Introduction

Enzymes are an important protein in living organisms essential for the existence of life. Their role is to speed up chemical reactions that are the foundation bodily functions, including digestion, cell formation, and even waste disposal. Without enzymes these chemical reactions would occur too slowly to support life. Thus, understanding enzymatic reactions and how they affect chemical processes is crucial to better understanding how many of our bodily functions happen.

An enzymatic reaction refers to a reaction in which an enzyme acts as a catalyst (Alberts et al, 2014). An enzyme is a specialized protein that increases the rate of a specific chemical reaction by lowering the activation energy. Activation energy is the energy a molecule requires to begin a chemical reaction (Alberts et al., 2014). An enzymatic reaction occurs in two steps (Artioli, 2008). The enzyme first binds the substrate, a reactant, at its active site to form a substrate-enzyme complex (Artioli, 2008). The substrate-enzyme complex then reacts (Artioli, 2008). The binding provides better chemical conditions to activate the reaction and, in turn, lowers the activation energy (Artioli, 2008).

The purpose of this lab was to observe the effect of enzyme concentration on the reaction time of an enzymatic reaction, as well as the effect of the concentration of reactants and products on the direction of enzymatic reactions. Because an enzyme’s role is to speed up a reaction, a useful hypothesis is that providing a greater concentration of an enzyme to a substrate (reactant) should increase the rate of reaction. However, enzymes only act when they bind to a substrate (Beals, Gross, & Harrel, 1999). Thus, when the concentration of enzymes exceeds the amount of substrate, these “extra” enzymes cannot act as catalysts. At this “saturation” point, increasing the concentration of enzymes should not affect the rate of reaction (Beals, Gross, & Harrell, 1999).

Salivary amylase catalyzes the reaction, acting on starch as the substrate [the other reactant] (Barrass, 1981). During the reaction, the alpha-1, 4 linkages between glucose units in starch are hydrolyzed (Sanderson & Walker, 1999) to form units of maltose, a disaccharide and reducing sugar (Rostogi, 2005). This maltose becomes a source of energy for the body. The aforementioned reaction occurs in the forward direction (meaning that the reactants, water and starch, collide to produce products) and is written as follows (Barrass, 1981).

By using different concentrations of salivary amylase, the effect of enzyme concentration on the reaction time can be observed. To confirm the presence of starch, a positive iodine test shows a change of colour, from blue to black (Harisha, 2006). The reaction’s end point is confirmed by a negative iodine test result, shown by the solution remaining the yellow colour of the original iodine solution (Harisha, 2006). Following these tests, a positive Benedict’s test confirms the presence of maltose, a reducing sugar (Kumar, 2007). The initial solution for Benedict’s test is blue in colour. A precipitate ranging in colour from green, yellow, brown to red then indicates the presence of maltose. If the solution remains the original blue colour.
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of Benedict’s solution, the test for presence of maltose is negative, meaning a reaction did not occur (Toole & Toole, 2004).

Another enzyme used for this experiment was phosphorylase, an enzyme found in plants that, like salivary amylase, also degrades starch (BeMiller & Whistler, 2009). The enzyme is crucial for a phosphorolysis reaction to occur. During this type of reaction (which is analogous to hydrolysis) phosphoric acid, rather than water, acts as a reactant to break down complex starch molecules into simpler subunits of glycosyl (Brody, 1999). The enzyme phosphorylase catalyzes a reaction between the starch and inorganic phosphate to remove single glucosyl units from the starch (BeMiller & Whistler, 2009).

Similar to rates of enzymatic reactions, concentrations of the substrate and the products of an enzymatic forward reaction should also have an effect on the reaction. In this case, following theories of degradative and synthetic reactions, a useful hypothesis is that an excess of product will encourage the building of substrate (synthesis); in contrast, an excess of reactant will encourage the breakdown of the substrate (degradation). By using different concentrations of starch, either in excess or in the primer form, the effect of the concentrations of reactants and products on the direction of the enzymatic reaction can be observed. Similar to the experiment with salivary amylase, an iodine test can confirm the presence of the longer starch – the synthetic reaction. In this case, the solution colour changes from yellow to blue-black (Harisha, 2006). A negative iodine test, shown by a yellow colour, confirms the presence of the shorter starch (starch primer) – the degradative reaction (Harisha, 2006).