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Original Research Article

Inhibitory effect of α-cyclodextrin on α-amylase activity

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Abstract

Purpose: *To explore the effect of α-cyclodextrin on the activity of α-amylase with a view to expanding its application range.*
Methods: *The concentration of α-cyclodextrin, temperature, pH and interaction time were used as single factors to explore the influence of α-cyclodextrin on the activity of α-amylase and endogenous fluorescence in the enzyme system.*
Results: *The results showed that the concentration, time, pH and temperature affect the interaction of them. The most obvious conditions for inhibition of α-amylase activity are as follows: 10 mmol/L concentration of α-cyclodextrin, pH 6.9, duration of 120 min and temperature at 55 ^{0}C. In addition, the fluorescence intensity of α-amylase changed as a result of the addition of α-cyclodextrin.*
Conclusion: *The activity of α-amylase can be inhibited by α-cyclodextrin. At the same time, the addition of α-cyclodextrin will lead to the transfer of tryptophan group in α-amylase, which cause the change of microenvironment and changes the endogenous fluorescence intensity of α-amylase.*

Keywords: *α-Cyclodextrin, α-Amylase, Fluorescence intensity, Inhibition*

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INTRODUCTION

Cyclodextrins (CDs) consists of α-D-glucose units linked by α-(1,4)-glucosidic bond, which can form a clathrate with a variety of substances because of its hydrophobic cavity [1]. CDs includes three types of CDs (α-, β-, γ-), which have the same cavity height but differ in size and volume that leads to their different properties [2]. Among the three, the application of β-CD is the most extensive, due to its many advantages such as low production cost, suitable cavity size, stable physical and chemical properties [3].

α-Amylase can hydrolyze polysaccharides into oligosaccharides, which is widely used in animals, plants and microorganisms [4]. The industrial use of α-amylase mostly comes from fungi and belongs to mesothermal amylase and has obvious application advantages, which have been used in beer brewing, paper industry and grain industry [5]. α-amylase could improve the product of wheat flour, by increasing the volume of bread, improving the quality of fermentation products and preventing excessive gelatinization of starch [6].

CDs can affect the catalytic reaction of enzyme by catalyzing the reaction group of enzyme or complex reaction substrate [7]. In some reactions, CDs can increase the rate of catalytic reaction, which may due to the fact that it can facilitate the dissolution or activation of substrate [8]. While the opposite effect has also been reported, for example, it can improve the browning of apple juice and banana juice by inhibiting enzymatic browning [9]. CDs can inhibit the activity of various enzymes, such as lipoxygenase, pullulanase and α-galactosidase [10,11]. At the same time, CDs have exhibited competitive inhibition against the activity of β-amylase produced by microorganisms and plants.

The interaction of CDs and enzyme can be characterized by the change of the properties of the enzyme. At present, the main detection methods include enzyme reaction kinetics, fluorescence spectra, circular dichroism, ultraviolet spectrum, etc. Fluorescence spectrometry was used to investigate the structure and microenvironment of fluorescence chromophore in enzyme molecules. Proteins produce different fluorescence spectra because they contain chromophoric groups include Try, Tyr and Phe [12]. Fluorescence spectra are commonly used to determine the variation of hydrophobic microenvironment, it can also investigation the interactions between molecules. This study aims to explore the inhibitory effect of α-CD on α-amylase and the change of fluorescence intensity in different conditions.

EXPERIMENTAL

Materials

α-amylase (from *Aspergullus oryzae*) aqueous solution, > 800 FAU/g), α-CD and starch were purchased from Sigma-Aldrich Trading Co. Ltd (Shanghai, China). All other reagents were of reagent grade.

α-amylase activity assay

The activity of α-amylase was determined by 3,5-Dinitrosalicylic acid colorimetric (DNS) method [12]. The enzyme activity unit was defined as the amount of enzyme needed to hydrolyze soluble starch to release 1 μmol reduction product in 5 min at 60 ^{o}C. 0.9 mL of the prepared solution was added to the test tube kept at 60 ^{o}C for 5 min. 1 mL of α-amylase was added to the system and then the system was kept at 60 ^{o}C for 5 min. 1 mL of DNS was added to the reaction system which was kept at 100^{o}C for 5 min. Then, distilled water was added to a volume of 10 mL. The absorbance at 540 nm was measured. α-amylase activity was calculated from a standard curve (y = 1.1889x+0.0672, R^2=0.9994) as in Eq 1.

