World Journal of Engineering Research and Technology
WJERT
www.wjert.org
SJIF Impact Factor: 5.218

CONTROL OF THE GENOTOXICITY LEVEL OF CERTAIN MYCOTOXINS BY A FIBER OPTICAL SOS-TYPE BIOSENSOR

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Article Received on 24/09/2018 Article Revised on 14/10/2018 Article Accepted on 04/11/2018

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ABSTRACT

In addition to their well-known general toxicity, mycotoxins have also been found to often exert genotoxicity. In turn, not only the presence of the mycotoxins is required to be determined, but such DNA-damaging effects by these substances are also needed to be detected in routine monitoring of mycotoxin. The main purpose of this article is to demonstrate the utility of a proposed special fiber optical SOS-chromotest type biosensor, based on the SOS-lux assay in recombinant *Escherichia coli* cells, in rapid measurement of the genotoxicity level of mycotoxins. The optrode-based fiber optic biosensor and its main operating conditions for such express analysis have been worked out, moreover, its efficacy in the determination of the effects of certain investigated mycotoxins e.g.,T2 mycotoxin, patulin, aflatoxin B2, zearalenone and ochratoxin on the genetic apparatus applied and their activation of the operon of the reparative genes is reported. The genotoxicity of the mycotoxins studied fell in the order: T2 mycotoxin > aflatoxin B2 > patulin >> ochratoxin > zearalenone.

KEYWORDS: Fiber optic-SOS biosensor, mycotoxin, genotoxicity, determination.

INTRODUCTION

Mycotoxins are a group of low molecular weight, non-immunogenic compounds, many of which are characterized by relative thermal stability. Currently known mycotoxins include more than 400 low molecular weight compounds produced by about 200 species of

micromycetes, but this number is likely to grow as further research identifies new toxin producer organisms.[1-4] Different genuses of micromycetes are capable of producing a wide range of mycotoxin substances including, among others, aflatoxins, rubra- and ochratoxins, fumonisins and trichothecenes. Currently, mycotoxins receive special attention due to numerous reasons, namely, their wide distribution (in the desert, solonchaks and highlands, although they are most typical for temperate latitudes), high toxicity for living organisms (embryotoxicity, teratogenicity, mutagenicity, carcinogenicity), and recently there emerged a danger of their use by bioterrorists (since such a representative of them as T2 exerts several orders of magnitude higher damaging effects than mustard gas or lewisite).[2,5,6] Different species of mycotoxins specifically affect organs and tissues: liver, kidneys, esophagus mucosa and intestines, as well as the brain and tissues of the genital organs. Obviously, therefore, mycotoxins are included in the list of substances subject to regulation of their content in food, feed and raw materials. A fairly large amount of information is available in the scientific literature about the environmental distribution of mycotoxins, yet data are limited, first of all, due to the lack of sufficiently simple and reliable methods for detecting this kind of toxins. The development of such methods lies at the heart of one of the most important directions in preventing undesirable environmental influences on human health. The use of highly sensitive, simple and reliable methods for the analysis of mycotoxins allows monitoring their level at all stages of food production and processing, from cultivation and harvesting to the food production chain and the final product that enters the human body. Today, strictly regulated permissible concentrations of mycotoxins in animal feed and human food exist in Europe, the United States and in Ukraine. In the European Union maximum level of 0.2 mg/kg has been set for the sum of T-2 and HT-2 toxins as a maximum level (ML) permitted in cereals.[3] Correspondingly, in accordance with the State Standard of Ukraine (DSTU 3768-98), the maximum allowable level of T-2 toxin content in wheat for food, technical purposes, exports and forage is set at 0.1 and 0.2 mg/kg of grain, respectively.[4]

Taking the general toxicity of mycotoxins to living organisms into consideration, various methods have been proposed for their screening in different environmental samples and for verification of previous results,[6-8] among which different approaches have been applied for assessing the effect on living organisms. Mycotoxins may exert general toxicity at corresponding concentrations (at levels that as a rule result in high and/or long exposures), endocrine disruption or genotoxicity even at low concentrations. DNA damage caused by xenobiotics can be detected by various bioassays on the basis of nucleotide recombination or

the detection of induced fluorescent or chemiluminescent signals.[5] Earlier we have developed rapid recombinant bacterial biosensors for detection of genotoxicity of various chemical substances,[9-11] and analyzed their efficiency in genotoxicity screening. In the present report we present detected levels of genotoxicity of selected mycotoxins to demonstrate their activity in affecting genome mutation processes.

