# COMPLETE AMINO ACID SEQUENCE OF PHOSPHOLIPASE $A_2$-II ISOLATED FROM *AGKISTRODON HALYS BLOMHOFFII* VENOM

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## 1. Introduction

Two forms of acidic and basic phospholipase $A_2$ (phosphatide acyl-hydrolase, EC 3.1.1.4) have been found in the venom of *Agkistrodon halys blomhoffii* [1]. The acidic phospholipase, designated as $A_2$-II, consists of a single polypeptide chain with molecular weight of about 13 700 [2]. It contains N-terminal pyroglutamic acid and C-terminal cystine and a total of 126 amino acid residues [3]. Following these works, we reported the amino acid sequence of first 17 residues from the N-terminal end of the enzyme [4]. In this communication we summarize the studies on the whole amino acid sequence of phospholipase $A_2$-II of *A. halys blomhoffii* venom. The sequence is complete except for assignment of a few amide groups.

## 2. Materials and methods

Phospholipase $A_2$-II was isolated from the venom of *A. halys blomhoffii* by the previous method [2] with a modification in the chromatographic step. Trypsin (TPCK-treated) and α-chymotrypsin (3 × recrystallized) were products of Sigma Chemical, Co., St. Louis. Thermolysin was a generous gift from Dr. K. Morihara, Shionogi Research Laboratory, Osaka. Carboxypeptidase A and B (both di-isopropylphosphorofluoridate treated) were from Worthington Biochemical Corp., Freehold.

The cyanogen bromide cleavage of whole protein was performed as reported previously and the resulting fragments, Br-III-A, Br-III-C and Br-I, were fractionated by gel-filtration in 30% acetic acid [4]. The amino acid sequences of the former two fragments having homoserine as their C-terminal ends and constituting the N-terminal portion of the whole protein have been established [4]. A large fragment, Br-I, consisting of 116 residues and C-terminal cystine was reduced and carboxymethylated [5]. Tryptic, thermolytic and chymotryptic digests of the alkylated Br-I were prepared using an enzyme:protein ratio of 1:50, in 0.2 M ammonium bicarbonate, pH 8.5. Digests were fractionated by ion exchange chromatography on Dowex-50-X2 and gel filtration on Sephadex G-25 (superfine). In many cases, the peptides were purified further by paper chromatography or high voltage paper electrophoresis at pH 3.5 and pH 6.5. The purity of the peptide was examined by thin-layer chromatography and electrophoresis on a cellulose plate (Merck Co., Darmstadt), according to the method of Kosakowski and Böck [6].

Amino acid compositions were determined, after hydrolysis in 5.7 N HCl (110°C, 24 hr, evacuated sealed tube) on a JEOL amino acid analyzer, Model JLC-5AH, according to the method of Spackman [7]. N-Terminal sequences of the isolated peptides were determined by the Edman's phenylisothiocyanate procedure (direct [8] and subtractive [9]). C-Terminal

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residues and sequences were investigated in some cases by digestions with carboxypeptidases A and B or by hydrazinolysis [10]. Amide groups in peptides were either detected by direct Edman degradation or by estimation of the net charge of the peptides during electrophoresis at pH 6.4.

## 3. Results and discussion

The sequence determined for snake venom phospholipase $A_2$-II is shown in fig. 1. Some amide assignments have not yet been completed.

The N-terminal pyroglutamyl residue and the sequence of the fragments, Br-III-A and Br-III-C, determined previously [4], were confirmed further by mass spectrometry (kindly made by Dr. K. Okada, Kanazawa University, Kanazawa). Much of sequence work was based on the 9 major and 6 minor tryptic peptides derived from the large cyanogen bromide fragment (Br-I). The order of these, and various other aspects of the sequence, was established by thermolytic and chymotryptic peptides. In the peptide sequence, the overlapping peptide fragments which provide the linkage between T-6-2 and T-9-1 could not be obtained, but these fragments must be located in the positions shown in fig. 1, because all other overlaps had been proved.

When we accomplished the sequence studies on the enzyme from *A. halys blomhoffii* venom, a report regarding the complete amino acid sequence of snake venom phospholipase A from *Bitis gabonica* and *Naja melanoleuca* was published [11,12]. Fig. 2 shows a compilation of the linear sequences of above three enzymes from different species of snakes, in addition to that of hog pancreatic phospholipase $A_2$ established by De Haas et al. [13]. Deletions and insertions were made placing the half-cystine residues at the same positions to obtain the most probable conformity.