A = M/0.01 …….. (1)

where A (U/mL) is the activity of α-amylase and M (μmol) is the amount of maltose produced under the action of α-amylase. The relative residual activity (RRA) was defined as the ratio of the activity of the α-amylase when α-CD was added and α-amylase activity without the addition of α-CD [11].

Sample preparation

α-amylase (50 μL) and α-CD (150 μL) reacted in phosphate buffer and set different conditions. The effect of α-CD on α-amylase was determined.

Determination of experimental conditions

In order to study the effect of α-CD on activity of α-CD, four key factors were selected, pH (3.9, 4.9, 5.9, 6.9, 7.9), concentration of α-CD (0.01, 0.1, 1, 10 mmol/L), time (0, 30, 60, 90, 120 min) and reaction temperature (25, 35, 45, 55, 65 ℃). Changes in α-amylase activity and fluorescence intensity were measured under different conditions.

Fluorescence spectroscopy

The fluorescence spectrum was measured in F-7000 fluorescence spectrometer (Hitachi, Japan). The emission wavelength was obtained in the range of 290 to 450 nm, and at the excitation wavelength of 227 nm (silt = 5.0 nm). The scanning speed was 12000 nm/min.

Statistical analysis

All experiments were repeated three times. The data were analyzed by SPSS version 22 (IBM ® SPSS ® Statistics, USA). Significant difference among the results was obtained by multiple sample comparison of the means (ANOVA) and the LSD, with significant level set at $p < 0.05$.

RESULTS

Effect of concentration of α-CD on the activity of α-amylase

As shown in Figure 1, the activity of α-amylase was affected by α-CD. The activity of α-amylase decreased slowly with the addition of α-CD and the activity was only 80 % when the

concentration of α-CD was 10 mmol/L. Yao and Yu studies with α-galactosidase and pullulanse also found similar phenomena [11]. According to Figure 2, the addition of α-CD also caused changes in the endogenous fluorescence of α-amylase. The fluorescence intensity of α-amylase decreased from 334.8 to 319.5 with increase in the concentration of α-CD from 0.01 to 1 mmol/L. α-CD of 10 mmol/L promoted fluorescence intensity.

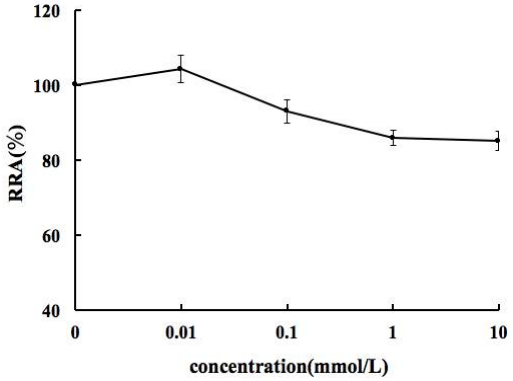

Figure 1: Effect of concentration on activity of α-amylase in the absence of β-CD (control) and in the presence of β-CD (0.01, 0.1, 1, and 10 mmol/L)

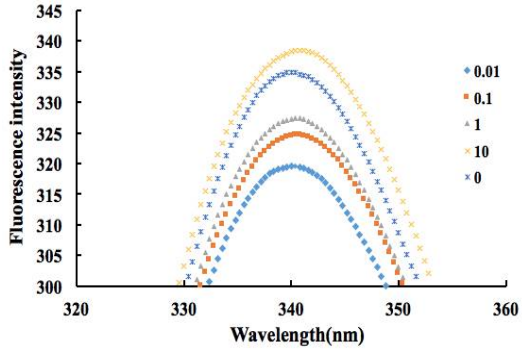

Figure 2: Effect of α-CD on different concentrations (0, 0.01, 0.1, 1 and 10 mmol/L) on the fluorescence intensity of α-amylase

Effects of reaction time on interaction of α-CD and α-amylase

As shown in Figure 3, activity of α-amylase decreased by 10 % to 90 % with the extension of incubation time. The activity decreased to 70 % when the reaction was 120 min. The change of fluorescence intensity was shown in Figure 4. Maximum fluorescence intensity increased from 349.7 to 381.7 as reaction time went on.

