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RESEARCH ARTICLE

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RAPID AND EFFICIENT PURIFICATION METHOD OF PHOSPHOLIPASE A2 FROM *ACANTHASTER PLANCI*

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ABSTRACT

Rapid and efficient purification method of the phopholipase A2 (PLA2) from the crown-of-thorns starfish *Acanthaster planci* was done. It was developed without using separation column and taken less time around one day purified. Compare to common procedure that was done by purification measurement using several columns chromatography and was known taken several days, this purification method is more simplify. This purification method use only 30 minutes heat treatment on 60^{o}C and then is fractionated by 20% saturated ammonium sulfate precipitation. The protein complex was analyzed by one dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis. PLA2 is obtained an excellent overall yield with a final enzyme activity was similar to result of common procedure using four columns chromatography.

KEYWORDS

Starfish, Simple method, Purification, Phospholipase A2, yield and activity

INTRODUCTION

The crown-of-thorns starfish *Acanthaster planci* caused widespread damage to many coral reefs[1, 2]. Among those, the *A. planci* is infamous for its dramatic outbreak that had devastated coral reefs, the treatment of the outbreaks are still not effective, only by taking the starfish and waste it in the land[3, 4]. Thereupon, the utilization of the resource is needed.

The *A. planci* has numbers of proteins in the spines venom of their surface body[5, 6]. It were reported there are at least three kinds of proteins/peptides in the venoms, plancinins that act as anticoagulant factor, phospolipase A2s (PLA2s) and placitoxins that is similar with mammalian deoxyribonuleases II[7, 8, 9]. In the three kinds of proteins/peptide, the phosholipase A2 is one of the protein that promising as antibacterial agent. PLA2s from other resources could against Gram-positive bacteria, including methicillin-resistant *staphylococcus* and vancomycin-resistant *enterococci*, which mean that the enzymes may be considered as future therapeutic agents against bacterial infections[10, 11].

The *A. planci* venom contain two PLA2, The molecular mass of PLA2-I was estimated to be 28 kDa by gel filtration and 15 kDa by SDS PAGE, indicating that PLA2-I is a dimer composed of the same subunits. In contrast, PLA2-II estimated to be a monomer with a molecular mass 12 kDa by gel filtration or 15 kDa (SDS-PAGE). Refer to result of previous research result [5, 6], it was known very difficult to purify PLA2 from *A. planci*. For purify PLA2, it was taken four column, CM cellulose, Phenyl Sepharose CL-4B, sepharacyl S-200 or superpose 12 and TSKgel Phenyl 5PW-RP or TSKgel ODS-120T[8]. This previous procedure was costly and taken more times. Current study is focused for developing a more simple and efficient method for PLA2 purification from *A. planci* and also clarifying content and properties of it's yield.

MATERIALS AND METHODS

(i) ***Starfish***

The specimens of crown-of-thorns starfish *A. planci* were captured from Ambon Island, Moluccas prefecture in September 2010. They were kept frozen at –40 °C before using.

(ii) ***Procedure of isolation phospholipids A2***

Spines (50 g) were collected from a specimes of A. planci. Toxin, firstly was extracted by spines sonication in 0.01 M phosphate buffer pH 7.0 and 0.001 M $CaCl_2$. Extract yield, secondly was centrifuged and it's supernatant yield that was consist crude venom was heated at 60^{o}C for 30 minutes. It's yield, then centrifuged again to produce supernatant that was called as heat extract. This heat

extract, thirdly was fractionated by 20, 40, 60, and 80% saturated ammonium sulfate precipitation. Finally, fractionated yield were diluted in 0.01 M phosphate buffer pH 7.0 and 0.001 M $CaCl_2$.

(iii) ***Assay of Phospholipase A2 activity***

Throughout above purification procedure, PLA2 activity, according to Marinetti method (1965)[12], was qualitatively analysis by measuring the clearing of egg yolk suspension. Egg yolk suspension is made in 0.01 M phosphate buffer (pH 7.0) at a concentration of 2 mg egg yolk/ml. At estimated 5 minutes interval in beginning, absorbance that was measured at 900 nm is performed to mixture of 0.2 ml above purification yield and 3 ml egg yolk suspension. Enzyme activity causing the decrease of 0.01 in absorbance/minute was defined as 1 (one) unit.

