Biochemical strategies for the detection and detoxification of toxic chemicals in the environment

Ferdinando Febbraio

Ferdinando Febbraio, Institute of Protein Biochemistry, National Research Council, 80131 Naples, Italy

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Correspondence to: Ferdinando Febbraio, PhD, Researcher, Institute of Protein Biochemistry, National Research Council, via P. Castellino 111, 80131 Naples, Italy. f.febbraio@ibp.cnr.it
Telephone: +39-081-6132611
Fax: +39-081-6132611

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Abstract

Addressing the problems related to the widespread presence of an increasing number of chemicals released into the environment by human activities represents one of the most important challenges of this century. In the last few years, to replace the high cost, in terms of time and money, of conventional technologies, the scientific community has directed considerable research towards the development both of new detection systems for the measurement of the contamination levels of chemicals in people’s body fluids and tissue, as well as in the environment, and of new remediation strategies for the removal of such chemicals from the environment, as a means of the prevention of human diseases. New emerging biosensors for the analysis of environmental chemicals have been proposed, including VHH antibodies, that combine the antibody performance with the affinity for small molecules, genetically engineered microorganisms, aptamers and new highly stable enzymes. However, the advances in the field of chemicals monitoring are still far from producing a continuous real-time and on-line system for their detection. Better results have been obtained in the development of strategies which use organisms (microorganisms, plants and animals) or metabolic pathway-based approaches (single enzymes or more complex enzymatic solutions) for the fixation, degradation and detoxification of chemicals in the environment. Systems for enzymatic detoxification and degradation of toxic agents in wastewater from chemical and manufacturing industries, such as ligninolytic enzymes for the treatment of wastewater from the textile industry, have been proposed. Considering the high value of these research studies, in terms of the protection of human health and of the ecosystem, science must play a major role in guiding policy changes in this field.

Key words: Biosensors; Biomonitoring; Bioremediation; Toxic compounds; Chemicals pollution; Human health; Environmental pollutants

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Core tip: The increasing focus on the presence of hazardous chemicals in the environment is directing scientific research towards the development of new and eco-sustainable strategies for their control. Such
Advances in technology are enabling scientists to improve the detection limits of these substances, in the environment, in food, and the human body, as well as to develop new strategies for their removal from their surroundings. However, further research is required to achieve the goal of a continuous monitoring of the environment and of providing, in real time, information on its current state.

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INTRODUCTION

Addressing the issue of chemicals in the environment is not easy. The world’s chemical production increased 400 fold in the last century, leading to the introduction of an increasing number of toxic substances into the environment, all directly related to technological progress and improvements in agricultural and industrial methods. The 2014 inventory of the Environmental Protection Agency includes approximately 85000 different chemicals available for use in just the United States[1], and only a small subset of these chemicals has been sufficiently characterized in order to draw conclusions about their toxicity[2,3]. While some chemicals may be harmless, others can be very toxic for human health. In particular, special attention has been focused on chemicals that persist in the environment and can accumulate in the human body, interfering with hormones (endocrine disruptors), causing cancer or damaging DNA.

In 2009, the “Fourth National Report on Human Exposure to Environmental Chemicals” of the Department of Health and Human Services (Centers for Disease Control and Prevention - National Center for Environmental Health - United States)[4], aimed at bio-monitoring the United States population by measuring the chemicals in people’s urine and blood, revealed for the first time the presence of 75 new toxic chemicals in the United States population. The update of this report in August 2014 (Updated Tables, July 2014)[5] presents data relating to 35 additional chemicals.

The priority list of hazardous substances (https://www.atsdr.cdc.gov/spl/) includes more than 700 compounds, belonging to groups such as Metals and Metalloids, Disinfection By-Products, Environmental Phenols, Pthalates, Polycyclic Aromatic Hydrocarbons, Volatile Organic Compounds, Fungicides, Herbicides and Pesticides (Carbamates, Organochlorines and Organophosphates).

