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DETERMINATION OF THE STEREOSPECIFICITY OF PHOSPHOLIPASE A FROM THE VENOM OF THE GREEN TOAD *Bufo viridis*

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The chemical composition of toad venom is responsible for the broad spectrum of its physiological action [1]. The main active principles of toad venom are usually considered to be its low-molecular-mass components, while its protein components have remained practically unstudied.

With the aim of studying the protein-peptide components of the venom of the green toad *Bufo viridis*, we previously [2] isolated by biospecific chromatography a protein possessing phospholipase activity. In order to determine the stereospecificity of the phospholipase isolated, we have carried out the enzymatic hydrolysis of a substrate, phosphatidylcholine (PC), that we isolated from cottonseed kernels as described in [3].

Enzymolysis was conducted in 0.1 M Tris-HCl buffer, pH 9.2 (1.5 ml) with the addition of 0.1 M $CaCl_2$ (0.3 ml), using a solution of 20 mg of PC in 10 ml of diethyl ether. The process was carried out at room temperature with constant stirring until the substrate had been hydrolyzed completely (50 min).

The course of hydrolysis was monitored by TLC in systems: 1) chloroform–methanol–25% ammonia (70:30:5) and 2) hexane–ether (7:3). After the end of enzymolysis, the ethereal layer was decanted off from the aqueous layer. The aqueous fraction was treated with ether (2 × 10 ml). The combined ethereal extracts were concentrated in a rotary evaporator at 40°C. The dry residue was dissolved in 2 ml of chloroform and chromatographed in a thin layer of silica gel in system 2. The free fatty acid (FFA) zone was eluted with chloroform (50 ml). The chloroform was evaporated off and the FFAs were methylated with diazomethane.

The aqueous fraction was also evaporated. The dry residue was dissolved in 2 ml of chloroform–methanol (1:1) and deposited on a TLC plate, with elution by system 1.

TABLE 1. Total and Positional Fatty Acid Compositions of Phosphatidylcholines, %

Experimental conditions, source of phospholipase	Acid		
	16:0 (palmitic)	18:1 (oleic)	18:2 (linoleic)
Control			
PC tot.	13.7	15.3	71.0
sn-2 posn.	5.1	17.4	77.5
sn-1 posn.	22.3	13.2	64.5
Experiment			
sn-2 posn.	5.2	16.7	78.1
sn-1 posn.	22.2	13.9	63.9

Control) products of the hydrolysis of PC by bee venom phospholipase A_2. Experiment) products of the hydrolysis of PC by the phospholipase A from the venom of the green toad *Bufo viridis*.

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The lyso-PC zone was eluted with 50 ml of a 1:1 mixture of chloroform and methanol. After elimination of the solvents, the residue was treated with 3 ml of 10% NaOH in methanol. The FFAs were isolated by means of diethyl ether after acidification of the hydrolysate with 20% aqueous HCl (2 × 10 ml). The ethereal extracts were combined and dried over anhydrous Na_2SO_4. The ether was driven off, and the FFAs were methylated with diazomethane. The FA methyl esters were analyzed by GLC on a Chrom-41 instrument (column 2 m long filled with Chrom W, 60-80 mesh, impregnated with 17% of PEGS; evaporator temperature 250°C, thermostat temperature 198-200°C, carrier gas helium).

As a check on the specificity of the phospholipase A from green toad venom we used enzymolysis by Sigma (USA) phospholipase A_2 from bee venom. The control enzymolysis was conducted under conditions analogous to those described above. The results are given in Table 1.

As can be seen from Table 1, the enzyme under investigation showed great similarity to the control. On this basis, it may be considered that the phospholipase under investigation possesses a strict stereospecificity in relation to PC — i.e., it hydrolyzes the FA in the sn-2 position of the glycerol residue in PC and is, therefore, a phospholipase A_2.

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