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STEREOSPECIFICITY OF A LYSOPHOSPHOLIPASE FROM THE VENOM OF *Vespa orientalis*

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In the majority of venoms of animal origin, in addition to specific neurotoxins, various enzymes are found the presence of which is a fairly important – if not the main – condition for the manifestation of biological effects. In particular, the venom of the hornet *Vespa orientalis* has been found to contain, in addition to neurotoxins with a presynaptic action, a phospholipase A_2 and a lysophospholipase [1, 2], thanks to enzymatic attack by which of the membranes of the primary terminal a high efficacy of the action of the neurotoxins is ensured.

When the hornet venom was incubated with a 1,2-diacyl-sn-glycero-3-phosphocholines (PCs) as substrate, regardless of the source of its isolation, we identified only fatty acids (FAs) and sn-glycero-3-phosphocholines (GPCs). Furthermore, in a study of the kinetics of the enzymolysis of PCs by the hornet venom we did not succeed in identifying lysophosphatidylcholines (L-PC) – unfailing product of the hydrolysis of PCs by phospholipase A_2 which may show that the lysophospholipase of the venom hydrolyzes L-PCs completely.

To evaluate the stereospecificity of the lysophospholipase we used substrates with various structures of the acyl radicals and different positions of them in the L-PC molecule. With the aid of purified phospholipase A_2 from the venom of the bee *Apis mellifera* and that of the kufi *Vipera lebetina* we obtained sn-1-acyl-sn-glycero-3-phosphocholines from the PCs of soybeans and cotton seeds and also hydrolyzed cottonseed PCs. Using phospholipase A_1 from the culture medium of *Rhizopus microsporus* [3] and the venom of the spider *Eresus niger* [4], we obtained sn-2-acyl-3-phosphocholines from the above-mentioned PCs. The L-PCs were purified by TLC on silica gel in the chloroform–methanol–25% ammonia (70:30:5) system. Enzymolysis was conducted for 10 min in Tris buffer, pH 8.4, at a ratio of enzyme to substrate of 1:50. It was monitored by TLC in the chloroform–methanol–25% ammonia (70:30:5) system and by chromatography on Whatman No. 1 paper in the butanol–acetic acid–water (5:3:1) system [5].

In the products of enzymolysis we identifed FAs and sn-glycero-3-phosphocholine. The FAs were purified by TLC in the hexane–ether (7:3) system, methylated with diazomethane, and identified with the aid of GLC. The GLC results (Table 1) for the methyl esters of the fatty acids from enzymolysis by the venom of the hornet *V. orientalis* (1-acyl-sn-glycero-3-PCs and 2-acyl-sn-glycero-3-PCs) proved to be very similar to the results obtained by ordinary alkaline saponification of the L-PCs investigated.

Thus, the results obtained unambiguously demonstrate that the lysophospholipase present in *Vespa orientalis* venom possesses no strict stereospecificity for the lysophosphatidylcholine substrate.

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TABLE 1. Overall and Position Compositions of the PCs

Acid	PC*			Native L-PC	Phospholipase A_2 (bee venom)			Phospholipase A_2 (kufi venom)			Phospholipase A_1 (UzLT-1)			Phospholipase A_1 (spider venom)		
					1-acyl-sn-glycero-3-PCs						2-acyl-sn-glycero-3-PCs					
	1	2	3		1	2	3	1	2	3	1	2	3	1	2	3
12 : 0	0.7	—	—	—	0.3	—	—	0.3	—	—	0.6	—	—	1.0	—	—
14 : 0	0.6	—	—	0.2	0.2	—	—	0.3	—	—	0.9	—	—	0.4	—	—
16 : 0	15.8	6.7	7.0	21.4	27.7	11.5	12.0	27.0	12.3	12.6	4.2	1.2	1.6	4.0	1.0	1.4
16 : 1	—	0.4	—	0.6	—	0.6	—	—	0.5	—	—	0.3	—	—	0.5	—
18 : 0	4.4	0.5	93.0	0.4	6.8	1.0	88.0	7.6	1.0	87.4	0.9	—	98.4	1.0	—	98.6
18 : 1	7.7	24.2	—	34.1	8.0	27.7	—	7.3	28.2	—	8.1	21.1	—	7.9	20.0	—
18 : 2	61.8	68.2	—	43.3	50.0	59.2	—	49.6	58.0	—	74.9	77.4	—	75.6	77.9	—
18 : 3	9.0	—	—	—	7.0	—	—	7.0	—	—	10.4	—	—	10.1	—	—
ΣS	21.5	7.2	100.0	22.0	35.0	12.5	100.0	35.2	13.3	100.0	6.6	1.5	100.0	6.4	1.5	100.0
ΣU	78.5	92.8	—	88.0	65.0	87.5	—	64.8	86.7	—	93.4	98.5	—	93.6	98.5	—

*1) Cottonseed phosphatidylcholine; 2) soybean phosphatidylcholine; 3) hydrogenated phosphatidylcholine

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