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
－ヘビ毒ホスホリパーゼ A_2のクロマフィン細胞に対する作用－

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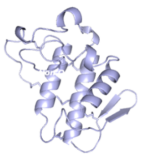
Introduction

Phospholipase A_2 …



PLA_2

- 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_2

Group I
- type IA　β-neurotoxins in the venom
- type IB　digestive enzymes in pancreas

Both PLA_2s are structurally-conserved

No relationship between toxicity and enzyme activity

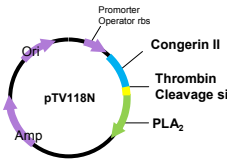
Purpose

To clarify the functional diversity, we have

- expressed the recombinant PLA_2 (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

Materials and Methods

Expression and purification of the expressed PLA_2



Construct

【FEATURE】

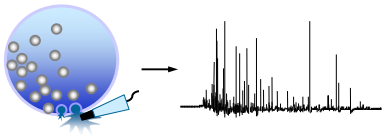
Congerin II is galectin with chaperone-like activity

⇨ express as soluble protein

The galectin bind specifically to agarose of Sepharose4B

⇨ affinity purification

Amperometric analysis of event-frequency and fusion kinetics



Event-frequency

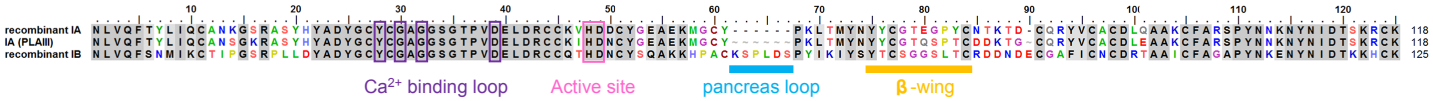
size of readily releasable pool and the vesicle recruitment

Kinetics

A chromaffin cell was stimulated by the recombinant PLA_2 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 catercholamine trasmitter was detected and digitalized. Finally, one exocytosis event is measured as a single spike.

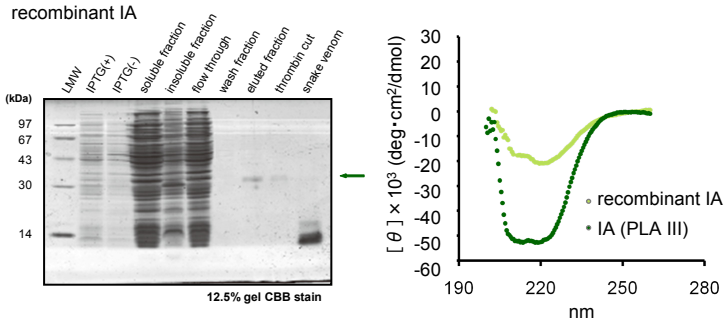
Results

Amino Acid sequence Alignment of Group IA and IB PLA_2s

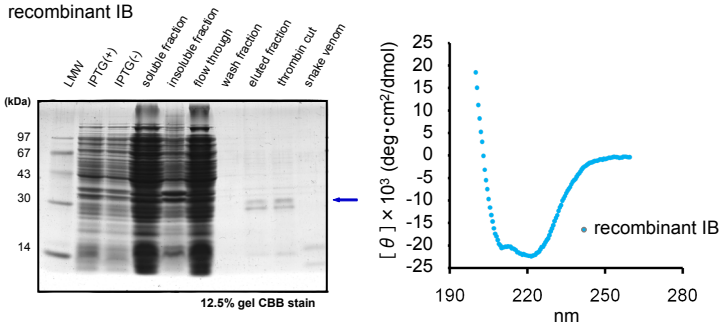


PLA_2 gene expression and protein structure of expressed PLA_2

recombinant IA

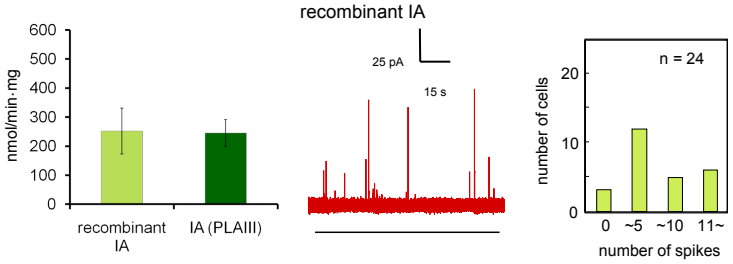


recombinant IB

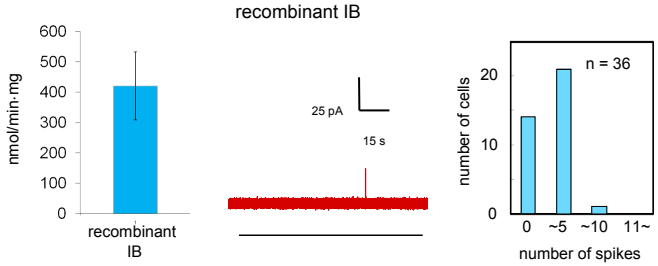


Effect of PLA_2 on the exocytosis of chromaffin cell

recombinant IA



recombinant IB



Conclusion

Type IA PLA_2

Deduced amino acid sequence showed 91% homology with the native PLA_2. 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_2s, both PLA_2s showed nearly the same activity. In conclusion, we have established the expression system of type IA PLA_2 in *E. coli*.

Type IB PLA_2

Unfortunately, we could not compare recombinant and native type IB PLA_2 because native type IB PLAs had not been isolated. Recombinant type IB PLA_2 CD spectrum also displayed minimum peak at 222 nm indicating the existence of α-helices. Moreover, recombinant type IB PLA_2 showed higher PLA_2 activity than type IA PLA_2 .
We have also established the expression system of type IB PLA_2.

Effect of recombinant PLA_2 on chromaffin cell

The recombinant type IA PLA_2 (toxic PLA_2) induced exocytosis of chromaffin cells. In contrast, recombinant type IB PLA_2 (non-toxic PLA_2) 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_2 activity. Induction of exocytosis from chromaffin cells may be caused by the neurotoxicity of PLA_2.