ENZYMATIC HYDROLYSIS OF PHOSPHATIDYLCHOLINES WITH

PHOSPHOLIPASE A FROM VARIOUS SOURCES

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In the present paper we give the results of a determination of the specificity of the action of phospholipase A from various snake venoms (dried in a desiccator over CaCl₂) (Central Asian origin: Naja oxiana Eich. (cobra), Vipera labetina L. (kufi), Echis carinatus Schneid. (saw-scaled viper), Ancistrodon halus Pall. (mamushi), and also Vipera berus (common adder), Vipera lebetina obtusa (Azerbaidzhan kufi), and also pure phospholipase A isolated from the venom of Naja oxiana Eich and the lipase of porcine pancreatic gland, on phosphatidylcholine (PC) from the thin-fibered cotton plant of variety S-6029 [1].

A criterion of the comparison of enzymatic hydrolysis was formed by the fatty-acid composition of positions 1 and 2 of the glyceride moiety of the PC molecule, and also the time of hydrolysis. For the reaction we took 60 mg of PC, 25 ml of ether, 1.5 mg of venom (in the case of the lipase, 30 mg, and in the case of the phospholipase A, 0.15 mg), in 0.3 ml of 0.1 M tris buffer, pH 10.2, and performed the hydrolysis at room temperature. The course of the reaction was followed by TLC on silica gel in the chloroform-methanol-water (65: 25:4) system. The hydrolysis products were separated and treated as described previously [2]. The fatty acids from both positions were methylated with diazomethane and analyzed by GLC.

It was established that position 2 of PC mainly contains the radicals of unsaturated fatty acids, and the ratio of the sums of the saturated and unsaturated acids was (%) 7.4/92.6 (common adder), 7.4/92.6 (mamushi), 6.4/93.6 (Azerbaidzhan kufi), 6.7/93.3 (saw-scaled viper), 7.5/92.5 (Central Asian kufi), 8.6/91.4 (cobra), 7.7/92.3 (phospholipase A), and 7.0/93.0 (lipase), which shows the specificity of the phospholipase A of all the sources investigated and agrees with previous results [2-4] for PC of both animal and plant origin.

The rates of complete hydrolysis under the experimental conditions for the phospholipases A from the sources given above were different and equal to, respectively (min): 10, 24, 30, 35, 40, 45, 45 and 120. The results given show that the sources of phospholipase A tested can be used for the enzymatic hydrolysis of PCs and the determination of their structure.

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