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ISOLATION AND PROPERTIES OF A PHOSPHOLIPASE A2 FROM THE VENOM OF THE SNAKE *BOTHROPS MOOJENI* (CAISSACA)

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ABSTRACT: BM-PLA$_2$, a phospholipase A2, was purified from the venom of the snake *Bothrops moojeni* by chromatography on Sephadex G-100, DEAE-Sephadex A-50 and rechromatography on DEAE-Sephadex A-50. BM-PLA$_2$ is an acidic protein of pI 4.6. The enzyme is homogeneous in polyacrylamide gel electrophoresis and ultracentrifugal analyses. The $s^o_{20,w}$ and $D^o_{20,w}$ are 2.08 S and 14.9 $\times$ 10^{-7} cm^2/sec., respectively. The molecular weight of 11,300 was calculated by s^o/D^o ratio. In SDS-polyacrylamide gel electrophoresis the denatured and reduced enzyme exhibits a single polypeptide chain of molecular weight of 11,900. The enzyme is cross-linked by four disulphide bridges and has histidine as N-terminal amino acid. Chemical modifications of BM-PLA$_2$ with p-bromophenacylbromide and N-bromosuccinimide cause complete loss of enzymatic activity. The purified phospholipase A2 is nontoxic, nonhemorrhagic and no edema forming.
KEY WORDS: Venom, *Bothrops moojeni*, phospholipase A2.

INTRODUCTION

Snake, bee and scorpion venoms and mammalian pancreas are rich sources of phospholipase A2. Snake venoms contain several phospholipase A2 enzymes which differ in enzymatic and pharmacological features. A great number of phospholipases A2 have already been purified from the venoms of snakes of the Elapidae and Viperidae families. However very little is known about phospholipases A2 from venoms of *Bothrops* species. The venoms of *Bothrops* species were considered by Marinetti[23] and Mebs[24] as poor sources of phospholipase A2. Vidal and Stoppani[34] and Vidal *et al*[33], on

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the contrary, observed a high phospholipase A2 activity in various venoms of *Bothrops* species, such as *Bothrops jararaca, Bothrops jararacussu* and *Bothrops atrox.* Until now phospholipases A2 of venoms of *Bothrops* species were isolated only from *B. neuwiedi* (Vidal and Stoppani[34]), *B. asper* (Alagon *et al*[1]; Gutierrez *et al*[11]) and *B. alternatus* (Nisenbon *et al*[25]).

In the venom of *Bothrops moojeni* was found a high phospholipase A2 activity associated to high blood-clotting, high proteolytic and low hemorrhagic activities. From this venom was isolated the coagulant enzyme batroxobin (Stocker and Barlow[30]). Also the major proteolytic enzyme, *moojeni* protease A, was already isolated (Assakura *et al*[2]).

In the present study, a phospholipase A2 from the venom of *Bothrops moojeni* was isolated to homogeneity and some biochemical, biophysical and physiopathological properties were characterized.

MATERIALS AND METHODS

Purification of phospholipase A2

Dried crude venom (1.8g) of *Bothrops moojeni* was dissolved in 0.05 M Tris-HCl buffer (pH 7.5) containing 0.5 mM $CaCl_2$ and 0.02% sodium azide (buffer A) and clarified by centrifugation. The supernatant solution containing 1.62g of protein in 12.5 ml was chromatographed on a column (2.6 × 80 cm) of Sephadex G-100 previously equilibrated in buffer A under the conditions described by Assakura *et al*[2] for the isolation of *moojeni* protease A. The fractions from peak II containing the phospholipase A2 activity were combined and lyophilized. A solution of 530 mg of protein in 17 ml, dialyzed against buffer A, was applied to a 2.5 × 50 cm column of DEAE-Sephadex A-50 equilibrated with buffer A. After washing the non adsorbed protein, a linear gradient up to 0.3 M NaCl in the same buffer (mixing vessels 500 ml) was applied. The fractions in the protein peak, which contained the phospholipase A2 activity, were combined, lyophilized and dialyzed against 0.05 M Tris-HCl buffer (pH 7.5) containing 0.5 mM $CaCl_2$, 0.02% sodium azide and 0.075 M NaCl (buffer B), and applied to a 2.5 × 50 cm column of DEAE-Sephadex A-50 previously equilibrated with buffer B. The fractions (45 to 60) with constant specific activity were combined and lyophilized.

