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Formation of the Surface Programmed Chemical Sites and Their Selectivity to Some Mycotoxins

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Abstract

It was used approach, early proposed by us, for the creation of electrochemical sensor with the potentiometric registration of signal at the detection of such mycotoxins as T2 and aflatoxin B1. The selective sites of this sensor were formed on the glass surface by the chemical way with the realization of so called template structures when the geometric space of the preliminarly sorbed analyte was limited by trimetylchlorsilane (3MeSiCl). This approach was used too in case of the formation of selective sites on the gold transducer surface of optical sensor based on the principle of surface plasmon resonance (SPR). In this case more effective is the preliminary treatment of the transducer by of the polyallylamine hydrochloride (PAA). For both types of sensors it was demonstrated the sensitivity of analysis and cross reactivity to others mycotoxins including searelenone and patulin. Taken into attention of these data it was recommend to use of the proposed way for the selective site creation if sensors are intended for preliminary screening of mycotoxins among of environmental objects. It is very effective since the transducer surface of the sensor may be renewed by simple way at its treatment with methanol or acetonitrile. At the repeated use more than 10 times the specific signal changes only within 10%.

Keywords: Chemical programmed; Selective sites; Sensors; Mycotoxins; Determination

Introduction

Today biosensor technology is very wide common since it gives a new and very effective approaches for the diagnostics, control of quality of foods, feeds and state of different environment objects [1,2]. In spite on that this technology provides us by the devices which are very sensitive, able for the express analysis and effective in case of the application in the regime of field conditions there have a number disadvantages and among them the main place belong to the necessity of the application of biological substances as main sensitive and selective elements. As a rule, biological materials are non stable, very expensive and cannot be reused for the number of analysis. That is why, to overcome this problem there is necessary to find ways for replacement biological selective sites on the artificial chemical structures which would be have a needed level of the specificity and allowed to fulfill analysis with the needed level of the sensitivity. In last time there is proposed a number of approaches and among them there is necessary to mention that which are connected with the application of calixarenes [3,4], photopolymerizable membranes [5], so called template technology [6] and others. Early we tried to use the last technology for the determination of the low molecular weight substances at their detection by electrochemical methods. This article is devoted to detailed analysis of the application of this technology to use as perspective approach at the creation of sensor devices for control of some mycotoxins with sensor devices based not only on the electrochemical principle of the selective signal registration and but optical one too, in particular, with the application of the effect of the SPR. There is necessary to mention that mycotoxins are very fairly common substances in environment and very dangers for the living organisms [7,8].

Material and Methods

In the investigations it was used a number of such mycotoins as: T2, patulin, aflatoxin B1 and searelenone from Sigma Aldrich (USA). The signal was registered by the electrochemical way according to our previous data [6] and by the device based on the principle of SPR produced in A.V. Glushkov Institute of the Cybernetics of the National Academy of Sciences of Ukraine [9]. Surfaces of transducers were formed on the glass plate [6] or on the glas with the preliminary deposited layers of chromium and gold. The plates were placed into so called «template»

solution (appropriate mycotoxin solved in acetonitrile at the concentration in 100μg/mL) during at last 6h at the room temperature. Then plates were dried at 120 °C during 3h and at last they were transferred into vacuum conditions in the presence of the frozen 3MeSiCl on 12h at the room temperature. After this procedure plates were washed by methanol and water. Such preliminary prepared plates were installed into the measuring cell in which were introduced the appropriate mycotoxin at the different concentrations started from 10ng/mL and up to 1μg/mL. The plates contained gold layer has hydrophobic properties and this is consistent with the analysis of mycotoxins, which are also generally hydrophobic. Mycotoxins were in acetonitrile solution, which allows for the inclusion of hydrophobic compounds. However, silane compounds, which are factors of limitation of geometric and structural surface, are intermediate in this respect. Reducing the level of hydrophilicity of the surface leads to a limitation of their sorption on it. In the special experiments the transducers surfaces for the SPR were preliminary treated by PAA. Immune biosensor analysis of mycotoxins was carried out as it was described by us early [10]. Specific antibodies to individual mycotoxins were obtained from Sigma-Aldrich (USA).

Results and Discussion

Efficiency of the selectivity of artificial template sites at the analysis by the electrochemical way

Table 1: Level of the cross-reactions of individual mycotoxins when used as selective binding sites of matrix-programmed surface to T2 mycotoxin.

