Pure Appl. Chem., Vol. 74, No. 12, pp. 2311–2316, 2002.

Chemical and biological safety. Biosensors and nanotechnological methods for the detection and monitoring of chemical and biological agents*

S. Varfolomeyev[1,‡], I. Kurochkin[1,2], A. Eremenko[1,2], and E. Efremenko[1]

[1]The Lomonosov Moscow State University, Faculty of Chemistry, Chemical Enzymology Department, Lenin's Hills, 1/11, Moscow, 119992 Russia; [2]Russian Center of Molecular Diagnostics and Therapy, Simferopolsky b-r, 8, Moscow 1131439, Russia

Abstract: The elaboration of highly sensitive and express methods for quantitative and qualitative detection and monitoring of chemical warfare agents (CWA), organophosphate and carbamate pesticides, compounds with delayed neurotoxicity, and pathogenic microorganisms and viruses is discussed. The application of potentiometric and amperometric biosensors, automatic biosensors discriminating the neurotoxins of different classes, is performed. The information about biosensors detecting the compounds with delayed neurotoxicity through the evaluation of "neurotoxic esterase" activity in the blood is presented. The use of immunochip technology for the detection of pathogenic microorganisms and viruses is demonstrated. The enzymatic methods of destruction of organophosphorus neurotoxins are considered as the base of new defense technology.

The contemporary level of investigations in chemistry and molecular biology notably extends the possibilities for the creation of new compounds and systems potentially applicable as agents of chemical and biological lesions. The factors promoting this situation are as follows:

- growing volume of available information concerning the structure of compounds of various classes and their physiological activity,
- broad application of urban and agricultural neurotoxins and pesticides comparable in toxicity with traditional toxicants,
- elaboration and development of new methods of synthesis including the enzymatic ones, and
- elaboration and broad application of gene-engineering methods for sufficiently simple transfer of genes of biological supertoxicants into the nonpathogenic human microflora.

Thereupon, a cardinal objective seems to be the creation of new reliable highly sensitive and express methods for control, qualitative and quantitative detection, and monitoring of supertoxicants of various classes. The important aim appears to be the elaboration of new methods for protection against chemical and biological terrorism.

*Lecture presented at the IUPAC Workshop, Impact of Scientific Developments on the Chemical Weapons Convention, Bergen, Norway, 30 June–3 July 2002. Other presentations are published in this issue, pp. 2229–2322.

‡Corresponding author

In terms of the overall task of chemical and biological safety, a pressing purpose seems to be a notable improvement of analytical methods and protocols allowing:

- 10–10^4-fold increase of efficiency of analytical instruments and assay methods and, hence, reduction of analytical procedure duration;
- 100–1000-fold enhancement of methods' sensitivity;
- remarkable reduction of analysis cost;
- arrangement of a system of nonstop control and monitoring of water, air, and foods; and
- elaboration of adequate methods for individual control.

At present, there are evident approaches that allow a solution to this problem. These approaches include the following analytical technologies: biosensors [1–8], bioluminescent analysis, immuno-analytical protocols [9–13], enzymatic, DNA- and immuno-biochip technologies, and nanotechnological methods based on scanning probe microscopy [14–19].

Recently, the Chemical Enzymology Department (Chemistry Faculty, The Lomonosov Moscow State University) jointly with colleagues from other institutions conducted a battery of researches on the creation of analytical methods for the detection of toxins of various classes and pathogenic microorganisms.

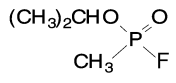
NEUROTOXINS AS CHOLINESTERASE INHIBITORS

Biosensors for the detection and discrimination of neurotoxic compounds

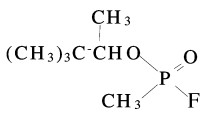
Presently, widely used pesticides are presumably the derivatives of two classes, i.e., organophosphorus pesticides, phosphoric, phosphonic, and thiophosphorilic acids, as well as carbamates, carbonic acid derivatives. CWA are extremely toxic compounds of these classes. Both organophosphorus neurotoxins and carbamates are highly effective inhibitors of cholinesterase (ChE). In some cases, the toxicity of carbamates is close to that of CWA [20].