MATERIAL AND METHODS

Five mycotoxins representing a wide range of chemical moieties, namely T2 mycotoxin (T2) , zearalenone (Zon), ochratoxin (Ochra), patulin (Pat) and aflatoxin B2 (AfB2) were selected as target substances. On the basis of our sample preparation optimization for mycotoxin analysis by an immunobiosensor approach reported previously.[12] acetonitrile as an extraction solvent was used in the current study. The method of detection was fiber optical SOS-biosensing with the application of recombinant C600 (pPLS-1) *Escherichia coli* cells on a cellophane membrane for the contact of the analytical unit with the transducer surface.[10] All chemical reagents were obtained from Sigma-Aldrich (USA). The fiber optics biosensor on the basis of *E. coli* cells combining the SOS system, indicative of DNA-damaging agents, as a receptor component and a bioluminescence (lux) system as a rapid reporter technique was constructed as described earlier.[4] This device works in a differential regime, which allows registering comparative levels of chemiluminescence between the presence of the analyzed substance in the measuring cell and in its physiological solution (control sample).[10,11]

RESULTS AND DISCUSSION

Initially, acetonitrile concentrations for mycotoxins extraction had to be established to prevent any solvent effect occurring on sample analysis. According to our preliminarily results,[12] acetonitrile did not effect on the level of the antigen-antibody reaction on certain mycotoxin immunosensors even at the concentration about 40%. In contrast, other organic solvents (e.g., methanol) occasionally affected the detected genotoxicity index at a concentration of 3.0% and above. The genotoxicity level of acetonitrile at concentrations from 0.1% to 1.0% detected in the current experiments indicated mild effects (Fig.1).

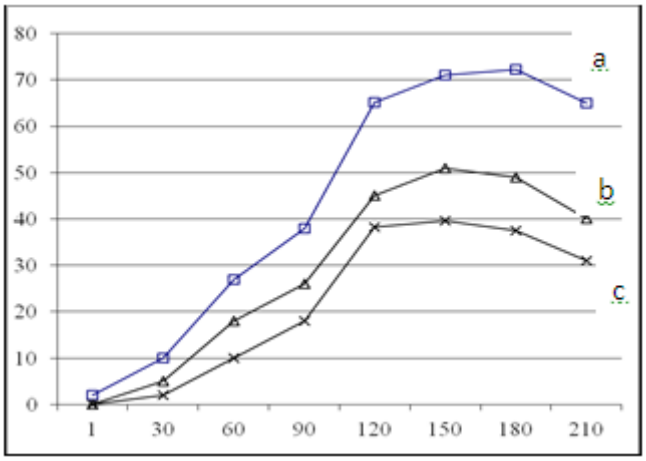

Figure 1: Dynamics of changes of the chemiluminescence level of the SOS-lux biosensor after the addition of acetonitrile at concentrations: 1% (a) 0.2% (b) and 0.1% (c) to the measuring cell. Ordinate – relative units of the chemiluminescence level in % and Abscise – time of measurement in min.

The specific signal increased with increasing concentrations of acetonitrile in the samples analyzed. Not only the intensity of fluorescence of the referent cells was observed as the result of the inclusion of their operon with the complex of the reparative genes and activation of their intensity of work, but this process was also accompanied by a shift of the fluorescence maximum in a short period of time, as simultaneously with the reparative genes, expression of the fluorescent protein gene introduced in the above mentioned operon was activated.

Using acetonitrile concentration chosen to be 0.2%, mycotoxin solutions were prepared. First the appropriate mycotoxins were dissolved in 20.0% of acetonitrile at concentrations of 1.0 and 2.0 µg/ml. These stock solutions were then diluted 1:100 times by the physiological solution and were used for assessing genotoxicity levels.

