AMINO-ACID COMPOSITION OF THE PHOSPHOLIPASES A_2 OF THE VENOM OF THE CENTRAL ASIAN COBRA

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Two fractions with phospholipase A_2 activity have been isolated from the venom of the Central Asian cobra by filtration through Sephadex G-75 gel followed by ion-exchange chromatography on CM-cellulose [1, 2]. The first – phospholipase A_2I – according to the results of rechromatography, horizontal electrophoresis in starch and polyacrylamide gels, and disk electrophoresis, proved to be homogeneous with a molecular weight of 12,000 (determined by gel filtration [3]). The second – phospholipase A_2II – was also an individual substance according to the criteria mentioned above, but it was less active and had a molecular weight of 19,000; it is assumed that the phospholipase A_2II fraction contains a component not separated by the use of the ordinary preparative methods and possibly inhibiting the enzyme [2, 4]. Below we give the amino-acid composition of both phospholipases A_2 of the venom of the Central Asian cobra.

The results of the investigation of the amino-acid composition of the enzymes isolated from the venom of the cobra are given in Table 1. The mean values of three determinations are given. The molecule of phospholipase A_2I consists of 115-117 amino-acid residues; the molecular weight calculated on this

TABLE 1. Amino-Acid Composition of Phospholipases A_2 of the Central Asian Cobra

Amino acid	Time of hydrolysis, h							
	phospholipase A_2I				phospholipase A_2II			
	24	48	72	rounded-off means	24	48	72	rounded-off means
Lysine	6,2	5,9	5,3	5—6	9,4	9,3	8,7	9
Histidine	0,7	0,88	0,9	1	2,34	3,7	3,16	2—3
Arginine	4,3	4,25	4,1	4	7,2	7,63	6,92	7
Aspartic acid	19,7	16,9	16,7	19	21,6	21,4	21,4	21
Threonine	4,6	4,5	4,3	5	10,1	10,45	9,98	10
Serine	6,2	5,9	5,6	6	16,7	16,7	14,8	17
Glutamic acid	7,4	7,8	7,3	7—8	18,1	17,0	18,5	18
Proline	5,1	5,5	5,4	5	10,4	10,02	10,7	10
Glycine	8,4	9,4	8,8	9	11,0	11,3	11,0	11
Alanine	10,0	11,0	11,5	11	11,1	11,96	10,35	11
1/2 Cystine	12,7	14,7	14,1	14	12,0	13,5	13,5	13—14
Valine	3,9	3,38	4,2	4	7,6	8,89	8,9	8—9
Methionine	1,2	1,32	1,32	1	2,4	2,17	3,15	2—3
Isoleucine	3,4	4,3	4,5	4	3,4	5,93	5,5	5
Leucine	5,8	5,9	5,9	6	8,8	8,1	9,2	9
Tyrosine	8,1	7,6	8,2	8	8,8	7,53	8,9	8—9
Phenylalanine	3,8	3,45	4,4	4	4,1	4,33	4,3	4
Tryptophan				2				3
Total number of amino acids				115—117				168—173
Mol. wt.: a) by gel filtration				12000				19000
b) from the amino-acid composition				11370—11627				18553—19186

Note. The numbers of residues of individual amino acids were obtained by extrapolation to zero time (threonine, serine, tyrosine); for valine, leucine, and isoleucine the results of the 72-hour hydrolysis were taken as a basis.