IMPACT ON HUMAN HEALTH OF LOW LEVEL EXPOSURE TO TOXIC CHEMICALS

The toxic effects of several chemicals on human health and on the environment (animals and plants) are well known. For instance, phthalates, causing epigenetic alterations, play an important role in adverse oxidative stress and pro-inflammatory responses[16,20], promote cancer and other pulmonary and cardiovascular diseases[21], the habit remains unacceptably prevalent in both developed and developing countries.

In relation to tobacco smoking, in order to avoid the...
introduction of harmful chemicals other than those that are really necessary to maintain the current technological status, we should simply reduce, or even better stop, the consumption of cigarettes.

In conclusion, we must agree with Vassallo et al[22] in their opinion that cigarette smoking is a leading cause of preventable mortality in the world.

THE CASE OF PESTICIDES

Currently, the most dangerous chemical pollution is represented by pesticides. The fact that five of the nine new persistent organic pollutants (POPs)[23-25], which bioaccumulate in humans and animals, are pesticides (https://www.environment.gov.au/protection/chemicals-management/pops/new-pops), highlights the importance that must be attached to the presence of these substances in the environment. Differently from other harmful chemicals, introduced into the environment by natural processes, or produced as by-products of industrial processes and other human activities, pesticides are designed to be toxic and are used as poisons to kill organisms. They are extensively applied over large areas in agriculture and in urban settings, becoming the most diffuse form of chemical pollution in the world, and one which is also very difficult to control. In the last 20 years the number of publications relating to pesticides has doubled to up to one thousand per year, and the majority of these articles concern their negative effects on human health, and focus, in particular, on neurological diseases in children[26-31]. Recently, Science, a high impact factor journal, has addressed the problems relating to pesticides in a special issue entitled “Smart pest control”[32]. The articles in this issue focus on many topics including the increasing use of pesticides in Asia, South America and Europe, their widespread diffusion and transformation in the environment, their involvement with neurodegenerative diseases and cognitive deficits in developing age, new strategies to reduce their negative impacts, the development of new synthetic chemicals with less collateral damage, and above all the major role that science could play in guiding policy changes in this field.

STATE OF THE ART IN CHEMICAL DETECTION AND DEGRADATION

The detection and monitoring of toxic compounds has improved significantly in the last few years. Technological advances, such as Surface-Activated Chemical Ionization[33-34] which increases the sensitivity and accuracy of GC-LC-MS measurements, in association with methodologies, such as Quick Easy Cheap Effective Rugged and Safe[35], allow the identification of hundreds of these substances in a single shot. However, their detection in the environment still remains difficult, because toxic chemicals are so widely distributed in the world and can be found in locations as diverse as pristine forests and the blood of arctic animals, wind, rain and other weather phenomena contributing to their diffusion.

The use of biomarkers in combination with sampling from water and soil will facilitate the tracking of the amount of chemicals in the environment[36-38]. However, any analysis using the technology currently available, although it is very powerful, generally requires several weeks to complete. In fact, it is necessary to collect many samples in order to have an accurate representation of an environment (randomly from air; soil, water; foods and human and animal fluids or tissues, such as the blood and urine). These samples must be transferred and stored for the necessary time for their analysis in authorized centers, without considering the possibility that very often an extraction and/or derivatization of the samples is also required. All this infrastructure has too high a cost in terms of money and time to allow a continuous monitoring of the environment or of the population.

In agreement with the United States Environmental Protection Agency[39], in vitro screening using biological pathway-based approaches has become central to 21st century toxicity testing. Furthermore, I would suggest that the establishment of a capillary network of environmental sensors for the on-line and real-time monitoring of toxic chemicals and the development of new eco-sustainable methodologies for the removal of these toxic compounds from the environment should now be considered among the most important challenges for the future.

In fact, the current chemical remediation methodologies are unsuitable because most of them use chemical or physical approaches which either simply shift the problem into the future (such as the storage of nerve agents in military bases, or the sequestration and storage of obsolete pesticides in inadequate facilities, particularly in third world countries) or introduce other harmful compounds into the environment, such as through the incineration of pesticides or the inactivation of reactive groups of chemicals using strong alkaline (sodium hydroxide) or acidic solutions[40-43]. Moreover, due to the wide heterogeneity of chemical pollution, none of the strategies currently employed is totally satisfactory. Therefore, the development of new environmentally-friendly strategies to support the current methodologies has become crucial.