(iv) ***Protein concentration assay***

The concentration of protein was determined by using *Lowry* method, using bovine serum albumin as standard[13].

(v) ***Gel electrophoresis***

Purified enzymes molecular weight were determined by 15% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSIONS

Purification of phospholipase A2

This work was set to make more simplify PLA2 purification process with high yield and reduce consumed time that relate to process cost reduction. At beginning, extraction step of crude venom from the spines of *A. planci* was performed by only spines sonicating in phosphate buffer and also by both of spines homogenization and sonication in phosphate buffer. Simplify analysis that shown qualitatively results were performed by extract ability test to prevent blood fluids from coagulation. Both of extraction procedure could prevent blood from the coagulation (data not shown), and for minimization of other protein contamination from spine cells, it is better to use spines sonication procedure without homogenization. Figure 1 showed that only 4 main proteins was existed in the crude venom and it suggested that Plancitoxin, unknown protein, PLA2 and plancinin have molecular weights around 22, 20, 15 and 7 kDa, respectively [8, 9, 14].

Furthermore, based on previous experimental result, PLA2 still have activity until 75^{o}C[8], and it means that PLA2 still stable in high temperature. Based on this reason, firstly, purification was done by heat treatment at 60^{o}C for 30 minutes and PLA2 could be separated from about 22 % of other protein, that were surely denatured.

Secondly, ammonium sulfate precipitation was utilized to fractionate heat treated extract by 20, 40, 60 and 80% saturated ammonium sulfate precipitation and final fractionated yield were diluted in phosphate buffer.

Table 1

Summary of PLA2 purification from A. planci

Sample	Protein Concentrations mg/ml	Volume (ml)	Units	Activity (unit/ml)	Total activity (unit)	Total Protein (mg)	Specific activity (unit/mg)	Purity
Crude venom	1,73	107	1,81	9,05	968,35	185,21	5,23	1
Heat extract	1,45	101	1,80	9,00	909,00	146,05	6,22	1,19
Fraction ammonium sulfate 0-20%	0,54	5	11,65	58,25	291,25	2,68	108,48	20,75
Fraction ammonium sulfate 20-40%	2,09	5	6,89	34,45	172,25	10,47	16,45	3,15
Fraction ammonium sulfate 40-60%	3,02	5	1,76	8,80	44,00	15,11	2,91	0,56
Fraction ammonium sulfate 60-80%	6,19	5	2,86	14,30	71,50	30,93	2,31	0,44

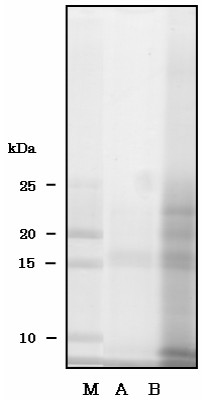

Figure 1

SDS-PAGE of the purified PLA2.

Lane M, standard protein; lane A, fraction ammonium sulfate of 20% saturation; Lane B, crude venom

The PLA2 activity were analyzed by using Marinetti method, it's activities were increased related to purification step. Crude venom have activity 5.23 unit/mg, and after heat treating, its extract activity increase to 6.22 unit/mg. Amazingly, further treating by 20% saturated ammonium sulfate precipitation, its fractionated enzyme activity increased until 20 fold compare to crude venom activity result (**Table 1**). This increasing fractionated enzyme activity result was similar to *Shiomi et al.* method that was performed using four columns chromatography[8]. To confirm the purity of extract, PLA2 molecular weight analysis was performed by one dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis, and obviously it shown that only PLA2 band at 15 kDa was detected (**Fig. 1**). Finally, PLA2 product yield was 1.4 % that ten folds compare to PLA2 yield that was produced by *Shiomi* method[8].

As a conclusion, this simplify

proposed method that was done around one day purified, could purify PLA2 from *A. planci* with an excellent result in both of overall yield and activity. In this moment, further studies in antimicrobial activities and other biological activities is on progress.

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