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ISOLATION AND CHARACTERIZATION OF PHOSPHOLIPASE A2 FROM THE SPINES VENOM OF THE CROWN-OF-THORNS STARFISH ISOLATED FROM PAPUA ISLAND

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ABSTRACT

The crown of thorns starfish (COT) *Acanthaster planci* spine venom contains phospholipase A2 (PLA_2). Recently, we have reported the rapid and efficient purification methods of it, especially for the PLA_2 from COT spine isolated in Ambon islands. The COT isolated in Papua islands has a different morphology compared with COT in Ambon islands, the spines were bigger and longer. To confirm the availability of PLA_2 in COT isolated in Papua, we isolated it by using our method. The activity of crude PLA_2 of it higher compare with crude PLA_2 isolated from COT spines from Ambon islands. Unfortunately, fractionated of ammonium sulfate at a concentration 20% is only slightly increased three times compared with the crude PLA_2. The result shows that the PLA_2 is calcium-dependent enzyme.

Key word : Crown of Thorns Starfish, Phospholipase A_2, Calcium-dependent, purification.

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INTRODUCTION

Crude venom spines of The crown of thorns starfish (COT) *Acanthaster planci* shows various biological activity such as haemolytic, myonecrotic, bleeding, increasing capillary permeability, odema, activity of histamine release from mast cells, anticoagulant and phospholipase-A_2 (PLA_2)[1,2,3,4]. Biological activity of PLA_2 might be applied in medicine and pharmacy. PLA_2 isolated from snake venom has as an antibacterial activity on *Burkholderia pseudomallei*[5]. PLA_2 from bee and snake venoms are potent as an anti-HIV agents[6]. Furthermore, contribution of PLA_2 based on its function in lipid metabolism is an enzyme that releases the acyl-2n from phospholipids and produce arachidonic acid and the lysophospholipid. Since cell membrane consists of cholesterol, proteins, glycolipids, oligosaccharides and phospholipids, therefore suggested PLA_2 can hydrolyze fats in the blood vessels and useful for cardiovascular therapy.

COT is a potential source of PLA_2. Recently, we proposed a rapid and simple method of how to purify PLA_2 from COT *A. planci*. COT from Papua seems different with from Maluku islands, its spines are bigger and longer. The differences of morphology might be influenced by habitat, age and even might be species. The PLA_2 from COT Maluku island was purified by using the combination of preheated venom with fractionated ammonium sulfate 20% and produce single band of protein. The specific activity of PLA_2increased twenty times comparing with the crude venom. Based on our previous results, we proposed to apply the methods to purify PLA_2 from spines COT isolated from Papua islands.

MATERIALS AND METHODS

(i) Crown of Thorns (COT) Starfish

COT was captured from Tanjung Kasuwary West-Sorong Papua in April 2011, then frozen and stored at -20^{o}C until used.

(ii) Isolation of Phospholipase A_2 (PLA_2)

The fifty grams of spines were collected from specimen COT. Firstly, toxin was extracted by sonicated of spines in 0.01 M phosphate buffer pH 7.0 and 0.001 M $CaCl_2$. Secondly, yield extract was centrifuged and the supernatant yield was heated or unheated at 60^{o}C for 30 minutes. Heated extract then centrifuged again to produce supernatant and called as heat extract. Thirdly, this heat extract 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)Phospholipase A_2 Activity Assay

PLA_2 activity assay according to Marinetti method (1965)[7], was qualitatively and quantitatively 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. Absorbance was measured at 900 nm is performed to mixture of 0.2 ml above purification yield and 3 ml egg yolk suspension in interval time 5 minutes. Enzyme activity causing the decrease of 0.01 in absorbance/minute was defined as 1 (one) unit.

(iv)Divalent-Cation dependent assays of PLA_2

PLA_2 isolated from COT *A. planci* is a cation dependent enzyme. To confirm the dependences of PLA_2isolated from the COT Papua, we characterize the activity of crude PLA_2 in several divalent-cations. Egg yolk suspension is made in 0.01 M phosphate buffer (pH 7.0) at a concentration of 2 mg egg yolk/ml. Absorbance was measured at 900 nm is performed to mixture of 0.2 ml crude venom preadded with 5 mM cations solution (Zn^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Fe^{2+}) and 3 ml egg yolk suspension in interval time 5 minutes.

(v) Determination of Protein Concentration

Protein concentration was determinate by using Lowry (1951) method. the bovine serum albumin (BSA) was a standard.

