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Extraction of acid phosphatase from mung bean sprouts and determination of its enzymatic properties

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Abstract: To investigate the enzymatic properties of acid phosphatase from mung bean sprouts and explore the optimal reaction conditions for this enzyme. This study extracted phosphatase from fresh mung bean sprouts and determined its enzymatic properties. It was found that the activity of acid phosphatase in 1ml of mung bean sprout enzyme solution was 8.68, the optimal pH was 5.4, and the temperature at which the activity was highest was 50 degrees Celsius. This study not only determined the enzymatic properties of the enzyme and obtained the optimal reaction conditions, laying the foundation for related research, but also providing theoretical guidance for subsequent industrial applications.

Keywords: Acid Phosphatase, Enzymatic Properties, Optimum Condition, Mung Bean Sprouts, Enzyme activity

1. INTRODUCTION

Acid phosphatase (ACPase) is widely distributed in animals and plants and is an important enzyme in biological phosphorus metabolism[1]. In addition to participating in phospholipid metabolism, it also participates in important life activities such as metabolic regulation and energy conversion. ACPase is involved in plant phosphorus metabolism and plays an important role in cellular regulation.

These enzymes play important roles in various biological processes within the organism, such as cellular signaling, energy metabolism, and disease development. In recent years, significant progress has been made in the study of acid phosphatase, particularly in structural biology, enzyme activity regulation mechanisms, and disease correlation. In terms of structural biology, scientists have revealed the three-dimensional structure of various acid phosphatase enzymes through X-ray crystallography and nuclear magnetic resonance (NMR) techniques. These structural information are of great significance for understanding the catalytic mechanism of enzymes and designing inhibitors. For example, studies have found that the active sites of ACP typically contain iron ions, which are crucial for the catalytic activity of enzymes[2].In terms of enzyme activity regulation mechanisms, researchers have explored how factors such as pH, metal ions, and substrate specificity affect the activity of ACP. For example, pH has a significant impact on the activity of ACP, with enzymes exhibiting the highest activity in acidic environments. In addition, certain metal ions can act as cofactors to enhance the activity of ACP[3].In

terms of disease relevance, an increasing number of studies have shown that ACP is closely related to the development of various diseases, such as prostate cancer, osteoporosis, and neurodegenerative diseases. Therefore, ACP is considered a potential biomarker and therapeutic target. For example, prostate acid phosphatase (PAP) is an important biomarker in the diagnosis of prostate cancer[4-8].

The enzymatic activity of ACP in mung bean sprouts is essential for plant growth. It is involved in the hydrolysis of phosphate compounds, releasing phosphoric acid and organic molecules, which are important for energy conversion and utilization in plants. In addition, ACP is involved in signaling processes in plants, especially during the seed germination and seedling growth stages, and changes in ACP activity may be an important factor in regulating these physiological processes. By regulating the activity of ACP, the growth rate and acclimatization of the plant, and consequently its ability to adapt to the growing environment, can be influenced.The importance of ACP in plant growth and development, and understanding and determining its optimal response conditions are of practical importance for improving and controlling plant growth. By optimizing these conditions, the activity of ACP can be increased to promote the growth of mung bean sprouts or, in the case of agricultural production, to improve crop yield and quality by regulating these conditions.

This experiment used mung bean sprouts as the experimental material, isolated and purified ACPase, and studied its enzymatic properties, providing theoretical reference for the cultivation of mung bean sprouts[9][10].This study not only determined the enzymatic properties of the enzyme and obtained the optimal reaction conditions[11], laying the foundation for related research[12], but also providing theoretical guidance for subsequent industrial applications[13].

2. MATERIALS AND METHODS

2.1 Acid phosphatase extraction

Pinch off the roots and leaves of mung bean sprouts to obtain mung bean sprouts and stems, weigh 50g of mung bean sprouts and stems, thoroughly grind them in a mortar, let them stand at room temperature for 30 minutes[14], and squeeze and filter with double-layer gauze in a culture dish to obtain the filtrate. Transfer the filtrate to two centrifuge tubes, balance them, and place them in a centrifuge. Centrifuge at a speed of 12000 r/min for 8 minutes[15]. Transfer most of the supernatant from the centrifuge tube to a graduated cylinder, and filter a small portion of the supernatant close to the sediment through filter paper.