Phospholipase A2 activity

This was determined using egg-yolk lecithin as substrate according to Kornalik and Master[17]. Lysolecithin released as a result of enzyme activity was assayed by the hemolytic effect on washed horse red blood cells. One unit of phospholipase A2 activity was defined as the amount of protein which causes 50% hemolysis corresponding to an increase in O.D. of 1.0 at 540 nm. Specific activity is expressed as Units per mg of protein.

Caseinolytic activity

Activity was determined by a modification of the method of Kunitz[18], as described by Mandelbaum *et al*[22]. One unit of caseinase activity is defined as the amount of enzyme yielding an increase in O.D. of 1.0 per min at 750 nm.

Blood-clotting activity

This activity was assayed on citrated horse plasma [1 ml of trisodium citrate 3.8% (w/v) for 10 ml of blood] and measured as described by Henriques *et al*[13].

Hemorrhagic activity

The hemorrhagic activity was assayed by intradermal injection into the backs of mice with doses varying from 2 to 20 μg of venom or isolated phospholipase A2 protein in a volume of 0.1 ml. Four Swiss mice (20-22g) were used for each dose. The mice were sacrificed 2 h later by ether inhalation, the skins removed and the hemorrhagic lesions were evaluated on the visceral side and also on the dorsal surface of the musculature.

Estimation of protein

Protein concentration was measured by the method of Lowry *et al*[20] using bovine serum albumin as standard, or by spectrophotometric determination of absorption at 280 nm.

Polyacrylamide gel electrophoresis

Polyacrylamide 10% gel electrophoresis in Tris-glycine buffer, pH 8.5 was performed according to the method of Maizel[21] with a 2.5% gel spacer following the method of Davis[4]. Runs were of 3 h with 1.25-1.50 mA per tube (0.5 × 8.0 cm), bromophenol blue being used as indicator. Gels were stained for 16 h at room temperature with 0.05% Coomassie brilliant blue R in 12.5% trichloroacetic acid, containing 40% methanol, and destained with several changes of 7.5% acetic acid -5% methanol. For glycoprotein detection the gels were stained with periodic acid-Schiff according to the procedure of Zacharius *et al*[40] as modified by Glossmann and Neville[9].

Molecular weight determination

Sodium dodecyl sulfate (SDS) 10% (w/v)-polyacrylamide gel electrophoresis was performed according to the method of Weber and Osborn[36]. Gels were cast in 0.5 cm i.d. tubes to a height of 8 cm, and the protein sample contained 1% SDS (w/v) and 1% 2-mercaptoethanol (v/v) in 0.01 M phosphate buffer (pH 7.4). The mobilities of ovalbumin, bovine serum albumin, ribonuclease and γ -globulin (H-and L-chains) were measured in comparison to that of bromophenol blue.

Analytical isoelectric focusing

Isoelectric focusing was conducted in a Pharmacia Flat Bed Apparatus FBE 3000 in a layer of 5% polyacrylamide gel with 2% Ampholine (LKB) in the pH range 3-6, at 200 V for 20 h and 4°C, according to the procedure of Vesterberg[32]. Ovalbumin (pI 4.6) and methyl blue (pI 3.6) were run as markers. The pH gradient in the layer was measured potenciometrically after 4 h diffusion of equal samples cut from the gel in 1 ml of water at 25°C.

Amino acid analyses

Amino acid analyses were carried out with a Technicon amino acid analyzer (Spackman *et al*[28]; Hamilton[12]). Lyophilized salt-free samples were hydrolyzed in 3N p-toluene sulfonic acid containing 0.2% 3(2-aminoethyl) indol at 110°C for 24,48 and 72 h according to the method of Liu and Chang[19]. Half-cystine content was determined as cysteic acid after performic acid oxidation (Hirs[14]) prior to hydrolysis for 16 and 24 h in constant

boiling HCl (6N) at 110°C. Tryptophan and tyrosine were also determined spectrophotometrically in 0.1N NaOH, as described by Goodwin and Morton[8].

Partial specific volume

$\bar{V}$ was calculated from the amino acid composition of the enzyme, according to the procedure of Cohn and Edsall[3].

