LITERATURE CITED

1. FRG Patent No. 1,142,365 of Jan. 17, 1963.
2. Japanese Patent No. 8310 of February 2, 1971.
3. A. S. Spirin, Biokhimiya, 23, No. 5, 655 [sic].
4. Z. Dische, in: Nucleic Acids (ed. E. Chargaff), [Russian translation, Moscow (1975), pp. 425 and 437].

IMMOBILIZATION OF SNAKE VENOM ON EPOXY-ACTIVATED SILOKHROMS

O. V. Ostapenko, V. V. Chupin,
G. A. Serebrennikova, and R. P. Evstigneeva

UDC 577.152.311*4'17:661.183.8

The production of immobilized phospholipase A_2 preparations by the covalent addition of the proteins of viper venom to Silokhroms containing epoxide groups is described. The dependence of the amount of protein bound to the support on its concentration and on the amount of epoxide groups in the sorbent has been investigated. The phospholipase activity of the preparations obtained did not depend on the amount of immobilized enzyme but was determined by diffusional factors.

Phospholipases A_2 (EC 3.1.1.4) – enzymes that catalyze the splitting out of the fatty acid from the second position of sn-glycero-3-phospholipids – are widely used in the chemical modification of various types of synthetic and natural phospholipids and in the study of their structures. In a number of cases the immobilization of the enzyme on various supports enables stable enzyme preparations for repeated use to be obtained. As a rule, the method of covalent attachment of proteins to the sorbents is used for the immobilization of lipolytic enzymes. Thus, the production of preparations of covalently bound phospholipases A_2 from cobra venom [1-3] and from the venom of the Far Eastern mamushi [4] and of cottonseed lipase [5] has been described. The inclusion of enzymes in polymers during their polymerization has been used to obtain preparations of C.cylindracea lipase [6] and of phospholipases A and D [7]. The properties of mitochondrial phospholipase A_2 bound adsorptively with an affinity phospholipid-containing sorbent have been studied [8].

The immobilization of lipolytic enzymes is accompanied by a considerable decrease in enzymatic activity. The choice of support and method of attachment ensuring the least inactivation of the protein molecule is the main task in the production of an immobilized enzyme. As supports for the attachment of phospholipases and lipases are used activated agarose [1, 3] and porous glass [2], organosilicon sorbents [4], and a water soluble copolymer of N-vinylpyrrolidone with glycide acrylate [5].

It appeared desirable to investigate the possibility of using a readily available modified silica – Silokhrom – containing epoxide groups (epoxy-Silokhrom) for the covalent attachment of phospholipases. Sorbents with epoxy groups (epoxy-Sepharose, water-soluble polymers) have previously been used successfully in the immobilization of various types of proteins [5, 9, 10] and, in particular, in the production of heat-stable preparations of cottonseed lipase [5].

Snake venoms possess a high phospholipase activity. This permits the use of the whole venom and not the purified enzyme for obtaining immobilized phospholipase preparations with a good activity. In the present paper we describe a method of immobilizing viper venom.

For the retention of the activity of the enzymes on their attachment to a support it is very important that the functional groupings present in the active center do not

M. V. Lomonosov Institute of Fine Chemical Technology, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 126-129, January-February, 1989. Original article submitted February 23, 1988.

 0009-3130/89/2501-0112 $12.50

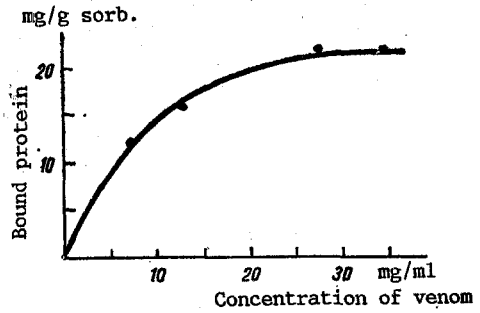
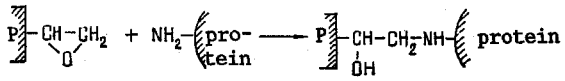


Fig. 1. Dependence of the amount of protein bound to the sorbent on the concentration of the venom in immobilization on epoxy-Silokhrom. Concentration of epoxide groups – 100 μmole/g of sorbent.

participate in the process of covalent attachment. It is known from the literature that in the phospholipases A_2 of the Viperidea family, to which the viper belongs, the terminal amino groups do not participate in the processes of enzymatic catalysis [11], and therefore we brought about the addition of the enzyme to the matrix by the alkylation of the amino groups of the protein with the epoxide groups of the matrix:



The opening of the epoxide ring of the sorbent by the NH_2 groups of the protein components of the venom was carried out at pH 8.5 and room temperature for 20 h [9, 10]. At higher pH values and higher temperatures denaturation of the enzyme and the breakdown of the matrix are possible.

