Advances in Biology Laboratory Education
Publication of the Association for Biology Laboratory Education
Volume 41, Article 44, 2020

A Simple, Quantitative Peroxidase Assay Demonstrating Enzyme Inhibition with L-cysteine

Michael Mitsch[1] and Bryan White[2]

[1]Okanagan College, Department of Biology, 7000 College Way, Vernon BC V1B 2N5 CAN
[2]Okanagan College, Department of Biology, 2552 10th Ave NE, Salmon Arm BC V1E 2S4 CAN
(**mmitsch@okanagan.bc.ca; bgwhite@okanagan.bc.ca**)

We present a simple enzyme assay using the robust horseradish peroxidase and the indicator guaiacol which, when oxidized to form tetraguaiacol, can be detected and quantified using a visible spectrophotometer at 470nm. This assay uses buffers ($NaPO_4$), substrates (H_2O_2) and equipment already widely used and available in most first-year laboratories and is a cost-effective addition to current experiments. In addition to determining and comparing specific activity under a variety of traditional variables (pH, temperature) participants will use L-cysteine, reported in the literature to be a non-competitive inhibitor of peroxidase. L-cysteine demonstrates a unique inhibition pattern in our assay losing its inhibitory characteristics in a concentration and time dependent manner. This gives the instructor a range of options to demonstrate enzyme inhibition as well as an opportunity for investigations and self-directed experimentation by the students with a more challenging result to consider and analyze.

Keywords: biochemistry, peroxidase, enzyme assay, guaiacol, inhibition, L-cysteine, inquiry-based learning

Introduction

We identified a need for a simple, economical quantitative assay to assist students in understanding enzyme activity. Most quantifiable enzyme assays utilize cofactor reduction and observation using UV spectrophotometers which is beyond the budget of most first-year biology classes. In the past oxidizable indicators such as guaiacol (Maehly and Chance, 1954, Koduri and Tien, 1995, Doerge *et al*. 1997) have been used in conjunction with the robust horse radish peroxidase and we sought to revive this old technique. We also wanted to modify it to include an experiment beyond traditional enzyme inhibition with abiotic factors such as temperature or pH which simply reduce or eliminate enzyme function. Instead, we wanted to explore enzyme inhibition from a cellular or metabolic perspective by including relevant enzyme inhibitors that were part of a negative feedback loop or substrate analogs which would reduce, but not eliminate enzyme function.

Inhibitors of Peroxidase

A brief literature search (Peroxidase Inhibitors, Sigma Bulletin) identified several potential inhibitors but some had no obvious cellular or metabolic significance (2-imidazolidinethione purum,sodium azide) and some would be problematic in an undergraduate laboratory (sodium cyanide). A potential substrate analog sodium orthovanadate was tested but showed no inhibition in our assay and at high concentrations produced a pigment which made spectrophotometric readings unreliable. We determined that L-cysteine may be a suitable candidate as it had been reported to inhibit peroxidase activity from avocado by 93% and reduced the V_{max} indicative of a non-competitive inhibitor (Rojas-Reyes *et al*. 2014). In our assay L-cysteine demonstrates a unique inhibition pattern (Fig.2) losing its inhibitory characteristics in a concentration and time dependent manner. Our initial hypothesis was that the L-cysteine was being oxidized to the dimer L-cystine and as its concentration was reduced it lost its inhibitive properties. This possibility is confusing as L-cystine is also listed as an inhibitor of peroxidases (Peroxidase Inhibitors, Sigma Bulletin). It is also possible that the oxidation of L-cysteine to L-cystine competes with the oxidation of guaiacol to tetraguaiacol thus reducing its formation resulting in the lack of absorbance at A_{470nm}. Another possibility is that peroxidase is known to mimic catalase in producing O_2 (Baker *et al*. 2000, Hiner *et al*. 2001) and we theorize that this O_2 could interact with L-cysteine converting it to cysteine sulfinic acid thus losing its inhibitory characteristics during the assay. Recently peroxidase has been used to modify, map and quantify