It was revealed that all used mycotoxins have demonstrated increasing levels of the fluorescence in the test system (Figs. 2 and 3), with the highest effect observed for T2, AfB2 and Pat. Genotoxicity levels were observed from the extent of peak chemiluminescence reached in the SOS-lux biosensoric analysis (absolute chemiluminescence values). However, when relative chemiluminescence levels were compared, it has been clearly indicated that all mycotoxins showed similar kinetics, moreover, peak chemiluminescence is reached more rapidly in the analysis with increasing mycotoxin concentrations in the test solutions. Thus,

peak chemiluminescence was reached in 180 and 120 min by all mycotoxins when applied at 10 and 50 ng/ml concentrations, respectively (Figs. 2 and 3).

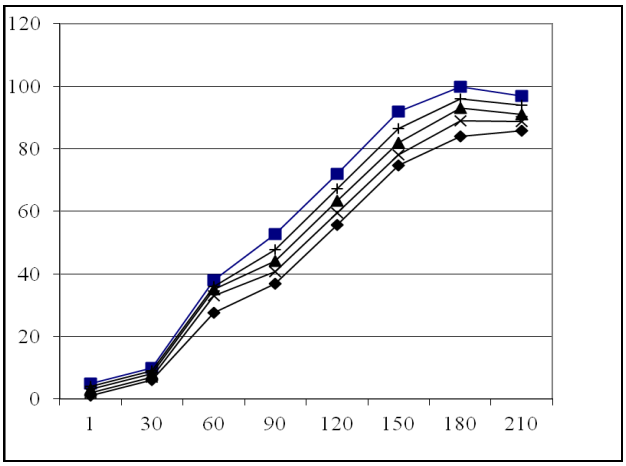

Figure 2: Dynamics of changes of the chemiluminescence level of the SOS-lux biosensor after the addition of mycotoxins (T2, AfB2, Pat, Ochra and Zon from above to below) at a concentration of 10ng/ml to the measuring cell. Ordinate – relative units of the chemiluminescence level in % and Abscise – time of measurement in min.

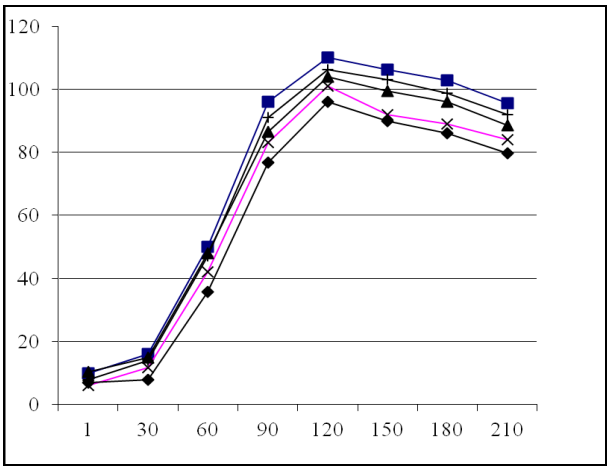

Figure 3: Dynamics of changes of the chemiluminescence level of the SOS-lux biosensor after the addition of mycotoxins (T2, AfB2, Pat, Ochra and Zon from above to below) at a concentration of 50ng/ml to the measuring cell. Ordinate – relative units of the chemiluminescence level in % and Abscise – time of measurement in min.

The difference in the genotoxic activity of individual mycotoxins may be related to a number of factors. First and foremost of all is the efficacy of these compounds to exert effects on the genome of cells including their reparative system. Additional factors may, however, also influence the genotoxicity level of the corresponding mycotoxin detected e.g., the organic

solvent used influences the apparent solubility of the given mycotoxin, and increases its bioavailability and ability to penetrate into reference cells of the SOS-lux biosensor, as seen in the similar kinetics for given concentrations for all mycotoxins studied. Molecular genotoxicity and physico-chemical availability to the active sites, therefore, act in concert.

CONCLUSION

Results seen in the current experiments allow several very important conclusions. First of all, it was demonstrated again that the quite simple experimental approach reported allows rapid and easy determination of the genotoxic effects exerted by chemical substances including mycotoxins. Secondly, the genotoxic activity of the five mycotoxins studied was analyzed at 10 and 50 ng/ml concentrations and compared to each other, revealing that T2, AfB2 and Pat exerted the strongest genotoxicity in these series, while Ochra and Zon were found to show lower genotoxic activity. Thirdly, solubility affected by the presence of an organic solvent (acetonitrile), in addition to its own mild genotoxic activity, occurred to exert a secondary effect on the genotoxicity level of these mycotoxins observed, possibly by affecting their bioavailability.

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