The structures of some the most known neurotoxins, being the inhibitors of ChE are given below.

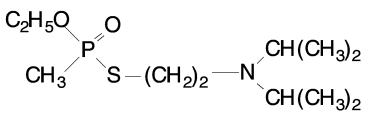
1) Chemical Warfare Agents



Sarin

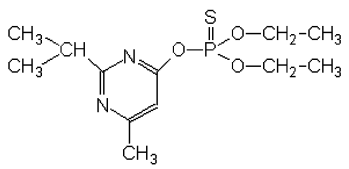


Soman

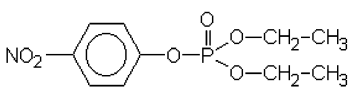


VX

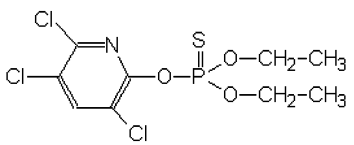
2) Organophosphorus pesticides



Diazinon

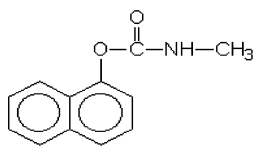


Paraoxon

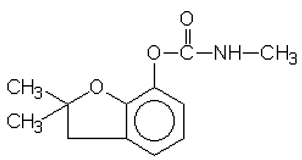


Chlorpyrifos

2) Carbamates



Carbaryl



Carbofuran

Potentiometric biosensors, using organophosphate hydrolases

Organophosphate hydrolase (EC 3.1.8.1) is capable of hydrolyzing the P-I, P-S, and P-O bonds in all presently known organophosphorus neurotoxins [21]. Since the hydrolysis products are strong acids, the detection of a starting compound can be done by the shift of potential of a pH-sensitive device (pH-sensitive electrode, field transducer and polyaniline-coated electrode) [22,23]. The advantages of a potentiometric biosensor are the following: a high rate of response (a few seconds) and simplicity and low cost of both production process and measuring electronic instruments. The potentiometric biosensor is an ideal sensor for monitoring toxicants in water and air; it can be recommended for wide-scale applications for monitoring of air flows in areas with high population density (such as mass transit stations).

The drawbacks of a potentiometric biosensor are a rather low sensitivity (up to 10^{-7} M) and a possibility of detecting only organophosphorus neurotoxins since the biosensor is insensitive to carbamate neurotoxins.

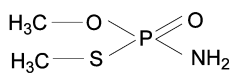
Amperometric biosensors for the detection and discrimination of neurotoxic compounds

Amperometric biosensors are used to detect the neurotoxins of various classes of multienzymatic systems comprising ChE and choline oxidase [24]. The level of butyryl choline esterase (BChE) inhibition is proportional to the quantity of neurotoxins in the sample. BChE activity is detected in accordance with the rate of appearance of hydrogen peroxide as a result of multienzymatic transformation of the substrate (butyryl choline). Two approaches for registration of hydrogen peroxide were elaborated.

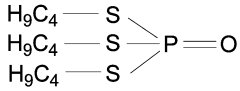
The first approach supposes the application of peroxidase at the last reaction step with direct electron transfer from the electrode to the enzyme-active site [24–26]. The second approach deals with amperometric registration of hydrogen peroxide with platinum electrode. The biosensor quantifies the neurotoxins (pesticides and toxins) in the range of extreme permissible concentrations and lower. The sensitivity limit for diisopropyl fluorophosphate (DFP) is up to 10^{-11} M. The neurotoxins of various chemical classes (ions of heavy metals, organophosphates, and carbamates) are discriminated by application of specific enzymatic and chemical kits. An automatic robot performing all analytical operations with no operator participation was elaborated. The biosensor was elaborated for chemical safety, environmental monitoring (water, soil, and air), qualitative control of agricultural production, and food safety.