Comparing their sequences, it is evident that a relatively high degree of homology exists among the amino-terminal portions of four enzymes. Especially,

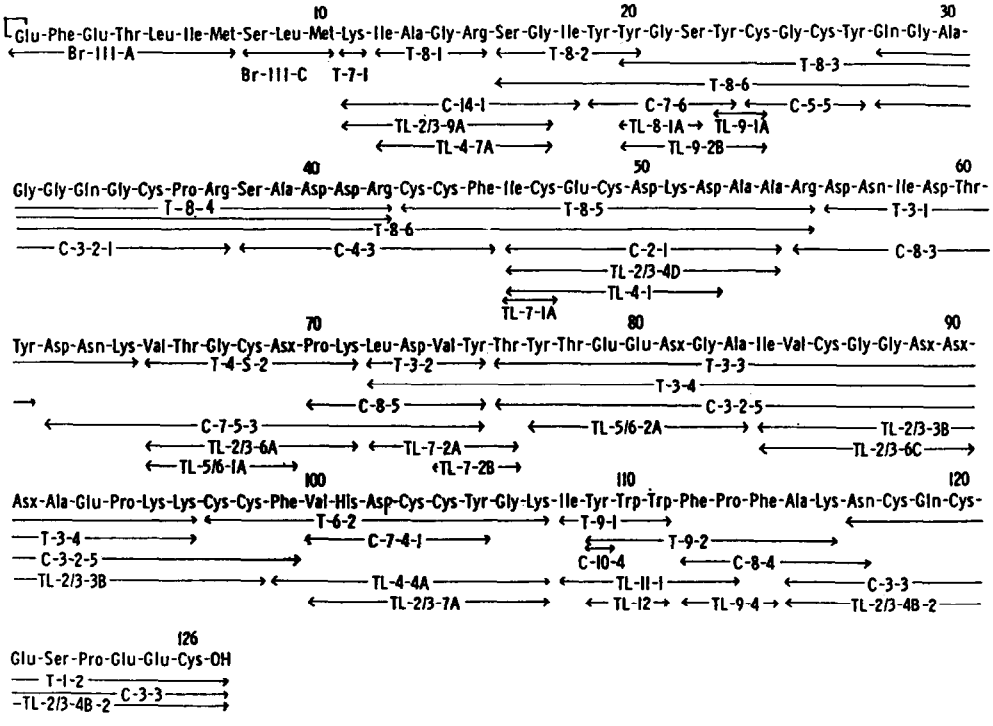


Fig. 1. Amino acid sequence of phospholipase $A_2$-II isolated from the venom of *A. halys blomhoffii*. The peptides obtained from digests with trypsin (T), α-chymotrypsin (C) and thermolysin (TL) are shown by arrows.

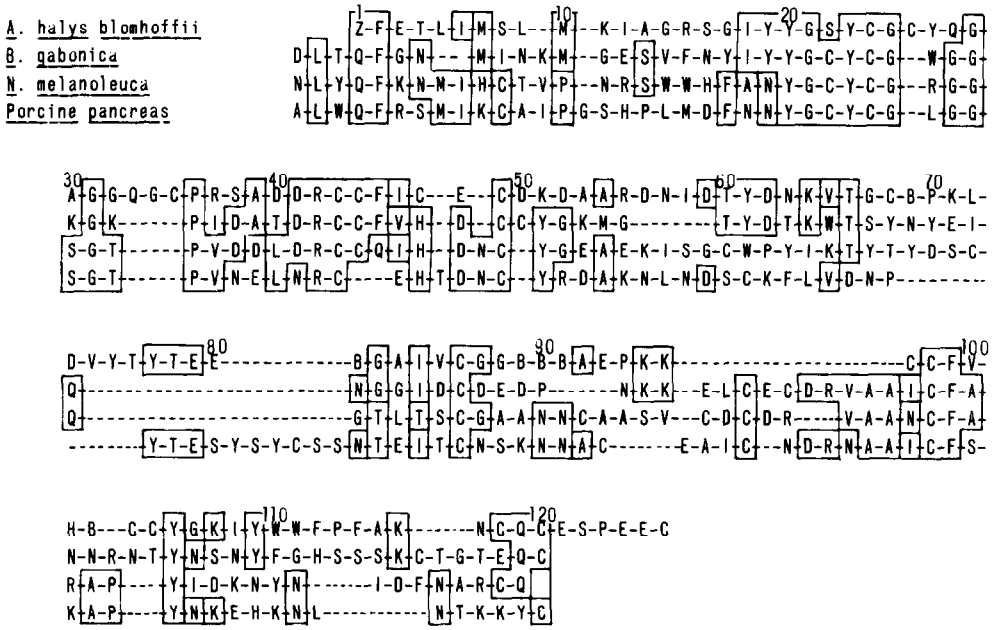


Fig. 2. Comparison of amino acid sequences of reptilian and mammalian phospholipase $A_2$. Boxes enclose identical residues in the sequences of the three snake venom enzymes and porcine pancreatic enzyme.

there is a high sequence conservation from the residues 20 to 25 and further the residues 41 to 44, suggesting that the regions are structurally or functionally important for the enzyme molecule. The location of their half-cystine residues are also similar, indicating that about 50% of total half-cystine occupies the same positions with some deletions and insertions of amino acid residues.

However, in spite of these similarities the overall sequence homologies among four enzymes seem to be very low. For example, a total homology of the enzyme from *A. halys blomhoffii* venom with those of *Bitis gabonica* venom, *Naja melanoleuca* venom and hog pancreas was 34, 20 and 22%, respectively. Moreover, the linear sequences of the reptilian and mammalian phospholipase A's differ greatly from that of the honey bee venom enzyme, recently determined by Shipolini et al. [14]. Such significant difference of the sequence suggest a rapid rate of molecular evolution of phospholipase $A_2$.

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