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The Effect of Co^{2+} in the Form of $CoCl_2$ Compounds on α-Amylase Activity

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Article Info	ABSTRACT
	This study aims to determine: (1) the optimum conditions for α-amylase enzyme activity with potato starch as a substrate including incubation time, pH, temperature, substrate concentration, and enzyme concentration, (2) the effect of Co^{2+} metal ions in the form of $CoCl_2$ compounds on α-amylase enzyme activity with potato starch substrate at optimum conditions. The determination of α-amylase enzyme activity with potato starch substrate was carried out using the *dinitrosalicylic acid* (DNS) method. The determination of α-amylase enzyme activity with and without the addition of $CoCl_2$ was carried out at the optimum conditions that have been obtained. The variation in the added concentration of $CoCl_2$ were 0.01; 0.02; 0.03; 0.04; and 0.05 M. The data obtained in this study were enzyme activity in units of mg/mL/minute at 37° C. The data analysis used was descriptive qualitative by comparing the activity of the α-amylase enzyme with and without the addition of $CoCl_2$ at optimum conditions. The results of the average α-amylase enzyme activity at optimum conditions (temperature 37° C, pH 7.2, incubation time 15 minutes, substrate concentration 20 mg/mL, and enzyme concentration 40 mg/mL), namely 0.00794 mg/mL at temperature 37° C. For the mean α-amylase enzyme activity with the addition of $CoCl_2$ at a concentration of 0.01; 0.02; 0.03; 0.04; and 0.05 M, respectively 0.0038; 0.0013; 0.0012; 0.0011; and 0.00031 mg/mL per minute at 37° C. Based on these data it can be concluded that the tendency of the Co^{2+} metal ion is inhibitory and greatly affects the activity of the α-amylase enzyme. ***Keyword:*** *enzyme activity, α-amylase, Co^{2+} metal ion, $CoCl_2$, inhibitor*

1. INTRODUCTION

Enzyme is a biocatalyst which will act as a catalyst (Rusli, 2012). Enzymes work specifically which is on particular substrate into a product. The reaction between the substrate and the enzyme occurs on the active side of the enzyme, which is the space used for the substrate to bind the enzyme. This substrate enzyme pair must be suitable, because if the type of substrate used does not match the active side of the enzyme, the reaction cannot occur. Enzyme and substrate reactions will form a temporary enzyme-substrate complex. This complex can later decompose to yield products and release enzymes (Poedjiadi, 2006).

In the human body there is an amylase enzyme, which consists of the enzymes α-amylase, β-amylase, and glucoamylase (Ao, et al, 2007). Amylase enzyme is an enzyme that is synthesized in cells,

but it works outside the cell (extracellular) (Van der Kaaij, et al, 2007). The α-amylase enzyme is able to hydrolyze amylose, resulting in simple forms of sugar such as dextrin, glucose and maltose. The α-1,4-glycoside bonds in starch were broken down by the α-amylase enzyme, but did not change the structure of the α-1,6-glycoside bond in amylopectin (Wahyuni et al, 2015). The function of this enzyme is to break down the starch contained in a food. Another function of this enzyme was valuable for plants e.g., giving sweet taste of fruit due to the enzyme synthesized during the ripening process (Ariandi, 2016).

Enzyme activity is defined as the amount of enzyme that causes one micromole of substrate per minute to be converted at the optimum measurement state (Lehninger, 1982). The α-amylase enzyme activity test is usually carried out under optimum conditions using starch substrate. Enzyme activity testing is usually expressed in a specific unit (Howard, 2015). Enzyme activity can be influenced by several factors including incubation time, pH, temperature, substrate concentration, enzyme concentration, and cofactors in the form of cations which is useful as activators or inhibitors. In this study, the DNS (Dinitrosalicylic Acid) method was used to determine the α-amylase enzyme activity.

The quantitative tests of α-amylase enzyme are sought to observe the reduced sugar content that produced by the enzyme on the substrate. The method that can be used to test the α-amylase enzyme activity is the DNS method (Miller, 1959). DNS reagent consists of several components, including rochelle salt (KNa-Tartrat), phenol, sodium bisulfite and sodium hydroxide. Some of these components have functions, namely: (1) 3,5-dinitrosalicylic acid to reduce glucose in an alkaline state assisted by sodium hydroxide, (2) rochelle salt to eliminate the influence of disturbing compounds so that the color complex remains stable, (3) phenol to stabilize the color that is formed, and (4) sodium bisulfite to remove the influence of dissolved oxygen which can oxidize the product glucose (Miller, 1959).