Delayed neurotoxicity

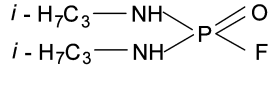
Certain organophosphates were found to induce a delayed neuropathy in humans and susceptible species several weeks after an initial toxic insult, this effect being unrelated to acetyl cholinesterase inhibition. To illustrate the substances of this class, mipafox and some other compounds are listed below.



metamidophos



butyphos



mipafox

Many organophosphates commonly used as insecticides reveal the delayed neurotoxicity. It is initiated by the organophosphorylation and specific modification of the neuronal protein known as neuropathy target esterase (neurotoxic esterase, NTE). A new biosensor method for NTE activity measurements based on the combination of NTE enzymatic hydrolysis of phenyl valerate with electrochemical phenol assay was developed [27,28].

Good correlation between brain and lymphocyte NTE inhibition, as well as between brain and blood NTE inhibition, and lymphocyte and blood NTE inhibition suggests blood NTE activity as a biochemical marker of neuropathic organophosphate exposure. This biosensor gives the unique method for the medico-biological monitoring of personal.

The NTE biosensor can be applied to the control of a broad group of compounds on their potential delayed neurotoxicity, for monitoring the occupational exposure of humans to neurotoxic organophosphates, particularly for field use and for developing and improving the methods of early diagnostics of delayed neuropathy.

Nanotechnological methods for the detection of superpathogenic microorganisms and viruses

The principles of express detection of microorganisms and viruses by the use of an atomic force microscope were elaborated [14–19]. The method is based on the creation of an immunochip with high-specific antibodies and the detection of adsorbed cells by the scanning probe microscope. The typical immunochip surface obtained by Langmuir–Blodgett (LB) method using amphiphilic polyelectrolytes is shown in Fig. 1.

The assay protocol includes: (1) obtaining of affinity surface [in the general case, the antibodies, receptors, and DNA(RNA)-probe can be employed]; (2) performance of specific adsorption of the searched object in the analyte; (3) scanning of the surface; and (4) image analysis and identification of the biological agent using pattern-recognition technique.

The possibilities of the method were demonstrated for the analysis of *Coxiella burnetti*, *Smallpox vaccine virus,* and *Yersinia pestis* (Fig. 2). The sensitivity of the method is up to single cells.

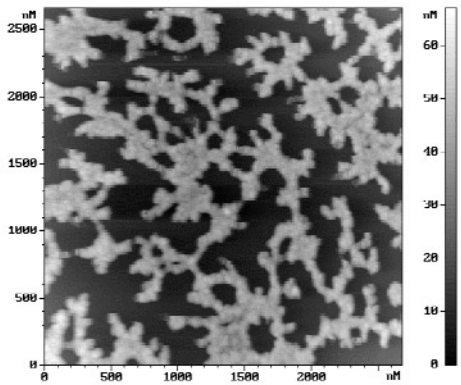


Fig. 1 Atomic force image of immunochip coated with LB films from antibodies and amphiphilic polyelectrolytes.