cysteine residues in proteins (Toledo *et al*. 2011) so its interaction with L-cysteine is not surprising. Despite no definitive cause to the inhibition, the assay we have adopted (Purdue University Instrument Van Project) and modified is an easy, fast (10 minutes per assay), reliable, and cheap experiment with most equipment (visible spectrophotometers, test tubes, micropipetters) and reagents (H_2O_2, $NaPO_4$) already available in most undergraduate labs with only the indicator guaiacol being an addition to inventory. The experiment as we have outlined it can be completed in a three-hour laboratory period and expands on traditional qualitative enzyme assays done at the first year/introductory level. The students who have conducted this experiment have found the explanation for the inhibition pattern to be much more challenging to formulate, explain and present. This leads to an enhanced appreciation for enzymes, assays, and generating theories to explain data in either question format or a complete laboratory report.

Future Possibilities

This assay lends itself to further development (kinetics and zymograms) and may be expanded to fulfill the needs of more sophisticated upper level biochemistry courses.

Student Outline

Objectives

- Use visible spectrophotometers and excel spreadsheets to calculate and compare enzyme activity
- Understand abiotic effects on enzyme activity
- Theorize how L-cysteine may affect peroxidase activity

Introduction

Living cells carry out a multitude of chemical reactions necessary for the continuance of life. Many of these reactions normally occur slowly or not at all at temperatures typical for cells. Enzymes serve as biological **catalysts** that decrease the amount of energy required to initiate a reaction, thereby enabling the reaction to proceed readily at moderate temperatures. The energy required is called the **activation energy**, so an enzyme lowers the activation energy for a reaction. The specific molecule or molecules that are acted upon by an enzyme are called **substrates**. In an enzyme-catalyzed reaction, the enzyme combines physically with the substrate(s) to form an **enzyme-substrate complex**; the act of combining may promote the reaction by distorting particular chemical bonds in the substrate making them easier to break, or it may bring specific areas of different substrate molecules close to each other so that a bond between them can be formed more readily. The products formed are then released from the surface of the enzyme. This process may be summarized by the following equation:

$$Enzyme + Substrate(s) \xrightarrow{yields} (Enzyme\ Substrate\ Complex) \xrightarrow{yields} Product(s) + Enzyme$$

Note that the enzyme molecule is released unchanged and can react with additional substrate molecules. It is important to recognize, however, that enzymes are not possessed of some mystical power to promote chemical reactions that otherwise would not occur. Enzymes do not change the thermodynamic equilibrium of a reaction; they merely increase the rate of reaction and thus speed the approach to chemical equilibrium. Consequently, they speed the reverse as well as the forward reaction.

Structurally enzymes consist of a long chain of amino acids and the amino acids interact with each other to form a complex coiled or folded structure that has a specific 3-dimensional shape. Each different enzyme has a unique shape and can catalyze only one particular reaction or class of reaction. Anything that disrupts the shape of an enzyme can alter its catalytic effectiveness. Usually an enzyme operates best at a particular temperature and pH. Higher temperatures generally speed up chemical reactions but for enzyme-catalyzed reactions, when the temperature becomes too great, the hydrogen bonds, which help to stabilize the enzyme's shape, are broken. When this occurs, the enzyme is said to be **denatured**. Changes in pH can also denature an enzyme and reduce its effectiveness. In addition to environmental factors such as pH or temperature compounds with similar structures to the substrate may compete for binding at the active site even though these compounds cannot be converted to a product by the enzyme resulting in **competitive inhibition** of the enzyme. These compounds are referred to as substrate analogs. Alternatively, some compounds may bind the enzyme away from the active site but their binding changes the three-dimensional shape of the active site of the enzyme (**allosteric regulation**) reducing or eliminating its activity resulting in non-competitive inhibition of the enzyme. In this experiment we will be testing **horseradish peroxidase** an enzyme responsible for catalyzing the oxidation of various organic substrates with hydrogen peroxide and its response to temperature, pH, and L-cysteine. You should compare the structure of the substrate H_2O_2 and L-cysteine and consider the fact that L-cysteine can be oxidized to L-cystine or absorb O_2 to form cysteine sulfinic acid (Fig. 1).

