

# The Influence of Al<sup>3+</sup> Metal Ion in AlCl<sub>3</sub> Compound On $\alpha$ -Amylase Enzyme Activity

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## ABSTRACT

The influence of Al<sup>3+</sup> metal ion in AlCl<sub>3</sub> towards  $\alpha$ -amylase enzyme activity have been studied. The activity of the  $\alpha$ -amylase enzyme on potato starch substrate was determined using the DNS method (3,5-dinitrosalicylic acid) at optimum conditions. The data analysis was conducted in a descriptive qualitative manner. The results obtained that  $\alpha$ -amylase enzyme activity with potato starch substrate was optimum at 20 mg/mL with pH 7.2, incubation temperature 37°C, incubation time 15 minutes, and enzyme concentration of 40 mg/mL with an average enzyme activity value of 0.0079 mg/mL/minute at 37°C. However, after the addition of Al<sup>3+</sup> metal ion, the average value of enzyme activity decreased. These means that empirically Al<sup>3+</sup> metal ion has a tendency to be inhibitory to the  $\alpha$ -amylase enzyme activity on potato starch substrates.

**Keyword:** :  $\alpha$ -amylase enzyme, DNS method, inhibitor, Al<sup>3+</sup> metal ion

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## 1. INTRODUCTION

Amylase is one of the most important enzymes in biotechnology field nowadays, which is a glycoside hydrolase enzyme that catalyzes the breakdown of starch into sugars (Souza & Magalhães, 2010). Amylase was prepared from various type of living things such as bacteria, fungi, plants, and humans (Pandey, et al, 2000). Amylase is a digestive enzyme, mainly carried out by the pancreas and salivary glands and divided into three types namely  $\alpha$ -amylase,  $\beta$ -amylase, and glucoamylase enzymes.

One of amylase enzyme that exists in human digestion is the enzyme -1,4-glucan-4-glucanohydrolase or  $\alpha$ -amylase.  $\alpha$ -amylase enzymes are a family of endoamylase enzymes that can randomly catalyze the initial hydrolysis of  $\alpha$ -(1,4) glycosidic bonds in starch to become a shorter oligosaccharides with low molecular weight, such as maltose, glucose, and maltotriose units (Souza & Magalhães, 2010).. The end products of the  $\alpha$ -amylase reaction are oligosaccharides of varying length with  $\alpha$ -configuration,  $\alpha$ -limit dextrans, containing of maltotriose, maltose, and branched oligosaccharides consisting of 6-8 glucose units containing  $\alpha$ -1,4 and  $\alpha$ -1,6 bonds (Souza & Magalhães, 2010).. The -amylase enzyme contains at least 1 calcium atom per molecule and is tightly attached to the enzyme molecule. The presence of calcium makes this enzyme is called as "calcium metalcoenzyme". These calcium ions are important for the stability and activity of enzymes. The calcium ion in  $\alpha$ -amylase has a stronger affinity than other cations. There is still being investigated whether the calcium ion can be replaced by other cations (Vihinen & Mantsala, 1989).

$\alpha$ -amylase enzyme can act on certain substrates, such as rice starch, potato starch, corn starch, and cassava starch. Enzymes can change the rate of chemical reactions, but have no effect on the final

equilibrium of the reaction. It is only need a small amount of enzyme to make a big changes and enzymes will only work under appropriate conditions, such as pH, concentration, cofactors, and so on (Bintang, 2010). The activity of the  $\alpha$ -amylase enzyme can be determined by several methods such as the 3,5-dinitroslylic acid (DNS) method, the fuwa method, and the nelson-somogyi method. Enzyme concentration and substrate concentration can affect enzyme activity, but it can also be influenced by cofactors, inhibitors, and the effect of activators in some circumstances. Bailey and Ollis (1988) explained that one of the characteristics of enzyme activity is that it requires a cofactor or non-protein group from the enzyme that can determine its catalytic activity. Cofactors can be in the form of coenzymes that are not strongly bound to enzymes in the form of organic molecules, and prosthetic groups that are strongly bound to enzymes in the form of metal ions, including metal ions  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Ca}^{2+}$  (Lehninger, 1997). An activator is a compound or ion that can increase the speed of an enzymatic reaction. While inhibitors are chemical substances that can inhibit enzyme activity (Wirahadikusumah, 1989). According to Winarno (1992), the inhibitors work by attacking the active site of the enzyme, so the enzyme cannot bind to the substrate, thus disturbing the catalytic function of the enzyme.