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TABLE 2. Amino-Acid Compositions of Phospholipases A_2

Amino acids	Central Asian cobra		N. nigricollis [13]		N. n. atra [7]	Lat. semifasciata [14]	N. naja [8]		Ag. halys blomhoffii [15]		Cr. adamanteus [9]		Bee venom [5]	Phospholipase from pancreatic gland [16]
	PL A_2I	PL A_2II	PL A_2I	PL A_2II			peak 1	peak 2	PL A_2I	PL A_2II	PL A_2 I	PL A_2 II		
Lysine	5–6	9	10	7	5,1	7	10,1 (5)	10,0 (5)	17	8	16 (8)	16 (8)	17	9
Histidine	1	2–3	3	3	1,0	2	2,3 (1)	2,2 (1)	2	1	5 (2–3)	5 (2–3)	10	3
Arginine	4	7	5	4	5,0	4	10,0 (5)	11,6 (6)	6	4	12 (6)	12 (6)	8	4
Aspartic acid	19	21	17	19	19,3	11	41,5 (21)	40,0 (20)	14	17	30 (15)	30 (15)	22	23
Threonine	5	10	5	4	4,7	6	8,2 (4)	7,9 (4)	6	5	13 (6–7)	13 (6–7)	14	7
Serine	6	17	2–3	5–6	5,2	7	9,7 (5)	9,4 (5)	5	4	13 (6–7)	13 (6–7)	12	10
Glutamic acid	7–8	18	4–5	10	7,9	9	15,2 (8)	15,0 (8)	6	13	24 (12)	24 (12)	10	7
Proline	5	10	6	6	4,9	5	7,5 (4)	6,3 (3)	5	5	16 (8)	16 (8)	8	6
Glycine	9	11	11	12	9,7	10	18,6 (9)	17,2 (9)	10	13	24 (12)	24 (12)	16	6
Alanine	11	11	10	9	10,5	8	22,5 (11)	21,5 (11)	5	7	15 (7–8)	15 (7–8)	7	8
1/2 Cystine	14	13–14	13	14	12,2	12	19,0 (10)	21,9 (11)	14	14	30 (15)	30 (15)	12	14
Valine	4	8–9	4	6–7	3,9	4	8,1 (4)	8,0 (4)	4	4	11 (5–6)	11 (5–6)	8	2
Methionine	1	2–3	2	1	0,9	1	None	None	3	2	2 (1)	2 (1)	4	2
Isoleucine	4	5	4	2	4,8	3	7,7 (4)	7,9 (4)	7	7	11 (5–6)	11 (5–6)	5	5
Leucine	6	9	5	9	4,9	5	10,0 (5)	10,0 (5)	5	5	11 (5–6)	11 (5–6)	13	7
Phenylalanine	4	4	5	3	4,0	3	8,0 (4)	8,0 (4)	5	5	10 (5)	10 (5)	7	5
Tyrosine	8	8–9	9	10	8,7	10	13,9 (7)	13,7 (7)	10	10	16 (8)	16 (8)	10	8
Tryptophan	2	3	1	5	2,6	1	7,2 (4)	5,7 (3)	2	2	7 (3–4)	7 (3–4)	—	2
	—	—	—	—	—	—	32,0 (16)	24,0 (12)	5	5	16 (8)	16 (8)	—	—
Total No. amino acids	115–117	168–173	117	130		108			126	126	266	266	183	128
Mol. wt.	12000	19000	13000	14600	12500	11100	24000		13130	13900	30000		18500	

Note. The figures are given for the molecular weight in the computation of which the number of individual amino acids was calculated.

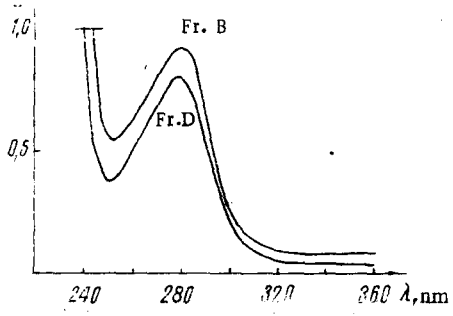

Fig. 1. Absorption spectra of phospholipases A_2 of the venom of the Central Asian cobra (0.05% solution of the enzymes in 0.05 M tris hydrochloride buffer, pH 7.5 containing 6 M guanidine chloride): Fr B) phospholipase A_2I; Fr D) phospholipase A_2II.