Ultracentrifugal analyses

These analyses were performed in a Spinco Model E ultracentrifuge equipped with RTIC control unit and phase plate. Both Schlieren and interference optics were employed. All measurements of ultracentrifuge patterns were made with a Zeiss small toolmaker's measuring microscope. Sedimentation and diffusion coefficients were determined at 20°C in 0.1 M Tris-HCl containing 0.1 M NaCl and 2 mM $CaCl_2$ buffer solution (pH 7.6), in double sector, capillary type, synthetic boundary cells. Sample concentrations were in the range of 0.9 to 3 mg per ml. Sedimentation velocity experiments were performed with Schlieren optics at 56,000 revs/min (D rotor). The sedimentation coefficients were calculated according to the method of Schachman[27]. Diffusion coefficients were determined at 6,000 revs/min (J rotor), and the pictures analysed by the height-area method of Ehrenberg[6]. Sedimentation and diffusion coefficients were corrected to standard conditions of density and viscosity of water at 20°C. Low speed sedimentation equilibrium experiments were performed at 20°C with double sector cells having a 12mm light path, fitted with quartz windows. The column heights of enzyme solution were of 3 mm (Van Holde and Baldwin[31]; Yphantis[39]). Fluorcarbon FC-43 was used as base fluid to give a transparent cell bottom. The rotor speeds were sufficiently low for the solute concentration at the meniscus not to be zero. Equilibrium was established when no measurable change occurred in fringe displacement with time. Molecular weights were determined with samples of 1.8 and 0.9 mg per ml.

Oxidation with N-bromosuccinimide (NBS)

The modification of the phospholipase A2 was perfomed essentially according to the procedure of Spande and Witkop[29]. The enzyme sample (0.72 mg which corresponds to O.D. at 280 nm of 1.0) was dissolved in 1 ml of 0.1 M acetate buffer, pH 4.0. To this solution 5 mM NBS were added in 10 μl portions. After each addition the change in absorbance at 280 nm was measured and the number of oxidized tryptophan residues was estimated from the decrease in the absorbance at 280 nm.

Modification with p-bromophenacylbromide

The enzyme (0.5 mg) was dissolved in 0.5 ml of 0.025 M Tris-HCl buffer, pH 7.5 containing 0.2 mM EDTA. To this solution 25 μl of 2 mM p-bromophenacylbromide were added. After 4 and 6 h at room temperature, phospholipase A2 activity was assayed in aliquots of the reaction mixture, as described above.

Free sulphydryl group

Free sulphydryl group was measured according to the method of Ellman[7]. The reactions were performed with 0.025 to 0.05 μmoles of the enzyme in 0.1 M phosphate buffer, pH 8.0 in presence of 1% SDS and 10^{-4}

M EDTA. The absorbance at 412 nm was measured 30 min and 1 h after addition of 5,5'-dithiobis (2-nitrobenzoic acid).

N-terminal amino acid analysis

This was carried out using dansyl chloride as described by Gros and Labouesse[10] with denatured and reduced enzyme according to the procedure of Weber and Osborn[36]. The DNS-amino acids obtained after acid hydrolysis were identified by the method of Woods and Wang[37].

RESULTS

Isolation of phospholipase A2

A significant phospholipase A2 activity was observed in the venom of *Bothrops moojeni* when it was fractionated by the procedure used for the isolation of *moojeni* protease A (Assakura *et al*[2]). The phospholipase A2 activity was found in the second peak of the gel filtration on Sephadex G-100. This fraction contained also various proteolytic enzymes, being however free from the blood-clotting and hemorrhagic activities present in the venom