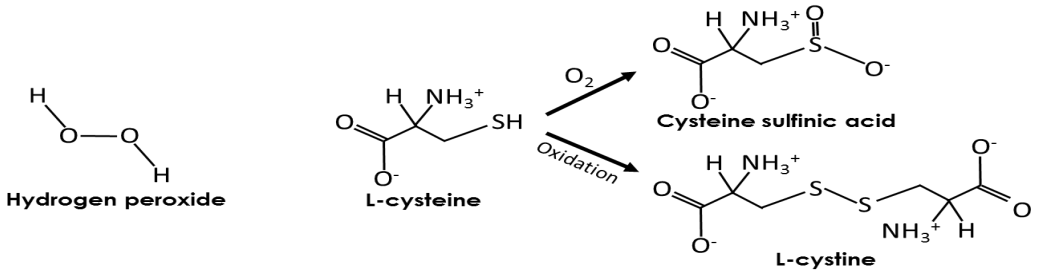
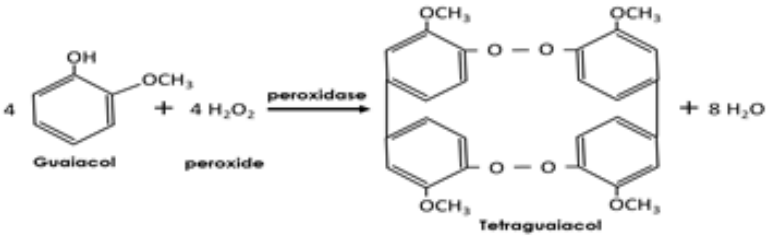


Figure 1. Comparison of structure between hydrogen peroxide and L-cysteine and the effect of O_2 and oxidation on L-cysteine to produce cysteine sulfinic acid or L-cystine

Methods and Data Collection

Part A: Detection and Quantification of Peroxidase Activity by Oxidation of Guaiacol

The peroxidase reaction can be monitored by using the compound guaiacol (2-methyoxyphenol) which can be oxidized to produce a brown product (tetraguaiacol) that can be detected and quantified by a spectrophotometer at 470nm wavelength:



A standard reaction (Purdue University Instrument Van Project) will contain:

10mM H_2O_2 substrate	2mL
25mM guaiacol indicator	1mL
0.1M $NaPO_4$ buffer pH5	2mL
Peroxidase 0.02 μg/μL	10μL

To simplify the set up for you the substrate and indicator guaiacol have been combined for you as 3mL substrate tubes (2mL, 10mM H_2O_2 and 1mL guaiacol). You will need:

- eight substrate test tubes
- six 0.1M $NaPO_4$ buffer pH5 test tubes

Part B: Determination of Peroxidase Activity (Positive Control)

1. Turn on the spectrophotometer and let it warm up. After self-calibration and start up set the wavelength to 470nm.
2. Set up a blank tube to zero the spectrophotometer. This will contain all the material except the peroxidase enzyme. Mix a 0.1M $NaPO_4$ buffer pH5 tube to a 3mL substrate tube. Wipe the tube with a Kimwipe® and insert the blank into the spectrophotometer and press the zero absorbance/100% transmittance. Keep this tube to re-blank the spectrophotometer at the beginning of each experimental run.
3. Set up the reaction tube by adding the 10μL of peroxidase enzyme to a 2mL 0.1M $NaPO_4$ pH5 buffer tube then transfer this to a 3mL substrate tube and mix thoroughly. Once the tubes are combined the reaction will begin. Wipe the tube with a Kimwipe® and immediately insert the tube into the spectrophotometer and record the absorbance at one-minute intervals for a total of 10 minutes. Record the absorbance values in Table 1 (positive control) or directly onto an excel spreadsheet.