basis is 11,370-11,627. In phospholipase A_2II we found 168-173 residues; its molecular weight is higher: 18,553-19,186. We have established that phospholipase A_2I contains less than 0.1 mole of free sulfhydryl groups per mole of protein. Consequently, the 14 semicystine residues in phospholipase A_2I form seven disulfide bonds. We have been unable to determine the number and state of the SH groups in phospholipase A_2II. According to the amino-acid analysis, this enzyme contains 13-14 semicystine residues, but one must consider the possibility of the presence in the enzyme preparation of another protein (or peptide) [2, 4]. The two phospholipases A_2 are distinguished by their contents of individual amino acids, particularly hydroxy amino acids (serine, threonine) and lysine, arginine and glutamic acid. If it is assumed that some of the dicarboxylic amino acids are present in the amide form, the difference in their isoelectric points – about 4.0-4.5 for phospholipase A_2I and 8.0-9.0 for A_2II – becomes understandable.

The absorption spectra of phospholipases A_2I and II of the venom of the Central Asian cobra are shown in Fig. 1. We observed an absorption maximum at a wavelength of 280 nm and a minimum at 257-258 nm. The molar extinction of phospholipase A_2I calculated for a molecular weight (mol. wt.) of 12,000 is $2.2 \cdot 10^4$ and that of A_2II (mol. wt. 19,000) is $2.88 \cdot 10^4$. The coefficient of molar extinction of phospholipase A_2 from bee venom (mol. wt. 18,500) is $3.6 \cdot 10^4$ [5], which is close to our values. The $E^{1\%}_{cm}$ values of phospholipases A_2I and II of the venom of the Central Asian cobra are 15.2 and 18.2, respectively. For comparison, we may mention that $E^{1\%}_{cm}$ of the phospholipase A_2 with a molecular weight of 18,500 is 19.9 and that of the phospholipases A_2I and II from the venom of the rattlesnake Ag. halus blomhoffii is 14.9 for a molecular weight of 13,800 ± 500 and 15.1 for a molecular weight of 13,700 ± 500 [6]. The phospholipase A_2II of this venom is the closest to the phospholipase A_2I that we have isolated in a number of properties.

Table 2 gives, for comparison, information on the amino-acid compositions of phospholipases A_2 from a number of different snakes, for bee venom, and for the enzyme of porcine pancreatic gland. The phospholipase A_2I with a molecular weight of 12,000 that we obtained is similar, in its quantitative amounts of individual amino acids, to phospholipases A_2 having molecular weights within the same limits – from 11,100 to 14,600 – and, particularly, to the phospholipase A_2 from the venom of the cobra Naja naja atra [7]. For the two phospholipases A_2 from the venom of the cobra N. naja, the amino-acid composition was calculated for a molecular weight of 24,000, and for the venom of the rattlesnake Cr. adamanteus for a molecular weight of 30,000 [8, 9]. Nevertheless, it has been shown that the molecular weight of 24,000-30,000 determined by ultracentrifugation is the molecular weight of the enzyme in a dimeric form; the molecular weight of phospholipase A_2 monomer is 11,000-15,000 [10, 11]. Consequently, for comparison we halved the figures for the amounts of individual amino acids in the phospholipases A_2 of the venoms of N. naja and Cr. adamanteus (the results of this operation are given in parentheses in the appropriate columns of Table 2). In this way it was possible to show that, calculated to the molecular weights of the monomers, all the phospholipases A_2 have similar amino-acid compositions. A common feature of them is a high content of semicystine (more than 10% of all the amino-acid residues). The number of semicystine residues varies from 12 to 15, but for the majority of phospholipases A_2 it is 14. In none of the enzymes were free SH groups found, i.e., all the semicystines are bound by disulfide bridges. Consequently, odd numbers are unsuitable as indices of the semicystine content. Also characteristic for the phospholipases A_2 are high amounts of aspartic and glutamic acids, tyrosine, and lysine, and also glycine and proline. The latter is responsible for the pronounced stability of the protein molecule of the enzyme. Small variations in the amounts of methionine may be due to errors of determination and calculation. In any case, the closest to phospholipase A_2I of the venom of the Central Asian cobra in its properties and composition are the phospholipases A_2II of the venoms of N. nigricollis and of N. naja atra; the venom of the sea snake Lat. semifasciata also has one methionine residue in its composition. In the majority of phospholipases A_2 two to three tryptophan residues have been found spectrophotometrically [12]; only in the phospholipase A_2II of the cobra of N. nigricollis were five residues found, but in this case a different method of calculation was used [13].