2.2 Determination of acid phosphatase activity

Disodium phenyl phosphate can be hydrolyzed by acid phosphatase to produce phenols and inorganic phosphorus. When there is a sufficient amount of substrate sodium diphenylphosphate present, the greater the activity of acid phosphatase, the more phenols and organic phosphorus produced. According to the definition of enzyme activity units, 1 is generated per minute under the optimal conditions of enzymatic reactions μ The enzyme required for the mol product is one activity unit, so the activity of acid phosphatase can be expressed by measuring the product phenol using the Folin phenol method or by measuring inorganic phosphorus using the fixed phosphorus method.

Six test tubes were taken for the production of standard curves, numbered from 00 to 05, with empty tubes numbered as 00. Reagents were added in sequence according to Table 1, and after adding the reagents, the absorbance test was performed after standing still for 35 minutes.

Table 1: Production of Standard Curve.

Reagent	Test tube number					
	00	01	02	03	04	05
0.4mmol/LPhenol standard solution	0	0.1	0.2	0.3	0.4	0.5
0.05mol/L pH5.0 citrate buffer	1	0.9	0.8	0.7	0.6	0.5
1mol/L sodium carbonate solution	5.0	5.0	5.0	5.0	5.0	5.0
Folin reagent	0.5	0.5	0.5	0.5	0.5	0.5

Using test tube 00 as a blank, read the optical density OD680nm of each tube at a wavelength of 680nm on a visible spectrophotometer. Use OD680nm as the x-axis and the volume (mL) of phenol standard solution as the y-axis to draw the standard curve using a first-order function fitting method.

Take 2 test tubes, number and operate according to Table 2, and measure the enzyme activity of acid phosphatase.

Table 2: Enzyme Activity Determination.

Procedure	Test tube number	
	01'	00'
Add 5mmol/L sodium benzoate solution	0.5 ml	0.5 ml
Preheat at 35 ℃ for 2 minutes		
Add enzyme solution preheated at 35 ℃	0.5 ml	0 ml
Accurate reaction for 10 minutes		
1mol/L sodium carbonate solution	5ml	5ml
Folin reagent	0.5ml	0.5ml
Add enzyme solution preheated at 35 ℃	0 ml	0.5 ml
Shake well, keep at 35 ℃ for 10 minutes to develop color		

Using test tube 00 'as a control, measure the OD680nm optical density value of test tube 01' at 680nm using a visible spectrophotometer. Calculate the volume (V) of the phenol standard solution corresponding to OD680nm based on the standard curve. Calculate the enzyme activity corresponding to 1ml of enzyme solution according to Equation 1[11].

$$\text{enzyme activity} = \frac{2 \times 0.4 \times V \times 1000}{10} \quad (1)$$

2.3 Determination of optimal temperature

Take 9 test tubes and number them from 20 to 28. The experimental design is shown in Table :3. Add 1.0mL of 1.2mmol/L NPP solution to test tubes 1-8 and keep at their corresponding temperatures for 2 minutes. After insulation, test tubes 1-8 were added to the enzyme solution in a constant temperature water bath at 10 ℃, 20 ℃, 30 ℃, 35 ℃, 40 ℃, 50 ℃, 60 ℃, and 70 ℃ for 2 minutes. After 15 minutes of precise reaction, add 3.0mL of 0.3mol/L NaOH solution to terminate the reaction. The reaction temperature of tube 20 is 50 ℃. First, 3.0mL of 0.3mol/L NaOH solution is added, and then enzyme solution is added. After each tube is terminated, OD405nm is measured sequentially using tube 10 as a control. The results are recorded according to different pH values.

Table 3: Enzyme Activity Determination Process.

Test tube number	20	21	22	23	24	25	26	27	28
Reaction temperature(℃)	50	10	20	30	35	40	50	60	70
1.2mmol/L NPP(ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Enzyme solution (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.3mol/L NaOH(ml)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

3. CONCLUSION

Using OD680nm as the x-axis and the volume (mL) of the phenol standard solution as the y-axis, perform a first-order function fitting to obtain the standard curve, as shown in Figure 1.

