Extraction of Castor Plant Peroxidase by Aqueous Two-phase Systems

Pakaphon Thongchum and Assoc. Prof. Dr. Lalida Shank

Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University

FACULTY OF SCIENCE
CHIANG MAI UNIVERSITY

Introduction

- **The castor bean** is a species of perennial flowering plant in the spurge family, Euphorbiaceae. It is the sole species in the monotypic genus, *Ricinus*, and subtribe, Ricininae, is a good source of castor beans oil for food, medication, skin care, lubricant and biodiesel.
- **Peroxidase** is an enzyme in the oxido-reductases group, widely found in nature. It catalyzes the oxidation of various substrates using hydrogen peroxide as an electron acceptor. Its molecular weight ranges between 30 and 55 kDa and peroxidase is useful for removing phenolic compounds and peroxides, as well as in chemical synthesis and wastewater treatment in industries
- **Aqueous two-phase systems (ATPS)** are clean alternatives for traditional organic-water solvent extraction systems, two phases are mostly composed of water and non-volatile components, thus eliminating volatile organic compounds.
- **SDS-PAGE** is a discontinuous electrophoretic system developed by Ulrich K. Laemmli which is commonly used as a method to separate proteins with molecular masses between 5 and 250 kDa. The combined use of sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) and polyacrylamide gel eliminates the influence of structure and charge, and proteins are separated by differences in their size.

Methodology

- **Preparation of crude extract**

  Weigh 25 g of chopped castor leaves , homogenize with extraction buffer 100 ml using a blender, filter the solution and centrifuge at 10,000 rpm 4 °C for 20 minutes.

- **Extraction using an aqueous two-phase systems**

  Mix the crude extract with polyethylene glycol (PEG) and sodium sulfate, adjust the volume with the crude extract to 10 ml in volumetric cylinder after phase separation for 3 hours measure the volume of the upper and lower phase solutions.

- **Determination of peroxidase activity**

  Mix 1% (v/v) hydrogen peroxide, 4% (v/v) guaicol, 0.1 M sodium acetate buffer pH 6.0 and enzyme extract after phase separation in a test tube, allow the reaction to proceed for 3 minutes add 5% TCA and measure the absorbance at 470 nm.

- **Determination of protein content**

  Bradfrod protein assay

  Mix the sample with 0.1 M sodium acetate buffer pH 6.0 and coomassie brilliant blue G-250 allow the reaction to proceed for 3 minutes and measure the absorbance at 595 nm , create a protein standard curve for calculate the protein content.

- **Determination of protein profile using SDS-PAGE and semi native-PAGE technique**

  SDS-PAGE

  Mix the sample with loading dye + 10% β-mercaptoethanol and heat to 95 °C for 10 minutes, after run gel for 3 hours incubate the gel with coomassie R-250 overnight and destain.

  Semi native-PAGE

  Mix the sample with loading dye and load the sample into the well, after run gel for 3 hours wash the gel with 2.5% triton x-100 for 40 minutes and stain with substrate, incubate the gel with coomassie R-250 overnight.

Abstract

This research aimed to extract peroxidase from castor plants using the aqueous two-phase systems (ATPS). Peroxidase activity was determined by using guaiacol as a substrate in the presence of hydrogen peroxide. Protein content was determined by Bradford method. In this aqueous two-phase extraction experiment, different amounts (%w/w) of polyethylene glycol (PEG) and sodium sulfate were used under five different combinations in crude extract giving the final volume of 10 mL as follows: the first 14% PEG 1500 and 12% sodium sulfate, the second 18% PEG 1500 with 8% sodium sulfate; the third 14% PEG 4000 and 8% sodium sulfate; the fourth 18% PEG 4000 with 8% sodium sulfate; and the last 18% PEG 6000) with 8% sodium sulfate. Phase separation was allowed to proceed for 3 hours. Results showed the total activity of peroxidase in the bottom sodium sulfate phase to be higher than that of the top polyethylene glycol phase and thus become the phase of interest. Results showed that the use of 18% PEG 1500 with 8% sodium sulfate gave the highest peroxidase purity. In the protein profile examination of the salt phase samples by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and semi-native polyacrylamide gel electrophoresis (Semi-native-PAGE), the salt phase extracts in all conditions showed colored bands of tetra-guaiacol after substrate staining, confirming that the extracts contained peroxidase among other proteins that can be further separated.