No	Compounds	Level of Response, %
1	Patulin	15-Oct
2	T2	90-95
3	Aflatoxin B1	15-Oct

It was stated that the detection effect of T2 mycotoxin at the application of appropriate template sites was approximately 95% of the pre-determined concentration, in accordance with the calibration curve constructed on the basis of the results of the immune biosensor analysis using the SPR principle. In the case of other types of mycotoxins, this level fluctuated within 10% but not exceeded 15% (Table 1), which indicated a rather high specificity of the created artificial sites on the basis of matrix-programmed structures, since, even immunoassay analysis of these mycotoxins at the using of the polyclonal antibodies was characterized by the level of cross-reactions approaching the above (Table 2). In the same way an analysis of the level of the selectivity of the created artificial sites based on matrix-programmed structures in determining aflatoxin B1 was carried out. The evaluation was based on the quantitative characteristics of the detection of the above-mentioned mycotoxin and a number of such toxins as zearelenoi, mycotoxin T2 and patulin. It was established that the effect of detection of aflatoxin B1 was close to 90% of the preset concentration determined according to the calibration curve constructed on the basis of immune biosensor analysis. In the case of other types of mycotoxins, this level fluctuated within 15%, but did not exceed 20%, which indicated a significant specificity of created and artificial sites based on matrix-programmed structures.

Table 2: Level of cross-reactions of individual mycotoxins when as selective binding sites were used of polyclonal antibodies specific for T2 mycotoxin.

No	Compounds	Level of Cross Reactivity %
1	Patulin	100
2	T2	5
3	Aflatoxin B1	10
4	Searelenone	7
5	Phthalate	0

It should be noted that, in the case of the creation of matrix-programmed structures for aflatoxin B1, their specificity to it was somewhat smaller than in the case of those created for T2 mycotoxin. Moreover, a similar situation is noted in the case of immune biosensor analysis of these mycotoxins using polyclonal antibodies, which was characterized by levels of cross - reactions close to those indicated above. The obtained data indicate the promising use of created artificial sites on the basis of matrix-programmed structures in sensory analytical devices, aimed, first of all, in case of the express screening observations.

Analysis of efficiency of artificial template sites at the analysis mycotoxins by SPR

At first, it was found that the sorption level of silane compounds significantly increases on the surface after its previous modification by polyelectrolytes, namely, PAA (Table 3). The obtained data indicate that the sorption of T2 mycotoxin decreases on the surface of the pretreated PAA, as compared to when it was in the initial state. However, the amount of silane sorbed on the PAA treated surface increases, indicating its possible participation in the formation of matrix structures for T2 mycotoxin.

Table 3: Level of cross-reactions of individual mycotoxins when as selective binding sites were used of polyclonal antibodies specific for T2 mycotoxin.

No	Compounds	Level of Cross Reactivity %
1	Patulin	100
2	T2	5
3	Aflatoxin B1	10
4	Searelenone	7
5	Phthalate	0

On the next stage of research it was estimated the sensitivity of T2 mycotoxin analysis using appropriate artificial template structures, as well as the level of their cross-reactivity to aflatoxin B1. These experiments were carried out with the surfaces of the gold layer pretreated using PAA. The obtained results testified that in this manner we are able to register T2 mycotoxin only at the minimum concentrations above 100ng/mL. The cross reactivity to aflatoxyn B1 reached only 15-25%. It should be noted that the obtained results were similar to that mentioned

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above, namely largely coincides with the ones in which aflatoxin B1 served as the base template structure. Some deviations in this case were observed only in the degrees of deflection of the reflex angles. There is necessary a special underline that the programmed surface structures can be restored for the reuse by washing them with acetonitrile or methanol. It has been determined that reuse may be more than 10 times with a decrease of a specific signal within 10% (Table 4).

Table 4: Changes in the reflection angle of SPR when silane compounds and T2 mycotoxin are introduced before and after processing of the gold layer with PAA.

No	Relatively Changes of the Reflection Angle of	
	SPR at the Sorption of:	
1	T2 mycotoxin	0.65
2	T2 mycotoxin and silane	‘ 0.70
3	PAA, T2 mycotoxin	0.49
4	PAA, T2 mycotoin and silane	0.80

Conclusion

Ways of forming matrix-programmed structures for the determination of such mycotoxins, such as T2 and aflatoxin B1, have been worked out. It has been established that the registration of the formation of a specific complex can be carried out potentiometrically and optically on the basis of the SPR. It was found that in the case of the registration of a specific complex formation by SPR the transducer surface must be pre-treated with PAA. The elaborate way of forming selective matrix-programmed surfaces can be recommended for the sensors aimed at screening mycotoxins among environmental. The programmed surface structures can be restored for re-use by washing them with acetonitrile or methanol. When repeated use more than 10 times the specific signal changes only within 10%.

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