Metal ions can enter the body and react with enzymes, one of which is the metal ion  $\text{Al}^{3+}$ . Aluminum that enters the human body can be overcome with a certain amount and remove it effectively. Based on the provisions of the World Health Organization (WHO), the maximum aluminum level that can be absorbed by the body is 40 milligrams per kilogram of body weight, that is, if a person weighs 60 kilograms, in one day that person can tolerate 2,400 milligrams of aluminum intake. According to Haugh [9] aluminum can accumulate in the body's organs, in a long time deposits will occur, and can cause poisoning or damage to organs. Research conducted by Nurrahman et al. (2016) showed that the presence of aluminum in the pancreas was more abundant than in other organs (heart, liver, lungs, kidneys, and bones). Whereas the pancreas is a vital organ, especially the producer of digestive enzymes, one of which is the  $\alpha$ -amylase enzyme.

Based on this explanation, it is necessary to conduct research on the effect of adding metal ions  $\text{Al}^{3+}$  in  $\text{AlCl}_3$  compounds so that empirically the activity of the -amylase enzyme using potato starch is carried out under optimum conditions. Through this research, it will be known the role of  $\text{Al}^{3+}$  metal ion as an activator or inhibitor of -amylase enzyme activity by looking at the tendency of its activity in adding various concentrations of  $\text{Al}^{3+}$  metal ion.

## 2. RESEARCH METHOD

### 2.1 Materials

The materials used were: phosphate buffer solution, -amylase enzyme, potato starch substrate, distilled water, DNS reagent, 0.1 M  $\text{Al}^{3+}$  metal ion solution, and ice cubes.

### 2.2 Determination of maximum wavelength and stability time

The determination of the maximum wavelength was measured using the 3,5-Dinitrosalicylic Acid (DNS) method. The test was started by inserting 0.25 mL of 20 mg/mL potato starch substrate into a test tube, pre-incubated for 5 minutes at 37°C. Then 0.25 mL of 0.2 M phosphate buffer pH 7 and 0.5 mL of 50 mg/mL -amylase enzyme solution were added, vortexed, and incubated for 10 minutes at 37°C. After incubation, 1 mL of DNS reagent was added (vortex again) then heated in boiling water for 5 minutes, then cooled in ice water. Added 8 mL of distilled water into a cooled test tube, homogenized with a vortex and read measuring the absorbance with a wavelength of 380-550 nm.

The determination of the stability time is carried out in the same way as the procedure for determining the maximum wavelength, only for the determination of the stability time, the absorbance reading uses the maximum wavelength that has been obtained previously, which is 400 nm. Absorbance readings were carried out every 5, 8, 10, 13, 15, 18, 20, 23, 25, 28, and 30 minutes from when the distilled water was poured into the solution.