Objectives

- To extract peroxidase from castor leaves using an aqueous two-phase systems (ATPS)
- To determine the optimum conditions for peroxidase extraction from castor leaves using an aqueous two-phase systems (ATPS)
- To obtain protein profile from castor leaves using SDS-PAGE

Results & Discussion

• Preparation of crude extract

Table 1 shows the total extract volume and specific activity

Sample	Total volume of extract (ml)	Peroxidase activity (unit/ml)	Protein content (mg)	Total peroxidase activity (unit)	Total protein content (mg)	Specific activity (unit/mg)
Crude extract	190.00	6.18 ± 0.15	0.26 ± 0.01	1174.20	988.00	1.17

• Determination of peroxidase activity and the protein content

Table 2 shows specific activity between the upper phase and lower phase of the different conditions using ATPS

Sample	Total peroxidase activity (unit)		Total protein content (mg)		Specific activity (unit/mg)	
	The upper phase	The lower phase	The upper phase	The lower phase	The upper phase	The lower phase
14% PEG 1500/12% Na_2SO_4	0.96	49.50	13.51	24.89	0.07	1.99
18% PEG 1500/8% Na_2SO_4	2.44	43.80	14.52	13.40	0.17	3.27
14% PEG 4000/8% Na_2SO_4	0.93	39.87	9.57	14.22	0.10	2.80
18% PEG 4000/8% Na_2SO_4	2.97	34.73	12.95	19.50	0.23	1.78
18% PEG 6000/8% Na_2SO_4	0.72	36.27	11.72	18.31	0.06	1.98

- The specific activity of the lower phase is higher than the upper phase.

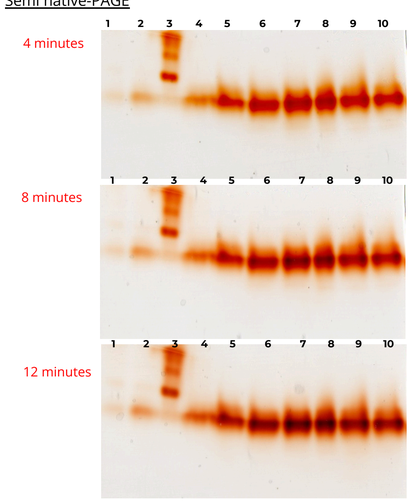
Table 3 summary of the purification of peroxidase extracted under different conditions using ATPS showing values for the lower phase

Sample	Total peroxidase activity (unit)	Total protein content (mg)	Specific activity (unit/mg)	Purification fold	% Recovery
Crude Extract	61.84	52.77	1.17	1.00	100.00
14% PEG 1500/12% Na_2SO_4	49.50	24.89	1.99	1.70	80.05
18% PEG 1500/8% Na_2SO_4	43.80	13.40	3.27	2.79	70.83
14% PEG 4000/8% Na_2SO_4	39.87	14.22	2.80	2.39	64.47
18% PEG 4000/8% Na_2SO_4	34.73	19.50	1.78	1.52	56.16
18% PEG 6000/8% Na_2SO_4	36.27	18.31	1.98	1.69	58.65

- The highest specific activity was observed in 18% polyethylene glycol 1500 with 8% sodium sulfate. The purification fold was 2.79 when compared to the crude extract.
- 14% polyethylene glycol 1500 with 8% sodium sulfate had the highest %recovery.

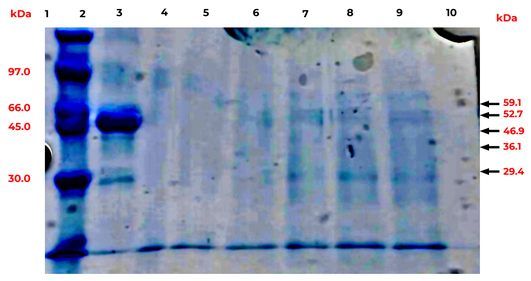
• Determination of protein profile using SDS-PAGE and semi native-PAGE

Semi native-PAGE



- The gel was stained using guaiacol and hydrogen peroxide.
- In the presence of tetra guaiacol with orange color would be formed and the color intensity increased when stained with more time.

SDS-PAGE



- Several protein bands appeared showimg that peroxidase was partially purified and other proteins can befurther separated.

Conclusion

- The lower phase showed higher activity than that of the upper phase for all conditions tested.
- 18% polyethylene glycol 1500 with 8% sodium sulfate was the optimal condition for extraction
- Semi-native PAGE confirmed the presence of peroxidase in the extracts, SDS-PAGE showed several protein bands suggesting that peroxidase can be further purified

References

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Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University.