



# 神経系細胞の情報伝達機構に及ぼす新規生物毒の作用機序 ーヘビ毒ホスホリパーゼ A<sub>2</sub>のクロマフィン細胞に対する作用ー

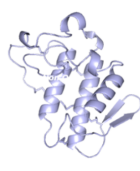


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## Introduction

### Phospholipase A<sub>2</sub> ...



PLA<sub>2</sub>

- ▷ is in the group of enzymes that hydrolyzes glycerophospholipid
- ▷ is found in wide species, animals or plants
- ▷ has a great diversity of functions, digestive enzyme upstream regulators of many inflammatory processes signaling cell lysis
- ▷ is one of a main toxic component of the snake venom neurotoxin myotoxin

### *Laticauda semifasciata*



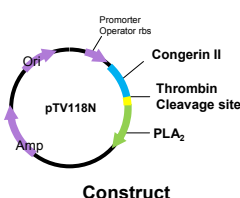
- has at least two types of PLA<sub>2</sub>
- Group I
  - type IA β-neurotoxins in the venom
  - type IB digestive enzymes in pancreas
- Both PLA<sub>2</sub>s are structurally-conserved
- No relationship between toxicity and enzyme activity

### Purpose

To clarify the functional diversity, we have

- expressed the recombinant PLA<sub>2</sub> (type IA and IB) in *E. coli*
- observed the interaction of the expressed recombinants and chromaffin cell which has the same embryologic origin as the neuron

### Expression and purification of the expressed PLA<sub>2</sub>

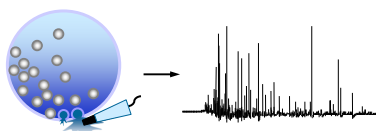


Construct

#### 【FEATURE】

- Congerin II is galectin with chaperone-like activity
  - ⇒ express as soluble protein
- The galectin bind specifically to agarose of Sepharose4B
  - ⇒ affinity purification

### Amperometric analysis of event-frequency and fusion kinetics



#### Event-frequency

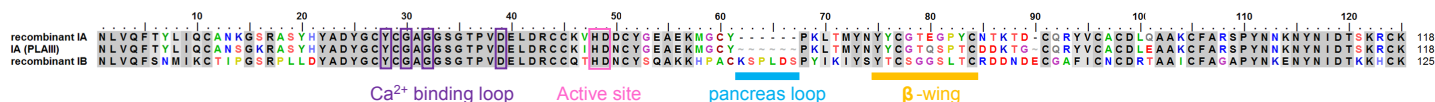
size of readily releasable pool and the vesicle recruitment

#### Kinetics

A chromaffin cell was stimulated by the recombinant PLA<sub>2</sub> with injection tip. Secretion of catecholamine from chromaffin cells was monitored by amperometry using carbon fiber electrodes at cell surface. The electrolytic current induced by the oxidized catecholamine transmitter was detected and digitalized. Finally, one exocytosis event is measured as a single spike.