RESULTS AND DISCUSSION

Crown of thorns (COT) from Papua appears different compare with from Ambon, its spines are longer and bigger compare with spines COT from Maluku, see figure 1. To confirm the PLA_2 activity and composition of the COT Papua, we isolated and characterized the PLA_2 by using previous method, the results describes below.

Figure 1
Spines of COT from Papua (A) and Maluku (B)

(i) Isolation of Phospholipase A_2

Isolation of PLA_2 performed as desribed[7]. First, the 50 grams of spines diluted in 100 ml 0.1 M phosphate buffer, sonicated and centrifuged, the supernatant was suggested contain the venom. To confirm that the venom was extracted, the sample was added to blood and observed the ability in preventing coagulation of blood. The result shows that the sample has anticoagulant activity. Anticoagulant activity of the venom can extended the formation of fibrin and prolonged bleeding time test in rats, and hemolytic activity against erythrocytes which is the lecithin added increasing its activity indicated the presence of PLA_2 activity.

The PLA_2 was purified as described[7]. In summary, the PLA_2 isolated from the COT Papua islands showed in tabel 1. Surprisingly, the PLA_2 activity of COT from Papua higher five times compare to the PLA_2 activity of COT from Maluku[8]. The fraction ammonium sulfate 20% has the highest PLA_2 activity compare with other fraction, similar with reported[7]. Unfortunately, The PLA_2 activity of fraction ammonium sulfate 20% only increase three times compare to the PLA_2 activity crude venom different with COT from Maluku islands which increased about twenty times. It is suggested, the preheated might be influenced by the denaturation process of enzyme. To confirm it, the PLA_2 isolated without heating at

60°C and separated by ammonium sulfate precipitation. The results show that the specific activities of the PLA_2 have no significant differences between with and without treatment by heating at 60°C. In conclusion, the PLA_2 from Papua islands COT also have stability in 60°C.

(ii) Coenzyme of divalent cations characterization

Divalent cations are common coenzyme for PLA_2. Ca^{2+} had increased 180% of the PLA_2 (I and II) activity from spines venom of *A. planci* whereas Cu^{2+} and Zn^{2+} reduced 10-20% of PLA_2 activity[4]. The PLA_2 activity of *Echisocellatus* venom was increased by the presence of Ca^{2+}, meanwhile Mg^{2+} and Co^{2+} very strong inhibited the activity and Cu^{2+} inhibited the PLA_2 activity in strong enough[11]. Affect of divalent cations on the activity PLA_2 in spine venom of COT from Papua was measured in the presence of Ca^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Fe^{2+}. The activity of PLA_2 in presence of Ca^{2+} was increased very strong from 32 to 234 unit/mg. whereas the other hand, Co^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+} and Fe^{2+} inhibited the PLA_2 activity (Figure 2 and table 2) and Zn^{2+} completely inhibited the activity of PLA_2. This data explained that Ca^{2+} was a coenzyme for PLA_2 venom of Papua COT similar with the previous results[4].

Tabel 1
Summary of PLA_2 Isolation from the Spine Venom of Papua COT

Sample	Activity (unit/ml)	Total activity (unit)	Total protein (mg)	Spesific activity (unit/mg)	Purity
Preheated venom					
crude venom (cv)	86.7	8670	261.65	33.1359	1
Heated cv	188.05	18805	264	71.2311	2.15
Fraction ammonium sulfate 20%	225.3	450.6	4.7742	94.3823	2.85
Fraction ammonium sulfate 40%	117.9	235.8	7.8564	30.0137	0.91
Fraction ammonium sulfate 60%	159.55	319.1	14.1388	22.5691	0.68
Fraction ammonium sulfate 80%	583.6	1527.8	21.4212	54.4843	1.64
Without preheated Venom					
crude venom (cv)	99.35	9935	268.71	36.9729	1
Fraction ammonium sulfate 20%	220.1	440.2	4.0094	109.792	2.97
Fraction ammonium sulfate 40%	142.95	285.9	7.633	37.4558	1.01
Fraction ammonium sulfate 60%	216.25	432.5	20.633	20.9616	0.57
Fraction ammonium sulfate 80%	763.8	1167.1	22.48	67.9614	1.84