The superfluous epoxide groups were then blocked with ethanolamine. The necessity for this stage is due to the fact that on cleavage by the enzyme preparation of phospholipids containing NH_2 groups (phosphatidylethanolamine, phosphatidylserine) their chemical interaction with the matrix is possible.

The viper venom was attached to epoxy-Silokhroms with different concentrations of epoxide groups. It can be seen from the figures given below that at the same concentrations of venom in the reaction mixture the amount of protein bound to the sorbent rose with an increase in the concentration of epoxy groupings in it (the concentration of the venom was 8.3 mg/ml):

Concentration of epoxy groups, mg/g of sorb.	5	100
Concentration of protein in the sorbent, mg/g of sorb.	6.9	12.8
Activity* of the preparation, μg/min·g of sorb.	5.7	5.8
Specific activity of the immobilized venom, μmole/min·mg of protein	0.83	0.45
Retention of the specific activity, %	10.8	5.7

The dependence of the binding of the proteins of the venom with the sorbent on its concentration was investigated. It can be seen from Fig. 1 that the greatest binding of the protein components with the sorbent was observed at a protein concentration of ~25 mg/ml, and a further increase in the concentration did not lead to an enhancement of the binding of the protein with the matrix.

The phospholipase activities of all the samples of immobilized venom obtained were determined. The specific activities of the preparations were different and amounted to 5.7 and 11.5% of the initial activity. The specific activities of the samples referred to 1 mg of protein on the sorbent decreased with an increase in the concentration of protein on the sorbent, while the activities of the preparations referred to 1 g of sorbent scarcely changed. Below, we give the characteristics of the preparations of immobilized phospholipase A_2 (concentration of epoxide groups 100 μmole/g of sorbent):

*Phospholipase activity of the preparation referred to 1 g of sorbent with the immobilized venom.

Concentration of protein on the sorbent, mg/g of sorb.	12.5	16.2	21.8
Activity of the preparation, μmole/min·g of sorb.	11.2	9.2	10.3
Specific activity of the immobilized venom, μmole/min·mg of protein	0.90	0.57	0.47
Retention of specific activity, %	11.5	7.3	6.1

The fact that the rate of the enzymatic process did not rise with an increase in the amount of phospholipase immobilized on the sorbent and was determined only by the amount of sorbent permits the assumption that the limited stage of the process is the mass transfer of the substrate from the solution to the surface of the sorbent. This finds its reflection in a rise of the specific activity of the enzyme preparation with a decrease in the amount of phospholipase immobilized on the sorbent. Apparently, an increase in the rate of mass transfer of the substrate from the solution to the surface of the sorbent and, consequently, in the activity of the preparation can be achieved by a modification of the surface layer of the support.

Viper venom immobilized on epoxy-Silokhrom used for the preparative cleavage of phosphatidylcholine isolated from eggs (400 mg of phospholipid cleaved completely by 300 mg of phospholipase preparation in 5 h) and also of synthetic 1-O-hexadecyl-2-O-palmitoyl-rac-glycero-3-phosphocholine retained its activity for several months.

EXPERIMENTAL

The support used was a silica sorbent – epoxide Silokhrom (d_{pore} = 250 Å, S_{sp} = 120 m^2/g) with different amounts of epoxy groups (50 and 100 μmole/g) produced by Biolar (Olaine). The venom of the viper Echis multisguamatus was obtained from the Central Asian zonal zoocombine. The specific activity of the venom was 7.83 μmole/min·mg. The phospholipase activities of the immobilized venom and of the dissolved venom were determined acidometrically [12], using egg phosphatidylcholine as substrate. The amount of protein on the sorbent was determined spectrophotometrically from the amount of dye adsorbed by the immobilized protein [13].

Immobilization of the Venom on Epoxy-Silokhrom. A weighed amount of the venom was dissolved in 3 ml of 0.1 M K phosphate buffer (pH 8.5), 200 mg of epoxy-Silokhrom was added and the suspension was shaken vigorously at room temperature for 20 h. Then the sorbent was washed on a filter with K phosphate buffer (100 ml) and 1% CH_3COOH (50 ml) until the unbound components had been eliminated completely, and then with distilled water. The sorbent was treated with 3 ml of 1 M ethanolamine (pH 9), the suspension was shaken for 4 h, and the sorbent was filtered off, washed with distilled water, and dried in the air.

Cleavage of Phosphatidylcholine by the Preparation of Immobilized Venom. Egg phosphatidylcholine (400 mg) was dissolved in 6 ml of diethyl ether and the solution was shaken vigorously with 300 mg of the phosphorylase preparation in 15 ml of buffer (50 mM Tris·Cl, pH 8, 25 mM $CaCl_2$, 0.2 M NaCl, 1 mM EDTA) at room temperature for 5 h. The sorbent was filtered off and was washed with 50 ml of the same buffer not containing $CaCl_2$. The lysophospholipid was extracted from the mother solution with 50% methanol in chloroform. The solvent was distilled off in vacuum and the residue was chromatographed on silica gel LM 100/160. The lysophosphatidylcholine was eluted with chloroform–methanol (1:4). This gave 180 mg of lysophosphatidylcholine.