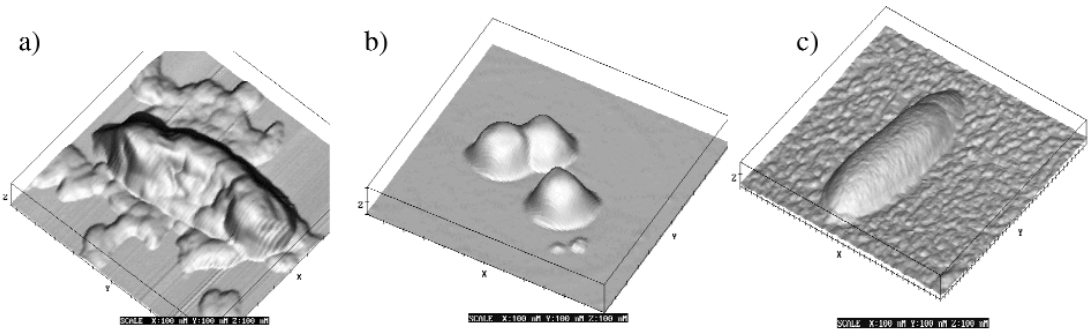


Fig. 2 Three-dimensional atomic force microscope images of *Coxiella burnetti* (a), *Smallpox vaccine virus* (b), and *Yersinia pestis* (c).

Enzymatic methods for the destruction of organophosphorus neurotoxins

The principles of application of enzymes for the degradation of toxins and pathogenic microorganisms are under development. A few gene-engineered constructions containing the gene encoding the synthesis of recombinant organophosphate hydrolase (OPH) have been constructed. Their transformation into various strains of *E. coli* host-cells increased the expression of OPH in the cells more than 10-fold compared to the best OPH producers known. The influence of different effectors on the OPH properties was investigated, and the role of amines as OPH activators was demonstrated [29].

The method of remarkable stabilization of OPH based on formation of enzyme-polyelectrolyte complex was elaborated [30]. Likewise, the possibility of OPH stabilization by additional intermolecular interactions was revealed [31]. Chemical immobilization of stabilized OPH on porous polymer carrier and on various samples of textile materials provided the obtainment of highly efficient and stable biocatalysts including the promising means for individual protection, degrading organophosphorus compounds.

REFERENCES

1. J. Wang, Y. Lin, A. V. Eremenko, I. N. Kurochkin, M. F. Mineyeva. *Anal. Chem.* **65**, 513–516 (1993).
2. J. Wang, E. Dempsey, A. Eremenko, M. Smyth. *Anal. Chim. Acta* **279**, 203–208 (1993).
3. J. Wang, Y. Lin, A. V. Eremenko, A. L. Gindilis, I. N. Kurochkin. *Anal. Lett*. **26**, 197–207 (1993).
4. A. Makower, A. V. Eremenko, K. Streffer, U. Wollenberger, F. W. Scheller. *J. Chem. Technol. Biotechnol*. **65**, 39–44 (1996).
5. F. F. Bier, E. Ehrentreich-Foerster, F. W. Scheller, A. Makower, A. Eremenko, U. Wollenberger, Ch. G. Bauer, D. Pfeiffer, N. Michael. *Sens. Actuators*, *B* **33**, 5–12 (1996).
6. Ch. G. Bauer, A. V. Eremenko, E. Ehrentreich-Foerster, F. F. Bier, A. Makower, H. B. Halsall, W. R. Heineman, F. W. Scheller. *Anal. Chem*. **68**, 2453–2458 (1996).
7. A. V. Eremenko, A. Makower, C. G. Bauer, I. N. Kurochkin, F. W. Scheller. *Electroanalysis* **9**, 1–5 (1997).
8. A. Makower, A. Barmin, F. Scheller. *Biospektrum* **6**, 119–121 (2000).
9. F. W. Scheller, A. Makower, F. Bier, U. Wollenberger, A. Ghindilis, A. Eremenko, D. Pfeiffer. *GIT Fachz. Lab*. **6**, 560–561 (1995).
10. A. Makover, A. Barmin, T. Morzunova, A. Eremenko, I. Kurochkin, F. Scheller. *Anal. Chim. Acta* **357**, 13–20 (1997).
11. F. F. Bier, E. Ehrentreich-Foerster, R. Doelling, A. V. Eremenko, F. W. Scheller. *Anal. Chim. Acta* **344**, 119–124 (1997).
12. A. V. Eremenko, C. G. Bauer, A. Makower, B. Kanne, H. Baumgarten, F. W. Scheller. *Anal. Chim. Acta* **358**, 5–13 (1998).
13. C. G. Bauer, A. V. Eremenko, A. Kuehn, K. Kuerzinger, A. Makower, F. W. Scheller. *Anal. Chem.* **70**, 4624–4630 (1998).
14. I. N. Kurochkin. *Adv. Biosensors* **3**, 77–109 (1995).
15. I. Budashov, I. Kurochkin, A. Denisov, V. Skripnyuk, G. Shabanov. *J. Microbiol. Epidemiol.* (Russ.) **6**, 11–15 (1997).
16. I. Kurochkin, I. Budashov, A. Pavelev, A. Denisov, V. Skripnyuk, G. Shabanov. *Sensornie Sistemi* (Russ.) **12**, 122–134 (1998).
17. A. Pavelev, I. Kurochkin, S. Chernov. *Biol. Membr.* (Russ.) **15**, 342–348 (1998).
18. I. A. Budashov, I. N. Kurochkin, V. V. Tsibezov, S. L. Kalnov, A. K. Denisov, O. I. Kiselyova, I. V. Yaminsky. *Biol. Membr.* (Russ.) **16**, 325–335 (1999).
19. I. A. Budashov, I. N. Kurochkin, V. V. Tsibezov, S. L. Kalnov, A. K. Denisov, O. I. Kiselyova, I. V. Yaminsky. *Membr. Cell Biol*. **13**, 397–409 (2000).