BIOCHEMICAL STRATEGIES TO DETECT CHEMICALS

The research into new technologies to replace conventional GC- and LC-MS methodologies for the environmental detection of chemicals has been intensifying in recent years. In particular, the development of sensors and biosensors for the precise detection and estimation of hazardous chemicals in different samples (water, human fluids and tissue, etc.) has been gaining momentum.

The advantages of biosensors compared to the current technological approaches could be summarized in a few words: Easiness, cheapness, and speediness.

Biosensors are characterized by a “bioreceptor”,...
which is the biological part recognizing the substance, a "transducer", that transforms the biological interaction into a measurable signal, and a "reader/recorder", that displays the results. Sometimes an "amplifier" can be used to amplify the signal improving the sensitivity of the biosensor. Biological scientists can play an important role in the development of biosensors, in that biosensors for toxic chemicals can be based on animals, microorganisms, antibodies, enzymes, and nucleic acids. The limit of detection (LOD) of a number of these biosensors is comparable with, if not greater than, standard technologies. Further details on the characteristics of the most recent biosensors and on the advances in the development of these technologies can be found in several recent articles. We can distinguish between two different applications of these biosensors, namely for diagnostic use in clinical settings and for the environmental monitoring of chemicals. The recent development of diagnostic biosensors permits us to hypothesize about their possible use in the near future for the measurement of chemicals in routine clinical analysis.

New emerging biosensors for the analysis of environmental chemicals have been proposed in order to offer a simple alternative means of assessment approach, such as VHH antibodies (the antigen binding fragment of heavy chain antibodies) that combine the comparable performance of conventional antibodies with the affinity for small molecules, or genetically engineered microbial whole-cells, that respond to target chemicals and produce detectable output signals. However, these advances in the field of environmental chemical monitoring are still far from producing a continuous real-time and on-line system for their detection.

In conclusion, it is necessary to highlight the fact that the use of biosensors is crucial not only for the detection of the presence of chemicals, but also for the detoxification monitoring which should always be used to evaluate the efficiency of a treatment technique.

DETECTION OF NEUROTOXIC CHEMICALS

Excluding neurotoxic poisons produced by certain fish, insects and reptiles (such as Bungarotoxin, Chloretoxin, Conotoxin, and Tetrodotoxin), or by certain plants, algae and bacteria (such as Anatoxin-a, Tetanus and the Botulinum toxins), as well as some metals, such as lead and mercury, that can affect the activities of the nervous system, the most diffuse synthetic chemicals are enzymes belonging to the carboxylesterase family, involved in the regulation of nerve signal transmission at the chemical synapses, by hydrolyzing acethylcholine and other choline ester neurotransmitters. The inactivation of this enzyme causes paralysis and even death. As a result, indeed, is the specific target of the above mentioned toxic chemicals, this family of enzymes remains the one most extensively studied for use as bioreceptors in the development of biosensors for neurotoxic chemicals. The principles of acethylcholinesterase biosensors are based on the measurement of the residual activity of the enzyme using different substrates, such as acethylcholine and thiocholine, that can be monitored using potentiometric, amperometric and optical devices. The LODs that can be obtained using acethylcholinesterase activities, in particular from insects, for the detection of these neurotoxic compounds, remain among the highest obtainable.

Recently, new alternative bioreceptors have been proposed, including new enzymes, microorganisms, antibodies, and aptamers, which represent a possible alternative to overcome the limitations involved in the use of acethylcholinesterases.

BIOREMEDICATION OF CHEMICALS

Exploiting the bioremediation lessons that the ecosystem teaches us, we advance to the development of strategies which use organisms (microorganisms, plants and animals) or metabolic pathway-based approaches (single enzymes or more complex enzymatic solutions) for the fixation, degradation and detoxification of chemicals in the environment.

