structure. In addition, not all enzymes have an acidic or basic amino acid in the active site, and therefore, binding would not necessarily be effected. This glove finger model could also serve as an analogy to other incremental detrimental changes to the enzyme, such as increases in solvent ionic strength or polarity of the solvent, each extreme resulting in enzyme denaturation. At the end of the exercise, students are asked to propose a macroscopic addition to this activity modeling the effects of temperature, with explicit instructions to not *use* temperature, but to *model* it, deterring the most common response of exposing the blindfolded student to warmer or cooler conditions in which to perform the activity.

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The three types of reversible inhibition (competitive, uncompetitive, and noncompetitive) are explored in Models 4−6. The types are not explicitly linked to each model, as one part of the procedure is for students to tie the models with each type of reversible inhibition through association with mode of inhibitor binding, either to free enzyme alone, to enzyme−substrate complex, or to either. Student pairs are encouraged to proceed through the numerous iterations in Models 4−6 in a random pattern so as to remove bias in results. This jumbled progression is more important when used in advanced biochemistry laboratories, as students have a stronger understanding of what patterns the data should embody. Model 4 investigates the effects of competitive inhibitors on the rate of reaction. The effects are observed by conducting another round similar to Model 1 with the addition of 10 nickels. A second round increases the inhibitor concentration by adding an additional 10 nickels to the system. Though the blindfolded player can tell they are holding a nickel, they must still attempt to place it into the tube. The nickel competes with the substrate for binding to the enzyme, but product is not formed as the nickel does not fit in the tube.

Model 5 investigates uncompetitive inhibition. In this model, the partner who is timekeeping also serves as the uncompetitive inhibitor by grabbing the hand of the blindfolded partner only when in possession of a penny, temporarily disallowing addition of the penny to the tube. Students observe how the rate of reaction changes due to the inhibitor. The students again start with 80 pennies and go through a full round with the added uncompetitive inhibitor. The timekeeper grabs the blindfolded partner's hand for 1−2 s a consistent number of times (at least four) at unpredictable intervals within the 30 s for each assay.

Finally, noncompetitive inhibition is investigated in Model 6. The timekeeper is again acting as the inhibitor, but in this case they may grab the hand of the blindfolded partner irrespective of penny possession, not allowing the penny to be added to the tube. The protocol set forth in Model 5 is again followed.

IMPLEMENTATION

The activity was piloted during both the spring (N = 55) and summer (N = 25) 2014 semesters in the laboratory of a one semester introduction of organic and biochemistry course for nonscience and prehealth (dietetics and nursing) students. The activity is designed to be completed in a single, 3-h period. In addition, the activity has been conducted in the lecture for the same population of students (N = 60) over two 50-min periods. On the first day of implementation in lecture, the teacher discusses Models 1−3: the impact of temperature, pH, and substrate concentration. The remaining three models on inhibition are discussed in the second lecture. A student assistant was present during implementation in both lecture and lab to aid the instructor in getting around to the different groups to answer questions and ensure students are on task; however, in smaller classes of 30−40 students, the activity has been successfully accomplished in class without the aid of a teaching assistant.

RESULTS

Typical Student Data

The plots created by students from the data in Models 1 and 3 are shown in Figure 2, panel A. These show the impact of substrate concentration and pH on the rate of reaction, respectively. Both plots show a similar trend of increasing rate of reaction upon increase in concentration of pennies, each appearing to approach an upper limit asymptotically, the maximal rate of reaction, V_{max}. Figure 2, panel B shows the graphs of typical data from students in Models 4−6. Within each model, students compare the impact of competitive, uncompetitive, and noncompetitive inhibitors to that of the uninhibited data collected in Model 1. This helps students to see how V_{max} and K_M change with each respective inhibitor.