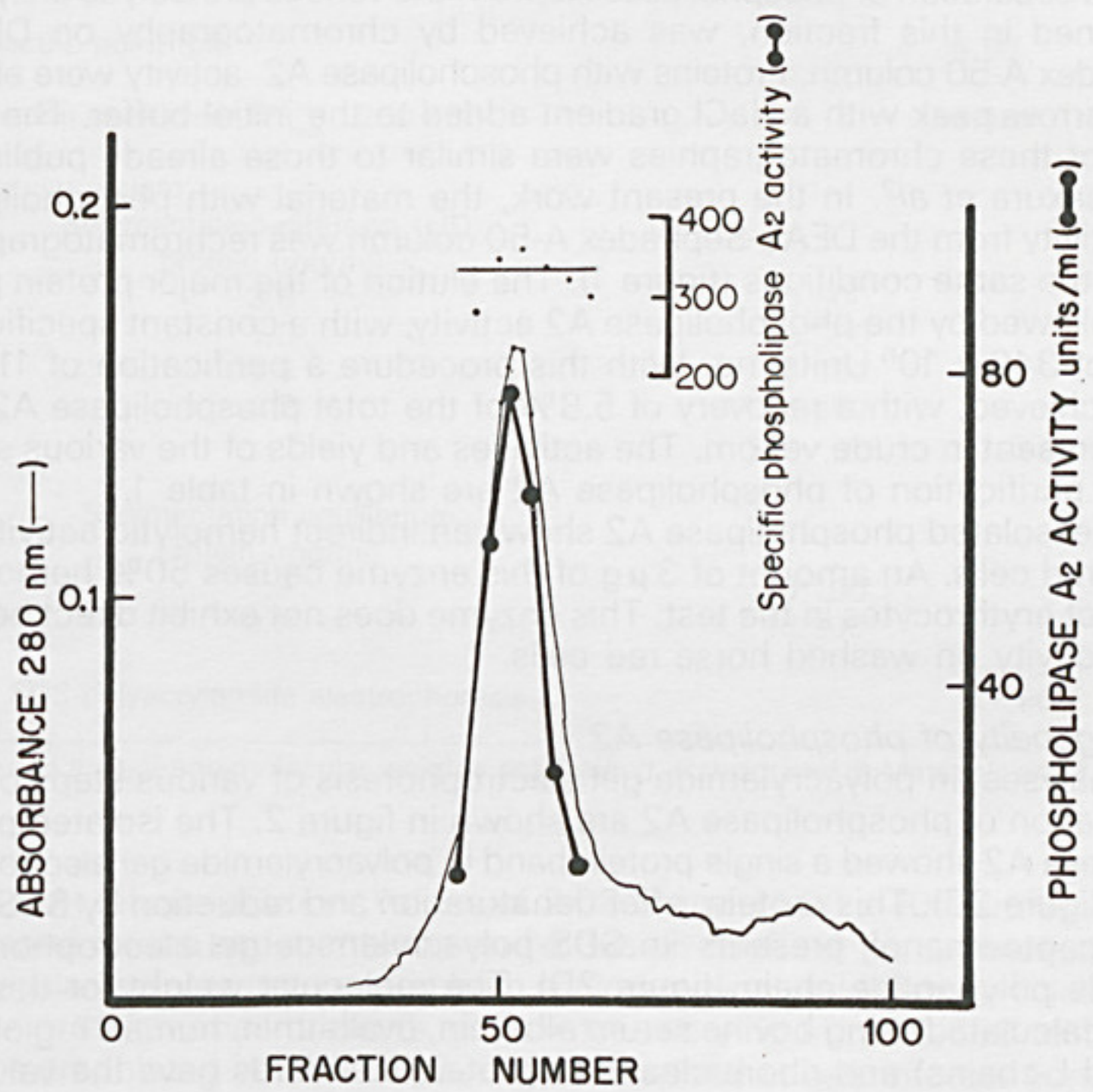

FIG. 1. ELUTION PROFILE OF BM-PLA$_2$ FROM RECHROMATOGRAPHY OF DEAE-SEPHADEX A-50.
A sample of phospholipase A2 of 22 mg in 7 ml, previously dialyzed against 0.05 M Tris-HCl — 0.5 mM $CaCl_2$ buffer, pH 7.5 containing 0.02% sodium azide and 0.075 M NaCl was applied to a DEAE-Sephadex A-50 column (2.5 x 50 cm) previously equilibrated with the same buffer solution.