In the enzyme activity we recognize cofactors, which are non-protein chemical compounds that are required for the biological activity of an enzyme (Winkler, Esselborn & Happe, 2013). The cofactors are inorganic molecules/ ions e.g., metal ions Zn^{2+}, Fe^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Co^{2+} (Rusli, 2012). In this research, Co^{2+} metal is used in the form of $CoCl_2$ compounds. Basically, $CoCl_2$ has sky blue in colour, but different amounts of hydrate can affect the color intensity. For example, the dihydrate compound has a purple color and the hexahydrate has a pink color. The most common hydrate compound on the market is the hexahydrate $CoCl_2.6H_2O$ compound that is widely used in laboratories (Greenwood & Earnshaw, 1997). In the Minister of Environment Regulation Number 05 of 2014 concerning Wastewater Quality Standards, the safe threshold for the concentration of metal Co is 0.4 mg/L. The metal Co has a high toxicological value when the body is frequently exposed to heavy metals Co.

Based on aforementioned explanation, it is necessary to conduct a research on the effect of adding Co^{2+} metal ions in the form of $CoCl_2$ compounds using potato starch under the optimum conditions. Through this research, it will be known the role of the Co^{2+} metal ion as an activator or inhibitor of the α-amylase enzyme activity by looking at the activity tendency in adding various variations in the concentration of Co^{2+} metal ions. The research began by determining the optimum conditions for the enzyme which include pH, temperature, incubation time, and substrate concentration, enzyme concentration; and followed by adding Co^{2+} metal ions to these optimum conditions. Information regarding the function of a metal ion which acts as a cofactor in the body as an activator or inhibitor is very important, so that we can investigate its amount in our body interferes with or not for the continuity of metabolism in the body and our health.

2. RESEARCH METHOD
2.1. Research Time and Place

This research was conducted at the Organic Chemistry Laboratory, Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta from January - June 2020.

2.2. Tools and Materials

The tools used in this study were a set of glassware, filter paper, incubator, analytical balance, pH meter, stopwatch, and Thermo Scientific Type Genesys 20 electronics. While, the materials used in this study were phosphate buffer solution, α-amylase enzyme, potato starch substrate, distilled water, 3,5-dinitrosalicylic acid, 0.2 N NaOH, K-Na-tartate, a solution of 0.1 M Co^{2+} metal ion, and ice cubes.

2.3. Procedure
2.3.1 DNS Reagent

As much as 1 gram of 3,5-dinitrosalicylic acid is dissolved in 50 mL of distilled water. Then 20 mL of 0.2 N NaOH and 30 grams of K-Na-Tartate (Rochella Salt) were added and stirred until homogeneous. Then put it in a 100 mL volumetric flask and add distilled water until the limit mark.

2.3.2 The Determination of Maximum Wavelength

Penentuan ini menggunakan metode DNS (*Dinitrosalisilic Acid*). Pertama menginkubasi 0,25 mL larutan amilum 20 mg/mL selama 5 menit pada suhu 37°C. Kemudian menambahkan 0,25 mL buffer fosfat pH 7 dan 0,5 ml larutan enzim α-amilase pH 7. Kemudian mengaduknya hingga tercampur sempurna. Pencampuran larutan ini dilakukan dengan *vortexmixer*, kemudian melakukan inkubasi kembali selama 10 menit pada suhu 37°C. Setelah itu menambahkan 1 mL reagen DNS 5% (v/v), kemudian mengaduknya dengan kuat menggunakan *vortexmixer*. Melakukan pemanasan tabung sampel ke dalam air mendidih selama 5 menit. Lalu mendinginkan larutan ke dalam air es. Kemudian menambahkan 8 mL akuades dan mengaduknya secara sempurna dengan *vortexmixer*. Mengukur nilai absorbansi pada panjang gelombang 390 - 550 nm. Panjang gelombang maksimum adalah panjang gelombang yang memberikan nilai absorbansi terbesar.

This determination uses the DNS method. First incubated 0.25 mL of starch 20 mg/mL solution for 5 minutes at 37°C. Then add 0.25 mL of pH 7 phosphate buffer and 0.5 mL of α-amylase enzyme of pH 7. Stir the solution using vortex mixer until completely mixed and incubated for 10 minutes at 37°C. After that, add 1 mL of 5% (v/v) DNS reagent, then stir it vigorously with a vortexmixer. Warm the sample tube into boiling water for 5 minutes and cooling the solution into ice water. Add a total of 8 mL of distilled water and mix thoroughly with a vortexmixer. Measure the absorbance value at a wavelength of 390 - 550 nm. The maximum wavelength is the wavelength that provides the greatest absorbance value.

2.3.3 The Determination of Time of Stability

Penentuan ini digunakan langkah yang sama dengan pengukuran penentuan panjang gelombang maksimum, tetapi pada penentuan ini dilakukan pencatatan dalam beberapa jeda menit selama 30 menit, dan diukur pada panjang gelombang maksimum.

