## Enzyme reaction - an overview: about the main in brief

Science, Biology



Enzymes, commonly known as proteins, are biological catalytic molecules that effect the rate at which chemical reactions take place within the cells of an organism. Generally speaking, these biological molecules are key players in the vitality, digestion and metabolism of all living organisms. The rate and direction at which enzymatic reactions are produced, depend on numerous factors. Variations in temperature, pH level, enzymatic concentration, activation energy and product concentration, are all among the influential factors that impose a great effect on the rate of synthesis and direction of enzymatic reactions. The purpose of this laboratory is to determine and understand influential conditions that effect enzyme reactivity.

Salivary amylase is an enzyme found in human saliva. This enzyme serves in the initiation of the starch-based digestive process of carbohydrates. This being said, by effectively using catalyzed hydrolytic reactions, salivary amylase hydrolyzes  $\alpha$ -1, 4 glycosidic bonds in starch polymers, ultimately leading to the production of maltose, a disaccharide sugar. In the first experimental section of this laboratory, various concentrations of salivary amylase are mixed into solution with tap water. The addition of water to the varied concentrations of salivary amylase, is needed to drive the hydrolytic breakdown of the glycosidic bonds found in starch polymers. With the use of the iodine test, a colour-reaction test that stains dark purplish-blue in the presence of starch components (amylose and amylopectin), it is possible to determine the presence of non-digested starch present in salivary amylase enzymatic reactions. This being said, because no starch components can be detected and digested in the mixture of salivary amylase and water, a negative iodine test result should be the case (yellow stain). On this note, it

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is easy to infer that salivary amylase in the presence of starch will first omit a positive reaction before the components of starch are digested, and a negative result (yellow stain) when the breakdown and digestion of starch into maltose sugars is complete. Maltose is a disaccharide sugar that can be detected by Benedict's Test for reducing sugars. The addition of Benedict's solution, a reduction of blue cupric ions, allows the formation of cuprous oxide by removing the functional groups accompanying reduced-sugar molecules like maltose. In the context of this experiment, Benedict's Test is used to determine the presence of maltose sugar molecules resulting from the breakdown of glycosidic bonds in starch by salivary amylase. It is to note that enzymatic reaction rate can also be determined by iodine testing. When salivary amylase is in reaction with a starch solution, the initial iodine test omits a dark purplish-blue stain, meaning there is a presence of starch. With time and in optimal conditions, while considering the influential factors of enzymatic reactions, the rate at which the enzyme digests the starch components may vary. As previously mentioned, a negative iodine test informs a complete digestion of starch components. lodine testing at set intervals allows investigators to determine the amount of time required for salivary amylase to completely catalyze amylose and amylopectin into maltose molecules.

The second experimental section of this laboratory focuses on the phosphorylase enzyme. Phosphorylase was discovered in potatoes, and much like salivary amylase, also belongs to the starch-digesting enzyme family. However, in contrast to salivary amylase, phosphorylase can either

contribute to the breakdown of starch into glucose-1-phosphate, to the synthesis of starch or to the equilibrium, depending on substrate and enzymatic concentrations. The reversible reaction between phosphorolysis and synthesis follows a multi-chain approach. Longer chains of starch (starch in excess) in reaction with phosphoric acid and an active enzyme (phosphorylase), is considered to create synthesis in terms of this reversible reaction. On the other hand, shorter chains (limited starch) in the presence of glucose-1-phosphate and an active enzyme (phosphorylase), is considered to create phosphorolysis in terms of this reversible reaction. As mentioned previously, the iodine permits investigators to determine whether or not starch components (amylose and amylopectin) are present in solution. In this case, the iodine test allows the investigation of the direction of this reversible reaction. On this note, a yellow stained iodine test converting to a dark purplish-blue colour will indicate a reaction in the direction of synthesis, inferring that the creation of starch has occurred (Whelan & Bailey, 1954). On the opposite side, a dark-purplish blue colour converting to a negative iodine result (yellow stain), indicates a reaction in the direction of phosphorolysis, meaning that starch has been catalyzed completely by the active enzyme and phosphoric acid. No colour change indicates an equilibrium.