Biochemical characterization of phospholipase A_2 (trimorphin) from the venom of the Sonoran Lyre Snake Trimorphodon biscutatus lambda (family Colubridae)

Ping Huang, Stephen P. Mackessy*

Department of Biological Sciences, University of Northern Colorado, 501 20th St., CB 92, Greeley, CO 80639-0017, USA

Received 3 December 2003; accepted 23 March 2004

Available online 18 May 2004

Abstract

Phospholipases A_2 (PLA_2), common venom components and bioregulatory enzymes, have been isolated and sequenced from many snake venoms, but never from the venom (Duvernoy's gland secretion) of colubrid snakes. We report for the first time the purification, biochemical characterization and partial sequence of a PLA_2 (trimorphin) from the venom of a colubrid snake, Trimorphodon biscutatus lambda (Sonoran Lyre Snake). Specific phospholipase activity of the purified PLA_2 was confirmed by enzyme assays. The molecular weight of the enzyme has been determined by SDS-PAGE and mass spectrometry to be 13,996 kDa. The sequence of 50 amino acid residues from the N-terminal has been identified and shows a high degree of sequence homology to the type IA PLA_2s, especially the Asp-49 enzymes. The Cys-11 residue, characteristic of the group IA PLA_2s, and the Ca^{2+} binding loop residues (Tyr-28, Gly-30, Gly-32, and Asp-49) are conserved. In addition, the His-48 residue, a key component of the active site, is also conserved in trimorphin. The results of phylogenetic analysis on the basis of amino acid sequence homology demonstrate that trimorphin belongs to the type IA family, and it appears to share a close evolutionary relationship with the PLA_2s from hydrophiine elapid snakes (sea snakes and Australian venomous snakes).

Keywords: Amino acid sequence; Catalytic site residue; Calcium binding site residues; Colubrid snake; Duvernoy's gland; Elapidae; Enzyme; Evolution; Mass spectrometry; Phospholipase A_2; Phylogenetic analysis

1. Introduction

Snake venoms are complex mixtures of components with a diverse array of actions both on prey and human victims, and they are generally rich sources of water-soluble enzymes and polypeptides. Among these enzymes, the secreted phospholipases A_2 are widely distributed among various species, and those from the venoms of reptiles and the pancreatic tissues of mammals are particularly well characterized (Danse et al., 1997). Phospholipases A_2 are esterolytic enzymes which hydrolyze acyl-ester bonds at the sn-2 position of 1,2-diacyl-3-sn-phosphoglycerides and release fatty acids and the corresponding 1-acyl lyso-phospholipids (van Deenen et al., 1963; Kini, 1997). Especially noteworthy are various types of phospholipase

(35 μg protein per lane; T. biscutatus) were also reduced. Gels were imaged using a Kodak DC-120 digital camera.

2.7. Reduction and alkylation

Purified trimorphin (approx. 250 μg) was dissolved in 1.0 ml of 0.1 M Tris buffer, pH 7.5, containing 1% SDS and 0.1 M dithiothreitol (DTT). The mixture was boiled for 3 min and then incubated under nitrogen for 1 h at room temperature. An aliquot of 40 μl of a freshly prepared 100 mM stock solution of 4-vinylpyridine was added to the solution and followed by incubation overnight under nitrogen at room temperature. The resultant mixture was transferred into washed dialysis tubing (3.5 kDa cutoff) and dialyzed against 1.0 l of 0.1% SDS for three changes.

2.8. Amino acid sequence analysis

The N-terminal amino acid sequence (first 50 residues) of the S-pyridylated PLA_2 enzyme was determined by automated Edman degradation using an Applied BioSystems 473a pulsed liquid-phase sequencer at the Protein

3.2. Purification of trimorphin

Like the venoms of most other snakes, the venom of T. biscutatus is a mixture of pharmacologically active proteins and polypeptides, including metalloproteases and phospholipase A_2. In order to isolate and purify PL;eh(26 02e)]TJ