TABLE 1

YIELD AND ACTIVITY OF PHOSPHOLIPASE A2 ISOLATED FROM THE VENOM OF ***BOTHROPS MOOJENI.***

Purification steps	Protein		Phospholipase A2 activity			
	mg	%	specific 10^{-6}x Units/mg	total x10^{-6}	%	purification factor
Crude venom	1620	100	31.2	50544	100	1.0
Sephadex G-100	530	32.8	43.5	23098	46	1.4
DEAE-Sephadex A-50 (first chromatography)	22	1.37	185	4096	8.1	5.9
DEAE-Sephadex A-50 (rechromatography)	8.7	0.54	340	2958	5.8	11

Specific activity is expressed as described in "Materials and Methods"

A good separation of phospholipase A2, from the various proteolytic enzymes contained in this fraction, was achieved by chromatography on DEAE-Sephadex A-50 column. Proteins with phospholipase A2 activity were eluted in a narrow peak with a NaCl gradient added to the initial buffer. The patterns of these chromatographies were similar to those already published by Assakura *et al*[2]. In the present work, the material with phospholipase A2 activity from the DEAE-Sephadex A-50 column was rechromatographed under the same conditions (figure 1). The elution of the major protein peak was followed by the phospholipase A2 activity, with a constant specific activity of 340 x 10^6 Units/mg. With this procedure a purification of 11-fold was achieved, with a recovery of 5.8% of the total phospholipase A2 activity present in crude venom. The activities and yields of the various steps of the purification of phospholipase A2 are shown in table 1.

The isolated phospholipase A2 shows an indirect hemolytic activity on horse red cells. An amount of 3 μg of this enzyme causes 50% hemolysis of intact erythrocytes in the test. This enzyme does not exhibit direct hemolytic activity on washed horse red cells.

Homogeneity of phospholipase A2

Analyses in polyacrylamide gel electrophoresis of various steps of the purification of phospholipase A2 are shown in figure 2. The isolated phospholipase A2 showed a single protein band in polyacrylamide gel electrophoresis (figure 2C). This protein, after denaturation and reduction by SDS and 2-mercaptoethanol, presents in SDS-polyacrylamide gel electrophoresis, a single polypeptide chain (figure 2D). The molecular weight for this enzyme calculated using bovine serum albumin, ovalbumin, human γ-globulin (H and L-chains) and ribonuclease as protein standards gave the value of 11,900.

Analytical ultracentrifugation using sedimentation velocity experiments yielded symmetrical single schlieren patterns. The values of the sedimentation and diffusion coefficients varied with the protein concentration. When concentration of phospholipase A2 varied from 3.6 to 0.9 mg/ml, the values for $s_{20,w}$ varied from 2.99 to 2.53, while $D_{20,w} \times 10^7$ varied from 12.55 to

TABLE 2

BIOPHYSICAL PROPERTIES OF *BOTHROPS MOOJENI* PHOSPHOLIPASE A2 (BM-PLA_2)

Parameter		Value
Sedimentation coefficient ($s_{20,w}$, S)		
protein (mg/ml)		
3.6	2.99	
1.8	2.99	
1.5	2.85	
0.9	2.53	
		2.08 ($s^o_{20,w}$)
Diffusion coefficient ($D_{20,w}x10^7$, cm^2/sec)		
protein (mg/ml)		
3.6	12.55	
1.8	12.70	
1.5	13.99	
0.9	14.21	
		14.90 ($D^o_{20,w}x10^7$)
Partial specific volume ($\bar{v}$, ml/g)		0.704
Isoelectric point (pI)		4.60
Extinction coefficient ($E^{1\%}_{1cm}$, 280 nm)		13.96
Molecular weight		
Sedimentation-diffusion (s/D)		
protein (mg/ml)		
3.6	19,600	
1.8	19,380	
1.5	16,800	
0.9	14,650	
		11,300 (s^o/D^o)
Sedimentation equilibrium		
protein (mg/ml)		
1.8	19,100	
0.9	13,900	
SDS-polyacrylamide electrophoresis		11,900

Values of s, D, $\bar{v}$ and molecular weights determined as described in Materials and Methods.

14.21. At infinite dilution, values of 2.08 S and 14.90 x 10^{-7} cm^2/sec were obtained for the sedimentation and diffusion coefficients, respectively. The molecular weights determined from sedimentation and diffusion values, with these protein concentrations, gave values varying from 19,600 to 14,600. The extrapolated value of 11,300 was obtained at zero protein concentration. Molecular weights were also calculated from sedimentation equilibrium experiments. By this method the molecular weights of phospholipase A2 in the concentrations of 1.8 and 0.9 mg/ml, gave values of 19,000 and 13,900, respectively.

By isoelectric focusing in thin layer of polyacrylamide gel, phospholipase A2 showed to be an acidic protein with a pI of 4.60.

