CHEMICAL MODIFICATION OF HISTIDINE IN THE ACTIVE CENTER OF PHOSPHOLIPASE A_2 OF THE VENOM OF *Vespa orientalis*

M. U. Tuichibaev

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The phospholipases A_2 from various sources have a histidine residue in the active center the modification of which by specific reagents — in particular, p-bromophenacyl bromide — leads to a loss of catalytic properties [1]. The phospholipase A_2 from the venom of the hornet *Vespa orientalis* [2] differs substantially in structure from the analogous enzymes isolated from other sources. Our aim was to determine the amino acid residues present in the active center of this enzyme.

The treatment of the phospholipase A_2 from *V. orientalis* with p-bromophenacyl bromide in sodium cacodylate buffer containing 0.1 M NaCl, pH 6.0, at 30°C [3] led to an only 50% inhibition of the activity of the enzyme in 2-3 h (Fig. 1, curve 1). Neither raising the concentration of the reagent nor increasing the time of incubation led to the complete inactivation of the enzyme, and the addition to the medium of Ca^{2+} ions or of lysolecithin, conversely, retarded the reaction. In view of the high tendency of the enzyme to form dimeric complexes, this phenomenon can be explained by the "half-site reactivity" of the histidine residue, as has been shown previously in the case of the modification of the histidine and aspartic residues in the phospholipases A_2 from cobra venom [4]. It may be assumed that the phospholipase A_2 from the venom of *V. orientalis* is present in solution in the form of an unsymmetrical dimer composed of two identical subunits. The functionally important histidine residue in one subunit is accessible for interaction with the modifying reagent and in the other it is included in an internal region and does not take part in the reaction. From the results of an analysis of the amino acid compositions of the native and modified protein it can be seen that, in actual fact, the number of histidine residues in the (p-bromophenacyl bromide)-inactivated phospholipase A_2 is approximately one less than in the native enzyme, calculated to the dimer, i.e., in the dimer molecule only one histidine residue of one of its subunits undergoes modification and in the other subunit the corresponding histidine residue remains unmodified. Consequently, the enzyme is only 50% inactivated:

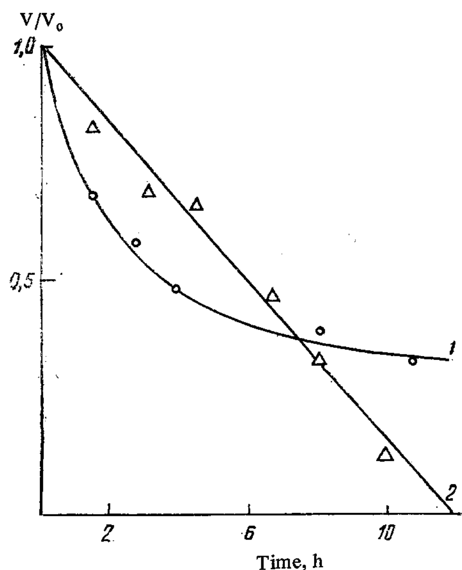

Fig. 1. Kinetics of the inactivation of phospholipase A_2 from *V. orientalis* by p-bromophenacyl bromide (1) and by bromomethyl adamantyl ketone (2).

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Phospholipase A_2	Number of histidine residues (per monomer)		Activity, %
	Total	Modified	
Native	3.96	0	100
Modified with p-bromophenacyl bromide	3.43	0.54	44
Modified with bromomethyl adamantyl ketone	2.87	1.12	1–2

On the other hand, as has been shown previously, in a series of phospholipases A_2 from various sources, the histidine residue located in the active center of the enzyme is surrounded by hydrophobic amino acid residues which probably interferes with the access of the modifying reagent to the histidine residue [5]. In the phospholipase A_2 from *V. orientalis*, however, there are considerably more hydrophobic amino acids than in the phospholipases A_2 from other sources [2]. In view of this, the more hydrophobic molecule of bromomethyl adamantyl ketone was used to modify the enzyme under investigation. It was found that this reagent leads to 100% inactivation of the enzyme, although after a longer period (Fig. 1, curve 2). At the same time, the kinetic curve of the inactivation of the enzyme with time has a linear nature. Analysis of the amino acid compositions of the native and modified proteins (see above) show that bromomethyl adamantyl ketone is capable of modifying the functionally important histamine residues in both subunits of the dimer of phospholipase A_2 from *V. orientalis* venom. The results obtained permit the conclusion that there is a functionally important histidine residue in the active center of the molecule of *V. orientalis* venom phospholipase A_2, as well.

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SYNTHESIS OF MINILULIBERIN

Yu. P. Shvachkin and Yu. N. Tikhonov

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Compounds possessing the capacity for regulating ovulation processes are of interest for a number of fields of medicine and agriculture. The development of methods for obtaining such compounds is an urgent problem.

The group of compounds capable of regulating ovulation processes by stimulating the liberation of the luteinizing hormone includes miniluliberin which is structurally related to the immunostimulator tuftsin [1, 2] and consists of the tripeptide of (I) [3] (here and below, all amino acids are of the L series).

(1)

We have performed the synthesis of miniluliberin (I) by schemes 1 and 2, involving the stepwise formation of the tripeptide in solution with the use as the amino components of benzyl (system 1) or p-nitrobenzyl (scheme 2) esters of the C-terminal amino acid and intermediate dipeptides, and as the activated carboxylic components the pentafluorophenyl esters [4], or mixed anhydrides of the protected lycine and threonine, respectively.

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