### 2.3 Determination Optimum Condition of $\alpha$ -amylase enzyme

Determination of the optimum conditions of  $\alpha$ -amylase enzyme with potato starch substrate used the same procedure with determination of maximum wavelength and determination of stability time. The optimum conditions determined were incubation time (5, 10, 15, 20, and 25 minutes), pH (6.6; 6.8; 7; 7.2; and 7.4), temperature (27°C, 32°C, 37°C, 42°C, and 47°C), substrate concentration (10 mg/mL; 20 mg/mL; 30 mg/mL; 40 mg/mL; and 50 mg/mL), and enzyme concentrations (30 mg/mL; 40 mg/mL; 50 mg/mL; 60 mg/mL; and 70 mg/mL). The maximum wavelength used to read the absorbance is 400 nm (previously obtained). In this procedure, it is done by determining the absorbance of the sample solution, control solution, and blank solution. The absorbance reading of the control solution was started with 0.25 mL of 20 mg/mL potato starch substrate and 0.25 mL of phosphate buffer pH 7, pre-incubated for 5 minutes at 37°C, added 1 mL of DNS reagent and 0.5 mL of 50 mg/mL  $\alpha$ -amylase enzyme solution, vortex the solution and heated in a boiling water bath for 5 minutes, then allowed to stand in ice water to cool and add 8 mL of distilled water and vortex again to mix thoroughly, measure the absorbance at the maximum wavelength. As for the blank solution, 1 mL of phosphate buffer solution pH 7 and 1 mL of DNS reagent were put into a test tube and added 8 mL of distilled water, vortex the solution and then measure the absorbance at the maximum wavelength. This procedure was repeated three times (triplo).

### 2.4 Determination of $\alpha$ -amylase Enzyme Activity at optimum condition

The procedure for determining the activity of the  $\alpha$ -amylase enzyme under optimum conditions was carried out in the same way as the procedure for determining the optimum conditions for the  $\alpha$ -amylase enzyme. This determination was carried out at incubation time, pH, temperature, substrate concentration, and optimum enzyme concentration obtained in the previous procedure (incubation time 15 minutes, pH 7.2, incubation temperature 37°C, substrate concentration 20 mg/mL, and concentration of 20 mg/mL). enzyme 40 mg/mL) and was repeated five times.

The influence  $Al^{3+}$  ion in  $AlCl_3$  was studied using the same procedure. The concentration of  $Al^{3+}$  ion was varying by the addition of 0.01 M; 0.02 M; 0.03 M; 0.04 M; 0.05 M  $AlCl_3$  to the sample solution after the addition of the  $\alpha$ -amylase enzyme. Meanwhile, in the control solution  $Al^{3+}$  ions were added after the addition of phosphate buffer

## 3. RESULTS AND DISCUSSION

This study aims to determine the effect of metal ions  $Al^{3+}$  in  $AlCl_3$  compounds at various concentrations on the activity of the  $\alpha$ -amylase enzyme with potato starch substrate at optimum conditions. This research was started by determining the maximum wavelength and optimum stability time of the  $\alpha$ -amylase enzyme. Then proceed to determine the activity of the  $\alpha$ -amylase enzyme with potato starch substrate using the DNS method at optimum conditions (incubation time, pH, temperature, substrate concentration, and enzyme concentration). Then determined the activity of the  $\alpha$ -amylase enzyme without and with the addition of  $Al^{3+}$  metal ions in the form of  $AlCl_3$  compounds. Data analysis was carried out in a qualitative descriptive manner, namely by making a graph of the relationship between the addition of  $Al^{3+}$  metal ions in the form of  $AlCl_3$  compounds to  $\alpha$ -amylase enzyme activity at optimum conditions compared to  $\alpha$ -amylase enzyme activity at optimum conditions. The determination of maximum wavelength resulted the highest absorption at 400 nm with the value 0.213. Determination of stability time aims to find out how long the enzyme is stable when measuring which can be known by making a curve of the absorbance relationship of the solution with the measurement time (Gandjar & Rohman, 2019). In this study, it was shown that the stable time for the  $\alpha$ -amylase enzyme was 10 minutes. Thus, the operational time for measuring the reaction results is carried out during this time period in order to obtain good reaction results.