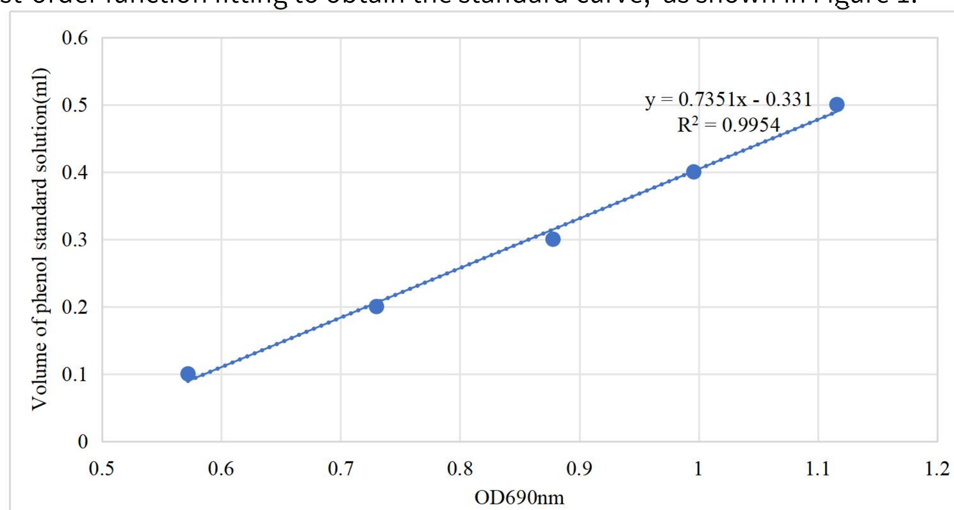

Figure 1. Standard curve

The volume corresponding relationship between OD680nm and phenol standard solution is shown in equation 2. The standard curve R^2=0.9954 after a single fitting in this experiment indicates that the curve is relatively accurate.

$$y = 0.7351x - 0.331 \tag{2}$$

In the formula, x represents the optical density value at 680nm, and y represents the volume of phenol standard solution (ml)

Using a visible spectrophotometer as a control, the optical density value OD680nm=0.598 at 680nm in test tube 01 'was measured. According to the standard curve, the optical density value at 680nm was calculated to be equivalent to 0.1085ml of phenol standard solution. Then, according to equation 1, the activity of 1ml of enzyme solution was calculated to be 8.68.

The results of the enzyme optimal pH determination experiment are shown in Table 4. In 10 groups of experiments, the pH of the buffer with the highest acid phosphatase activity was 5.5, and OD405nm=1.371.

Table 4: Results of Enzyme Optimal pH Determination Experiment.

Test tube number	11	12	13	14	15	16	17	18	19	110
OD405nm	0.050	0.219	0.386	0.668	1.073	1.313	1.371	1.149	0.843	0.445

Based on this experimental data, curve fitting was performed using Excel. The results are shown in Figure 2, indicating that the optimal pH for acid phosphatase is 5.4, with OD405nm=1.382.

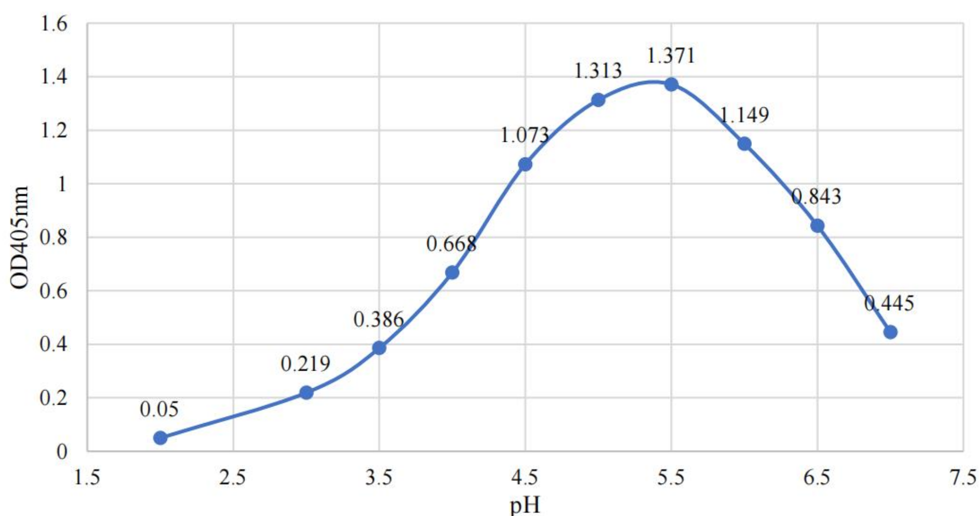

Figure 2: Fitting curve of enzyme optimal pH measurement experiment results

The results of the enzyme optimal temperature determination experiment are shown in Table 5. Among the 8 groups of experiments, the temperature at which acid phosphatase activity is highest is 50 degrees Celsius, at which OD405nm=1.371.