The cleavage of 1-O-hexadecyl-2-O-palmitoyl-rac-glycero-3-phosphocholine by the preparation of immobilized venom and the isolation of the lysophospholipid were performed similarly.

SUMMARY

Viper venom has been immobilized on epoxy-Silokhroms with the retention by the immobilized venom of 5.7-11.5% of its initial phospholipase activity. The enzyme preparations obtained have been used to cleave diacyl- and alkylacylphosphatidylcholines.

LITERATURE CITED

1. D. Lombardo and E. A. Dennis, J. Biol. Chem., 260, No. 30, 16114 (1985).
2. M. Adamich, H. F. Voss, and E. A. Dennis, Arch. Biochem. Biophys., 189, No. 2, 417 (1978).

3. U. R. Apsalon, O. G. Shamborant, and A. I. Miroshnikov, Bioorg. Khim., 3, No. 11, 1553 (1977).
4. N. G. Evstratova, O. V. Ostapenko, G. A. Serebrennikova, and R. P. Evstigneeva, Bioorg. Khim., 10, No. 6, 844 (1984).
5. M. Kh. Tadzhiev, F. Ibragimov, Kh. G. Khasanov, N. R. Dzhanbaeva, and M. M. Rakhimov, Prikl. Biokhim. Mikrobiol., 14, No. 5, 703 (1978).
6. Y. Kimura, A. Tanaka, K. Sonomoto, T. Nihira, and S. Fukui, Eur. J. Appl. Microbiol. Biotechnol., 17, No. 1, 107 (1983).
7. H. Jiro and M. Satiko, Kobunshi, High Polym. Jpn., 35, No. 6, 565 (1986).
8. Kha Zen Te, R. Akhmedzhanov, and M. M. Rakhimov, Uzb. Biol. Zh., No. 6, 3 (1985).
9. O. V. Lebedeva, I. G. Frumkina, and N. N. Ugarova, Prikl. Khim. Mikrobiol., 22, No. 5, 669 (1986).
10. G. S. Murthy and N. R. Moudgal, J. Biosci., 10, No. 3, 351 (1986).
11. N. Oda, M. Yoshida, S. Tanaka, H. Kihara, and M. Ohno, J. Biochem. (Tokyo), 100, No. 6, 1551 (1986).
12. E. A. Dennis, J. Lipid Res., 14, No. 1, 152 (1973).
13. N. I. Polikarpova, Lab. Delo., No. 7, 447 (1986).

TRAIL PHEROMONE OF Kalotermes flavicollis

S. G. Klochkov and A. N. Pushin

UDC 595.7

The trail pheromone of the termite Kalotermes flavicollis Fabr. has been isolated with the aid of column, liquid, and gas chromatography. Four of its components have been identified by PMR and chromato-mass spectrometry: nonan-1-ol, decan-1-ol, undecan-1-ol, and dodecan-1-ol, which are present in the natural trail pheromone in a ratio of 1:2:4:4. This has been confirmed by comparison with standard substances by means of gas chromatography and biotesting.

Termites are serious pests of articles, materials, and structures [1]. Their chemical signals have acquired exceptional diversity and regulate practically all aspects of their lives. Chemical signaling is considered as one of the conditions of the appearance of social behavior in termites [2], and it therefore appears desirable to use it in the development of effective methods for the fight against these harmful insects. The most promising in this respect are the trail pheromones. At the present time, the trail pheromones of a number of species of termites have been isolated and identified [3]; however, the trail pheromones of termites living in the USSR are unknown.

The trail pheromone from a population of the termite Kalotermes flavicollis Fabr. living in France has been isolated; it was identified as hex-cis-3-en-ol [4]. However, a number of facts cast doubt on the correctness of this determination, at least in relation to a population of this species of termite living on the territory of the USSR. Hex-cis-3-en-1-ol is a widely distributed substance characteristic of many plants and an attractant for many insects [5]. Furthermore, the hypothesis of the multicomponent nature of the pheromones of insects in general [6] and of termites in particular [3] is generally accepted. In view of the facts presented, we have carried out work on the isolation and identification of the trail pheromone of K. flavicollis.

To isolate and identify the trail pheromone we prepared an extract from 10,000 pseudoergates of the K. flavicollis. After two-stage column chromatography of the extract a single active fraction was obtained which, as GLC analysis showed, consisted of several

Institute of the Physiology of Active Substances, Academy of Sciences of the USSR, Chernogolovka. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 129-132, January-February, 1989. Original article submitted March 3, 1988.

0009-3130/89/2501-0115 $12.50