Part C: Effect of Temperature on Peroxidase Activity

1. Place two substrate tubes in a test tube rack at room temperature on your bench.
2. Obtain two $NaPO_4$ buffer pH5 tubes and add 10μL of peroxidase enzyme to each tube. Incubate one tube at 4ºC and the second at 65ºC for 15 minutes.
3. Use the blank you produced to zero the spectrophotometer.
4. After incubation add the 2mL of incubated enzyme + buffer to the substrate reaction tube (this will be time zero) and mix thoroughly. Wipe the tube with a Kimwipe® and immediately insert the tube into the spectrophotometer and record the absorbance at one-minute intervals for a total of 10 minutes. Record the absorbance values in Table 1 (4ºC and 65ºC) or directly onto an excel spreadsheet.
5. Repeat with the second temperature sample. By staggering the start times (time zero and time 30 seconds) you should be able to measure both samples over the same time course.

Part D: Effect of pH on Peroxidase Activity

1. Place two substrate tubes in a test tube rack at room temperature on your bench.
2. Obtain one $NaPO_4$ buffer pH4 tube and one $NaPO_4$ buffer pH7 tube and add 10μL of peroxidase enzyme to each tube. Incubate the tubes at room temperature for 15 minutes.

3. Use the blank you produced to zero the spectrophotometer.
4. After incubation add the 2mL of incubated enzyme + buffer to the substrate reaction tube (this will be time zero) and mix thoroughly. Wipe the tube with a Kimwipe® and immediately insert the tube into the spectrophotometer and record the absorbance at one-minute intervals for a total of 10 minutes. Record the absorbance values in Table 1 (pH4 and pH7) or directly onto an excel spreadsheet.
5. Repeat with the second pH sample. By staggering the start times (time zero and time 30 seconds) you should be able to measure both samples over the same time course.

Part E: Effect of L-cysteine on Peroxidase Activity

1. Place two substrate tubes in a test tube rack at room temperature on your bench.
2. Obtain two $NaPO_4$ buffer pH5 tubes and add 10µL of peroxidase enzyme to each tube. Add 5µL 0.05M of L-cysteine to one tube and 10µL of 0.05M L-cysteine to the second tube. Incubate the tubes at room temperature for 15 minutes.
3. Use the blank you produced to zero the spectrophotometer.
4. After incubation add the 2mL of incubated enzyme + L-cysteine + buffer to the substrate reaction tube (this will be time zero) and mix thoroughly. Wipe the tube with a Kimwipe® and immediately insert the tube into the spectrophotometer and record the absorbance at one-minute intervals for a total of 10 minutes. Record the absorbance values in Table 1 (L-cysteine) or directly onto an excel spreadsheet.
5. Repeat with the second L-cysteine sample. By staggering the start times (time zero and time 30 seconds) you should be able to measure both samples over the same time course.

Table 1. Peroxidase Activity.

Time (minutes)	**Positive control Absorbance (at 470nm)**	**Absorbance (at 470nm) at 4ºC**	**Absorbance (at 470nm) at 65ºC**	**Absorbance (at 470nm) at pH 4**	**Absorbance (at 470nm) at pH 7**	**Absorbance (at 470nm) with 5µL 0.05M L-cysteine**	**Absorbance (at 470nm) with 10µL 0.05M L-cysteine**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Data Analysis

To analyze the data you are collecting today, you will need to calculate **initial velocity** $(v)_0$ of the enzyme. This initial rate of reaction can be expressed simply as a change in absorbance per unit of time: for tetraguaiacol formation this would be ΔA_{470nm}/min which corresponds to the slope on your absorbance vs. time graph.

