Enzymatic activity is the driving force for all biological systems. Enzymes allow for the most specific and efficient use of nutrients and energy required for living systems to maintain their homeostatic environment while other stresses in the environment threaten them. One such environment humans in industrialized societies face is the oxidation caused by pollution. The burning of carbon containing substances releases carbon dioxide (CO2) and water (H2O), but if the compounds burned were processed with other chemicals, the products may be more harmful than the natural CO2 and H2O human bodies are accustomed to dealing with. These products may contain oxidizing agents that can cause unnatural oxidation, which is the removal of electrons from one species or chemical to another. This is often done by the introduction of oxygen, as with rust. Iron is oxidized naturally by an oxygen rich environment, because iron is oxidized to iron oxide. This can also happen with biological species as well. Enzymes are one example of these biological species.

Enzymes work by acting as catalysts for the biochemical reactions that occur within a given biological system. Reactions that would occur naturally would still occur within the body, but enzymes help facilitate and speed up the reaction by acting as the sites of reactions. Enzymatic activity can be activated and inhibited in many ways, because each enzyme is specific for a single species or group of species. Lactate, for example, aids in the breakdown of the sugar lactose. An enzyme can be likened to a well organized and stocked operating room. If a surgeon has all the tools and other materials necessary to perform the operation within easy access, efficiency increases while keeping the chance of error at a minimum. Therefore, all that is really necessary for the operation to happen is to have the surgeon and the patient come together, and having this occur in an operating room makes a successful surgery more likely and in some cases more possible. Other factors affect the procedure just as the presence of nurses also greatly improves the surgeon’s efficiency, but in the end, certain key requirements are needed for surgeries (or chemical reactions) to occur and the operating room (or enzyme) can aid in making the process the fastest and easiest possible.

The enzyme this study analyzed was elastase. Elastase is a type of protease, which is a class of enzymes that break down proteins, and it gets its name from its ability to break down the protein elastin. Elastin is a key component in keeping parts of the body elastic, such as the elasticity in the skin and in the lungs. The loss of this elasticity can have drastic effects on the body. The lungs rely on their elasticity to be able to expand and retract, giving a person the ability to breathe regularly. If elasticity is lost, the lungs can no longer function properly, which ultimately leads to death. Mild loss of elasticity is a key component to Chronic Pulmonary Obstructive Diseases (COPDs) such as emphysema and bronchitis. Each disease has its own deadly nature, but both are linked to the inhalation of pollutants such as cigarette smoke. As mentioned earlier, these pollutants often contain oxidizing agents that create an oxidative environment in the lungs. Elastase, an enzyme that cleanses the body of damaged proteins, is naturally activated by oxidation via the presence of hydrogen peroxide (H2O2). The pollutants can also activate elastase, activating it unnaturally at a times when the body did not intend to do so. This leads to unnatural break down of healthy proteins such as elastin, reducing the amount
of elasticity in that region of the body. Smoking, then, by causing an oxidative environment within the lungs, promotes the destruction of elastin, which eventually leads to COPDs.

How does elastase get activated by oxidation? The answer lies within the inhibition of elastase. Elastase is a type of enzyme that will continue to work unless inhibited. This inhibition can be likened to a muzzle on a hungry animal. While inhibited, elastase will not break down proteins, because the inhibitor occupies the active site or site of reaction on elastase. Some inhibitors bind to another part of the enzyme, often called an allosteric site, to change the shape of the active site. This, however, is not the case for elastase. The active site is where the compounds meet to undergo the required reaction. Elastin would bind to the active site on elastase if the inhibitor were not there. Active sites are very specific, which is why enzymes are very specific, so that a single change to the site causes the intended response to be halted. The inhibitor specifically involved in the natural inhibition of elastase is alpha-1 protease inhibitor (α1PI). Inhibition only works as long as the inhibitor is bound to the enzyme, therefore to activate elastase, α1PI must be removed from the active site. Just as a change to the enzyme via bound species changes its shape and role, oxidation of the α1PI causes it to change and thus fall off the enzyme. Thus oxidation is the mode in which α1PI is removed and elastase is activated.

The inhibition of enzymes has been categorized into three groups. The first group is competitive inhibition. Here, the inhibitor and the substrate, which is the species to be reacted, compete for the active site. If the inhibitor reaches the active site before the substrate, the enzyme is considered to be competitively inhibited since the reaction of the substrate will not occur. Competitive inhibition is the type of inhibition that α1PI exhibits on elastase. Another group of inhibition is uncompetitive inhibition. With this group, the inhibitors bind to another site on the enzyme, causing a conformational change and thus changing the shape of the binding site. Due to the change in shape, the substrate can no longer bind since the specificity of the site no longer matches the substrate. Competitive inhibition is stronger than uncompetitive; the active site is not completely blocked off in uncompetitive. With uncompetitive, the active site, though changed, may still bind to another substrate. Therefore, the third group of inhibition is called noncompetitive inhibition. Here, a mixture of competitive and uncompetitive inhibition occurs. These types of inhibition can be studied by analyzing the rates in which the reaction will occur at various concentrations of substrate and inhibitor in the presence of the enzyme.

This study uses the substrate n-succinyl-ala-ala-ala-p-nitroanilide (SANA), due to its release of color when hydrolyzed by elastase. When elastase hydrolyzes SANA, there is a change in the color of the SANA solution from a clear solution to yellow. This allows for Ultraviolet-Visible (UV-Vis) spectroscopy to be used in order to study the kinetics, or reaction rate, of the reaction between enzyme and substrate. In this study, the kinetics experiment allowed for the collection of absorbance data at the given wavelength over time. This wavelength was found by scanning a range of wavelengths in order to determine which wavelength yields the maximum absorbance. All subsequent scans were run at this absorbance.