This determination uses the same steps as the measurement of the maximum wavelength determination, but in this determination, it is recorded within interval time of 30 minutes, and is measured at the maximum wavelength.

2.3.4 The Determination of Optimal Incubation Time

It takes 3 solutions in this determination, namely the control, sample, and blank solution. For the control solution was 0.25 mL of 2% potato starch substrate and 0.25 mL of phosphate buffer solution pH 7, pre-incubated for 5 minutes at 37°C, added 1 mL of 5% (v/v) and 0 DNS reagent, 5 mL of α-amylase enzyme solution (pH 7), vortex the solution and heat it in a boiling water bath for 5 minutes, then let it stand in ice water until cold and add 8 mL of distilled water and vortex again to mix completely, measure the absorbance at maximum wavelength. In addition, for the blank solution, 1 mL of pH 7 phosphate buffer solution and 1 mL of DNS reagent were put into the test tube and added 8 mL of distilled water, the vortex of the solution was then measured for absorbance at the maximum wavelength. This treatment is done in triplo. The next is the sample solution that are the same as determining the maximum wavelength, with variations in the incubation time (5 minutes, 10 minutes, 15 minutes, 20 minutes, and 25 minutes).

2.3.5 The Determination of Optimum pH

This determination was carried out in the same way as the optimum incubation time, but the pH of the phosphate buffer was varied (6.6; 6.8; 7; 7.2; and 7.4).

2.3.6 The Determination of the Optimum Temperature

This determination is carried out the same as determining the optimum incubation time, but the incubation temperature was varied (27°C, 32°C, 37°C, 42°C, and 47 C).

2.3.7 The Determination of Optimum Substrat Concentration

Penentuan ini dilakukan sama dengan penentuan waktu inkubasi optimum, namun dilakukan variasi konsentrasi substrat (10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, dan 50 mg/mL).

This determination was carried out in the same way as the optimum incubation time determination, but the substrate concentration was varied (10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL).

2.3.8 The Determination of Optimum Enzyme Concentration

This determination was carried out in the same steps as the optimum incubation time, but with variations in enzyme concentrations (30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, and 70 mg/mL).

2.3.9 The Determination of Enzyme Activity at Optimal Conditions

Determination of enzyme activity is the same as determining the optimum incubation time. It's just done at optimum conditions (incubation time, pH, temperature, enzyme concentration and substrate concentration). This treatment was carried out in 5 times.

2.3.10 The Determination of Enzyme Activity by the Addition of Co^{2+} Metal Ions

This determination is carried out in the same way as the determination of enzyme activity at optimum conditions. It's just that the addition of $CoCl_2$ solutions with various concentrations (0.01 M, 0.02 M, 0.03 M, 0.04M, and 0.05M).

3. RESULTS AND DISCUSSION

In this section, it is explained the results of research and at the same time is given the comprehensive discussion. Empirically, this study aims to determine the effect of the addition of Co^{2+} metal ions in the form of $CoCl_2$ compounds at various concentrations of α-amylase enzyme activity with potato starch substrates at optimum conditions covering the maximum wavelength, time of stability, optimal incubation time, optimum pH, optimum temperature, optimum substrat concentration, optimum enzyme concentration, and enzyme activity at optimal conditions. After the optimum conditions have been meet, thus it followed by the determination of enzyme activity by the addition of Co^{2+} metals.

The first determination of optimum condition was the maximum wavelength. The maximum wavelength is the wavelength that provides the highest absorbance value. In determining the maximum wavelength, the highest absorbance value was obtained at a wavelength of 400 nm with an absorbance value of 0.213. The next is examining the optimal time of the α-amylase enzyme stability. The stability time is the time when the enzyme is stable to react with other substrates or reagents. The results obtained at 8-10 minutes; the absorbance was stable at an absorbance of 0.222. Thus, the stability time used in this study is 10 minutes. The following optimum condition is the determination of the α-amylase enzyme optimum incubation time which obtained at 15 minutes with an enzyme activity value of 0.00703 mg/mL/minute at 37°C. The results that obtained are confirmed the work from Farida (2010), which produces an optimum incubation time of 15 minutes. After that, in determining the optimum pH, the maximum activity results obtained are 0.0045 mg/mL/minute at 37°C, namely at pH 7.2. This is in accordance with the theory of the α-amylase enzyme which works optimally in the pH range of 5.6 - 7.2, at this pH the enzyme will work optimally (Poedjiadi, 2006). Furthermore, the determination of the optimum temperature results in a maximum activity of 0.0020 mg/mL/minute at 37°C, which lies at a variation of 37°C. The results obtained are consistent with research by Sumrin et al. (2011) where the optimum temperature was 37°C. Furthermore, the determination of the optimum substrate concentration obtained the optimum activity results of 0.0024 mg/mL/minute at 37°C, that is, at a

