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WAYS OF FORMATION OF ARTIFICIAL SELECTIVE SITES FOR INSTRUMENTAL ANALYTICAL DEVICES INTENDED FOR FOOD QUALITY CONTROL

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. 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 it has 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, photopolymerizable membranes, so called template technology and others [1-3]. Early we tried to use the last technology for the determination of the low molecular weight substances at their detection by electrochemical methods.

In this report it will be given detailed analysis of the application of three types of technologies (based on the template principle, calixarenes and aptamers) to use as perspective approach at the creation of sensor devices for control of some mycotoxins with the selective signal registration by the optical way with the help of surface plasmon resonance (SPR). There is necessary to mention that mycotoxins are very fairly common substances in environment and very dangers for the living organisms. As analytes it was taken T2 mycotoxin, aflatoxin B1 searelenone and patulin.

It was shown that at the formation of template selective sites the SPR transducer surface should be treated by the polyallylamine hydrochloride (PAA). The obtained results testified that in this manner we are able to register T2 mycotoxin at the concentrations above 100 ng/mL. The cross reactivity to aflatoxyn B1 reached only 15-25%. It should be noted that the obtained results were similar to that, 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 too that the programmed surface structures can be restored for the re-use 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%.

Number of calix[4]rezortsynarenes with the different structures: R(H)-CH₃, R(H)-C₃H₇, R (H)C₇H₁₅, R(H)-C₁₁H₂₃ and R(OH)-C₁₁H₂₃ were selected by the computer modeling for the investigation of their absorption properties in respect of such mycotoxins as patulin, T2, aflatoxin B1 and zearelenon. It was found that all used calix[4]rezortsynarenes interact with the mentioned mycotoxins but the structure type R(H)C₁₁H₂₃ was characterized by the highest level of sorption activity. Based on the level of the concentration of the tested mycotoxins and on the determined association coefficient in the reaction of certain mycotoxins ith the calix[4]rezortsynarenes it should be considered that the level of specificity

or too low, or nonexistent. Application of the calix[4]resortsynarenes as selective sites can be recommended only for general screening the presence of mycotoxins in the environment.

For the experiments with the aptamers it was selected the structures based on the SELEX method, namely: oligonucleotide structures: (A) 5'-GATCGGGTGTGGGTGGCGTAAAG-GGAGCATCGGACA-3' and (B) 5'-ACTGCTAGAGATTTTsCAAT-3', which turned out to be most suitable for selectivity to ochratoxin A and aflatoxin M1, respectively. Immobilization of these aptamers was carried out on the original metal (gold) surface of the transducer and pre-treated with polyamines such as PAA and polyallylseryl sulfate. When direct immobilization of aptamer on the transducer surface was used as their usual forms, and modified by thiol groups. It turned out that the response of the SPR of the sensor significantly depended on the realized method of immobilization of the above aptamer on the transducer surface. Response was most prominent in the case of thiolated aptamer when their thiol group was covalently fixed by gold ions. At the same time, the smallest response of the SPR sensor was observed when sorption of the polynucleotide structures of the aptamer on the golden surface of the transducer, due to the possibility of blocking the metal surface of its active centres. When using pre-preparation of the transducer surface of polyamines, the registered response was only slightly lower, especially when using polyallyl steril sulfate, indicating on the level of hydrophilicity of the aptamer structures and their charge characteristics. It turned out that their selectivity to the specified above mycotoxin is within 80% of the calculated level of the used concentration of analyte. The magnitude of the cross-reaction between these types of aptamer to ochratoxin A and aflatoxin M1 is within 35-40%. However, the level of mycotoxin that can be detected in the model solution is only for the A-type 65%, and for the B-type - 70%. It is concluded that the study aptamers can be used as selective sites for the identification of individual mycotoxins in the screening regime with an overall estimate of the simultaneous availability of a number of their individual variants.

As general conclusion there is possible to the next approvals. Artificial, chemical created selective sites on the basis of template, calixarenes and aptamer technologies can be used in sensor devices and they allow to provide mycotoxin detection among of different environmental objects. But it is recommended to fulfill analysis in screening regime with the possibility to renew transducer surface for multi-time reusing after its preliminary washing by methanol, or acetonitrile. But verification of the screening results there is necessary to do by devices contained selective sites of biological nature, for example, specific antibodies.

Citation:

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