To obtain the slopes of your data using excel:

1. Select/highlight your data
2. Select insert and under charts insert a scatter plot
3. A graph should appear on your spreadsheet. Select a data set and using the right mouse button and add a linear trend line and make sure to select display equation on chart so that you will be able to see the slope value
4. Use this value for your calculations
5. For some of your graphs only a portion may have a positive slope. You can calculate a slope from this region by typing (in the textbox at the top of excel) "=slope (select y coordinates, select x coordinates)". The slope calculation will now appear in the selected cell(s)

It is more useful to express the rate in terms of the amount of tetraguaiacol formed per unit time. This can be calculated as nanomoles per minute (nmol/min) or micromoles per minute (µmol/min). The absorbance value at 470nm can be converted to an actual concentration using the Beer-Lambert law:

$$C = \frac{A}{\varepsilon (l)}$$

C is the concentration of the absorbing material (tetraguaiacol, in our case), **A** is the absorbance measured at 470nm, and **l** is the length of the light path (1.0 cm for our spectrophotometers). The extinction coefficient (ε) for tetraguaiacol at 470nm is 26.6 mM^{-1} cm^{-1} (Sigma Guaiacol data sheet). Using this value and the Beer-Lambert equation, we can convert an absorbance reading into an actual amount of product formed. Assume your slope was 0.5/min:

$$\text{Concentration} = \frac{0.5/\text{min}}{(26.6/\text{mM}) * 1\text{cm}} = 0.019\ \text{mM/min} = 19\ \mu\text{M/min}$$

The units, μM, are micromoles per liter, so a concentration of 19 μM, will be 19 micromoles per liter, or 19 nanomoles/mL. The reaction occurred in a total volume of 5.01 mL, so to determine the total amount of tetraguaiacol product formed by the enzyme in one minute in the reaction instead of a concentration:

19 nmoles/ml/min x 5.01ml = 95.19 nmoles/min

In summary, you'll need to follow three steps to get from your graph of absorbance vs. time to the amount of product formed per minute:

1. Determine the slope of the straight portion of your curve (units are ΔA_{470}/min).
2. Convert this slope from ΔA_{470}/min to μM/min by using the Beer-Lambert equation.
3. Convert this concentration to the total amount of product formed in the cuvette by taking the total volume into account. Report your result in terms of nmol/min.

We can also determine the product formation rate as a function of the amount of enzyme protein present or **specific activity**. To calculate this, divide the rate of product formation x reaction volume divided by the amount of enzyme used in the reaction (enzyme concentration x volume used in the reaction):

$$\text{Specific activity} = \left(\frac{\text{Slope of } 0.5/\text{min}}{(26.6/\text{mM}) * 1\text{cm}}\right) \times 5.01\text{ml} \Big/ (0.02\text{mg/ml} \times 0.01\text{ml used in reaction})$$

=471 nmoles/min/mg protein

Discussion

1. Submit your graph of enzyme activity for peroxidase as if it were part of a formal lab: make sure it is properly formatted with a descriptive title.
2. Complete and submit table of peroxidase specific activity as if it were part of a formal lab: make sure it is properly formatted with descriptive title.
3. Describe the profile of the graph produced from exposing the peroxidase to the different concentrations and volumes of L-cysteine. Propose a hypothesis as to why the graph appears the way it does (hint: consider L-cysteine's shape and the fact it can absorb O2 or be oxidized). What type of inhibition does L-cysteine have on peroxidase? Explain.

Cited References

Purdue University Instrument Van Project: Factors Affecting Enzyme Activity. Obtained from: https://www.chem.purdue.edu/teacher/table_of_contents/Spectronic%20Educator/ENZYME.pdf

Sigma Guaiacol data sheet. Obtained from: https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Product_Information_Sheet/2/g5502pis.pdf

Table 2. Recording of slope (ΔA_{470nm}/min), determination of nmoles of product formed in one minute and specific activity (nmoles/min/mg protein) of peroxidase.