In particular, microbial remediation strategies for soil ecology have attracted increasing interest since they are environmentally friendly and cost-effective. Several examples of such strategies can be found in literature, but none are totally satisfactory. A limitation in the use of strains isolated from the environment or genetically engineered in the laboratory, for the treatment of soil contaminated with chemicals, is related to their reduced growth capacity when sharing the environment with other microorganisms that use better substrates. Indeed, differently from results obtained in the laboratory where these microorganisms grow in optimized conditions and in the absence of competitors, the yields in chemical remediation are weak in operative conditions. A valid alternative is represented by the use of plants, but the introduction of new species into the environment requires considerable time before any significant results are obtained.

A different approach that overcomes the limitation consisting in the length of time required for decontamination using organisms could be the use of enzymes, which can bind with a high affinity the chemical compounds and catalyze their hydrolysis in hours if not minutes. Unfortunately, the use of enzymes in soil remediation is often handicapped by the large amount of enzyme required, by the absence of a sufficient quantity of water, generally needed for the solubilization and the hydrolysis...
of substrates, by the low stability of enzymes outside cells, and by the presence of microorganisms, that inactivate them. For these reasons the bioremediation of ground represents one of the most important challenges of our century, but, unfortunately, we are still far from achieving important results.

A different situation applies in relation to the treatment of contaminated water, since it is possible to extract a large quantity of water from watercourses or lakes, transferring it into bioreactors containing enzymes and reducing the number of contaminants in a short time. The use of very stable enzymes, genetically engineered to be resistant to proteases or to the presence of organic solvents and detergents, and produced in a large quantity at a low cost by over-expression in an appropriate host, could be the right answer to the problem. A formulation of different enzymatic activities or a number of bioreactors containing different enzymes and connected in a series could treat several chemicals at the same time. In particular, as proposed for the detoxification by liginolytic enzymes of wastewater from the textile industry with a yield from 69% up to 87%[71], the enzymatic detoxification and degradation of toxic agents in wastewater from the chemical industry could possibly be used to reduce drastically the quantity of chemicals released into the environment.

**BIOREMEDIATION OF NEUROTOXIC COMPOUNDS**

For the bioremediation of neurotoxic compounds, such as organophosphates, other and efficient enzymes have recently been studied. Differently from the activities of acetylcholinesterase that are inhibited by neurotoxins, some enzymes belonging to the Aryldialkylphosphatase family, such as the *Pseudomonas diminuta* phosphotriesterase[72] and *Sulfolobus solfataricus* paraoxonase[73,74], are able to hydrolyze these chemicals. Considering the importance of this field, in particular in relation to the possible use of enzymes in the decontamination of nerve agents in military actions, all the enzymes are under patent, and some have already been commercialized individually or in formulation. Despite the great potential of these enzymes, several limitations still remain. Indeed, mesophilic enzymes, that show a high activity towards several nerve agents, have at the same time a very low stability and lose their activity in minutes in the environment. Differently, alternative enzymes isolated from thermophilic organisms, showing a high stability not only towards temperature but also towards organic solvents, detergents and proteases, present a lower catalytic performance, if compared to the mesophilic ones[75].

Although the production of nerve agents should be discontinued, large amounts already produced during the cold war are still stored and require degradation into less dangerous chemicals. Moreover, some of these enzymes are also active on a number of pesticides, and therefore developments in this field are undoubtedly much needed.

**CONCLUSION**

Human activity has resulted in severe environmental pollution, which has now emerged as a major global issue. However, it is a utopian idea to hope for a world without any chemical pollutants, as technological and scientific progress requires an extensive use of chemicals. Therefore, the requirement is not to reduce industrial activities, but to realize that we must optimize current technologies in order to reduce the quantity of contaminants released. An example could be the amount of pesticides used on crops, which could be significantly diminished simply by monitoring the quantity still present in the field, so avoiding an excessive use. On the other hand, we need to develop new eco-sustainable processes of production in order to avoid a massive introduction of harmful chemicals into the environment. Meanwhile, the use of metabolic pathways present in or extracted from microorganisms and plants could be the right approach for future strategies of bioremediation. To achieve this goal, it will be necessary to strengthen both national and international engagement, enabling scientists from different fields to collaborate in the development of innovative strategies to address the problems related to toxic chemicals.

To put it simply, we should arguably have had an antidote before introducing a poison into the environment. That antidote may well be enzymes, which catalyze almost all of the reactions on the Earth.

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