3.3. Effect of EDTA and pH on enzyme activity

At concentrations above 50 μM, the metal ion chelator EDTA completely inhibited PLA_2 activity, demonstrating the requirement of divalent cation for activity (likely Ca^{2+}, as for other PLA_2s); the IC_{50} is approximately 15 μM. Fig. 5 presents the pH-activity profile of trimorphin. The enzyme shows a broad pH optimum (7.0−9.0) with an apparent peak of activity at pH 7.5. No enzymatic activity was detected at

loss of enzymatic activity in equine pancreatic PLA_2, even though the binding of monomeric substrate and cofactor Ca^{2+} to the active site remains unaffected (Verheij et al., 1980). Furthermore, a majority of residues involved in the formation of a hydrophobic channel (Leu-2, Phe-5, and Ile-9) (Scott et al., 1990b) are also conserved in trimorphin with the exception of Trp-19, which has been substituted (somewhat conservatively) by Leu-19.

3.5. Evolutionary relationships

An analysis of sequence relatedness was conducted by comparing the N-terminal amino acid sequence of trimor-

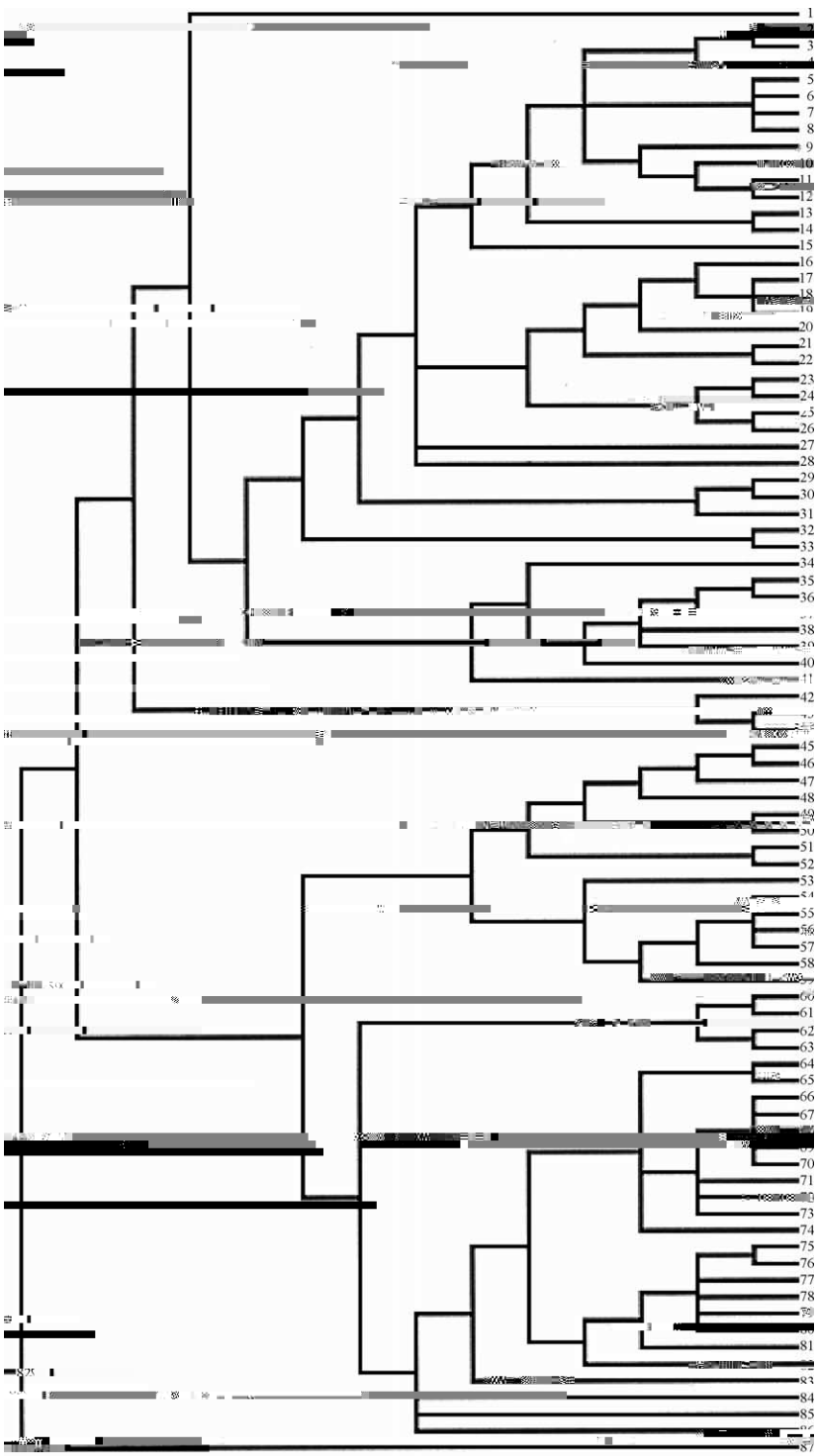

Fig. 6. Cladogram of relationship between T. biscutatus PLA_2 (trimorphin-1) and other snake venom group IA PLA_2

Acknowledgements

This work was partially supported by grant GM52665-01 from the National Institutes of Health, National Institute of General Medical Sciences (to SPM) and by the UNC Sponsored Programs and Academic Research Center. Snakes were donated by B. Tomberlin and W. Sherbrooke, and their help is greatly appreciated.

Appendix A

Phospholipase A_2 toxins and snake species included in cladistic analysis of PLA_2 relationships (Fig. 6). Sequences are available in Danse et al. (1997) and via the National Center for Biotechnology Information's NR Protein Database (FASTA programs: Pearson and Lipman, 1988)

Number	Snake species	Toxin name
1	Trimorphodon biscutatus	Trimorphin
2	Enhydrina schistosa	Myotoxin
3	Enhydrina schistosa	Myotoxin homolog
4	Hydrophis lapemoides	PLA_2
5	Notechis scutatus scutatus	Notechis II-5
6	Notechis scutatus scutatus	Notexin Np
7	Notechis scutatus scutatus	Notexin isoform Ns
8	Notechis scutatus scutatus	Scutoxin
9	Pseudonaja textilis	Textilotoxin A subunit
10	Laticauda semifasciata	Ls PLA I