**Tabel 1.** Optimum activity of  $\alpha$ -amylase enzyme

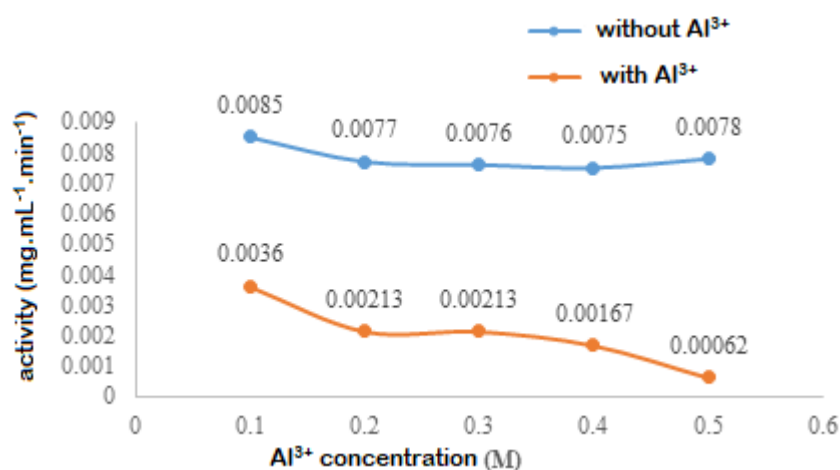
sample	Activity at 37°C (mg/mL.min)
1	0,0085
2	0,0077
3	0,0076
4	0,0075
5	0,0078

Determination of the optimum conditions for the  $\alpha$ -amylase enzyme obtained the following results: (1) optimum incubation time for 15 minutes, (2) optimum pH 7.2, (3) optimum temperature 37°C, (4) optimum substrate concentration of 20 mg/ mL, and (5) the optimum enzyme concentration was 50 mg/mL. The result was listed at Table 1, which indicated that average activity of  $\alpha$ -amylase enzyme is around 0.0079 mg/mL/ menit pada 37°C from five repetition.

**Tabel 2.**  $\alpha$ -amylase enzyme activity with addition of  $Al^{3+}$  ion

Concentration of $Mg^{2+}$ (M)	Enzyme activity (mg/mL per menit) at 37°C
0,01	0,0036
0,02	0,0021
0,03	0,0021
0,04	0,0016
0,05	0,0006

The procedure for determining the activity of the  $\alpha$ -amylase enzyme with the addition of metal ions  $Al^{3+}$  compounds was carried out in the same way as determining the activity of the  $\alpha$ -amylase enzyme at optimum conditions. The difference is the addition of metal ions  $Al^{3+}$ , the sample solution is added after the addition of the enzyme and the control solution is added after the addition of phosphate buffer. Based on the results of the absorbance measurement of the sample and control with the addition of metal ions  $Al^{3+}$  with varying concentrations of 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M, the results of  $\alpha$ -amylase enzyme activity were obtained respectively (Table 2). The result before and after the addition of  $Al^{3+}$  is showed in Figure 1.

Figure 1. The relation of  $Al^{3+}$  addition with  $\alpha$ -amylase enzyme activity

Based on Figure 1, the activity of the  $\alpha$ -amylase enzyme with the addition of metal ions  $Al^{3+}$  in the form of  $AlCl_3$  compound with a concentration of 0.01 M; 0.02 M; 0.03 M; 0.04 M; and 0.05 M lower

than the activity of the  $\alpha$ -amylase enzyme without the addition of  $Al^{3+}$  metal ions under optimum conditions. This indicates that the metal ion  $Al^{3+}$  in the form of  $AlCl_3$  compounds can inhibit the activity of the  $\alpha$ -amylase enzyme. In other words, metal ions  $Al^{3+}$  in the form of  $AlCl_3$  compounds are inhibitors of  $\alpha$ -amylase enzyme activity with potato starch as substrate.