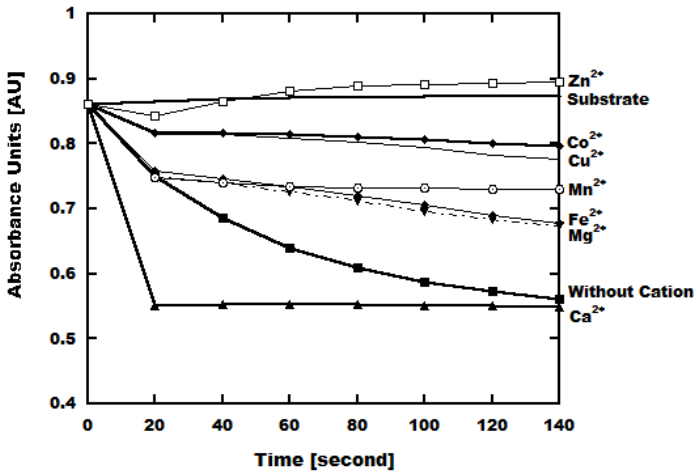

Figure 2
Cations Influenced on PLA_2 Decreasing Absorbance Value

Tabel 2
Activity of PLA_2 Influenced by Kation

Sample	Activity (unit/ml)	Total Activity (unit)	Total protein (mg)	Specific activity (unit/mg)
PLA_2	64.16	6415	199.88	32.09
PLA_2-Ca^{2+}	468.3	46830	199.88	234.29
PLA_2-Co^{2+}	13.82	1382	199.88	6.91
PLA_2-Cu^{2+}	18.39	1839	199.88	9.20
PLA_2-Fe^{2+}	39.47	3947	199.88	19.75
PLA_2-Mg^{2+}	40	4001	199.88	20.02
PLA_2-Mn^{2+}	28.16	2816	199.88	14.09
PLA_2-Zn^{2+}	0	0	199.88	0

CONCLUSION

In conclusion, our method could not apply to isolate PLA_2 from spines venom COT isolated from Papua. The COT isolated from Papua islands might have different venom expression compare with COT isolated from Ambon islands, it caused by different habitat between them.

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REFERENCE

1. Shiomi, K., Itoh, K., Yamanaka, H., Kikuchi, T. 1985. *Biological Activity of Crude Venom from the Crown-ofThorn starfish Acanthasterplanci.* Nippon Suisan Gakkaishi 51 : 1151-1154
2. Shiomi, K., yamamoto, S., Yamanaka, H., Kikuchi, T. 1988. *Purification and Characterization of Lethal Factor in Venom from Crown-of-Thorn strafihAcanthasterplanc.* Toxicon 26, 1077-1083.
3. Karasudani, I., Koyama, T., Nakandakari, S. and Aniya, Y. 1996. *Purifcation of anticoagulant factor from the spine venom of the crown-of-thorns starfish Acanthasterplanci*. Toxicon 34, 871-879.
4. Shiomi, K., Kazama, A., Shimakura, K,.Nagashima, Y. P. 1998. *Purification and Properties of Phospholipases A2 From the Crown-of-Thorns Starfish (Acanthasterplanci) venom*. Toxicon 36(4): 589-599
5. Samy, R. P., Pachiappan, A., Gopalakrishnakone, P., Thwin, M. M., Hian, Y. E., Chow, V. T. 2006. *In Vitro Antimicrobial Activity of Natural Toxins and Animal Venoms*. BMC Infectious Diseases. 6:100.
6. Fenard, D., Lambeau G., Valentin, E., Jean-Claude Lefebvre, Lazdunski,M.,Doglio, A., 1999. *Secreted phospholipases A2, a new class of HIV inhibitors that block virus entry into host cells.* NCBI
7. Marinetti, G. V. 1965. *The action of phospholipase A on lipoproteins*. Biochim. Biophys. Acta 98: 554-556
8. Savitri I.K.E., Ibrahim, F., Sahlan, M,.Wijanarko, A. 2011. *Rapid and Efficient Purification Method of Phospholipase A2 from Acanthaster planci*. International Journal of Pharma and Bio Sciences 2 (2): B401 –B405
9. Yara A. 1992. *Cardiopvascular effects of Acanthasterplanci venom in rat.* Toxicon. 30: 1281-1289
10. Shiomi, K., Kazama, A., Yamanaka, K, H.,Kikuchi, T. 1989. *Inhibitory Effect og Antiinflammantory Agent on Cutaneous Capillary Leakage Induced by Sea Marine Venom. Nippon Suisan Gakkaishi 55: 131-134*
11. Sallau, A.B., Ibrahim, M. A., Salihu, A. Patrick, F. U. 2008. *Characterization of Phospholipase A_2 (PLA_2) from Echisocellatus Venom.* African Journal of Biochemistry Research Vol. 2 (4) : 098-101
12. Koyama, T., Noguchi, K., Aniya., and Sakanashi, M. 1998. *Analysis for Sites of Anticoagulant Action of Placinin, a New Anticoagulant Peptide Isolated from the Starfish Acanthaster planci, in the Blood Coagulant Cascade*, Gen. Pharmac. 31(2): 227-228.