The figures for the amino-acid composition of the phospholipase A_2II cannot be discussed in depth, since the material was possibly contaminated with another protein for the separation which a special procedure is required [4]. Nevertheless, tendencies are observed that are characteristic for phospholipases

A_2 in general: high amounts of cysteine, aspartic and glutamic acids, lysine, proline, and glycine. In its molecular weight and numbers of amino-acid residues phospholipase A_2II is close to the phospholipase A_2 of bee venom, the polypeptide chain of which consists of 183 residues and has mol. wt. 18,500 [5]. However, later work has shown that the phospholipase A_2 of the bee venom contains 129 amino-acid residues and its molecular weight calculated on the basis of the composition is 14,629 [17]. The differences in the molecular weights are due to the presence of carbohydrates in the phospholipase A_2 of bee venom [5, 17]. It is not excluded that carbohydrates may also be present in the phospholipase A_2II that we have investigated.

Analysis of the N-terminal amino acids has shown the purity of both the fractions with phospholipase activity isolated from cobra venom. Using the fluorodinitrobenzene method, asparagine was isolated when phospholipase A_2I was chromatographed; the dansyl method enabled asparagine to be identified at the N-end. Asparagine has been isolated from the N end of phospholipase A_2 of the venom of N. naja atra [7]. Lysine was found at the N end of phospholipase A_2II by the fluorodinitrobenzene method. In connection with the hypothesis of the presence of another component in this fraction, the N-terminal amino acid of phospholipase A_2II requires confirmation.

EXPERIMENTAL METHOD

The preparations of phospholipase A_2 were obtained from the venom of the cobra by a method described previously; their purity was checked by horizontal electrophoresis in starch and polyacrylamide gels at various pH values, by disk electrophoresis, by rechromatography, and by terminal amino-acid analysis [1, 2].

The amino-acid compositions of the phospholipases A_2 were determined on a AAA-881 amino-acid analyzer (Czechoslovakia). The proteins were hydrolyzed twice with redistilled hydrochloric acid in tubes sealed under vacuum. Hydrolysis was performed at 105°C for 24, 48, and 72 h. The amounts of tryptophan were determined on a Specord UV-Vis spectrophotometer by Edelhoch's method [12]. Solutions of the native protein with concentrations of 0.1 and 0.050% in 0.05 M tris hydrochloride buffer (pH 7.5) containing 6 M guanidine chloride were used. The amount of free SH groups was measured by amperometric titration [18]. The amino acids at the N end were identified by the fluorodinitrobenzene and dansyl methods [19, 20].

SUMMARY

1. The amino-acid compositions of two phospholipases A_2 isolated from the venom of the Central Asian cobra have been determined. It has been shown that phospholipase A_2I has no free sulfhydryl groups. The molecule of phospholipase A_2I consists of a single polypeptide chain stabilized by seven disulfide bonds.

2. The absorption spectra of the phospholipases A_2 isolated have been recorded. The absorption maxima of both phospholipases are located at a wavelength of 280 nm and the minima at 257-258 nm. The coefficient of molar extinction of phospholipase A_2I is $2.2 \cdot 10^4$ and of A_2II $2.88 \cdot 10^4$.

3. Asparagine has been identified at the N end of phospholipase A_2I by the fluorodinitrobenzene and dansyl methods. The amino acid at the N end of phospholipase A_2II is probably lysine.

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