This study is in line with the results of research conducted by David et al. [13], that the effect of  $Al^{3+}$  10 mM on the  $\alpha$ -amylase enzyme from the isolation of *Bacillus subtilis* bacteria using 1% corn starch as a substrate indicated that  $Al^{3+}$  inhibited the activity of the  $\alpha$ -amylase enzyme. However, these results are different from the research of Prakash, Jaiswal, and Pandey [14] which showed that the effect of adding  $Al^{3+}$  metal ions to the activity of amylase enzymes with 2% starch as an activator as a substrate. In this study, the concentration of  $Al^{3+}$  metal ion used was 0.0005 mM; 0.25 mM; 0.5 mM; 1 mM; 2 mM; 3 mM; 4 mM; and 5 mM, where the activity of the amylase enzyme decreased with the addition of  $Al^{3+}$  metal ion concentration. With these two research results, it can be stated that the higher the concentration of  $Al^{3+}$  metal ions, the lower the activity of the amylase enzyme. Although at a concentration of 0.0005 mM – 5 mM it still shows that the enzyme requires  $Al^{3+}$  metal ions to work, but if the concentration is too high it will affect the enzyme's work in product formation. This is because the active site of the enzyme is not saturated enough to bind to the substrate in producing the product, because the substrate competes for the active site with the  $Al^{3+}$  metal ion which occupies the active site of the enzyme.

#### 4. CONCLUSION

It can be concluded that the optimum conditions for  $\alpha$ -amylase enzyme activity with potato starch substrate as much as 2 mg/mL were at incubation time of 15 minutes, pH 7.2, temperature 37°C, and enzyme concentration 40 mg/mL. The effect of adding metal ions  $Al^{3+}$  in the form of  $AlCl_3$  to the activity of the  $\alpha$ -amylase enzyme empirically shows that it can significantly reduce the activity of the  $\alpha$ -amylase enzyme, so that the metal ion  $Al^{3+}$  is designated as an inhibitor of the activity of the  $\alpha$ -amylase enzyme.

#### REFERENCES

- Bailey, J. E., & Ollis, D. F. (1988). *Biochemical Engineering Fundamental (2nd ed.)*. New York: Mc Graw-Hill Book Company.
- Bintang, M. (2010). *Biokimia Teknik Penelitian*. Jakarta: Erlangga.
- David S, Femi B, Gbenga A, Saanu AB. (2017). Purification and Characterization of  $\alpha$ -Amylase from *Bacillus subtilis* Isolated from Cassava Processing Sites. *Jurnal Bioremediat Biodegrad*, 8: 417.
- Gandjar, I. G., & Rohman, A. (2019). *Kimia Farmasi Analisis*. Yogyakarta: Pustaka Pelajar, pp: 253-254.
- Haugh, A. (1983). *Molecular Aspects of Aluminium Toxicity*. *CRC Critical Review in Plant Sciences*, 1(4) p: 345-373.
- Lehninger, A. L. (1997). *Dasar-Dasar Biokimia Jilid 1*. Alih bahasa oleh Maggy Thenawidjaya. Jakarta: Erlangga.
- Miller, G. (1959). Use of Dinitrosalisilyc Acid Reagent for Determination of Reducing Sugar. *Anal Chem*, 31, 426-428.
- Nurrahman, Murwani, R., & Yazid, N. (2016). Pengaruh Penggunaan Tawas pada Pakan terhadap Toksisitas dan Kadar Aluminium Organ Tikus *In Vivo*. *Jurnal Pangan dan Gizi*, 3(5).
- Souza P. M. and Magalhães P. O. (2010). Application Of Microbial  $\alpha$ -Amylase In Industry. *A Review*. Departamento de Ciências Farmacêuticas, Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, DF, Brasil.
- Pandey, A.; Nigam, P.; Soccol, C.R.; Soccol, V.T.; Singh, D.; Mohan, R. (2000). Review: Advances in Microbial Amylases, *Biotechnol. Appl. Biochem*, 31, 135– 152.
- Prakash, O., Jaiswal, N., & Pandey, R. K. (2011). Effect of Metal Ions, EDTA and Sulphydryl Reagents on Soybean Amylase Activity. *Asian Journal of Biochemistry* 6 (3): 282-290.
- Vihinen MM, Mantsala P. (1989). Microbial amytolitic Enzymes. *Critical Rev Biochem Molec Biol*, 24: 329-418.
- Wirahadikusumah, M. (1989). *Biokimia: Protein, Enzim, dan Asam Nukleat*. Bandung: ITB.

Winarno, F. G. (1992). *Kimia Pangan dan Gizi*. Jakarta: Gramedia.