The scans were plotted in terms of absorbance versus time, where absorbance is directly proportional to the concentration of the product from the enzyme/substrate reaction. From this graph the initial rate of the reaction was measured through taking the slope of the initial straight line portion of the graph. The Michaelis-Menten equation: [Your browser may not support display of this image.] ;where [S] is the concentration of the
substrate, SANA, V0 is the initial rate of the reaction, Vmax is the maximum rate of the reaction, and KM is the Michaelis constant; was then transformed into the Lineweaver-Burk equation: [Your browser may not support display of this image.]. Using this equation, the inverse of the initial rate was plotted against the inverse of the concentration of the substrate. From a series of these graphs produced from the use of several concentrations of the inhibitor, the mode of inhibition was determined.

A buffer was created using Trizma base with concentrated hydrochloric acid to adjust the pH to 8.0. Several liters of buffer were made up at a specific time. This buffer was then used as the solvent for the SANA and inhibitor solutions. The SANA and inhibitor solutions were made up each day that the trials were run. For the trials, all chemicals were left at room temperature, which means that the solids were left to stand for one hour prior to massing and that the buffer was also allowed to reach room temperature before diluting the solutions. Once stock solutions of the substrate and inhibitor were prepared to their proper concentrations, the data collection began. Using a Varian Cary 100 Bio Ultraviolet-Visible spectrometer, the absorbance of the reaction was measured for a thirty-second interval. In a given session, there were four concentrations of inhibitor run with five concentrations of SANA, which were kept constant across inhibitor concentrations. These data were then processed through the Lineweaver-Burk plot to determine the mode of inhibition.

The inhibitors used came from the cruciferous family of vegetables. The effects that each inhibitor had was tabulated with reference to both the mode of inhibition, competitive, uncompetitive, or noncompetitive, and the value of \( \alpha \), which is a measure of the inhibitor's affinity for the enzyme. The larger the \( \alpha \) value, the greater the affinity the inhibitor has, which results in greater binding of the inhibitor to the enzyme, and therefore lowering its concentration in the given sample. The different forms of inhibition all have a different equation for how to calculate the value of \( \alpha \). This study aimed to locate inhibitors that were competitive with a large value of \( \alpha \).

Beers Law, a key concept of this study, comes with a hefty price. In order for the equation, and the assumptions that come from it, to be valid, the value of the absorbance must be less than 1. This is very important because of its relation to other factors such as concentration and temperature. This was taken into account when first choosing the concentrations. In order to maintain the absorbance less than 1, concentrations of both enzyme and substrate were adjusted.

Temperature played an important part in the data collection of this study. By adjusting the temperature, the rate at which the reaction took place changed. Therefore, choosing the temperature at which to run the experiment was crucial. However, each temperature range had its own consequences associated with it. The choice to run at a lower temperature meant that the rate proceeded more slowly, which allowed for a larger number of viable points. The problem associated with running at a low temperature was that as the machine ran, the temperature in the cell compartment rose, which caused a sufficient temperature difference between cell and cell surroundings. This caused the formation of condensation on the surface of the cell. With the formation of this layer, the ability of light to pass through was impeded which caused the absorbance readings to become inaccurate.

In response to this concern, the temperature was raised to eliminate the creation of condensation. However, with this adjustment came the problem of the increased rate of the reaction. An increase in the rate of the reaction gave way to the problem of the absorbance reaching a value of 1 more quickly, which limited the amount of data points used to calculate the initial rate of the
reaction. Another possible source of error was that the temperature could still vary in each subsequent trial with no efficient way of measuring the change.

It was the nature of massing out tiny amounts of the solid chemicals that resulted in reproducibility becoming very difficult. A change in concentration among trials made comparisons more difficult. As a result, maintaining a constant concentration of both the inhibitor and the substrate took some adjusting. This problem was solved by either varying the volume added to the cuvette during spectroscopy readings or by varying the volume of buffer used to create the solutions. Varying the volume of the buffer used involved the use of a class A buret, which accurately measured a volume to 0.02 milliliters. This failed to be an efficient method of compensating variability in massing, mainly due to the maintenance and calibration required to quantitatively use a buret. However, by varying the volume added to the cuvette, another issue was encountered in adjusting the volume pulled by the Eppendorf pipettes. The reliability and tolerance allowed by these pipettes limited the choices of usable volumes. The latter method did, however, prove to be a better balance of limiting systematic error and improving efficiency in order to compensate for the changes in substrate and inhibitor masses.

This study offers a collection of inhibitors that have been studied in vitro in the presence of elastase and a model substrate, n-succinyl-ala-ala-ala-p-nitroanilide (SANA). SANA was chosen as the model for its release of color when hydrolyzed by elastase. The color was then measured via UV-Vis spectroscopy, and using Beer's Law a relationship between concentration and time was correlated. This allows for the study of the effectiveness of each inhibitor, in order to find a suitable replacement for α1PI, whose uses are hindered by an oxidative environment. Smoking and pollution eventually build an oxidative environment in the lungs, leading to COPDs, so this study builds a path to finding a pharmaceutical way to help those who suffer from COPDs caused by over activation of elastase. Though inhibition will not actively repair the damage, it will prevent or lessen further damage from occurring, allowing more time for the body to heal. The inhibitors in this study were isolated from cruciferous vegetables, and if a suitable inhibitor is found in them, the consumption of these vegetables would most likely decrease one's chances for acquiring a COPD. This research opens the door for possible relief for those who suffer from COPD, as well as for a greater understanding of elastase activity and COPD prevention.*
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