This homogeneous phospholipase A2 isolated from *B. moojeni* venom, was named BM-PLA_2. The biophysical constants of BM-PLA_2 are shown in table 2.

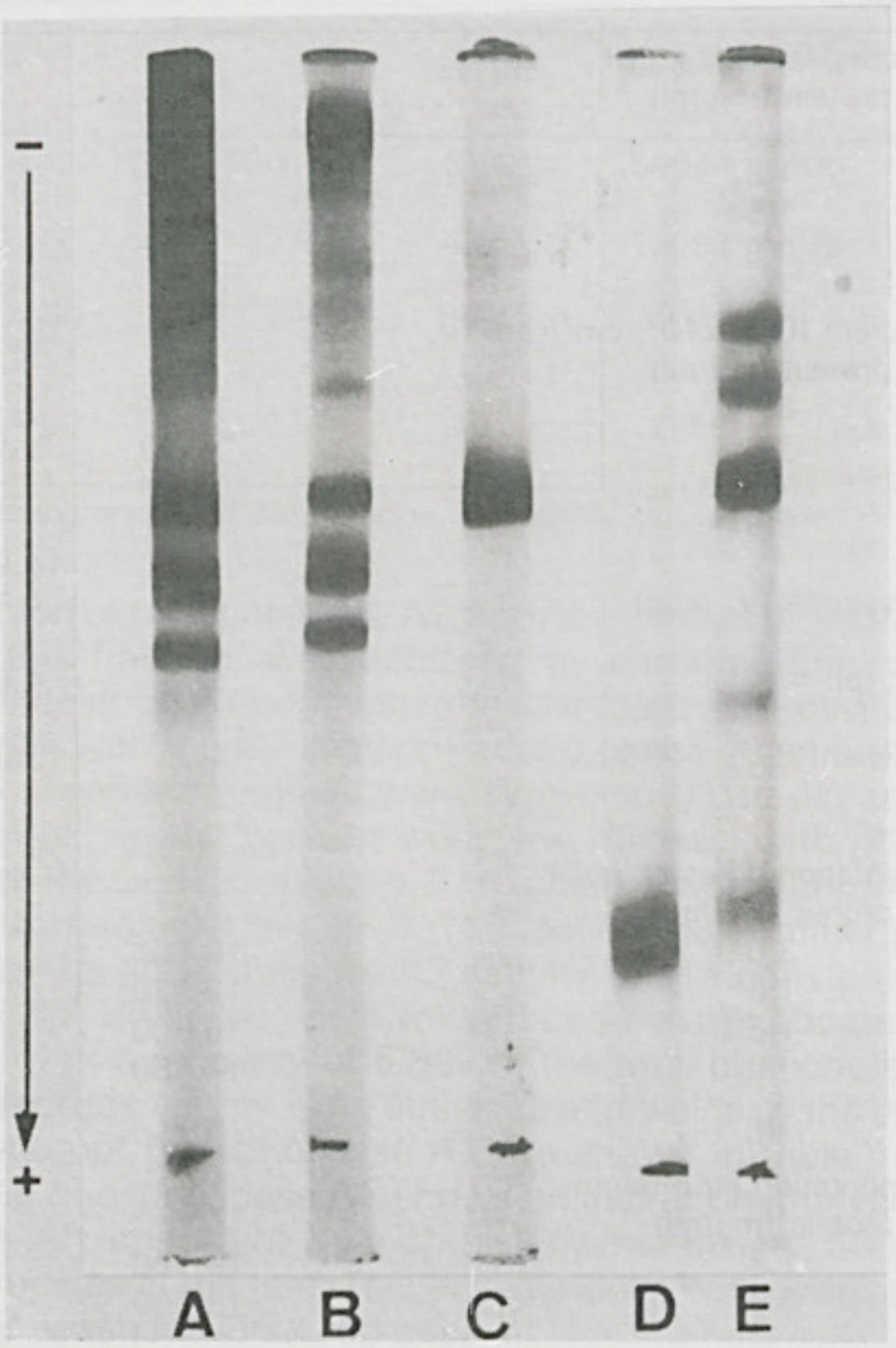

FIG. 2. POLYACRYLAMIDE GEL ELECTROPHORESIS.
(A) Crude venom (80 μg). (B) Proteins from peak II of Sephadex G-100 column (30 μg). (C) Isolated *B. moojeni* phospholipase A2 (15 μg). (D) reduced enzyme (12 μg). (E) mixture of reduced bovine serum albumin, ovalbumin, γ-globulin (H and L-chains) and ribonuclease used as standards for molecular weight measures.