Effect of reaction temperature on interaction of α-CD and α-amylase

As shown in Figure 5, the interaction between α-CD and α-amylase was affected by temperature; the inhibitory effect first increased and then decreased with change in temperature. The inhibitory effect was strongest at 55 ^{o}C. As can be seen from Figure 6, the maximum fluorescence intensity varied with temperature, as it increased from 349.7 to 371.3, when the temperature increased from 25 to 55 ^{o}C.

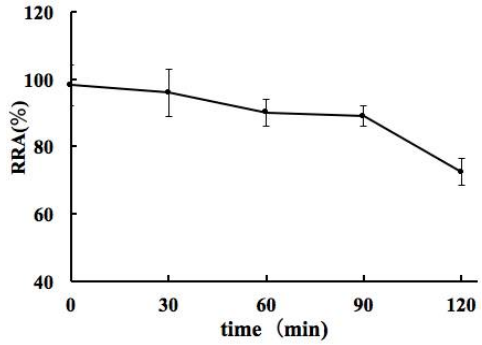

Figure 3: Effect of reaction time on activity of α-amylase under different reaction times (0, 30, 60, 90 and 120 min)

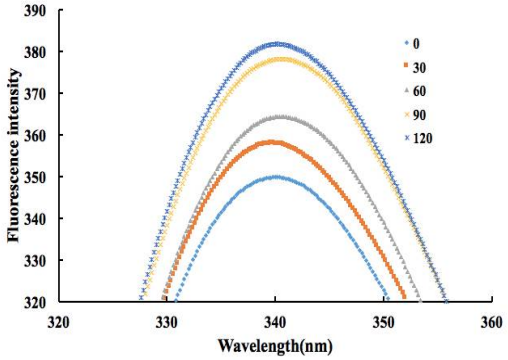

Figure 4: Effect of various duration (0, 30, 60, 90, 120 min) on the fluorescence intensity of α-amylase

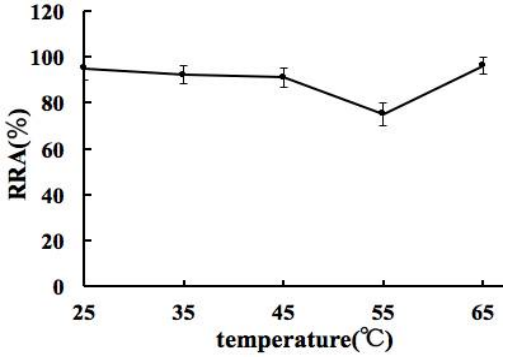

Figure 5: Effect of reaction time on activity of α-amylase under different reaction times (25, 35, 45, 55 and 65 ^{o}C).

Effect of pH on interaction of α-CD and α-amylase

As shown in Figure 7, α-CD has varying inhibitory effect on α-amylase at different pH. The

activity of α-amylase first decreased and then increased, the lowest activity being at pH 6.9. Figure 8 shows the relationship between pH and fluorescence intensity. Fluorescence intensity increased from 289.9 to 338.2 when pH was adjusted from 3.9 to 7.9.

