LYSOPHOSPHOLIPASE ACTIVITY OF ORIENTOTOXIN FROM THE VENOM OF THE HORNET *Vespa orientalis*

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A number of neurotoxins with presynaptic action (notexin, β-bungarotoxin) possess phospholipase activity, and some include subunits with a high enzymatic activity (crotoxin, taipoxin) [1-3]. Orientotoxin from the venom of the hornet *Vespa orientalis*, which also exhibits a presynaptic action [4] is capable of catalyzing the hydrolysis of 1-acyllysophospholipids. In the present paper we consider results relating to this enzymatic fuction of orientotoxin.

The lysophospholipase activity of orientotoxin was determined by a titrimetric method using as the substrate egg 1-acyllysophosphatidylcholine or 1-palmitoyllysophosphatidylcholine. It was found that the two lysophospholipids were hydrolyzed by orientotoxin at approximately the same rate. The greatest enzymatic activity of the toxin under investigation was observed in a very narrow pH range with its maximum at 7.5 (Fig. 1), which is uncharacteristic for the majority of lysophospholipases from other sources that have been studied [5]. Ions of bivalent metals — in particular, Ca^{2+} — had no appreciable influence on the activation of the catalytic function of orientotoxin. Thus, orientotoxin exhibited a fairly high enzymatic activity even without the addition of exogenous calcium, but the addition of Ca^{2+} ions to the incubation medium led to some acceleration of the reaction. At the same time, an increase in the concentration of Ca^{+} ions in the incubation medium (from 1 to 10 mM) with the addition of 1 mM EDTA did not change the rate of the reaction, i.e., the degree of activation of the lysophospholipase function of orientotoxin did not depend on the concentration of added Ca^{2+} ions. The results obtained indicated that Ca^{2+} does not play a fundamental role in the manifestation of the catalytic activity of orientotoxin. This conclusion is an additional confirmation of the fact that, as we have shown previously [4], its presynaptic effect in the absence of Ca^{2+} and Mg^{2+} ions is stronger than in their presence.

The orientotoxin from the venom of *V. orientalis* as an enzyme — a lysophospholipase — was less stable to the action of high temperatures than the phospholipase A_2 from the same venom. Thus, when the toxin was first treated at various temperatures for 5 min its catalytic activity was retained at the initial level up to 60°C, but above this temperature it began to fall sharply. At the same time, the phospholipase A_2 from this venom retained its activity in similar conditions up to 70-75°C.

Inhibition by detergents is a universal property of all known lysophospholipases [5]. In actual fact, the lysophospholipase activity of orientotoxin was likewise inactivated when the incubation medium contained detergents — sodium deoxycholate and Triton X-100 (Fig. 2). While in the case of the nonionic detergent Triton X-100 the degree of inactivation of the catalytic activity of orientotoxin depended on the concentration of the detergent, the anionic detergent — sodium deoxycholate — proved to be the most effective inhibitor, i.e., when 0.5-1 mM of this detergent was present in the incubation medium no orientotoxin-catalyzed hydrolysis of a lysophospholipid whatever took place. Furthermore, it was shown that an inhibiting effect was also possessed by high concentrations of Tris-HCl buffer. For example, at a concentration of 4 mM this buffer system caused 50% inhibition of the enzymatic activity of orientotoxin. This phenomenon is possibly connected with the fact that like many lipolytic enzymes and, in particular, phospholipase A_2 [5], the orientotoxin molecules are capable of aggregating in solutions of high ionic strength.

On investigating the dependence of the initial rate of the orientotoxin-catalyzed hydrolysis of egg 1-acyllysophosphatidylcholine on the concentrations of the toxin and of the substrate, we found that the initial rate of the hydrolysis of the substrate increased in proportion to a rise in the concentration of the toxin and at low concentrations of it. At higher concentrations of the toxin the rate of the reaction fell. The curve of the dependence of the initial rate of hydrolysis on the concentration of the substrate had the usual hyper-

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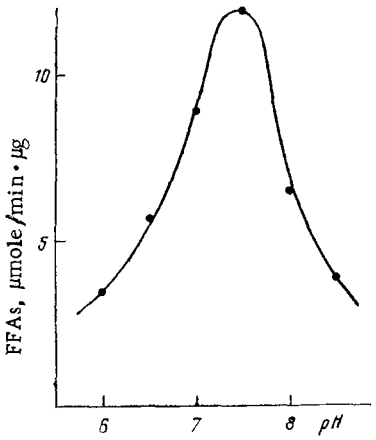

Fig. 1

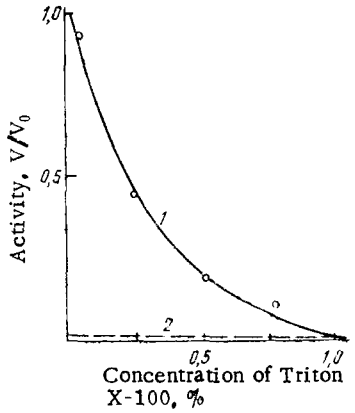

Fig. 2

Fig. 1. Influence of the pH of the medium on the catalytic activity of orientotoxin. The reaction mixture with a total volume of 2 ml contained (mM): NaCl, 150; $CaCl_2$, 1; Tris-HCl, 1; egg 1-acyllysophosphatidylcholine or 1-palmitoyllysophosphatidylcholine, 16; and orientotoxin, 0.1-1 μg/ml.

Fig. 2. Action of the detergents Triton X-100 (1) and sodium deoxycholate (2) on the lysophospholipidase activity of orientotoxin. Rate of the reaction in the presence (V) and in the absence (V_0) of the detergents.

bolic form that is characteristic for enzymatic reactions. The dependence of 1/V on 1/S was linear in the Lineweaver–Burk coordinates. The apparent Michaelis constant K_M and the maximum rate of the reaction V_{max} calculated from the points of intersection of the curves with the axes of ordinates and abscissas were 2.83 mM and 12.5 μmole/min·μg of the protein, respectively. These values are comparable with the analogous values obtained for the lysophospholipase from snake venom [6].

Thus, the experimental results given above permit the conclusion that orientotoxin from the venom of the hornet *Vespa orientalis* belongs, with respect to its catalytic properties, to the lysophospholipids. This enzyme, like known lysophospholipases from other sources, is less heat-stable than phospholipase A_2, and Ca^{2+} ion play no fundamental role in the manifestation of its catalytic activity; the enzyme investigated is also inactivated by detergents — Triton X-100 and sodium deoxycholate.

LITERATURE CITED

1. K. Kondo, K. Narita, and C. Y. Lee, J. Biochem. (Tokyo), 83, 101 (1978).
2. J. Fohlman, P. Lind, and D. Eaker, FEBS Lett., 84, 367 (1977).
3. B. D. Howard and C. B. Gundersen, Ann. Rev. Pharmacol. Toxicol., 20, 307 (1980).
4. M. U. Tuichibaev, B. A. Tashmukhamedov, L. G. Magazanik, and I. M. Gotgil'f, Bioorg. Khim., 10, No. 3, 318 (1984).
5. H. Brockerhoff and R. G. Jensen, Lipolytic Enzymes, Academic Press, New York (1974).
6. C. Takasaki and N. Tamija, Biochem. J., 203, 269 (1982).