11	Laticauda semifasciata	Ls PLA III
12	Laticauda semifasciata	Ls PLA IV
13	Notechis scutatus scutatus	PLA_2 11′2
14	Notechis scutatus scutatus	Notechis II-1
15	Australaps superba	Platelet aggregation inhibitor
16	Aipysurus laevis	PLA_2-like
17	Pseudechis australis	Pa-13
18	Pseudechis australis	Pa-15a
19	Pseudechis australis	Pa-15b
20	Laticauda colubrina	Lc-PLA-II
21	Laticauda laticauda	PLA_2-like
22	Laticauda colubrina	Lc-PLA-I
23	Pseudechis australis	Pa-1Ga
24	Pseudechis australis	Pa-1Gb
25	Pseudechis australis	Pa-3a
26	Pseudechis australis	Pa-3b
27	Pseudechis papuanus	PPV PLA_2, neutral
28	Pseudechis australis	Pa-10a
29	Pseudechis australis	Pa-11
30	Pseudechis australis	Pa-12a
31	Pseudechis australis	Pa-12c
32	Pseudechis australis	Pa-5a
33	Pseudechis australis	Pa-5b
34	Pseudechis porphyriacus	Pseudexin A
35	Bungarus fasciatus	Toxin Va cardiotoxin
36	Bungarus fasciatus	Toxin Vb-2 cardiotoxin
37	Bungarus fasciatus	Toxin V-I cardiotoxin
38	Bungarus fasciatus	Toxin X-I basic
39	Bungarus fasciatus	Toxin II-2 basic
40	Bungarus fasciatus	Toxin III neutral
41	Bungarus fasciatus	Nonenzymatic acidic mutant PLA2
42	Pseudonaja textilis	Textilotoxin C subunit
43	Pseudechis porphyriacus	Pseudexin B
44	Pseudechis porphyriacus	Pseudexin C
45	Oxyuranus scutellatus scutellatus	Taipoxin α chain
46	Pseudonaja textilis	Textilotoxin B subunit
47	Oxyuranus scutellatus scutellatus	Taipoxin β1 chain
48	Oxyuranus scutellatus scutellatus	Taicatoxin PLA_2 1.6.4.2
49	Oxyuranus scutellatus scutellatus	Taicatoxin PLA_2 1.6.4.3
50	Oxyuranus scutellatus scutellatus	OS_2
51	Notechis scutatus scutatus	PLA_2 24′2
52	Pseudechis australis	Pa-9c
53	Bungarus multicinctus	Phospholipase A
54	Bungarus multicinctus	β-bungarotoxin, A1 chain
55	Bungarus multicinctus	β-bungarotoxin, A2 chain
56	Bungarus multicinctus	β-bungarotoxin, A2 chain variant
57	Bungarus multicinctus	β-bungarotoxin, A3 chain
58	Bungarus multicinctus	P11 PLA_2 isoform
59	Bungarus multicinctus	B. multicinctus A4 chain
60	Maticora bivirgata	PLA_2 I
61	Maticora bivirgata	PLA_2 II
62	Micrurus nigrocinctus	PLA 2.5
63	Micrurus nigrocinctus	PLA 3.6
64	Micrurus nigrocinctus	PLA 1.3
65	Aspidelaps scutatus	CM-II
66	Micrurus corallinus	PLA_2-V2
67	Naja naja atra	Acidic PLA
68	Naja naja atra	Acidic PLA, isoform