substrate concentration of 20 mg/mL. This is the same as research by Wahyuni et al. (2015) which also used a substrate concentration of 2% or 20 mg/mL as the substrate concentration in determining α-amylase enzyme activity. The next is the determination of the enzyme concentration which resulting in the optimum enzyme concentration at a concentration of 40 mg/mL with an activity value of 0.00593 mg/mL/minute at 37°C. Furthermore, the determination of the α-amylase enzyme activity at optimum conditions (incubation time of 15 minutes, pH 7.2, temperature 37°C, substrate concentration 20 mg / mL, and enzyme concentration 40 mg / mL). This determination was carried out by pentaplo, resulting in an average α-amylase enzyme activity of 0.00794 mg/mL/minute at 37°C. Determination of the α-amylase enzyme activity with the addition of Co^{2+} metal ions was carried out by the DNS method. The Co^{2+} metal ion used is in the form of a $CoCl_2$ compound with a concentration variation of 0.01 M; 0.02 M; 0.03 M; 0.04 M; and 0.05 M. The results obtained in this research can be summarized in Figure 1.
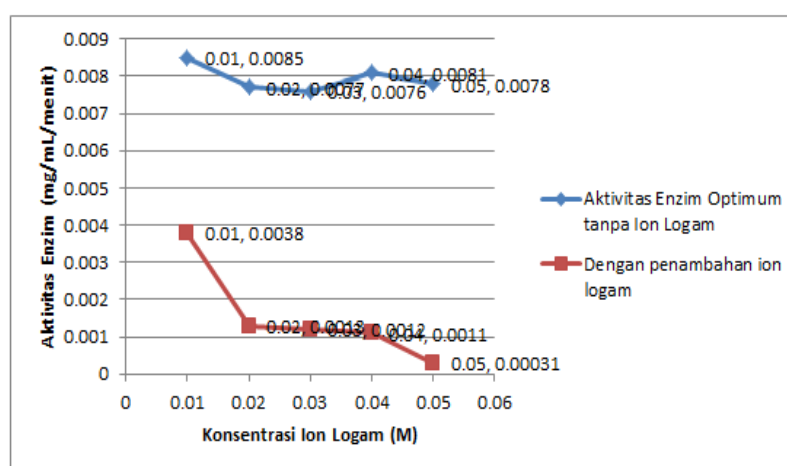

Figure 1. The effects of the addition of Co^{2+} metals in the form of $CoCl_2$ compounds

Based on Figure 1, the optimum activity of the α-amylase enzyme is above the α-amylase enzyme with the addition of the metal ion Co^{2+}. The activity of the α-amylase enzyme was seen to decrease with the addition of metal ions in the form of a $CoCl_2$ compound with a concentration variation of 0.01 M; 0.02 M; 0.03 M; 0.04 M; and 0.05 M. At optimum conditions the α-amylase enzyme resulted in an average activity of 0.00794 mg/mL/minute at 37°C. After the addition of Co^{2+} metal ions there is a decrease in activity so that it can be concluded as an inhibitor. This is consistent with research of (Soeka, 2015) that the Co^{2+} metal ion as an inhibitor can affect the activity of the α-amylase enzyme. Based on the research that has been done, it can be seen that with the addition of Co^{2+} metal ions in the form of a $CoCl_2$ compound, the activity of the α-amylase enzyme with pro-analysis potato starch substrate has decreased and there is an inhibition. However, in certain concentrations the Co^{2+} metal does not act as an inhibitor, but can act as an activator. This is stated in the research of Lestari, et al. (2011) that at low concentrations (1 mM) the Co^{2+} metal ion does not inhibit enzyme activity but becomes an activator. However, if the activator compound is excess, it will act the opposite. Because the activator compound increases the enzyme catalytic reaction speed to a certain amount. If the activator is too large, it causes competition between the free activator and the substrate activator complex against the enzyme (Microbiology, 2015).

4.   CONCLUSION

The activity of the α-amylase enzyme at optimum conditions includes at pH 7.2, temperature 37°C, incubation time 15 minutes, substrate concentration 20 mg/mL, and enzyme concentration 40 mg/mL is 0.00794 mg/mL/minute at 37°C. The addition of Co^{2+} metal ions in the form of $CoCl_2$ compounds in determining the activity of the α-amylase enzyme at optimum conditions reduces the activity of the α-amylase enzyme. The greater the concentration of the addition of $CoCl_2$ compound, the lower the α-amylase enzyme activity, therefore it can be said to be an inhibitor.

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