20. Yu. G. Zhukovskii, S. A. Kutsenko, L. P. Kuznetsova, E. E. Sochilina, E. N. Dmitrieva, M. L. Fartseiger, V. D. Tonkopii, N. N. Korkishko, V. I. Kozlovskaya, V. E. Fel'd. *J. Evol. Biochem. Physiol.* (Russ.) **30**, 177–184 (1994).
21. E. N. Efremenko and V. S. Sergeeva. *Russ. Chem. Bull, Int. Ed.* **50**, 1826–1832 (2001).
22. E. I. Rainina, E. N. Efremenco, S. D. Varfolomeyev, A. L. Simonian, J. R. Wild. *Biosens. Bioelectron.* **11**, 991–1000 (1996).
23. A. L. Simonian, E. N. Efremenko, J. R. Wild. *Anal. Chem. Acta* **444**, 179–186 (2001).
24. A. L. Ghindilis and I. N. Kurochkin. *Biosens. Bioelectron.* **9**, 353–357 (1994).
25. A. L. Ghindilis, T. G. Morzunova, A. V. Barmin, I. N. Kurochkin. *Biosens. Bioelectron.* **11**, 873–880 (1996).
26. S. D. Varfolomeyev, A. Y. Yaropolov, I. N. Kurochkin. *Biosens. Bioelectron.* **11**, 863–871 (1996).
27. L. V. Sigolaeva, A. V. Eremenko, A. Makower, G. F. Makhaeva, V. V. Malygin, I. N. Kurochkin. *Chem.-Biol. Interact.* **119–120**, 559–565 (1999).
28. L. V. Sigolaeva, A. Makower, A. V. Eremenko, G. F. Makhaeva, V. V. Malygin, I. N. Kurochkin, F. W. Scheller. *Anal. Biochem.* **290**, 1–9 (2001).
29. V. S. Sergeeva, E. N. Efremenko, G. M. Kazankov, S. D. Varfolomeyev. *J. Molec. Catalysis B: Enzymatic* **10**, 571–576 (2000).
30. V. S. Sergeeva, E. N. Efremenko, G. M. Kazankov, S. D. Varfolomeyev. *Biotechnol. Tech.* **13**, 479–483 (1999).
31. E. N. Efremenko, V. I. Lozinsky, V. S. Sergeeva, F. M. Plieva, T. A. Makhlis, G. M. Kazankov, A. K. Gladilin, S. D. Varfolomeyev. *J. Biochem. Biophys. Methods* **51**, 195–201 (2002).