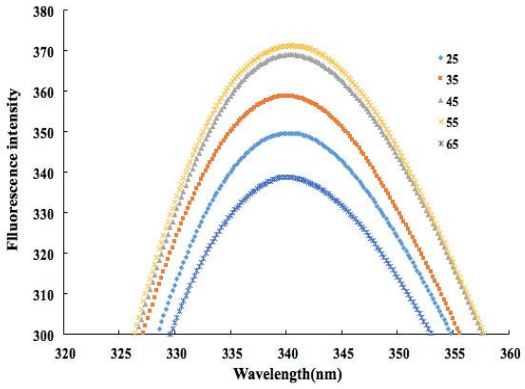

Figure 6: Effect of temperature (25, 35, 45, 55 and 65 ^{o}C) on the fluorescence intensity of α-amylase

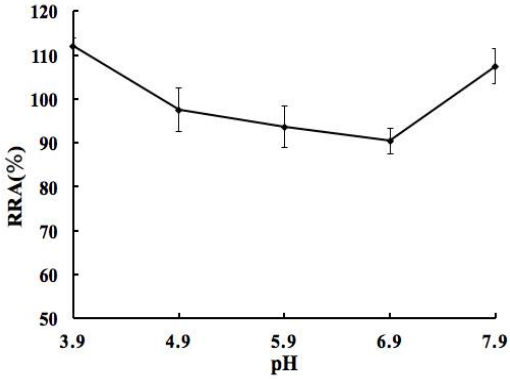

Figure 7: Effects of reaction time on activity of α-amylase under different pH (3.9, 4.9, 5.9, 6.9 and 7.9)

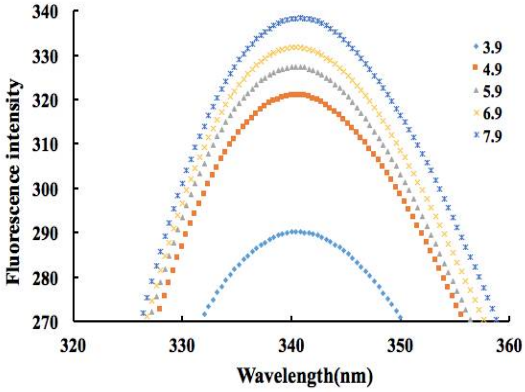

Figure 8: Effect of pH (3.9, 4.9, 5.9, 6.9 and 7.9) on the fluorescence intensity of α-amylase

DISCUSSION

Different concentrations of α-CD had an effect on the activity of α-amylase. The reason may be that the low concentrations of α-CD cannot cause a change in α-amylase molecular conformation. With increase of α-CD, more cavity and α-amylase molecules interact with each other, causing a change in enzyme molecular structure and resulting in decreased activity. Similarly, different concentrations of α-CD also caused changes in the fluorescence intensity of α-amylase. It may be that the presence of α-CD caused the microenvironment of the enzyme molecule to be altered. α-CD was added to the system to cause the transfer of tryptophan groups. However, tryptophan can be protected and detected when the concentration of α-CD is high. The interaction of α-CD and α-amylase can also be affected by fluorescence spectra.

The effect of α-CD on α-amylase activity and fluorescence intensity varied with time. The results were similar to those of Rajalakshmi *et al* in their kinetics studies on α-amylase [13]. The reason may be that the structure of α-amylase was loosened as reaction time was extended. The polarity of the α-amylase environment was destroyed by α-CD, and the exposure of tryptophan group increased fluorescence intensity. The results showed that the influence of α-CD on α-amylase changed with time.

Reaction temperature can affect the interaction between α-CD and α-amylase. Alline also found that the temperature was too low or high to have a detrimental effect of α-amylase [8]. Fluorescence was reduced to 338.7 at 65 ^{o}C. The possible reason is that the interaction of α-CD and α-amylase increased with temperature rise, which resulted in the enhancement of fluorescence as tryptophan residues are exposed more at higher temperature. However, it has to be noticed that too high temperature could cause tryptophan to be inactivated.

The interaction of α-CD and α-amylase can be affected by pH. This change may be because the change of pH leads to the changes of amino acids charge, which results in the changes of electrostatic interaction and hydrogen bonding in the system. In addition, the change of pH also causes the change of the enzyme molecule conformation.

CONCLUSION

The findings of this study show that α-CD inhibits the activity of α-amylase under certain conditions. α-CD can also change the displacement of tryptophan to cause microenvironmental change in enzyme molecule. These results provide a platform for extending the application of α-CD and α-amylase.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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