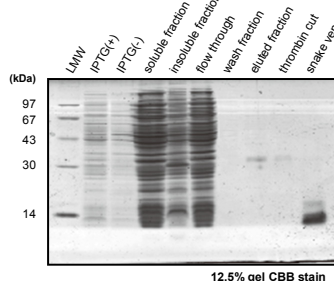
## Results

### Amino Acid sequence Alignment of Group IA and IB PLA<sub>2</sub>s

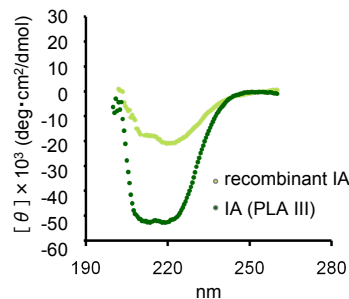


### PLA<sub>2</sub> gene expression and protein structure of expressed PLA<sub>2</sub>

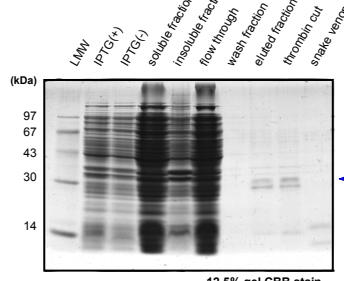
#### recombinant IA



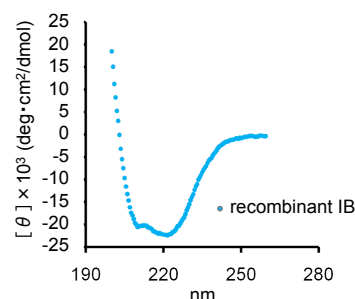
12.5% gel CBB stain



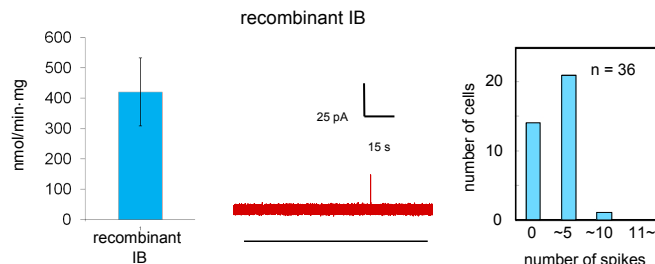
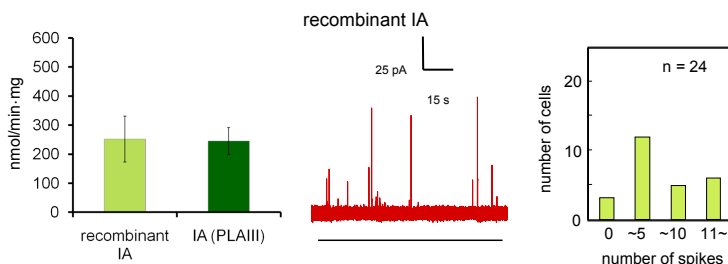
#### recombinant IB



12.5% gel CBB stain



### Effect of PLA<sub>2</sub> on the exocytosis of chromaffin cell



## Conclusion

### Type IA PLA<sub>2</sub>

Deduced amino acid sequence showed 91% homology with the native PLA<sub>2</sub>. Native and recombinant CD spectra displayed minimum peak at 222 nm indicating the existence of α-helices. However, the magnitudes of CD minimum peaks were different from each other. Thus the contents of α-helices were different from recombinant and native form. Comparing the enzymatic activity of native and recombinant PLA<sub>2</sub>s, both PLA<sub>2</sub>s showed nearly the same activity. In conclusion, we have established the expression system of type IA PLA<sub>2</sub> in *E. coli*.

### Type IB PLA<sub>2</sub>

Unfortunately, we could not compare recombinant and native type IB PLA<sub>2</sub> because native type IB PLAs had not been isolated. Recombinant type IB PLA<sub>2</sub> CD spectrum also displayed minimum peak at 222 nm indicating the existence of α-helices. Moreover, recombinant type IB PLA<sub>2</sub> showed higher PLA<sub>2</sub> activity than type IA PLA<sub>2</sub>. We have also established the expression system of type IB PLA<sub>2</sub>.

### Effect of recombinant PLA<sub>2</sub> on chromaffin cell

The recombinant type IA PLA<sub>2</sub> (toxic PLA<sub>2</sub>) induced exocytosis of chromaffin cells. In contrast, recombinant type IB PLA<sub>2</sub> (non-toxic PLA<sub>2</sub>) showing more than twice of the enzymatic activity did not induce exocytosis of chromaffin cells. Thus, there was no relationship between exocytosis of chromaffin cells and PLA<sub>2</sub> activity. Induction of exocytosis from chromaffin cells may be caused by the neurotoxicity of PLA<sub>2</sub>.