Amino acid composition

The amino acid composition of BM-PLA_2 is shown in table 3. The protein has a high content of acidic amino acids, 11 Asp and 8 Glu residues. BM-PLA_2 presents also high contents of 1/2 Cys, Gly and Lys. Tryptophan and tyrosine were also determined spectrophotometrically. The number of residues given by this procedure were the same as those obtained by hydrolysis with p-toluene sulfonic acid. The amino acid analyses showed that BM-PLA_2 contained 8 cysteine residues. Free sulfhydryl groups were not found. The method of Ellman[7] indicated less than 0.1 residues per mole of pro-

TABLE 3

AMINO ACID COMPOSITION OF *BOTHROPS MOOJENI* PHOSPHOLIPASE A2 ($BM\text{-}PLA_2$). HYDROLYSIS WITH 3 N p-TOLUENE SULFONIC ACID.

Amino Acid	Residues/mole of enzyme	nearest integer
ASP	11.32	11
THR[a]	3.98	4
SER[a]	4.75	5
GLU	7.91	8
PRO	3.74	4
GLY	7.64	8
ALA	3.62	4
VAL[b]	3.96	4
1/2CYS[c]	7.76	8
MET	1.15	1
ILEU[b]	2.45	2
LEU	2.12	2
TYR	4.00	4
PHE	4.38	4
LYS	5.80	6
HIS	1.68	2
TRP	1.01	1
ARG	3.61	4
NH_3[a]	12.21	7
TOTAL		89

Average values of duplicate analyses of 24,48 and 72 h acid hydrolysis. Number of residues based on molecular weight of 11,900.
a — extrapolated to zero time hydrolysis.
b — values of 72 h hydrolysis.
c — determined as cysteic acid after perfomic acid oxidation.

tein. Based on amino acid composition, the minimum molecular weight of $BM\text{-}PLA_2$ corresponds to 9,400. This value is 20% lower than those estimated by ultracentrifugal analyses and SDS-polyacrylamide gel electrophoresis. Considering that this low value given by the amino acid composition could be due to the presence of carbohydrates, $BM\text{-}PLA_2$ was assayed for glycoprotein. However, no Schiff's stain was observed.

The N-terminal amino acid of $BM\text{-}PLA_2$ was found to be histidine.

Stability of $BM\text{-}PLA_2$ at pH variation

The effect of pH on the enzyme was tested with solutions adjusted with dilute NAOH or HCl to various pHs. After 6 h at room temperature, the phospholipase A2 activity was measured. The enzyme is stable, as no significant alteration in enzymatic activity was observed at pHs ranging from 4 to 8. However, an increase of 30% of its activity was observed when the enzyme was submitted to pHs 8 to 10 and loss of 25% of the activity was observed at pHs below 4.

Chemical modifications

$BM\text{-}PLA_2$ lost completely its activity after oxidation with N-bromosuccinimide. The tryptophan residue was modified without denatu-

ration of BM-PLA_2 with urea. The modified enzyme gave a single band on SDS-polyacrylamide gel electrophoresis, with the same mobility as the native enzyme, excluding the possibility of a split in the polypeptide backbone. Treatment for 4 h of BM-PLA_2 with p-bromophenacylbromide resulted also in total loss of activity. Only one of the two histidine residues of the enzyme was alkylated. The other histidine was not modified and was found as N-terminal amino acid.

Biological activities

Some biological tests were assayed with the isolated BM-PLA_2 in mice. BM-PLA_2 was not toxic when injected intraperitoneally. No deaths after 24 h were observed in the injected mice with various doses up to 1 μg/g body weight (a minimum of 6 animals were used per dose). However crude venom in dose of 7.5 μg/g body weight, was enough to kill mice at the end of 3 h. Also no edema was observed when injected in the foot pads of the hind limbs with doses up to 25 μg of BM-PLA_2 following the method of Yamakawa *et al*[38]. Hemorrhage was also not detected even with doses of 20-25 μg of BM-PLA_2 injected intradermally, intraperitoneally or into the foot pads. With crude venom hemorrhage and edema were already observed with doses of 2 μg. This shows that BM-PLA_2 possesses no lethal, hemorrhagic or edema-forming activities.

