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CHARACTERIZATION OF TOXINS FROM VENOM OF *Vespa orientalis*

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The toxic properties of the venom of the hornet *Vespa orientalis* are due to the presence in it of lysophospholipase A<sub>1</sub>, which possesses a presynaptic action [1], and of highly toxic phospholipase A<sub>2</sub> [2]. In the present paper the properties of these two toxins, designated as orientotoxin-I (ORT-I) and orientotoxin-II (ORT-II), are compared:

Parameters and acting agents	ORT-I	ORT-II
Toxicity, LD <sub>50</sub> , mg/kg on intravenous injection	0.5	0.65
Molecular mass, kDa	16	15
Electrophoretic mobility, R <sub>f</sub>	0.47	0.53
N-Terminal amino acid	phenylalanine	phenylalanine
C-Terminal amino acid	lysine	lysine
Concentration necessary for complete hemolysis, µg	0.075*	0.75*
Specific substrate	lysolecithin	lecithin
Optimum pH	7.5	8.5
Optimum temperature, °C	45	50
Optimum Ca <sup>2+</sup> concentration, mM	5	10
Specific activity, units/mg	5480	29,400
Michaelis constant, mM	0.27	1.61
Inactivation constants, min <sup>-1</sup>		
by trypsin	0.11	0.08
by p-bromophenacyl bromide	3·10 <sup>-2</sup>	2.9·10 <sup>-2</sup>
by bromomethyl adamantyl ketone	2.1·10 <sup>-2</sup>	2.0·10 <sup>-2</sup>
by heat treatment at 50°C	0.012	0.012
by urea (8 M)	8.3·10 <sup>-2</sup>	8.3·10 <sup>-2</sup>
Inactivation by detergents, mM		
sodium deoxycholate	11	16
sodium dodecyl sulfate	10	15
Number of peptides on hydrolysis		
by trypsin	13(2)	15(2)
by chymotrypsin	23(3)	22(3)
by glutamine proteinase	15(5)	16(5)

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Area occupied by a molecule, Å <sup>2</sup>	380	596
Surface activity, dynes/cm	22.5	8.0

As we see the two toxins have close LD<sub>50</sub> values but differ considerably in hemolytic activity: The concentration of ORT-I causing the complete hemolysis of erythrocytes is 100 times [sic] lower than the corresponding concentration of ORT-II. The catalytic properties and substrate specificities of the two enzymes are different and so are the shapes of their kinetic curves: That for ORT-I has a lag period, while that for ORT-II is a normal curve with saturation in relation to fatty acid, the specific activity of ORT-II being 5.1 times higher than that of ORT-I.

At the same time, the similarity in the behaviors of the two enzymes on inactivation by various agents shows that they possess close molecular parameters. Their N- and C-terminal amino acid residues are identical, but analysis of the peptide maps of tryptic, chymotryptic, and proteinase hydrolysates shows a small difference in the peptide compositions of ORT-I and ORT-II. Experiments on the adsorption of the toxins on phospholipid monolayers have shown that, with a similar molecular weight to ORT-II, the ORT-I molecule occupies a 1.5 times smaller area at a phase separation boundary and possesses a greater surface activity.

Thus, the venom of the hornet Vespa orientalis contains two toxins having similar structures but differing considerably in functional properties, and this appears to be of particular value for a study of the interrelationship between the structure and the catalytic and toxic properties of lipolytic enzymes.

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