Number	Snake species	Toxin name
69	Naja naja kaouthia	CM-II
70	Naja naja sputatrix	PLA_2 clone 1
71	Naja naja kaouthia	CM-III
72	Naja naja sputatrix	PLA_2 clone 2
73	Naja naja sputatrix	PLA_2 clone 3
74	Naja melanoleuca	DE-II
75	Naja mossambica mossambica	CM-I
76	Naja mossambica mossambica	CM-II
77	Naja mossambica mossambica	CM-III
78	Naja mossambica pallida	III
79	Naja nigricollis	Basic PLA
80	Naja nigricollis	Nigexin, cytotoxin
81	Naja melanoleuca	DE-I
82	Naja melanoleuca	DE-III
83	Hemachatus hemachatus	DE-I
84	Naja naja naja	Acidic
85	Naja naja naja	Acidic PLA2
86	Naja naja oxiana	Phospholipase A E3
87	Crotalus scutulatus scutulatus	Mojave toxin-b, basic subunit

.

Refe e ce

Bradford, M.M., 1976. A rapid and sensitive method for the quantitatiion of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Chang, C.C., 1985. Neurotoxins with PLA_2 activity in snake

Scott, D.L., White, S.P., Otwinowski, Z., Yuan, W., Gelb, M.H., Sigler, P.B., 1990b. Interfacial catalysis: the mechanism of phospholipase A_2. Science 250, 1541–1546.

Serrano, S.M., Reichl, A.P., Mentele, R., Auerswald, E.A., Santoro, M.L., Sampaio, C.A., Camargo, A.C., Assakura, M.T., 1999. A novel phospholipase A2, BJ-PLA2, from the venom of the snake Bothrops jararaca: purification, primary structure analysis, and its characterization as a platelet-aggregation-inhibiting factor. Arch. Biochem. Biophys. 367, 26–32.

Slowinski, J.B., Knight, A., Rooney, A.P., 1997. Inferring species trees from gene trees: a phylogenetic analysis of the Elapidae (Serpentes) based on the amino acid sequences of venom proteins. Mol. Phylogenet. Evol. 8, 349–362.

Tamiya, N., Yagi, T., 1985. Non-divergence theory of evolution: sequence comparison of some proteins from snakes and bacteria. J. Biochem. 98(2), 289–303.

Tsai, I.H., 1997. Phospholipases A_2 of Asian snake venoms. J. Toxicol. Toxin Rev. 16, 79–113.

Tu, A.T., Passey, R.B., Toom, P.M., 1970. Isolation and characterization of phospholipase A from sea snake