Table 5. Experimental Results of Enzyme Optimal Temperature Determination.

Test tube number	21	22	23	24	25	26	27	28
OD405nm	0.165	0.556	1.055	1.387	1.835	2.461	1.967	1.675

Based on this experimental data, curve fitting was performed using Excel. The results are shown in Figure 3, indicating that the optimal temperature for acid phosphatase is 50 degrees Celsius, at which OD405nm=1.371.

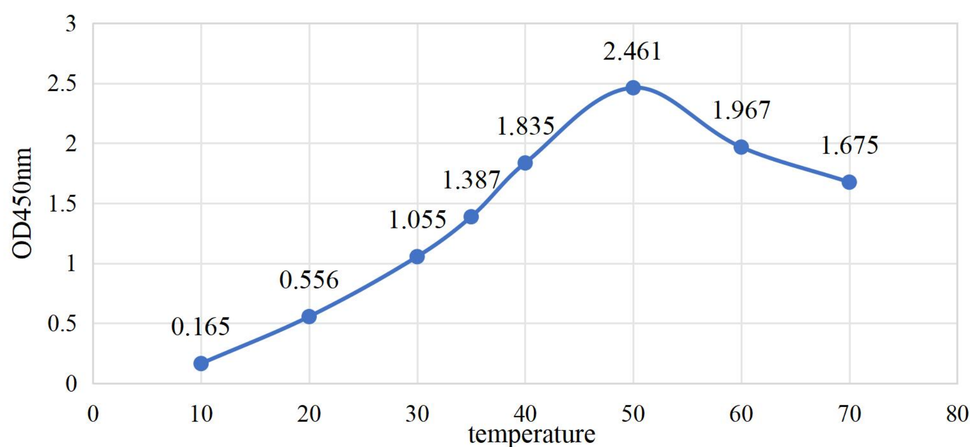

Figure 3: Fitting curve of enzyme optimal temperature measurement experiment results.

4. DISCUSSION

This study investigated the optimal temperature and pH of acid phosphatase (ACP) in mung bean sprouts, and calculated its optimal enzyme activity, providing important information for understanding the role of ACP in plant growth and development. Meanwhile, substrate concentration can affect enzyme saturation and reaction rate, so our team will determine its optimal concentration through experiments

in the future. In addition, the future will also explore the influence of certain metal ions as cofactors on ACP activity.

After completing the above tasks in the future, we will delve deeper into the following content.

Functional research: Further explore the specific biological functions of ACP in the growth and development of mung bean sprouts. For example, the effects of ACP on nutrient transport, energy metabolism, and plant hormone signaling pathways can be studied through gene silencing or overexpression techniques.

Genetic variation research: To study the genetic diversity of ACP genes in different mung bean varieties, and analyze how these variations affect enzyme activity and plant growth phenotype. This helps to screen out mung bean varieties with excellent agronomic traits.

Environmental adaptability research: examining the changes in ACP activity under different environmental conditions, such as temperature, light, soil nutrition, and moisture. This helps to understand how ACP helps plants adapt to environmental stress and may reveal new stress resistance genes or pathways.

Inhibitor/Activator Screening: Discover or design specific ACP inhibitors or activators, and study their effects on the growth and development of mung bean sprouts. This not only helps to gain a deeper understanding of the functions of ACP, but may also provide new growth regulators for agricultural production.

Biotechnology application: Using genetic engineering technology, genes with high ACP activity are introduced into other crops to study whether it can improve crop growth rate and yield, or enhance their adaptability to adverse soil conditions such as phosphorus deficiency.

Molecular mechanism research: At the molecular level, investigate how ACP is regulated by intracellular signaling pathways, including the effects of protein modification methods such as phosphorylation and ubiquitination on ACP activity.

Disease association research: As ACP may play a role in plant disease resistance, it is possible to investigate whether ACP affects the resistance of plant pathogens and whether it participates in systemic acquired disease resistance (SAR) in plants.

5. TASK DIVISION

Quan Zhou has completed the experimental design, data analysis and image drawing. At the same time, Quan Zhou wrote the Chinese version of the paper.

Lei Chen completed the specific experimental content and translated the Chinese version of the paper.

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