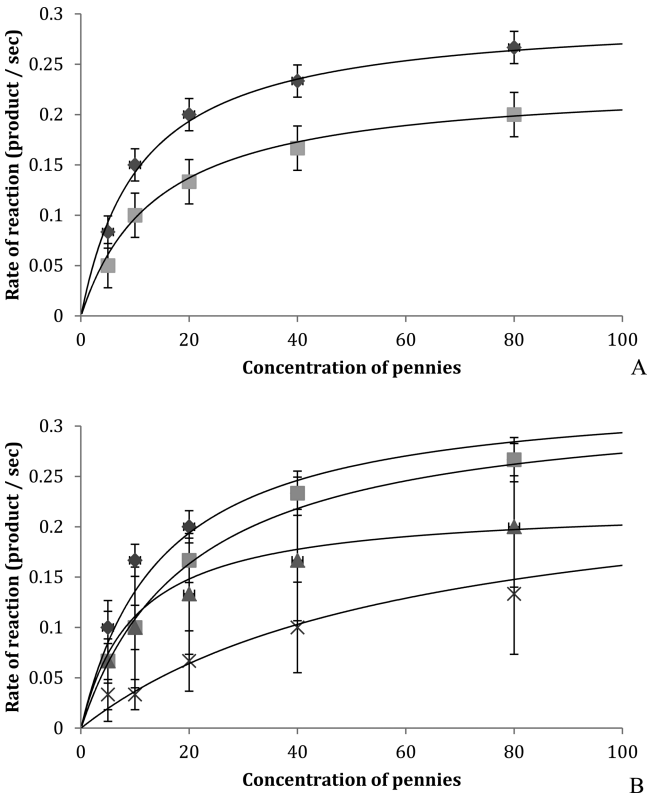

Figure 2. (A) Typical data (N = 4) from Models 1 and 3 with best fit hyperbolic curves. The impact of a deviation from optimal pH on rate of enzymatic reaction (squares, Model 3) compared to a reaction at optimal pH (diamonds, Model 1). (B) Typical data (N = 4) from Models 1, 4, 5, and 6 with best fit hyperbolic curves. The impact of competitive (squares, Model 4), uncompetitive (triangles, Model 5), and noncompetitive (crosses, Model 6) inhibitors compared to an uninhibited reaction (diamonds, Model 1). Error bars are standard deviations and generally not required in the exercise, shown here to illustrate the level of difficulty in obtaining ideal results.

Impact on Student Learning

To determine the impact on students' conceptual understanding of enzyme kinetics and inhibition, a six-item short answer survey (see Supporting Information) was administered to the students before and after the activity in the laboratory. In the spring 2014 laboratory sections (N = 55), 65% of students' increased their score on the assessment by an average of 0.89 points. Of the remaining students, 31% of students' scores did not change, and only three students had a decrease in score by no more than 1 point.

A separate survey was administered evaluating the activity itself. Students indicated that they had a good understanding of enzyme kinetics and inhibition and that the activity was fun. However, they also indicated that the experiment was too long as it occupied the entire 3 h laboratory time as opposed to the other experiments, which only require students to collect data over half the allotted time and then leave to answer questions at

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home. This experiment directs them to answer questions as they go to facilitate thinking about the data as they are collected.

CONCLUSION

The primary goal of this project was to create a guided-inquiry activity that provided a concrete analogy for enzyme kinetics and inhibition principles to allow students to gain a more conceptual understanding. The activity succeeded in providing an enjoyable modeling experience of topics that typically are taught through traditional kinetics experiments. Students not only felt that they had grasped the concepts, but also a large majority of students did slightly improve their understanding of enzyme kinetics and inhibition.

Additional models could be added to address irreversible inhibition using rubber bands irreversibly binding the thumb and forefinger together. This activity has been designed for nonscience and prehealth (dietetics and nursing) students; however, it could easily be adapted for high school classrooms and for biochemistry students. For instance, high school classes could focus primarily on Models 1−3 in a single 60−90 min session. Alternatively, biochemistry students have created Lineweaver−Burk plots to show the impacts on K_M and V_{max} or use quarters and half-dollars to show the effect of rapid ligand recognition versus potency of inhibition. In the latter, the use of larger diameter coins and the allowance of the blindfolded student to evaluate the coin prior to attempting to place it in the slit more closely models the behavior of enzymes selecting for specific substrates or embodies the variation in inhibitory effectiveness for competitive inhibitors. Finally, changing the lid of the tube for ones with different size slits could simulate site-directed mutations designed to alter substrate specificity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00562.

Answers to the assigned questions, student handout, and the surveys used to evaluate the effectiveness of the activity (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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