DISCUSSION

The isolated BM-PLA_2 showed to be homogeneous by polyacrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis, isoelectrofocusing, ultracentrifugation and N-terminal amino acid analysis.

BM-PLA_2 in ultracentrifugal analyses showed that both sedimentation and diffusion coefficients varied with the protein concentration. The decreasing values of the molecular weight calculated by s/D ratio or sedimentation equilibrium with the decreasing of the protein concentration indicate that BM-PLA_2 is found in reversible associated form. The extrapolated value of 11,300 daltons at zero protein concentration for the molecular weight of BM-PLA_2 is close to the molecular weight of 11,900 determined for the single polypeptide chain in SDS-polyacrylamide gel electrophoresis. Concentration-dependent aggregation was also observed in phospholipases A2 from the venoms of *Agkistrodon halys blomhoffii* (Kawauchi *et al*[16]) and of *Naja naja naja* (Deems and Dennis[5]).

BM-PLA_2 is an acidic protein of pI 4.6, with predominance of Asp and Glu residues over the basic amino acids. BM-PLA_2 contains 8 half-cystine residues, a lower content when compared to the phospholipases A2 isolated from elapidic venoms, which contain, in general 12 to 14 residues. Our results are close to the half-cystine content of phospholipases P_1 and P_2 isolated from the venom of *B. asper* (Alagon *et al*[1]). Compared to the phospholipases A2 of the elapidic venoms, the low content of half-cystine residues of phospholipases A2 of venoms of *Bothrops* species suggests a different cross-linking.

The loss of activity by chemical modification of BM-PLA_2 by N-bromosuccinimide is correlated to the destruction of the single tryptophan residue of the enzyme molecule. The oxidation of this tryptophan without

prior denaturation of the enzyme suggests that this amino acid must be on the surface of the native enzyme. Modification of the histidine residues of BM-PLA_2 with p-bromophenacylbromide also caused complete loss of enzyme activity. Volwerk *et al*[35] demonstrated that the histidine residue located at the active site of pancreatic phospholipase A2 is modified by p-bromophenacylbromide. Considering that one histidine of the modified BM-PLA_2 was found as the N-terminal amino acid we concluded that the histidine essential for the catalytic activity was alkylated by p-bromophenacylbromide.

The acidic phospholipase A2 isolated in this study, although having a high indirect hemolytic activity, is nonhemorrhagic, nontoxic and no edema forming.

Acid phospholipases A2 from snake venoms are usually less toxic in contrast to the basic ones (Rosenberg[26]). In spite of this fact, a phospholipase A2 with lethal activity was isolated from the venom of *Bothrops alternatus* (Nisenbon *et al*)[25]. On the other hand, not all basic phospholipases A2 are highly toxic. One of the phospholipases A2, VRV PL-VIIIa, isolated from the venom of *Vipera russelli*, with a low indirect hemolytic activity, is less toxic compared to the whole venom (Kanturi and Gowda[15]).

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RESUMO: Do veneno de *Bothrops moojeni* foi isolada uma fosfolipase A2, BM-PLA_2, por cromatografia em Sephadex G-100, DEAE-Sephadex A-50 e recromatografia em DEAE-Sephadex A-50. BM-PLA_2 é uma proteína ácida de pI 4,6. A enzima mostrou ser homogenea por eletroforese em gel de poliacrilamida e análises de ultracentrifugação. Os valores para $s°_{20,w}$ e $D°_{20,w}$ são de 2,08 S e 14,90 x 10^{-7} cm^2/ seg, respectivamente. O peso molecular calculado da relação $s°/D°$ é de 11.300 daltons. A enzima desnaturada e reduzida apresenta, por eletroforese em SDS-gel de poliacrilamida, uma única banda de peso molecular 11.900. BM-PLA_2 possui quatro pontes dissulfeto e seu N-terminal é histidina. Modificações químicas da enzima com p-bromofenacilbrometo e N-bromosuccinimida resultaram na perda total da atividade enzimática. A fosfolipase A2 isolada não causa hemorragia, não é tóxica e não é edematogênica.
UNITERMOS: Veneno, *Bothrops moojeni*, fosfolipase A2.

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