Enzyme and buffer tube	Substrate reaction tube	Incubation conditions	Slope (ΔA_{470nm}/min)	nmoles of product formed/minute	Specific activity (nmoles/min/mg protein)
2mL $NaPO_4$ buffer pH5 + 10µL peroxidase	2mL H_2O_2 + 1mL guaiacol	Positive control			
2mL $NaPO_4$ buffer pH5 + 10µL peroxidase	2mL H_2O_2 + 1mL guaiacol	4ºC for 15 minutes			
2mL $NaPO_4$ buffer pH5 + 10µL peroxidase	2mL H_2O_2 + 1mL guaiacol	65ºC for 15 minutes			
2mL $NaPO_4$ buffer pH4 + 10µL peroxidase	2mL H_2O_2 + 1mL guaiacol	22ºC for 15 minutes			
2mL $NaPO_4$ buffer pH7 + 10µL peroxidase	2mL H_2O_2 + 1mL guaiacol	22ºC for 15 minutes			
2mL $NaPO_4$ buffer pH5 + 10µL peroxidase + 5µL 0.05M L-cysteine	2mL H_2O_2 + 1mL guaiacol	22ºC for 15 minutes			
2mL $NaPO_4$ buffer pH5 + 10µL peroxidase + 10µL 0.05M L-cysteine	2mL H_2O_2 + 1mL guaiacol	22ºC for 15 minutes			

Materials

Equipment and Supplies:
A computer with Microsoft Excel®
Visible wavelength spectrophotometer (Thermospectronic Genesys 20)
13mm Test tubes and snap caps
Water bath(s)
Volumetric dispensers or pipettes
20 µL Micropipetters
Lab coats
Safety glasses

Solutions:
A 0.1M $NaPO_4$ buffer:
1M stocks of mono (Na) and dibasic (Na_2): 13.8g monobasic in 100ml H_2O, 14.2g dibasic in 100ml H_2O.

Make 0.1M stock at pH5: 2.1mL 1M dibasic + 97.9mL monobasic to a final volume of 1L.

Make 0.1M stock at pH4: 1mL 1M dibasic + 99mL monobasic to a final volume of 1L.

Make 0.1M stock at pH7: 57.7mL 1M dibasic + 42.3mL monobasic to a final volume of 1L.

Horse radish peroxidase (Fisher/alfa aesar j60026): stock is: 0.0014g (1.4mg) in 70ml $NaPO_4$ buffer pH5, gives final concentration of 0.02mg/mL or 0.02µg/µL, can be aliquoted and stored at -20ºC. Use 10µL per reaction.

0.05M L-cysteine HCl Monohydrate (Fisher/USB 14035): 0.01g of powdered L-cysteine dissolved in 1.138mL of H_2O. Affect starts at ~5µL of stock solution (5µL = final concentration of 50µM L-cysteine in 5mL reaction volume), 60µL will stop the reaction for the full 10min.

25mM guaiacol (Fisher/ Acros Organics 120192500): 0.28mL 100% guaiacol + 100mL isopropanol. 10mM H_2O_2 substrate (Fisher 7722-84-1): 0.074ml 30% H_2O_2 + 100mL H_2O.

Required volumes of solutions for all experiments listed above:

Reagent	Per group
10mM H_2O_2	16mL
Peroxidase (0.02µg/µL)	70µL
L-cysteine	15µL
25mM guaiacol	8mL
0.1M $NaPO_4$ pH5	12mL
0.1M $NaPO_4$ pH4	2mL
0.1M $NaPO_4$ pH7	2mL

Notes for the Instructor

We have found it is better to set up $NaPO_4$ buffer tubes (2mL) and separate guaiacol and H_2O_2 (3mL) substrate tubes into pre aliquoted 13mm clear test tubes. Students just add the peroxidase enzyme to the buffer tube and incubate for required time/condition then add to substrate tube (3mL). By combining these tubes and vigorously mixing the solutions together (5mL final volume) they are ready for spectrophotometer reading/time course. The assays can be done individually or staggered if time is limited. The use of different colored snap caps for each group of tubes (buffer and substrate) eases identification, limits mistakes and also reduces the amount of guaiacol odor released. Guaiacol is very pungent with the aroma of burnt wood or coffee, ventilation during and after the lab is recommended. We have found that an excel spreadsheet file already set up to display the graph and calculate the slope from the predicted linear portion of the graph is useful for students to visualize the results and analyze the data (Fig. 2).

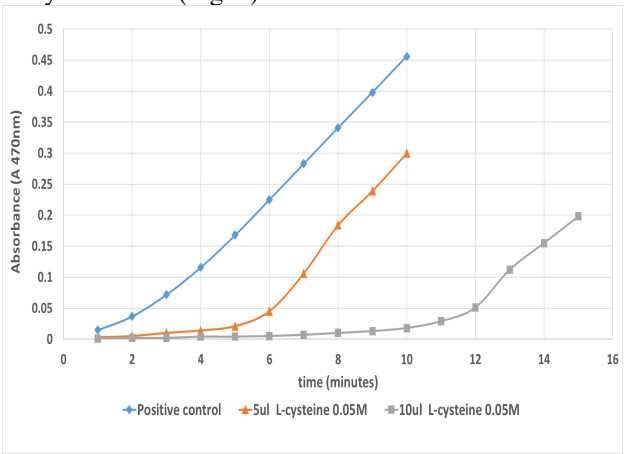


Figure 2. Typical peroxidase assay results with varying amounts of L-cysteine showing inhibition in time and concentration dependent manner.

Cited References

Baker CJ, Deahl K, Domek J, Orlandi EW. 2000. Scavenging of H_2O_2 and production of oxygen by horseradish peroxidase. Arch Biochem Biophys. 382(2):232-7.

Doerge DR, Divi RL, Churchwell MI. 1997. Identification of the colored guaiacol oxidation product produced by Peroxidases. Anal Biochem 250(1):10-17.

Hiner AN, Hernández-Ruiz J, Williams GA, Arnao MB, García-Cánovas F, Acosta M. 2001. Catalase-like oxygen production by horseradish peroxidase must predominantly be an enzyme-catalyzed reaction. Arch Biochem Biophys. 392(2):295-302.

Koduri, RS and Tien, M. 1995. Oxidation of Guaiacol by Lignin Peroxidase. J. Biol. Chem. 270: 22254–22258.

Maehly AC, and Chance B. 1954. Methods of Biochemical Analysis, New York: Interscience. 357–424p

Peroxidase inhibitors, Sigma bulletin. Obtained from: https://www.sigmaaldrich.com/life-science/biochemicals/biochemical-products.html?TablePage=15821520

Purdue University Instrument Van Project: Factors Affecting Enzyme Activity. Obtained from: https://www.chem.purdue.edu/teacher/table_of_contents/Spectronic%20Educator/ENZYME.pdf

Rojas-Reyes JO, Robles-Olvera V, Carvajal-Zarrabal O, Castro Matinez C, Waliszewski KN, and Aguilar-Uscanga MG. 2014 Purification and characterization of peroxidase from avocado (*Persea americana* Mill, cv. Hass). J Sci Food Agric. 94(9):1844-53.

Sigma Guaiacol data sheet. Obtained from: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/2/g5502pis.pdf

Toledo JC Jr, Audi R, Ogusucu R, Monteiro G, Netto LE, Augusto O. 2011. Horseradish peroxidase compound I as a tool to investigate reactive protein-cysteine residues: from quantification to kinetics. Free Radic Biol Med. 50(9):1032-1038.

Acknowledgments

We would like to thank Dr. Carl Doige for information regarding this assay, Dr. Morgan Martin for reviewing the manuscript and procedure, the Okanagan College Salmon Arm Biology 121 lab students for trouble shooting this experiment and the 2019 ABLE participants for their feedback.

About the Authors

Michael Mitsch has been an Instructor at Okanagan College since 2004, where he teaches first year biology and anatomy and physiology classes and second year microbiology and genetics. Bryan White has been an Instructor at Okanagan College since 2014, where he teaches first year biology and anatomy and physiology classes.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Advances in biology laboratory education is the peer-reviewed publication of the conference of the Association for Biology Laboratory Education. Published articles and extended abstracts are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board. Published abstracts are evaluated and selected by a committee prior to presentation at the conference.

Citing This Article

Mitsch, M and White, B. 2020. A simple, quantitative peroxidase assay demonstrating enzyme inhibition with L-cysteine. Article 44 In: McMahon K, editor. Advances in biology laboratory educaiton. Volume 41. Publication of the 41st Conference of the Association for Biology Laboratory Education (ABLE). **https://doi.org/10.37590/able.v41.art44**